

**Avocado Infection(s)**  
**by**  
**Botryosphaeria Branch Canker and Dieback**  
**and by**  
**Anthraco nose Blight Diseases**

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## Executive Summary

Avocado is one of the most influential crops to California's economy. However, it is susceptible to Anthracnose blight and Botryosphaeria branch canker and dieback diseases, caused by *Colletotrichum* spp and *Botryosphaeria* spp., respectively. In order to apply appropriate control measures to reduce crop losses, it is vital to have an understanding of the etiology and epidemiology of these pathogens (Avenot et al., 2019). For these reasons, samples were taken from avocado groves in the main counties of California where avocados are grown. These include San Diego, Riverside, Ventura, Santa Barbara and San Luis Obispo (Appendix 1). From these samples, isolations were made from various avocado tissue to identify the most common pathogen species involved, and then which ones are more aggressive to avocado.

## Project Objectives

Botryosphaeriaceae family members is a complex of fungal pathogens that causes branch canker and dieback in avocados.

In addition to the Botryosphaeriaceae family, *Colletotrichum* spp., specifically *Colletotrichum gloeosporioides*, are typically postharvest pathogens that can cause avocado fruit rot, postharvest. It also occurs in orchards, as a causal agent of anthracnose on leaves and fruits, mostly as latent infections (Avenot et al., 2019). *Botryosphaeria* and *Colletotrichum* spp. can attack the host tree through pruning wounds, natural and or mechanical wounds. Environmental stresses, such as drought, hail, freeze and sun damage, can also increase tissue weakness thus exacerbate the plant susceptibility to the pathogens. In addition, the pathogens have the ability to penetrate and infect healthy tissue through the formation of appressoria.

Anthracnose symptoms can occur on any part of the avocado tissue. Unhealthy or dead leaves are the most visible symptom (Figure 2B) but spots on leaves and necrosis on fruit and twigs are other common symptoms (Figure 2A).



**Figure 2A.** Left photo shows lesioned fruit caused by *Colletotrichum* spp.

**Figure 2B.** Right photo shows diseased, dying leaves in the field caused by Anthracnose diseases

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From previous isolations conducted by McDonald and Eskalen (2011) it has been found that various species of Botryosphaeriaceae family were consistently present in the margins of these canker. In this project, symptomatic and healthy tissues were collected from the fields and used analyze in the laboratory (1) to determine the extent of Botryosphaeria Canker and Anthracnose diseases, (2) the occurrence of latent infections and potential sources of pathogen inoculum in old and young avocado orchards; to identify the most common and aggressive species of *Botryosphaeria* (and *Colletotrichum*) species present in avocado orchards and (4) to study the life cycle of these pathogens.

### **Project Approach**

Inoculations with artificially-applied and naturally-occurring inocula, were carried out to examine the abiotic conditions for infection. Symptomatic tissues from plants were isolated on potato dextrose agar (PDA) medium and incubated at 30°C for a week.

Asymptomatic tissues from leaves, twigs, fruits, peduncles and pedicels were collected and were examined for latent infections. First, the tissue was sterilized with 70% ethanol and 1% sodium hypochlorite (NaOCl). Then for each sample, two different procedures were performed. The first procedure was plating on acidified potato dextrose agar (APDA) and the second was

incubating the tissue overnight at -20°C (ONFIT) than plating on APDA. For the overnight freezing incubation technique (ONFIT), the tissue was placed in clean crispers with a layer of autoclaved water at the bottom and incubated at room temperature for 1-3 weeks. After the incubation period was over, isolations of the fungi grown on the samples were performed by transferring part of the mycelium to APDA, in order to identify potential latent infections.

Artificial inoculations of asymptomatic tissues were done with spore suspension and mycelium plugs of one *Botryosphaeria* isolate (*Lasiodiplodiatheobromae*) and one *Colletotrichum* isolate (*C. gleosporioides*). The samples inoculated with spore suspension were incubated overnight at -20°C and at 25°C for a week. Then, the symptomatic tissue was plated on APDA and incubated for a week.

From APDA plates, mycelium was collected and used for DNA extraction and then in polymerase chain reaction (PCR) protocol in order to identify the fungus at the species level by using specific-specific primers.

### **Project Outcomes**

It was found that *Botryosphaeria* was the primary causal pathogen of avocado canker and dieback in the sampled orchards, while *Colletotrichum* spp. was secondary (Appendix 2). During the study, the extent of Anthracnose disease in avocado orchards, the most virulent and occurring *Botryosphaeria* and *Colletotrichum* species in the branch wounds and the phenology of these pathogens in the fields were investigated in order to clarify their lifecycles and impact of avocado health. Also, the time of sampling and the environmental conditions were considered to identify the most susceptible phenological phases of avocado trees. It is important to know in what conditions during the year the trees are most susceptible to infection as this is important for implementing disease management strategies.

Going into this, I did not know much about the effects of the different species of *Botryosphaeria*, *Colletotrichum* and *Alternaria* in avocado plants. But by the end, I had a minute understanding of the negative effects that fungal diseases can cause not only avocados but in other crops.

### Conclusions

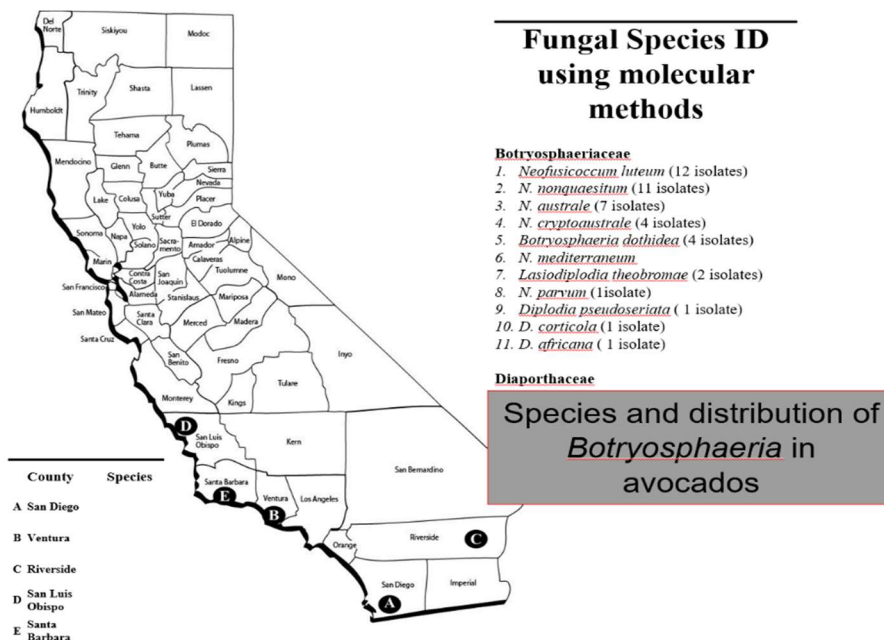
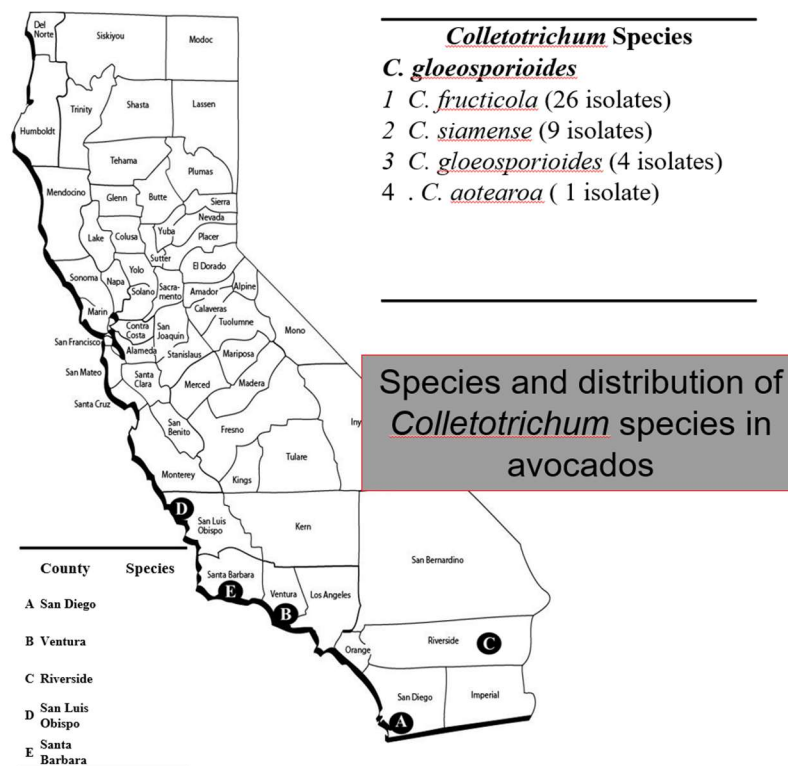
It has been found that different *Botryosphaeria* species are one of the leading causes of branch canker disease in avocados. While *Colletotrichum* spp. is a secondary invader of the xylem tissues being seen mostly post-harvest (Avenot et al., 2019). Molecular identification of the recovered fungi revealed that among *Botryosphaeria* species (*Lasiodiplodiatheobromae*, *Neofusicoccum nonquaesitum*, *N. luteum*, *N. parvum*, *N. australe*, *Botryosphaeria dothidea* ...), found in avocado orchards, *Neofusicoccum* species were the most common species associated with the cankered tissues (Avenot & Michailides, 2019, CAC Annual Report). Among the *Colletotrichum* spp., *C. fructicola* appeared to be the most prevalent one. Results from field survey and pathogenicity experiments pointed out the importance of *Botryosphaeria* species as the main pathological factor in avocado branch canker formation, and the potential role of *Colletotrichum* as secondary invaders of the xylem tissues (Avenot & Michailides, 2019). All recovered *Botryosphaeria* species from our survey were pathogenic on avocado shoots, with *L. theobromae*, *N. nonquaesitum* being the most aggressive species.

Further research is being conducted to identify the most aggressive fungal pathogens, the effects of water stress in accentuating the disease symptom in avocado stems and to test the efficacy of fungicides to control *Botryosphaeria* canker in nurseries and in the field. As well as, developing other methods for managing the diseases.

### **Acknowledgements**

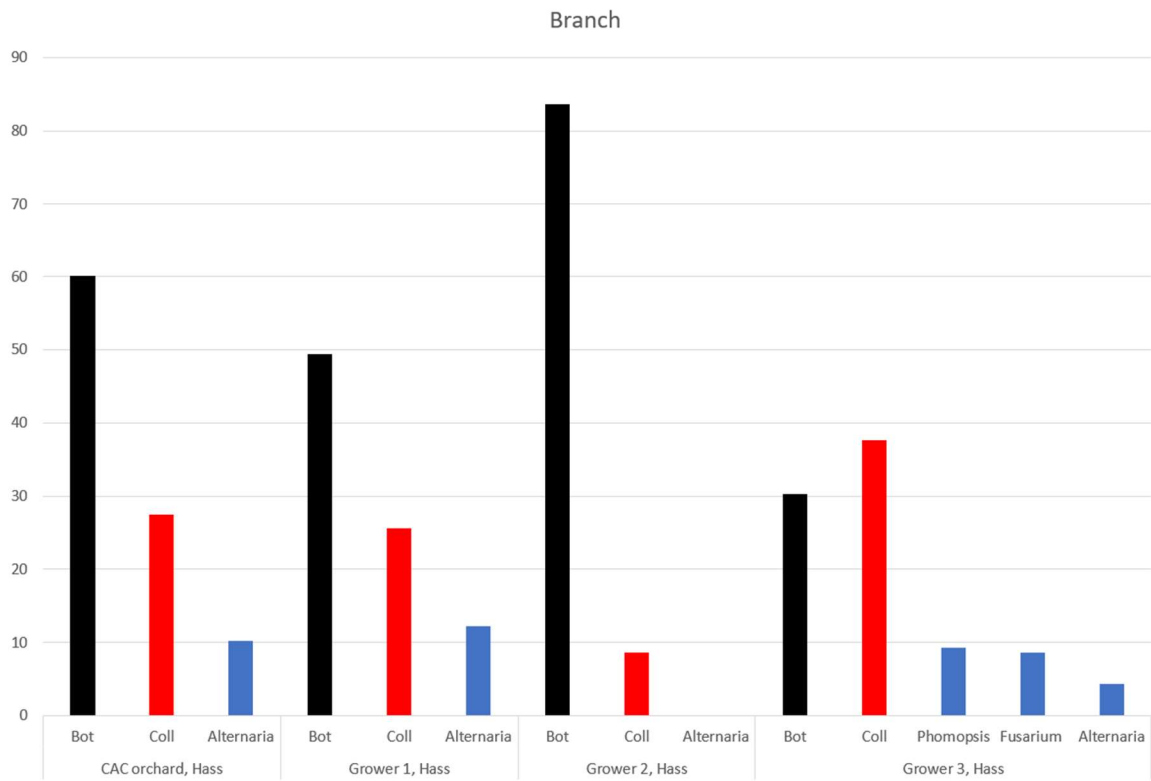
I would like to thank Dr. Themis J. Michailides and Dr. Hervé Avenot for giving me the opportunity to research avocados at the Kearney Agriculture Center. As well as John Lake for guiding me and providing me additional facts about plant pathology. Additionally, this project was supported by Hispanic-Serving Institution's Education Program Grant no. 2015-3842224058 from the USDA National Institute of Food and Agriculture.

## Appendices



**Appendix 1:** Top panel: Map of the counties that contained a sample of different *Colletotrichum* species. Bottom panel: Map of the counties that contained a sample of different *Botryosphaeria* species. Map created by Hervé Avenot.





**Appendix 2:** Results recovered showing that Botryosphaeria is the main invader in twig tissue.  
Graph created by Hervé Avenot.

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