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Geographical Distribution of Botryosphaeriaceae and *Phomopsis/Diaporthe* Canker Pathogens of Avocado in California

Botryosphaeriaceae and *Phomopsis/Diaporthe (P/D)* spp. are fungal pathogens known to cause cankers on a variety of woody hosts such as avocado, grapevine, almond, citrus, cherry, plum, mango, and coast live oak. On avocado (*Persea americana* Mill.), the disease came to be known as Dothiorella canker because the pathogen most often isolated at the time was known as *Dothiorella gregaria* (2). However, new research has shown that avocado canker is due to a complex of fungal species, of which the most common belong to the family Botryosphaeriaceae and to a lesser extent, the genera *P/D*.

Cankers are localized areas of dead bark caused by fungal infection that can occur on trees and other woody hosts (Figure 1). Symptoms of canker disease also include dead or dying shoots and branches, sometimes with dead leaves still attached, scattered among healthy foliage (Figure 2). On trees, cankers are usually found on branches, but can also occur on the trunk. The bark of a tree is composed of several layers of tissue surrounding its woody core. The outer bark consists of corky, dead plant cells which serve as a barrier to reduce water loss and to protect the inner bark tissue and sapwood. When the outer bark is not compromised, it prevents the entrance of most pathogens. Entry points through the outer bark can be caused by injuries such as pruning, grafting, girdling, phosphonate injections or insect wounds, split branches from sunburn, wind or frost damage (8).

Once the outer bark layer is breached by a canker pathogen, other bark tissue, such as the phloem and vascular cambium, can be colonized. The resulting infection can lead to disruption of nutrients, assimilates and water movement

resulting in weakening and decay of the wood at the infection site, which eventually can lead to branch or trunk death (8). The severity of infection is likely to be greater if the tree is already stressed from root rot, drought, flooding, insect attack, nutrient deficiencies or any other factor which weakens the tree.

Cankers often appear as sharply-defined, slightly sunken, depressed areas.

Figure 1. A. Bleeding cankers on avocado tree. B. Branch canker extending into the xylem.

Figure 2. A. Branch dieback B. Canker symptoms on avocado trees.

Cankered tissue is usually darkly discolored by shades of red, brown or black, as compared to the surrounding healthy bark (Figure 3A). The canker may exude a reddish sap, giving it a bleeding, water-soaked appearance, which will gradually dry to a whitish, sticky mass on the bark (Figure 1A). On older, thicker bark areas, surface discoloration may not be visible but the area will be sunken or flattened with the overlying bark appearing cracked. The underlying canker can usually be seen by carefully shaving off the outer bark with a knife (Figure 1B). Cankers tend to be oval or elongated in shape because canker pathogens tend to colonize the bark most rapidly along the main axis of the limb (8, 10). When severe, cankers can extend deep into the xylem, sometimes with a characteristic wedge-shaped discoloration visible on cross section (Figure 3B).

Figure 3. A and B. Cross sections of an avocado branch showing canker symptoms extending into the xylem tissue.

There are two types of fruiting bodies (perithecia and pycnidia), representing the sexual and asexual stages of the fungus, respectively. These structures produce the infective spores and appear as tiny black bumps that protrude from the bark in or around the canker tissue as well as on surrounding dead bark and twigs. In Botryosphaeriaceae, perithecia are known to eject their spores into the air and are known to be wind disseminated. Pycnidial spores ooze out in a ribbon-like gelatinous matrix and are usually disseminated by rain splash (6). Pycnidial spores are far more frequently observed in nature than perithecial spores.

The objective of our studies has been to identify the species of Botryosphaeriaceae and other fungi associated with the avocado canker disease complex in California and evaluate their distribution. Identifying and characterizing the primary causal agents of this disease is needed before developing the appropriate control measures to mitigate the disease and reduce eventual yield loss. Morphological and molecular methods were used to characterize these species.

Current Research and Discussion

Between September 2008 and March 2009, four or five avocado trees (cv. Hass) with branch canker in each of eight avocado groves in five major avocado production areas in California were sampled in order to identify the associated fungi. Botryosphaeriaceae and *P/D* fungi were present in all eight groves sampled and were isolated from 82 and 20 of 187 total cankers, respectively (Table 1).

This corresponded with the results from our spore trap study (Fig. 6-10) which was done to assess the type of wind-disseminated or rain-splashed spores that were present in the groves that could potentially initiate infection on any

open wounds. The most common fungal spores trapped were Botryosphaeriaceae and *P/D* species.

Table 1. Incidence of Botryosphaeriaceae and *Phomopsis/Diaporthe* (*P/D*) spp. isolated from cankers in the main avocado production areas of California, Sep 2008-Mar 2009.

County	No. groves[a]	No. branch cankers sampled	No. (%) Bot.[b]	No. (%) *P/D* spp.[c]
San Luis Obispo	2	46	18 (39)	7 (15)
Santa Barbara	1	48	18 (38)	8 (17)
Ventura	2	71	34 (48)	3 (4)
Riverside	2	12	10 (83)	1 (8)
San Diego	1	10	2 (20)	1 (10)
Total:	8	187	82 (44)[d]	20 (11)[e]

[a]Number of groves sampled.
[b]Number of branch cankers (and percentage of the total number of branch cankers sampled per county) yielding Botryosphaeriaceae.
[c]Number of branch cankers (and percentage of the total number of branch cankers sampled per county) yielding *P/D* spp.
[d]Total number of branch cankers (and percentage of the total number of branch cankers sampled) yielding Botryosphaeriaceae.
[e]Total number of branch cankers (and percentage of the total number of branch cankers sampled) yielding *P/D* spp.

Morphological and molecular methods were used to identify the Botryosphaeriaceae and *P/D* fungi to the species level. Six different Botryosphaeriaceae species and five different *P/D* species were found on avocado in California (Table 2).

Table 2. Number of each Botryosphaeriaceae and *Phomopsis/Diaporthe*[a] species, by county, in the main avocado production areas of California, Sep 2008-Mar 2009.

County	N. australe[b]	N. luteum[c]	N. parvum[d]	New Neo. sp.[e]	F. aesculi[f]	D. iberica[g]	P. theicola[h]	D. phaseo.[i]	D. viticola[j]	D. rhusicola[k]	D. eres[l]
San Luis Obispo	8	0	4	1	3	2	0	1	3	1	1
Santa Barbara	1	17	0	0	0	0	0	2	0	1	0
Ventura	13	9	1	2	8	1	2	1	0	0	0
Riverside	3	7	0	0	0	0	1	0	0	0	0
San Diego	1	0	1	0	0	0	1	0	0	0	0
Total:	26	33	6	3	11	3	4	4	3	2	1

[a]*Diaporthe* is the sexual stage of *Phomopsis*. [b]*Neofusicoccum australe*. [c]*Neofusicoccum luteum*. [d]*Neofusicoccum parvum*. [e]*New Neofusicoccum species*. [f]*Fusicoccum aesculi*. [g]*Dothiorella iberica*. [h]*Phomopsis theicola*. [i]*Diaporthe phaseolorum*. [j]*Diaporthe viticola*. [k]*Diaporthe rhusicola*. [l]*Diaporthe eres*.

Preliminary results from a greenhouse pathogenicity test of these six Botryosphaeriaceae species, and one of the *P/D* species tested, indicated all species were pathogenic on one year-old avocado (*Persea americana* Mill. cv Hass) (Fig. 4). The pathogenicity of the additional four *P/D* species is still to be assessed.

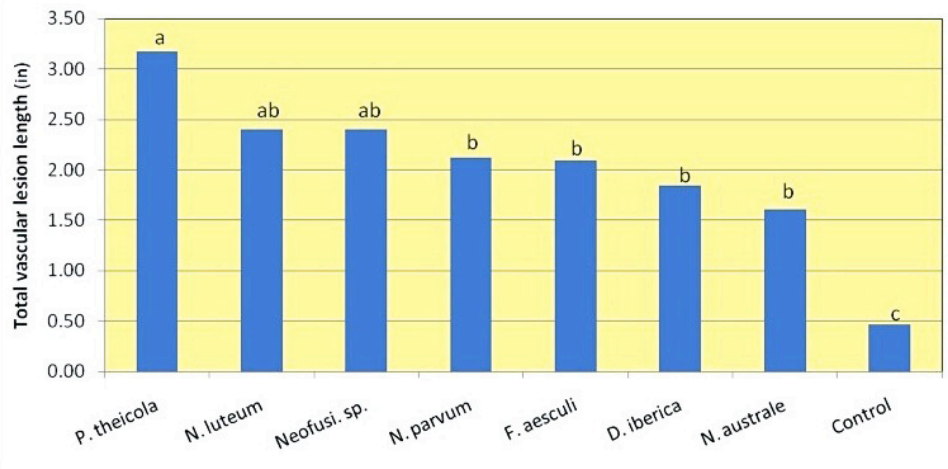

Fig. 4. Mean vascular lesion lengths of six *Botryosphaeriaceae* and one *Phomopsis* species on one-year-old 'Hass' avocado, 2009. Each bar represents the mean of 10 avocado seedlings. Means with the same letter are not significantly different at the 0.05 Probability level. *P. theicola=Phomopsis theicola; N. luteum=Neofusicoccum luteum; Neofusi. sp.=Neofusicoccum species; F. aesculi=Fusicoccum aesculi; D. iberica=Dothiorella iberica; N. australe=Neofusicoccum australe.*

At least two Botryosphaeriaceae species and one *P/D* species were found in all counties sampled in this study (Table 2, Fig. 5). *Neofusicoccum luteum* was the most frequently isolated species from cankers followed by *Neofusicoccum australe, Fusicoccum aesculi, Neofusicoccum parvum, Phomopsis theicola, Diaporthe phaseolorum, Dothiorella iberica,* a new *Neofusicoccum sp., Diaporthe viticola, Diaporthe rhusicola and Diaporthe eres.* Not all species were found in all counties. *N. australe* was found in all five counties. *N. luteum* was found in all but Riverside County. *N. parvum* was found in the northern counties of San Luis Obispo and Ventura counties, in addition to the southern county of San Diego. *F. aesculi, D. iberica* and the new *Neofusicoccum* sp. were found only in the two northern counties of San Luis Obispo and Ventura (Table 2, Fig. 5). *P. theicola*

was found in the Ventura, Riverside and San Diego counties. *D. phaseolorum* was found in the Ventura, Santa Barbara and San Luis Obispo counties. *D. viticola* and *D. eres* were only found in the northern county of San Luis Obispo. *D. rhusicola* was found in San Luis Obispo and Santa Barbara counties (Table 2, Fig. 5).

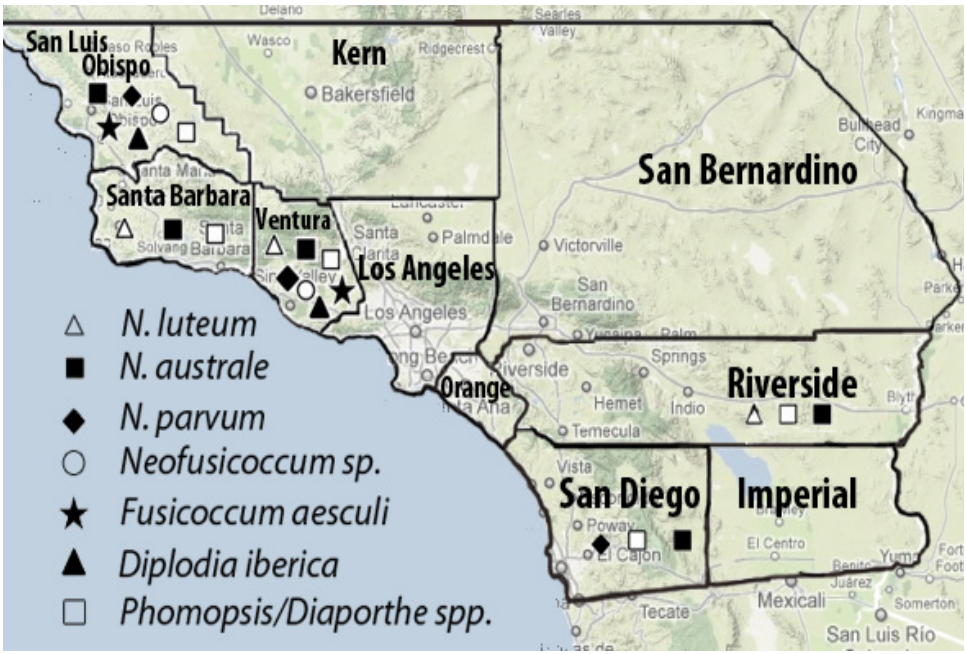

Fig. 5. California counties in which Botryosphaeriaceae and *Phomopsis/Diaporthe spp.* were detected in this study.

The rainy season in California extends from October to April with the heaviest rainfall usually occurring from December to February. Our spore trap studies showed that Botryosphaeriaceae and *P/D* spore release occurred most frequently during and following rain events in California avocado groves (Figs. 6-10). This was consistent with Botryosphaeriaceae spore trap studies in California vineyards which also showed the greatest spore release during and following rain events (9). The effects of irrigation on Botryosphaeriaceae spore release would depend on the type of irrigation system used. A previous study on pistachio showed that the release and dissemination of *Botryosphaeria dothidea* (*Fusicoccum aesculi*) pycnidial spores was triggered by sprinklers with high water throw. By simply lowering the trajectory of the sprinklers, panicle and shoot blight was

reduced (7). In avocado groves, a sprinkler irrigation system that uses sprinklers with horizontal water throw and do not produce significant water splash within the canopy would be recommended to avoid splash dispersal of pycnidial spores.

Humid conditions, such as might occur with fog, can stimulate spores to ooze out from the pycnidia in a ribbon-like structure called a cirrhus (7); however, the actual dispersal of these spores requires the impact of water droplets. A previous study on pistachio showed *B. dothidea* to be a splash-dispersed pathogen (1). These results coincide with our spore trap study in which most of the spores captured were during the rainy season. These results could also indicate that the sprinkler irrigation in the sampled avocado groves had minimal impact on spore release, as the spore numbers were low to zero during the dry season between May to September; however, most of our spore traps were located about 6 ft. above the sprinklers (Figs. 6-10).

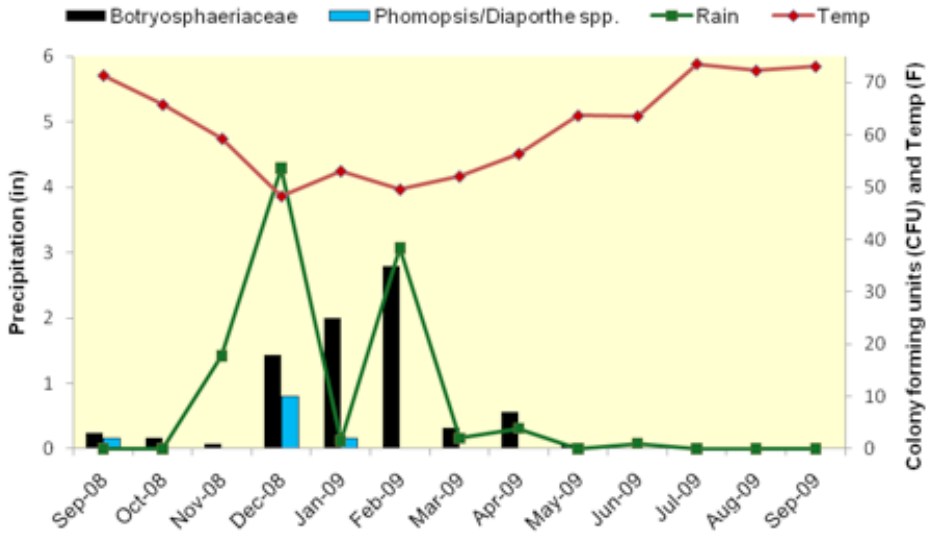

Fig. 6. Air-borne spores of Botryosphaeriaceae and P/D spp., monthly precipitation (inches) and average monthly temperature in Riverside County avocado groves, Sep. 2008 - Sep. 2009.

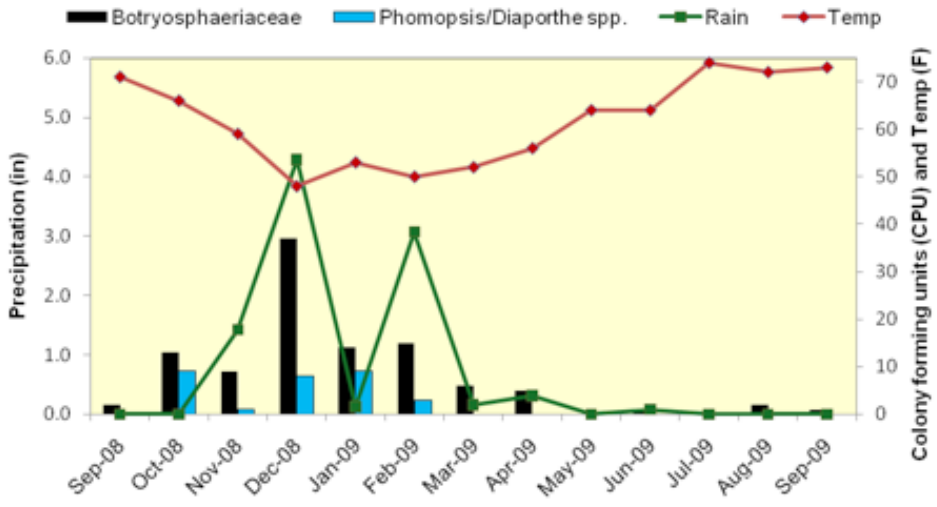

Fig. 7. Air-borne spores Botryosphaeriaceae and P/D spp., monthly precipitation (inches) and average monthly temperature San Diego County avocado groves, Sep. 2008 - Sep. 2009.

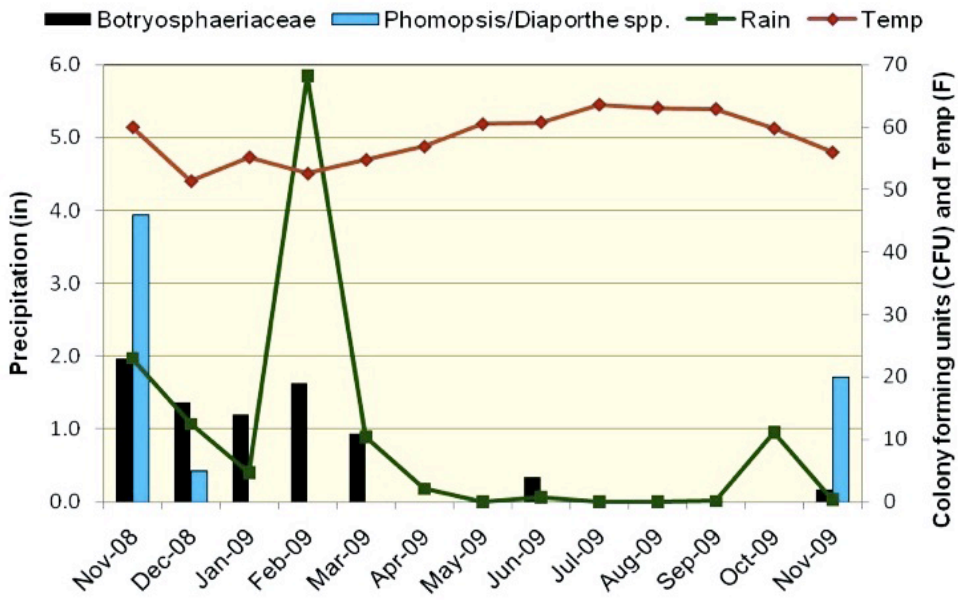

Fig. 8. Air-borne spores Botryosphaeriaceae and P/D spp., monthly precipitation (inches) and average monthly temperature Santa Barbara County avocado grove, Nov. 2008 - Nov. 2009.

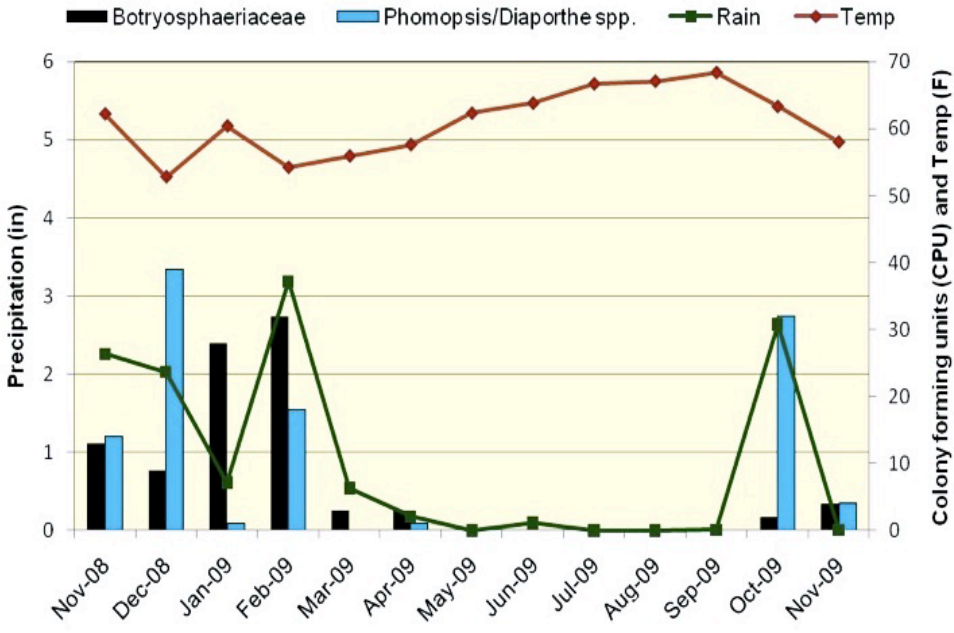

Fig. 9. Air-borne spores Botryosphaeriaceae and P/D spp., monthly precipitation (inches) and average monthly temperature Ventura County avocado groves, Nov. 2008 - Nov. 2009.

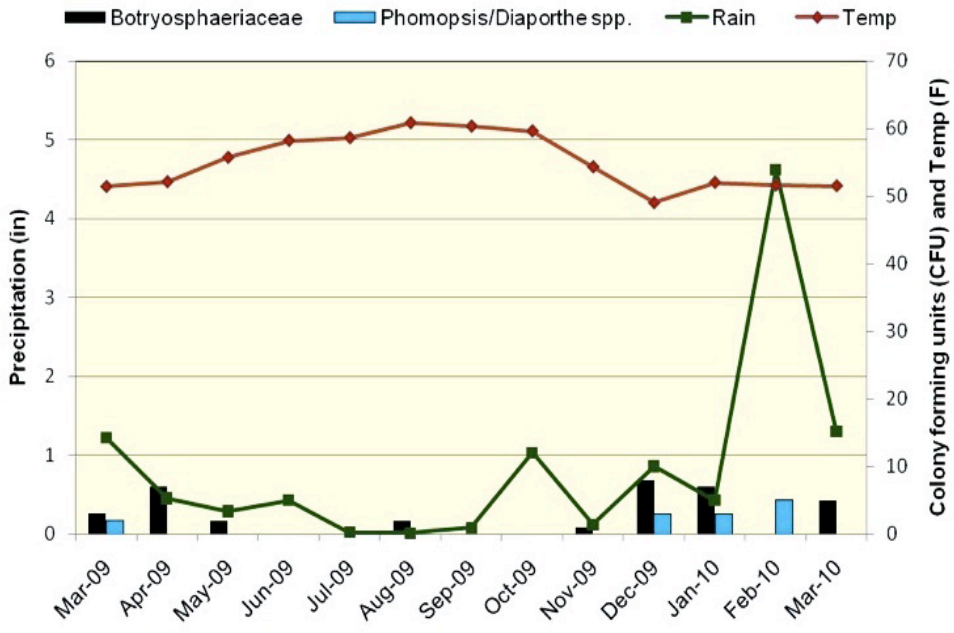

Fig. 10. Air-borne spores Botryosphaeriaceae and P/D spp., monthly precipitation (inches) and average monthly temperature San Luis Obispo County avocado groves, Mar. 2009 - Mar. 2010.

On avocado, common cultural practices which compromise the outer bark and could lead to infection by canker-causing pathogens include pruning, grafting, girdling and injecting. There is increased interest in high density plantings of avocado in California, a planting system already employed in Chile (3, 4). This planting strategy, which decreases the spacing between trees from 20 x 20 ft to 10 x 10 ft., requires more intensive canopy management, thereby increasing the risk for canker development. Grafting of young trees or top-worked stumps can lead to infection, with a canker forming around the graft union. Alternatively, the fungus can remain inside the tree after the graft union heals and suddenly cause collapse when a later stress occurs (6). Girdling is used to increase productivity in many fruit trees and involves cutting through and removing the bark in a circular pattern around the branch (5). This practice is usually done in October and may increase the risk for pathogen infection if the wound does not heal by the onset of the rainy season. Our results indicate that any activity causing mechanical damage to the outer bark during the rainy season and especially in December, January and February should be avoided. In addition, protecting wounds with a fungicide application may help prevent invasion by canker-causing pathogens. Preliminary studies in our lab have shown there are chemicals on the market that are effectively prevent infection by Botryosphaeriaceae and *P/D* spp. In fact, even though there were six different Botryosphaeriaceae species identified in this study, they were all sensitive to the five fungicides we are currently testing in field trials. Upon completion of our field trials we hope to recommend a specific fungicide treatment. We recommend additional cultural practices to avoid infection including:

a. pruning of cankered limbs at least five inches below the infection site;
b. pruning of dead limbs and twigs that carry pycnidia and perithecia (spore forming structure) of these species;
c. sterilization of pruning tools with either 25% household bleach or full strength of Lysol;
d. disposal of dead wood and old fruit beyond the avocado grove sites.

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