Prevalence, Impact, Significance, and Chemical control of *Botryosphaeria* fungi in Avocado Nurseries and Newly established Orchards in California.

Project start date: 1 November 2019
Project end date: 31 October 2021. Final project report – **Part 2**.
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: 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 (Avocado Branch Canker, ABC) poses a serious threat to the health and productivity of avocados. Increasing reports of failed young grafts in nursery and of young avocado trees dying in new plantings of California within few years after establishment prompted us to assess the phytosanitary status of propagation materials and nursery trees as well as the prevalence of canker pathogens in young orchards. Wood pieces or sections from asymptomatic tissues and/or margin of active necrosis of avocado seeds, scion and rootstock budwood from mother trees, grafted plants, liners, and graft union of potted trees as well as symptomatic branches of avocado trees collected from 1- to 3-year-old orchards showing canker or dieback symptoms were sampled, superficially disinfected using 10% commercial bleach, and plated on acidified potato dextrose agar (PDA) plates. Our preliminary analyses of the health status of avocado showed that *Botryosphaeria* spp. can exist as latent infections in asymptomatic propagating materials (seeds, budwoods, grafted plants) from California-based avocado nurseries and be ubiquitous in symptomatic tissues (failed graft plants, infected twigs, dead young trees) collected from nursery and young orchards across the main avocado-producing counties. The use of molecular-based identification techniques revealed that the same and common *Botryosphaeria* species were isolated from 1-year-old diseased avocado trees, nursery avocado trees, and the propagation material, an indication that inoculum in new orchards can originate from the nursery as latent infections on young trees, although spread from adjacent cankered old orchards cannot be excluded depending on the site. Pathogenicity trials were conducted by inoculating wounded, excised shoots ('Hass' and 'Gem' scions; 'Dusa' and 'Toro canyon' rootstocks) and one-year-old branches of 2-year-old scion ('Hass' and 'Gem' on 'Dusa' or 'Toro canyon') potted trees, with mycelium plugs from a culture of a *Lasiodiplodia theobromae* isolate. The pathogen caused lesions on all avocado scions and rootstocks while significantly smaller lesions were observed on the non-inoculated control plants. This study indicates the presence of canker pathogens in avocado propagation materials as well as nursery avocado trees. It further shows that wound created during grafting in propagating nurseries provide infection courts that, in optimum conditions, can lead to latent or successful *Botryosphaeria* infections. Our data suggest that nursery inoculum can serve as possible inoculum sources for canker pathogen infection in young orchards, with drought and

bark sunburn injuries in commercial orchards as triggering factors for successful symptom expression. Overall, this study emphasized the importance of implementing ABC management practices in nurseries and new plantings.

Introduction

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. 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 (Avenot et al. 2021; McDonald and Eskalen 2011). 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. Symptoms worldwide include death of graft union, dieback, and canker. More recently, avocado growers have reported frequent failed grafted 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%. 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 suggest that 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 nursey 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 canker 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 (CAC), 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 (IPM) which 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 nursery and orchards, and ideally 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 tree against sunburn injury in the field, respectively.

Materials and methods

Phytosanitary status of avocado propagation and planting materials from nursery.

A preliminary survey of avocado nursery 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, whether or not latent infections occur in avocado asymptomatic propagation materials which 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 nursery and sanitary analysis was performed to determine the associated fungi. Nursery graft samples were classified based on visual symptoms into 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 2-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 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 potato dextrose agar (PDA) plates. 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 to genus three to seven days after incubation at 25C and their incidence in the surveyed materials subsequently calculated.

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**). 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) 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.

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 suggested 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 were determined by sequencing of partial regions of specific genomic regions followed by comparison with established databases.

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, provided more informative results on the aggressiveness of selected isolates on the main avocado cultivars and rootstocks. Healthy budwood material originating from mother tree blocks (scions: Hass and GEM; and rootstocks (Toro Canyon and Dusa) were tested for their susceptibility after inoculations with isolates of *Neofusicoccum nonquaesitum* and *Lasiodiplodia theobromae*. The same *L. theobromae* isolate was used to assess its virulence on potted trees (Hass, GEM on Toro canyon or Dusa) in the greenhouse. Canker lesion lengths on twigs were recorded 2 weeks or 1 month after inoculation.

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 studied 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 were continually irrigated for about 5 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 canker or dieback symptoms. Subsequently, pieces of symptomatic and asymptomatic twigs were plated on acidified PDA plates followed by isolations of the associated fungi.

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 causing diseases in other tree species (Adaskaveg & Michailides, 2021) 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 four 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 percentage 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, *in-vivo* fungicide tests were conducted on potted avocado (Hass on Duck7) trees in the lathhouse at Kearney Center (Parlier, CA) and on Hass on Toro Canyon trees located at an experimental orchard (Pine Tree Ranch) in Santa Paula, CA. 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 6 weeks and 3 months in lathhouse and field experiments, respectively. Fungicide efficacy was then 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**).

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, 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 wound. Safe and anti-sunburn products such as kaolin clay, which is a natural mineral, which treatments have successfully 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 2-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 was determined after 5 weeks according to a 1-4 sunburn injury scale.

Results

Phytosanitary status of avocado propagation and planting materials.

Sanitary analyses of seeds and grafted plants showed that species of the genera of *Fusarium*, *Alternaria* and *Colletotrichum* were the most frequently isolated fungi in levels 2, 3 and 4, of the severity scale, while *Botryosphaeria* sp. were most prevalent in grafts of severity scale 2 (**Figure 2**). Although the main pathogen recovered from decayed seeds was *Geotrichum candidum*, it seems that seeds can also be contaminated by Botryosphaeriaceae fungi (*B.*

dothidea or *L. theobromae*) which 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 liners. Both *Botryosphaeria* sp. and *Fusarium* sp. were also the fungi mostly isolated from symptomatic twigs (**Table 2**).

Plating of wood pieces collected from healthy budwood of mother-plants of avocado scions and rootstocks 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 both water-splashed and aerial inoculum present in orchards.

Among 37 wood pieces the graft unions of potted avocado trees (Hass-Duck7 combination), 43.2, and 5.4 from the scion (Hass) part yielded *Botryosphaeria* and *Phomopsis* fungi while 25, 5, and 5% of the *Botryosphaeria, Phomopsis*, and *Fusarium* sp., respectively, were recovered out of 20 pieces of the Duck7 rootstock. This showed that the plants can be infected at the graft union which probably occur through the wound created during grafting.

Prevalence of *Botryosphaeria* and other fungi in newly established avocado orchards in major growing regions of California.

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 cvs across all counties (**Table 5; Figure 3**). Other fungi, including species of *Colletotrichum*, *Fusarium*, and *Phomopsis* were also 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 3 and 4**). Several saprophytic fungi, mainly *Alternaria* spp., were also encountered (**Table 5; Figure 3**).

Species identity, diversity, and genetic relatedness among recovered fungal pathogens in nurseries and orchards.

Molecular identification of the recovered fungi confirmed that several species of *Botryosphaeria* are involved in ABC in both commercial avocado nursery 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 commonly found species throughout the surveyed areas. The molecular techniques revealed that 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, conducted on healthy avocado budwood originating from mother tree blocks (scions: Hass and GEM; and rootstocks (Toro Canyon and Dusa) showed that, two weeks after inoculation 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 that they all appeared to be susceptible to infection by *L. theobromae* following Koch's postulates confirmation.

Effects of abiotic stress factors on disease initiation and expression.

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.

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) provided better inhibition of the mycelial growth of *Botryosphaeria* fungi while Luna Privilege (a.i. fluopyram; FRAC#7) seemed 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 sustained a good activity during the duration of the trial. It was followed by Merivon; Flint, Aprovia, and Approach, which 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.

Effect of kaolin against sunburn injury.

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.

Discussion and Conclusions.

This study showed the presence of canker pathogens in avocado propagation materials as well as nursery avocado trees and further indicated that wound created during grafting in propagating nurseries can provide infection courts that, in optimum conditions can lead to latent or successful *Botryosphaeria* infections. Our data suggest that nursery inoculum can serve as possible inoculum sources for canker pathogen infection in young orchards, with drought and bark sunburn injuries in commercial orchards as triggering factors for successful symptom expression. In nurseries, Botryosphaeriaceae infections of propagating grape and blueberry plant material may take place

in the field and during propagation; these species have been isolated from asymptomatic nursery plants with early infections taking place during the propagation of planting material, staying latent until the plants undergo abiotic or biotic stress (Tennakoon et al. 2017; van Niekerk et al. 2006). These reports along with the present data are consistent with Bot canker epidemiological studies in avocado production in Israel, where a spate of canker outbreaks, especially in young avocado orchards showed that infections caused by *Lasiodiplodia theobromae* originated from the nurseries that supplied the avocado plants. When stem sections were examined, there was extensive staining of the xylem vessels, and these Botryosphaeriaceous species were consistently isolated from symptomatic scion cutting, failed graft unions of avocado plants, and symptomatic tissues of young avocado plantings (Shtienberg, 2019, personal communication).

In summary, this study shows that:

1. *Botryosphaeria* (pathogens causing ABC) and *Colletotrichum* (pathogens causing anthracnose) can infect avocado plant materials during the propagation process in nurseries. As illustrated in **Figure 5**, latent infections of these pathogens can also take place during the propagation process and potentially serve as source of inoculum in newly established orchards.
2. ABC pathogens exist in a phase of latency early on in the budwood material 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 may also 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. Survey of young orchards showed the occurrence and predominance of *Botryosphaeria* species across all sampled counties.
5. A 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 M (thiophanate methyl) as an effective fungicide for protecting pruning and/or grafting wounds, but activity can be reduced or lost with time. It will also depend on the initial pathogen inoculum dose.
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 diseases-free avocado materials. Future studies will also test and validate molecular diagnostic procedures 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 manage 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 material 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.

Acknowledgments

We thank the California Avocado Commission for funding this project and special guidance by Dr. Tim Spann. We thank the avocado growers for extending their kind cooperation and permission to survey their nursery and orchards, nursery and field managers for their kind assistance, and Alexander Tako, Daina Grinbergs, and Giorgio Gusella for their technical assistance. We also thank Brokaw nursery for donating a large number of trees for stress and fungicide inoculation studies.

References

Herve Avenot, James Davis, Ben Faber, Christopher Greer, Mary Lu Arpaia, and Themis J. Michailides. 2021. New Findings on Botryosphaeria Branch Canker and Dieback of Avocados in California. 'From the Grove'. Fall 2021.

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

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.

K. M. S. Tennakoon, et al. "Botryosphaeriaceae Species Associated with Blueberry Dieback and Sources of Primary Inoculum In Propagation Nurseries In New Zealand." European journal of plant pathology, v. 150,.2 pp. 363-374. doi: 10.1007/s10658-017-1283-9

van Niekerk, J. M., Fourie, P.H., Haleen, F., and Pedro, W.C. (2006). *Botryosphaeria* species as Grapevine Trunk Disease Pathogens, Phytopathologia Mediterranea, 45 (Supplement), S43-54.

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

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

*budwoods were sterilized in bleach as a standard procedure by the nursery.

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 Rootstock
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

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 group age	Year on the ground
Colletotrichum	31.0	Young	3
Fusarium	38.1		
Phomopsis	14.3		
Alternaria	95.2		

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.

Table 7. Chemical and physical products used in lathhouse and field fungicide trials.

Fungicide trade name [x]	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

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

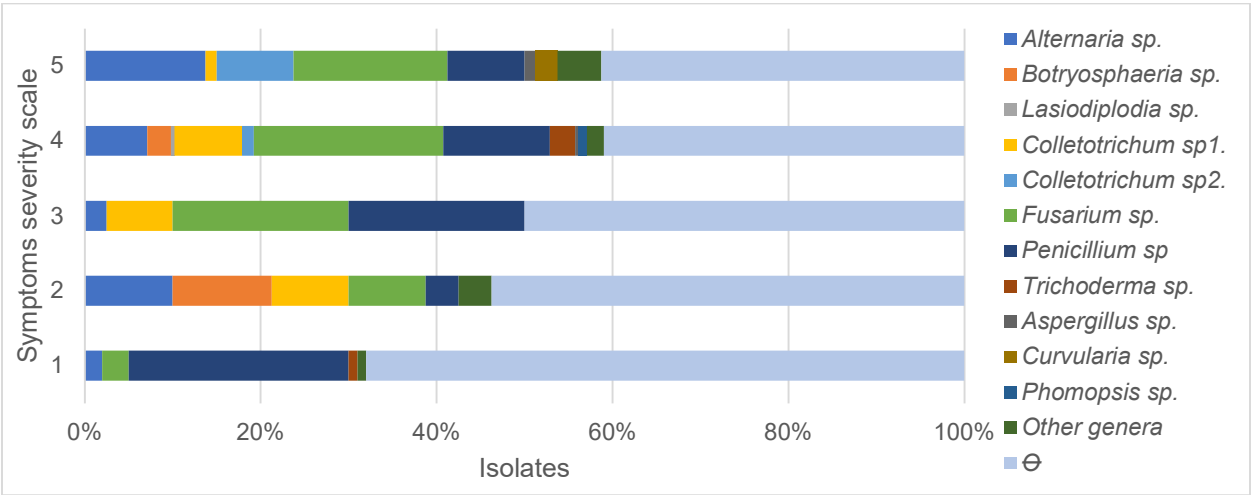

Fig. 2. Fungal genera isolated from grafted avocado plants in relation to the necrosis symptoms severity scale.

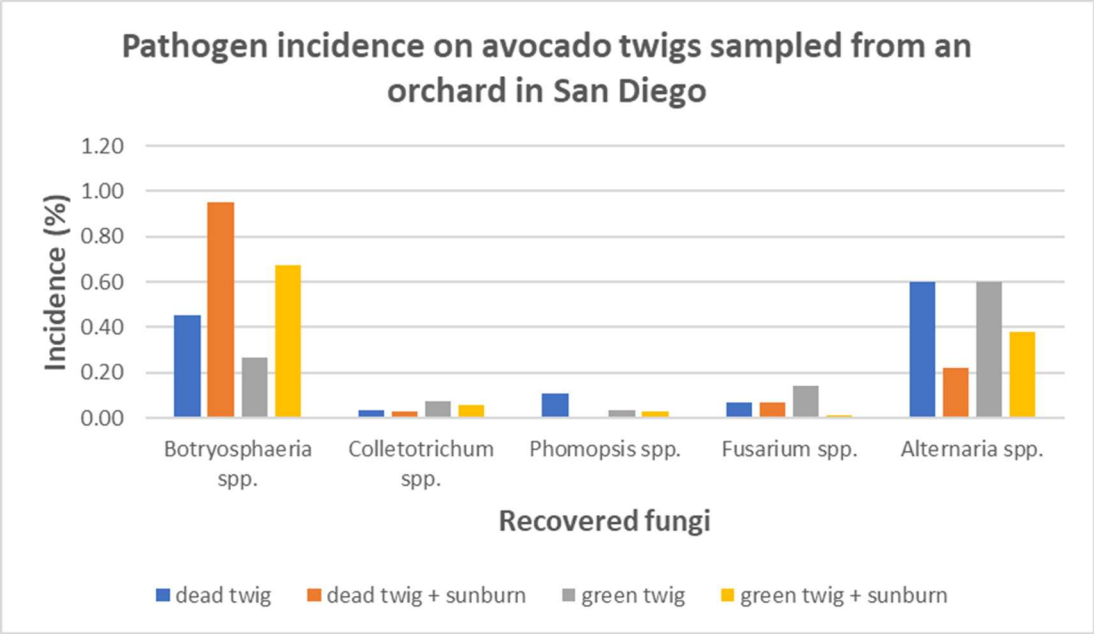

Fig. 3. Incidence of *Botryosphaeria, Colletotrichum* and other fungi in infected twigs (with or without sunburn damage) collected from a young avocado orchard in San Diego County.

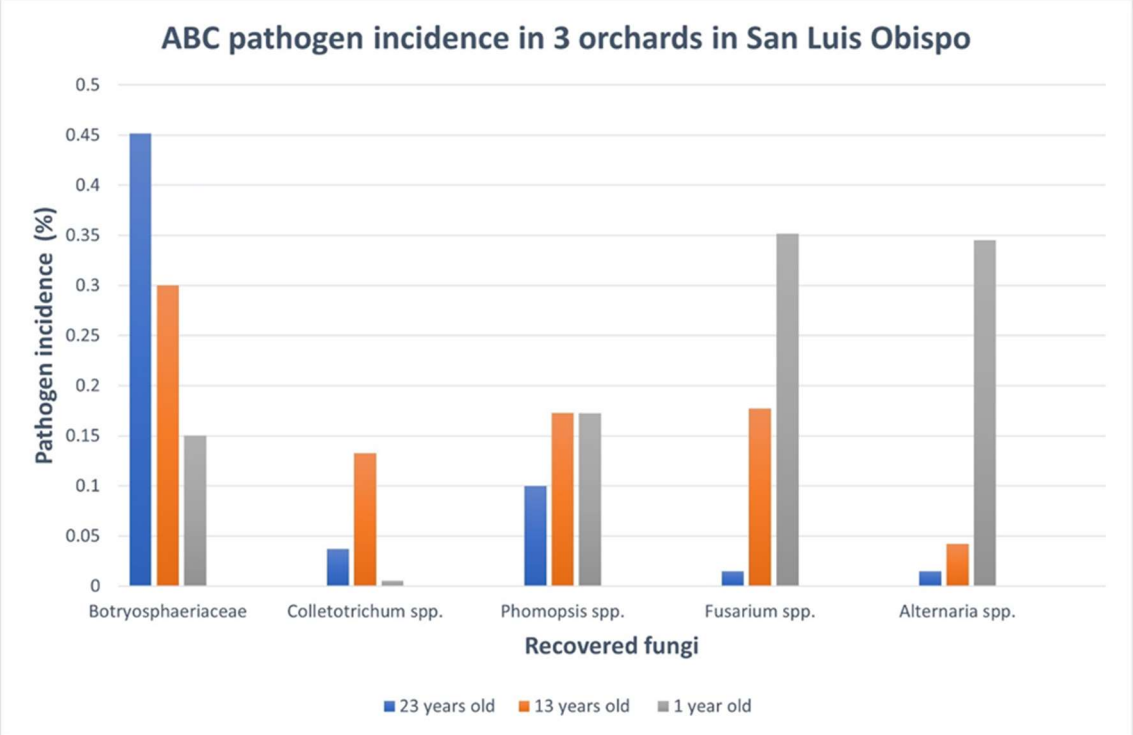

Fig. 4. Incidence of *Botryosphaeria, Colletotrichum* and other fungi in infected twigs (with or without sunburn damage) collected from a young avocado orchard in San Diego County.

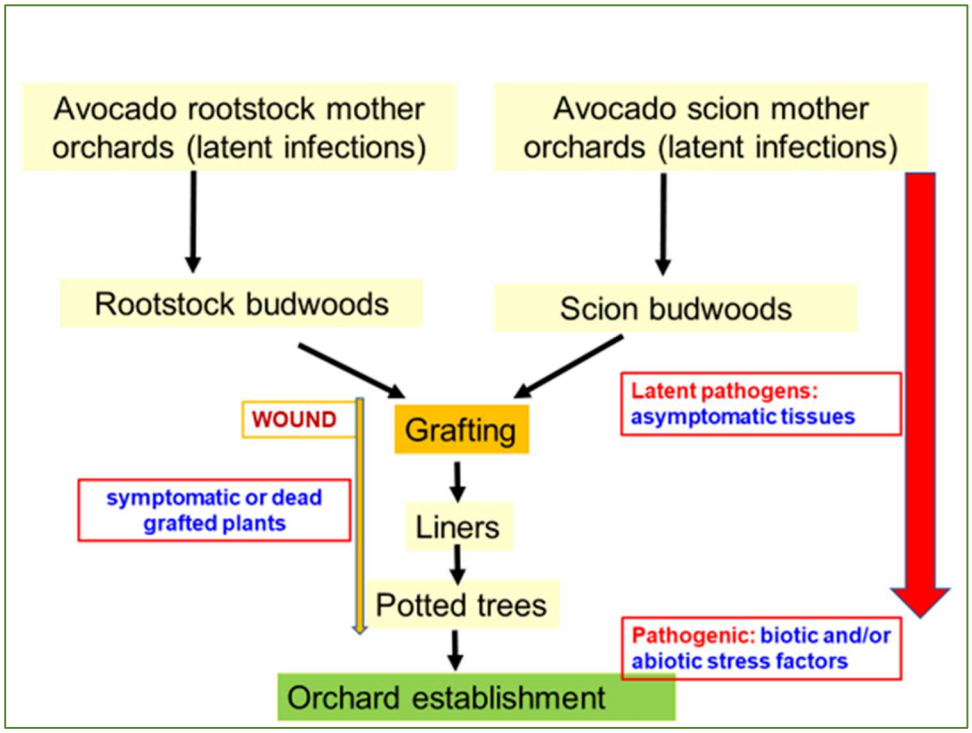

Fig. 5. Avocado nursery production process and potential sources of inoculum and point of infections.