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Prevalence, Impact, and Significance of Botryosphaeria Fungi in Avocado Nurseries and New Plantings in California

Propagating, planting, and growing disease-free avocado (*Persea americana* Mill.) materials are crucial for expanding avocado production areas in California while preventing economic losses. Avocado Branch Canker (ABC) and anthracnose blight, caused by *Botryosphaeria* and *Colletotrichum* species, respectively, are two diseases threatening avocado health and productivity worldwide. Although California anthracnose of avocados —as a disease affecting fruit on the tree, leaves, and twigs —is unusual, ABC is increasingly being recognized as a major issue for avocado production in California. Symptoms worldwide include death of graft union, dieback, and canker. More recently, avocado growers have reported frequent failed graft plants possibly dying due to graft union failure in California avocado nurseries. In addition, cases of initially healthy-looking young avocado trees, dying in the year or a few years after planted, were reported. In some instances, loss can reach 10%.

Past surveys of mature avocado orchards in California have confirmed the widespread occurrence of ABC and that several species in the Botryosphaeriaceae family are the primary causal agents of this disease. These fungi are generally considered to be wound parasites, infecting young and mature trees through pruning and grafting wounds. In addition, other causes such as drought and bark sunburn injuries in commercial orchards can provide optimum conditions for successful fungal infections.

Little information is presently available on the disease etiology and epidemiology of the fungi involved in the decline of grafted avocado plants in nursery and young trees in new plantings. Although the causal fungi may spread from nearby, old, infected tree orchards, circumstantial evidence suggests the potential causal pathogens (*Botryosphaeria* spp.) might have means of spread other than by external inoculum and be present as latent infections (without symptom development) in nursery stocks. Injuries occurring during grafting might provide entry points for initial infections leading to graft union failure in nursery while in newly established orchards, young trees sold by nurseries potentially carrying inocula remain symptomless. These early latent infections would only develop cankers years after planting when the plants undergo abiotic stress, such as drought or sunburn, which provide conditions for successful infections. These reports are consistent with ABC epidemiology in avocado productions in Israel, where a spate of canker outbreaks in young avocado orchards was found to be caused by *Botryosphaeria* species and originated from nurseries.

Following meetings with avocado nursery managers, consultants, and representatives from the California Avocado Commission, our plant pathology group at the University of California, Kearney Agricultural Research and Extension center (UC, KARE, Parlier, CA), received funding from the Commission to assess the phytosanitary status of avocados in California propagation nurseries and newly established groves. The overall goal was to gain insights about the modes and times of infection, sources and movement of pathogen inocula in nurseries, potential significance of nursery inoculum in ABC epidemiology in young orchards, factors triggering symptom initiation and expression in the field, and eventually develop appropriate integrated management practices that must be initiated at the nursery level. The lack of products registered for use against ABC in avocado nursery and orchards is a serious concern for California avocado farmers and nurserymen. Hence, preventative measures are critically needed to optimize the production of disease-free plants in nurseries and orchards, and ideally Integrated Pest Management (IPM) practices should include chemical control for grafting and pruning wound protections.

The specific objectives of this project, supported by the California Avocado Commission, were to survey avocado nurseries and newly established orchards and: 1) investigate the occurrence and extent of ABC and anthracnose problems during propagation in nurseries and young orchards; 2) determine whether or not latent infections occur in young asymptomatic plants and the potential sources of inoculum in nurseries and young groves; 3) identify the causal agent(s) in diseased materials in nurseries and infected tissues in young trees in orchards and assess the diversity and genetic relatedness of the recovered fungal species; 4) test the susceptibility of avocado scions and rootstocks to selected species of *Botryosphaeria* present in orchards and nurseries; 5) assess the impact of heat and water stresses as contributing factors in ABC disease expression; and 6) conduct laboratory, lathhouse, and field trials to test the efficacy of chemical and physical control products to protect wounds against pathogens causing ABC in nurseries and trees against sunburn injury in the field, respectively.

Phytosanitary Status of Avocado Propagation and Planting Materials from Nursery.

A preliminary survey of avocado nurseries was initiated to determine the possible stages of initiation and extent of ABC and anthracnose infections in symptomatic avocado materials used during the propagation process in nurseries, and whether or not latent infections occur in avocado asymptomatic propagation materials that eventually can serve as sources of inoculum for infections in grafted plants and young trees. Healthy and diseased seeds and grafted plants were collected from nurseries and sanitary analysis was performed to determine the associated fungi. Nursery graft samples were classified based on visual symptoms into a severity scale **(Table 1; Figure 1)**. Healthy liners of various scions (Hass and GEM) and rootstocks (Toro canyon, Duck 7 and Dusa), budwoods from avocado mother trees (Hass, GEM, Toro Canyon, Duck 7, and Dusa), and two-year-old potted avocado trees (Hass and GEM on Toro canyon, Duck 7, or Dusa) were provided by cooperating nurseries. Wood pieces or sections from asymptomatic tissues and a margin of active necrosis of the above seeds, grafted plants, liners, and graft union of potted trees were superficially disinfected using 10% commercial bleach and plated on acidified

Table 1. Symptom's severity scale of asymptomatic and symptomatic avocado graft plants.

Necrosis location	Description
1	Healthy. Control.
2	Necrosis progressing from the scion. Sharp margin. Healthy scion proximal end, graft and rootstock.
3	Necrosed scion. Healthy graft and rootstock.
4	Necrosed graft.
5	Complete necrosis. Graft + scion + rootstock.

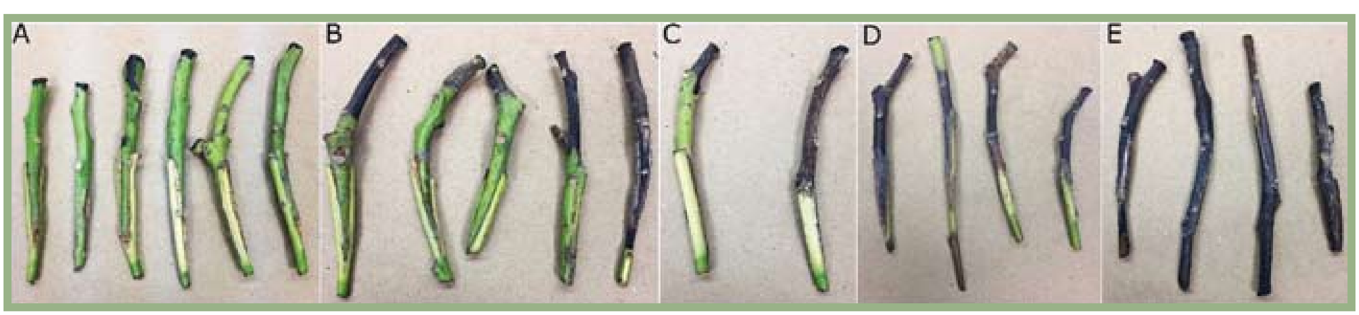

Fig. 1. Avocado necrotic symptoms severity scale. A: 1 in the severity scale, B: 2, C: 3, D: 4 and E: 5.

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

potato dextrose agar plates **(Figure 2)**. Sanitary analysis was performed to determine if any/which pathogen(s) were present in the above asymptomatic and symptomatic avocado tissues. Growing fungi were identified by genus three to seven days after incubation at 25˚C and their incidence in the surveyed materials subsequently calculated.

Sanitary analyses of seeds and grafted plants showed that *Fusarium* sp., *Alternaria* sp. and *Colletotrichum* sp. were the most frequently isolated fungal genera in levels 2, 3 and 4 of the severity scale, while *Botryosphaeria* sp. were most prevalent in grafts of severity scale 2 **(Figure 3)**. Although the main pathogen recovered from decayed seeds was *Geotrichum candidum*, it seems that seeds also can be contaminated by Botryosphaeriaceae fungi (*B. dothidea* or *L. theobromae*) that easily develop on mature avocado fruit. Interestingly, we did not recover any *Colletotrichum* sp. from seeds.

Sanitary analyses of healthy and diseased avocado liners showed that *Botryosphaeria* sp. and *Fusarium* sp. can exist as latent infections in asymptomatic samples. Both *Botryosphaeria* sp. and *Fusarium* sp. also were the fungi mostly isolated from symptomatic twigs **(Table 2)**.

Plating of wood pieces collected from healthy budwood of avocado scion and rootstock mother plants showed that no *Botryosphaeria* sp. were recovered from most batches of budwood, except a low incidence observed on GEM **(Table 3)**. In contrast, *Colletotrichum* sp. were consistently isolated from all batches of budwood, but at low incidences **(Table 3)**. The healthy-looking budwoods used for grafting underwent a bleach-sterilization step, as a routine procedure performed in the nursery, and explained the low levels of budwood contamination by *Botryosphaeria* and *Colletotrichum* fungi. Although we did not visit these orchards, sources of inoculum in mature mother trees are likely present consisting of fruiting structures of *Botryosphaeria* that can produce spores contributing to the aerial inoculum present in orchards.

Among 37 wood pieces gathered from the graft union region **(Figures 4A, B)** of potted avocado trees (Hass-Duke7 combination), 43.2%, and 5.4% of samples from the scion (Hass) part yielded *Botryosphaeria* and *Phomopsis* fungi, respectively. Whereas from 20 pieces of Duke7 rootstock tissue *Botryosphaeria, Phomopsis*, and *Fusarium* sp. were recovered from 25%, 5%, and 5% of pieces, respectively. This showed the

Table 2. Incidence of fungi recovered from avocado liners.

Avocado Liners	Material/ Organ	Symptom	#Pieces	# Pieces yielding specified fungi				
				Botryosphaeria	Colletotrichum	Phomopsis	Fusarium	Alternaria
Hass on Dusa (Cheravo seed)	Scion/Twig	Diseased	10	0	0	0	8	2
		Healthy	30	0	0	0	0	1
Hass on Toro canyon (Criollo seed)	Scion/Twig	Diseased	10	0	2	1	2	0
		Healthy	30	0	1	0	0	0
GEM on Toro canyon (Criollo seed)	Scion/Twig	Diseased	10	8	0	0	0	1
	Rootstock/ Twig	Healthy	20	3	0	0	0	0
	Scion/Twig	Healthy	30	1	0	0	0	0
Ungrafted clonal Dusa on Standard (Criollo)	Scion/Twig	Healthy	40	0	0	0	0	0
Ungrafted clonal Toro Canyon on Standard (Criollo)	Scion/Twig	Healthy	50	1	0	0	7	0

Table 3. Incidence of fungi recovered from avocado budwood collected from mother trees of different scions and rootstocks.

Cultivar / Rootstock *	Total tissues with colonies	Pathogen Incidence (%)								
		Botryosphaeria	Colletotrichum	Phomopsis	Fusarium	Alternaria	Cladosporium / Penicillium	Neurospora	Aspergillus	Epicoccum
Hass	272	0	2.6	0	0	69.5	15.4	0.4	0	1.5
Duke7	283	0	3.9	0	1.1	70.3	15.5	0.4	0.4	0.4
Dusa	256	0	3.9	0	0	78.9	9.4	0	0.4	0.4
GEM	330	0.3	3.9	0	0	62.7	20.0	1.2	0	3.6
Toro Canyon	314	0	4.1	0	0.3	54.8	25.8	0.3	0	1.9

*budwood was sterilized in bleach as a standard procedure by the nursery.

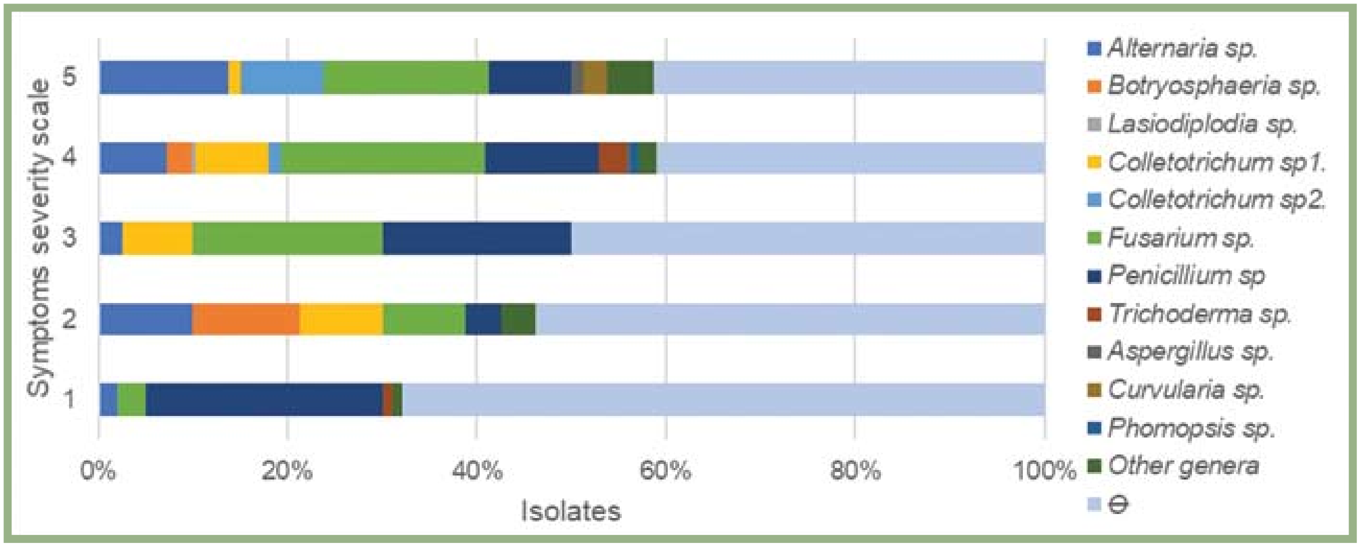

Fig. 3. Isolated genera grafted avocado plants in relation to the necrosis symptoms severity scale.

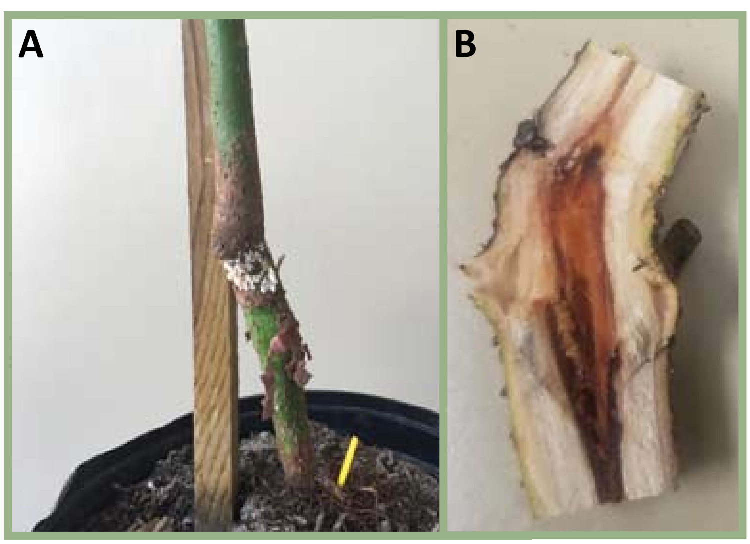

Fig. 4. Problems of infection of the graft union by inoculum contaminating the clonal part of the graft or the edible variety (budwood) (A) and vascular lesion (B).

plants can be infected at the graft union, which probably occurs through the wound created during grafting.

Prevalence of Botryosphaeria and Other Fungi in Newly Established Avocado Orchards in Major Growing Regions of California.

To determine the prevalence of *Botryosphaeria* and *Colletotrichum* in young avocado orchards, infected and symptomless avocado tissues (twigs, grafting union, scion, rootstock, and dead trees with or without sunburn damage) were collected from several young orchards located in San Diego, Riverside, Ventura, and San Luis Obispo Counties

Table 4. Characteristics of the sampled young avocado orchards in major California counties of production.

County	Location/City	Orchard			
		Code/location	Years on the ground	Date of sample collection	Cultivar
San Diego	Fallbrook	BAL-F	~ 1	3/10/2020	GEM
			~ 3		Hass
			~ 3		Bacon
	Escondido	JOH-B	8		Hass (high density)
		JOH-B	7		Hass on Dusa (high density)
	Valley Center	Red Mountain	3	10/30/2019	Hass
	Pauma Valley	Mesa Drive	8 months		Hass
		Starbeam	< 3		Hass
		Old Cole Grade Rd	< 3		Hass
	Fallbrook	AC-FW	~ 3		Hass
Ventura	Santa Paula	Block D, Butler	3	1/15/2020	Hass
		Dom-G	1		GEM on Dusa
Riverside	Rancho	Deluz, Hen-A	2	3/10/2020	Hass on Dusa
		Deluz, Hen-A	5-6		Hass
San Luis Obispo	Morro Bay	POP	1	6/12/2020	Hass

Fig. 5. Young 'Hass' avocado tree with typical branch canker and dieback symptoms in newly established planting.

(Table 4). The orchards varied in age and consisted mainly of Hass, but also GEM or Lamb Hass cultivars **(Table 4)**. Asymptomatic and infected tissues (with or without sunburn damage) **(Figure 5)** were sampled between October 2019 and June 2020. Laboratory analyses of healthy and symptomatic branches and twigs were carried out by plating twig or branch sections on acidified PDA plates. Infections were detected by direct isolation of fungi growing out of the symptomless and infected plant organs. Fungal identity was determined to genus through cultural and morphological characteristics.

Examination of the morphology of the growing fungi isolated from necrotic and cankered branches/twigs and dead trees sampled from the young orchards showed that Botryosphaeriaceae were the fungal pathogens predominantly associated with these symptoms on Hass and GEM across all counties **(Table 5; Figures 6 and 7)**. Other fungi, including species of *Colletotrichum*, *Fusarium*, and

Table 5. Prevalence of fungi recovered from cankered tissues collected from a newly established avocado orchard in Red Mountain, San Diego County.

Pathogen genus	Samples with infection /disease (%)	Orchard #1	
Botryosphaeria	97.6	Plant age group	Year in the ground
Colletotrichum	31.0	Young	3
Fusarium	38.1		
Phomopsis	14.3		
Alternaria	95.2		

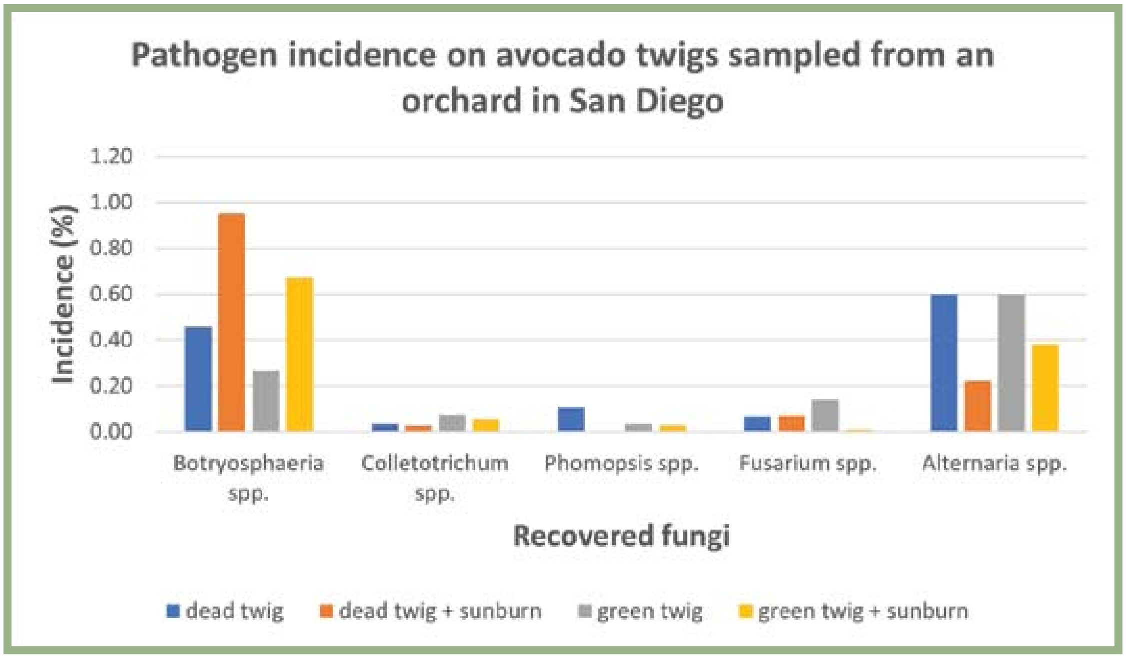

Fig. 6. Incidences of Botryosphaeria, Colletotrichum and other fungi in infected twigs (with or without sunburn damage) collected from a young avocado orchard in San Diego County.

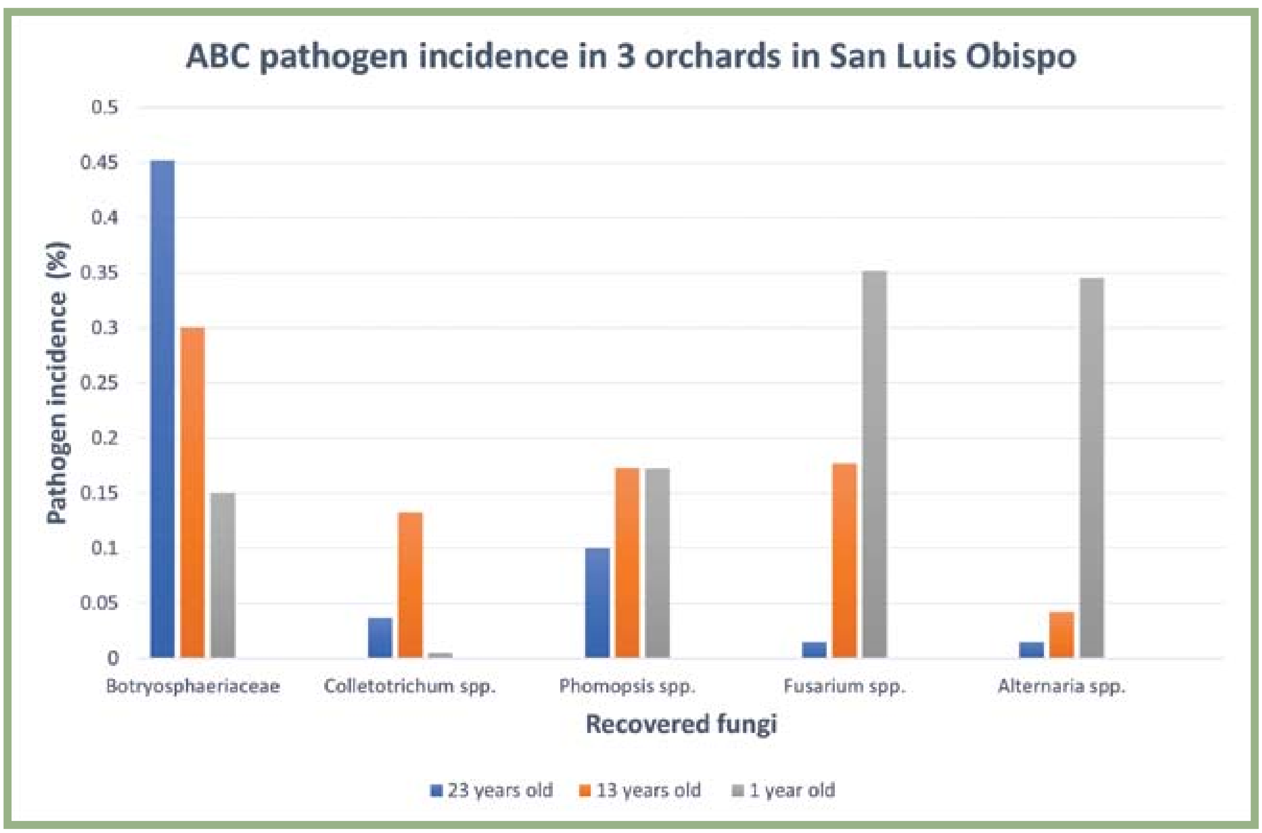

Fig. 7. Incidences of Botryosphaeria, Colletotrichum and other fungi in infected twigs (with or without sunburn damage) collected from a young avocado orchard in San Diego County.

Phomopsis also were isolated from the samples, but at lower proportions. *Botryosphaeria* species were recovered at high frequencies from infected twigs or dead tissues affected or not by sunburn damage, indicating that sunburn injury can trigger *Botryosphaeria* latent infections on the tissues to develop disease symptoms, but it is not required for infections to occur **(Figures 6 and 7)**. Several saprophytic fungi, mainly *Alternaria* spp., also were encountered **(Table 5; Figure 6).**

Species Identity, Diversity, and Genetic Relatedness Among Recovered Fungal Pathogens in Nurseries and Orchards.

Morphological observations of *Botryosphaeria* and *Colletotrichum* isolates recovered from the survey suggest the occurrence of several species. Molecular methods were used to classify the fungal isolates at the species level, analyze their diversity and compare genetic relatedness among the pathogen species recovered from both nursery and orchard settings. Representative isolates of the identified genera were selected for maintenance and mycelia used for DNA extractions. Species identification of *Colletotrichum* and *Botryosphaeria* isolates was determined by sequencing of partial regions of specific genomic regions followed by comparison with established databases.

Molecular identification of the recovered fungi confirmed that several species of *Botryosphaeria* are involved in ABC in both commercial avocado nurseries and orchards throughout California. These species include *Lasiodiplodia theobromae*, *Botryosphaeria dothidea*, and *Neofusicoccum* species (*N. luteum*, *N. parvum*, *N. australe*), the latter group being the most common species throughout the surveyed areas. The molecular techniques revealed the same species were recovered from nursery and young orchards, an additional indication that inoculum in new orchards can originate from the nursery as symptomless infection on young trees, although spread from adjacent cankered old orchards cannot be excluded depending on the site.

Impact of Pathogen on Avocado Scions and Rootstocks.

Pathogenicity experiments, using a mycelium plug inoculation method, were performed in laboratory on excised avocado budwood shoots and in greenhouse conditions on potted (Hass and GEM) avocado trees, providing more informative results on the aggressiveness of selected isolates on the main avocado cultivars and rootstocks. Healthy budwoods originating from mother tree blocks (scions: Hass and GEM; and rootstocks (Toro Canyon and Dusa) were tested for their susceptibility after inoculations with isolates

Table 6. Incidence of fungi recovered from healthy and infected avocado tissues of potted avocado trees.

Avocado plants	Symptoms/ tissues*	Pieces (#)	Number yielding specified fungi							
			Botryosphaeria	Colletotrichum	Phomopsis	Fusarium	Alternaria	Cladosporium	Aspergillus	Penicillium
Hass on Toro Canyon	Infected twigs	100	17	5	6	0	13	0	1	0
	Healthy twigs	170	8	0	5	7	10	0	6	0
Hass on Dusa	Healthy twigs	150	2	0	1	7	10	0	6	1
	Infected twigs	310	46	29	10	6	28	2	10	1
GEM on Dusa	Healthy twigs	100	0	0	1	1	33	0	1	0
	Fruit mummy	10	0	1	0	0	0	0	0	0
	Infected twigs	180	52	27	5	0	17	1	11	0
	Graft union of dead tree	30	12	0	0	2	6	0	0	0
GEM on Toro Canyon	Healthy twigs	120	25	1	0	1	25		0	2
	Infected twigs	60	44	16	0	0	4	0	0	0

*** Infected avocado twigs were recovered from potted avocado trees subjected to water stress.**

of *Neofusicoccum nonquaesitum* and *Lasiodiplodia theobromae*. The same *L. theobromae* isolate was used to assess its virulence on potted trees (Hass, GEM) in the greenhouse. Canker lesion lengths on twigs were recorded two weeks or one month after inoculations.

Pathogenicity, conducted on healthy avocado budwoods originating from mother tree blocks (scions: Hass and GEM) and rootstocks (Toro Canyon and Dusa), showed that two weeks after inoculations with mycelial plugs, all cultivars and rootstocks were susceptible to infections by *Neofusicoccum nonquaesitum* and *L. theobromae*. Inoculations of wounded shoots of potted Hass and GEM avocado trees showed they all appeared to be susceptible to infection by *L. theobromae* following Koch's postulates confirmation.

Effects of Abiotic Stress Factor on Disease Initiation and Expression.

Botryosphaeriaceae fungi can live, grow, and develop asymptomatically as endophytes within the plant tissues, prior to symptom expression. To assess the influence of water stress factor on ABC disease initiation and development, we analyzed its effect in lathhouse experiments. Initially healthy looking young potted avocado trees (GEM and Hass on Dusa or Toro Canyon combinations) were placed near the greenhouse at Kearney Center. A set of trees was continually irrigated for about five weeks while the other set was not. Non-irrigated healthy young potted avocado plants (GEM or Hass on Dusa or Toro Canyon) developed canker and dieback symptoms after the applied water stress event while the irrigated plants did not develop any symptoms. Subsequently, pieces of symptomatic and asymptomatic twigs were plated on acidified Potato Dextrose Agar (PDA) plates followed by isolations of the associated fungi.

Isolations from pieces of asymptomatic twigs revealed that *Botryosphaeria* fungi could be recovered among other pathogens, indicating that they can exist latently in twig tissues prior to exposure to water stress. Infections could be detected by direct isolation in twig or branch tissues collected from trees subjected to water stress, which therefore can induce the disease **(Table 6)**. These results indicate that detection of latent infections is very important in order to define the risk of the disease before the expression of symptoms and thus take the proper actions to alleviate the expression of the disease.

Effects of Selected Fungicides on the Incidence of Botryosphaeria Pathogens.

No fungicides are currently registered for use against ABC pathogens. Different active ingredients found in fungicides target different groups of fungi; so, testing and knowing the ones that are effective against *Botryosphaeria* can guide the selection and development of an efficient chemical control program. Selected fungicides were assessed for their efficacy and utility in protecting grafting and pruning wounds against infection by *Botryosphaeria* and other fungi in nursery and in the field. We first used *in vitro* tests to evaluate the inhibitory activity of selected fungicides belonging to two important chemical groups (Topsin, FRAC# 1; Switch, FRAC# 9 and 12; Rhyme, FRAC#3; and Scholar, FRAC#12) against the mycelial growth of target pathogens using a discriminatory fungicide concentration of 10 ppm. Representative isolates of the identified genera/species (*Botryosphaeria*, *Colletotrichum*, *Phomopsis*, and *Fusarium* species) were selected and used in this preliminary study. PDA plates amended with no fungicides were used as controls. The percentages of mycelial growth inhibition relative to the control were calculated and isolates were then classified as resistant (R) or sensitive (S) to the fungicide. We also performed *in vitro* mycelial growth test on agar media using various concentrations of fungicides belonging to two important chemical groups (i.e., FRAC codes 7 and 11). Furthermore, *invivo* fungicide tests were conducted on potted avocado (Hass on Duck7) trees in the lathhouse at Kearney Center (Parlier, Calif.) and on Hass on Toro Canyon trees located at an experimental orchard (Pine Tree Ranch) in Santa Paula, Calif. Solo fungicide products or fungicide mixtures belonging to key chemical groups (i.e., FRAC codes (FRAC#1 = Benzimidazoles; FRAC#3 = DMI; FRAC#7 = SDHI; FRAC#9 = Anilinopyrimidines; FRAC#11 = QoI; and FRAC#12 = Phenylpyrroles) were tested **(Table 1)**. Attached shoots on potted and planted trees were wounded artificially and fungicides were then applied at maximum label rates as used for *Botryosphaeria* shoot blight control on pistachio. Wounded shoots were inoculated with *Lasiodiplodia* spore suspension 24 h after fungicide sprays. Positive control consisted of wounded shoot inoculated with *Lasiodiplodia* spore suspension while negative control shoots were sprayed with sterile water. Inoculated and non-inoculated shoots were collected after six weeks and three months in lathhouse and field experiments, respectively. Fungicide efficacy was then

Table 7. Chemical and physical products used in lathhouse and field trials.			
Fungicide trade name	**Active ingredient/**	**FRAC group**	**Used in Lathhouse (L) or field (F) experiments**
Water (negative control)	-		L and F
Merivon	Pyraclostrobin+Fluxapyroxad	11/7	L and F
Aprovia	Benzovindiflupyr	7	L and F
Topsin M	Thiophanate-methyl	1	L and F
Approach	Cyproconazole+Picoxystrobin	3/11	L and F
Flint	Trifloxystrobin	11	L and F
Rhyme	Flutriafol	3	L
Switch	Fludioxonil+Cyprodinil	12/9	L
Fontelis	Penthiopyrad	7	L
Scholar	Fludioxonil	12	L
Luna Experience	Fluopyram + Tebuconazole	7/3	L
Luna Privilege	Fluopyram	7	L
Surround® WP	Kaolin	-	L

measured by recording lesion lengths or percent of recovery of the pathogen. Eleven and five fungicide products were tested in lathhouse and field experiments, respectively **(Table 7)**.

Our results from mycelial growth assays using a single discriminatory concentration showed that the four fungicides tested (Topsin, FRAC# 1; Switch, FRAC# 9 and 12; Rhyme, FRAC#3; and Scholar, FRAC#12) were effective against *Botryosphaeria*, *Colletotrichum*, and *Phomopsis* species, but overall, they were ineffective against *Fusarium* species. Results from *in-vitro* mycelial growth at various concentrations of fungicides showed that new SDHI fungicide Aprovia (a.i. benzovindiflupyr; FRAC#7) provides better inhibition of the mycelial growth of *Botryosphaeria* fungi while Luna Privilege (a.i. fluopyram; FRAC#7) seems ineffective. Flint (a.i. trifloxystrobin; FRAC#11) was effective against *Colletotrichum* species.

Our Pine Tree Ranch trial results showed that Topsin M was the most effective fungicide protecting wounds against infection by *Lasiodiplodia* and that it sustained good activity during the duration of the trial. It was followed by Merivon; Flint, Aprovia, and Approach did not perform well in comparison to the control treatment after the trial period. Our lathhouse trial results showed that Topsin M provided the best efficacy, followed by Merivon, Fontelis, and then Flint and Luna Privilege. The remaining products, including Rhyme, Approach, Switch, and Aprovia appeared ineffective, or lost activity with time.

Effect of Kaolin Against Sunburn Injury.

Bark sunburn damage or injuries caused by high temperature and direct solar radiation in tree orchards are known to provide optimal conditions for successful fungal infections. To prevent these heat injury-related infections avocado growers use white paint **(Figure 5)**, which efficacy has yet to be proven; it may be phytotoxic to the avocado plant and with time weather and the pathogen may penetrate through unhealed wounds. Safe and anti-sunburn products such as kaolin clay, which is a natural mineral, have successfully been applied in different tree fruit orchards to prevent sunburn damage. We tested the efficacy of 95% kaolin clay particles (Surround® WP, Engelhard Corporation; applied at 4% concentration) treatments in protecting young avocado trees against sunburn injury **(Table 7)**. Eighteen two-year-old potted avocado (Hass on Duck 7) trees were randomly placed near our greenhouse at KARE center during the summer of 2021 (June to July 2021) in a completely randomized design. Nine random trees were treated with Surround WP at a standard rate of 0.4 kg product to 10 L water while the remaining trees served as untreated controls. Measurements of sunburn damage severity on each tree were determined after five weeks according to a one to four sunburn injury scale.

Our results showed that the preventive application of Surround reduced sunburn damage severity in Surround-treated trees while untreated trees sustained about 50% more damage.

Conclusions and Recommendations.

This study shows that:

1. *Botryosphaeria* (ABC pathogens) and *Colletotrichum* (anthracnose pathogens) can infect avocado plant materials during the propagation process in nurseries. As illustrated in **Figure 8**, latent infections of these pathogens also can take place during the propagation process and potentially serve as a source of inoculum in newly established orchards.
2. ABC pathogens exist in a phase of latency early on in the budwood from mother trees and enter the plants through wounds during the grafting process, which can lead to canker lesions or graft-union failures.
3. These early infections also may stay latent up to several years after the trees are transplanted in the orchard, until the young plantings undergo abiotic stress such as drought and sunburn, which trigger latent infections to become active infections leading to symptom expressions, including the potential killing of the young trees.
4. Surveys of young orchards showed the occurrence and predominance of *Botryosphaeria* species across all sampled counties.
5. Molecular method showed that same fungal species were found in nursery and young planting settings. Thus, nursery inocula can play an important role in avocado canker epidemiology in young orchards.
6. Development of best disease management strategies in nursery and young plantings should include the use of effective fungicides as adequate paints for protecting grafting/pruning wounds against ABC pathogen infection and trees against sunburn injuries.
7. All varieties and rootstocks appear susceptible to ABC after wounding.
8. Our fungicide efficacy trials on attached shoots in lathhouse and field conditions identified Topsin as an effective fungicide for protecting pruning and/or grafting wounds, but activity can be reduced or lost with time. It also will depend on the initial pathogen inoculum dose.

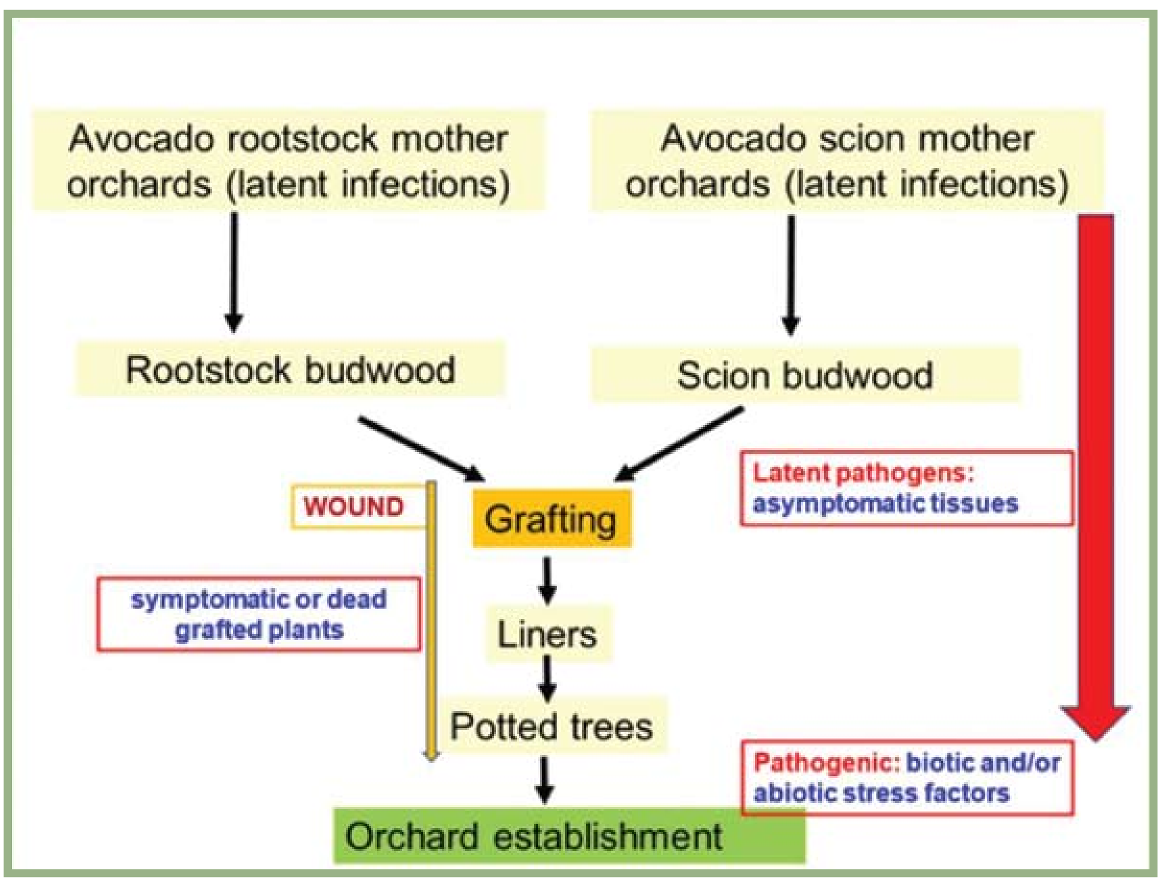

Fig. 8. Avocado nursery production process and potential sources of inoculum and point of infections.

9. Surround (Kaolin) is effective in reducing tree sunburn damage.

10. This study provides the groundwork for future chemical registrations/recommendations for managing ABC pathogens, propagating, and growing disease-free avocado materials. Future studies also will test and validate molecular diagnostic procedure for early detection of the latent, asymptomatic stage in avocado plant material and young trees.

11. Adherence to recommended best management practices for the management of ABC pathogens in avocado nurseries and groves should also be followed and include the following:

a. collect budwood from properly managed mother tree orchards (i.e., avoid pruning during or immediately after rain, dew or heavy fog; properly prune dense canopies to increase air flow and reduce humidity);

b. propagate avocado materials under high nursery sanitary conditions;

c. sanitize grafting equipment;

d. treat budwood prior and during propagation with effective fungicides when available, but especially wounds created during grafting;

e. reduce tree stress and maintain trees in good condition through proper irrigation and fertilization practices;

f. make every effort to source good quality water and manage rootzone salinity, especially for new plantings;

g. reduce planting stress of new trees by ensuring new trees are properly planted and irrigated;

h. avoid planting during periods that will cause tree stress — heatwaves, Santa Ana winds, etc.

i. use tree sunburn protectant product like kaolin potentially in combination with an effective fungicide that helps to reduce the incidence of ABC fungi.

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