Year-end progress report

Agency: California Avocado Commission (CAC) **Agreement No.**
Fiscal Year: 2008-2009 **Project Completion Percentage:** 40%

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Project Title: Identification, biology, epidemiology and geographical distribution of fungal and bacterial pathogens associated with avocado in California

Progress Report (November 2008 – September 2009)

PROJECT OBJECTIVES:
Objective 1: Conventional and molecular based identification and characterization of current fungal and bacterial pathogen populations associated with avocado and their rootstocks in California.
Objective 2: Determine the geographical distribution of fungal and bacterial pathogens on avocado in California.
Objective 3: Determine the pathogenicity and aggressiveness of isolated fungal and bacterial species associated with avocado in California.

PROGRESS:

1. DOTHIORELLA CANKER

Branch and trunk canker on avocado was formerly attributed to *Dothiorella gregaria*, hence the name Dothiorella canker. To our knowledge, *Botryosphaeria dothidea* (anamorph: *Neofusicoccum aesculi*) is the only reported species causing Dothiorella canker on avocado in California. Symptoms observed on avocado with Dothiorella canker include shoot blight and dieback, leaf scorch, fruit rot, and cankers on branches and bark (Fig.1, 2). Identifying and characterizing the causal agents of this disease will assist in applying the appropriate control measures to reduce yield loss. A spore trap study is ongoing in all surveyed groves to assess the spore loads of *Botryosphaeria* spp. and other potential pathogens over several growing seasons to determine when and under what conditions spores are released to re-infect plants.

Materials and Methods

To date, eight avocado groves in five California counties have been surveyed by sampling five symptomatic trees per grove. Symptomatic tissue was plated onto potato dextrose agar amended with tetracycline (0.01%) (PDA-tet) from an average of 50 symptomatic branch and trunk canker samples per tree (250 samples per grove). Spore traps were established within each sampled tree

and are changed every two to four weeks to assess species presence and spore loads in these groves. Morphological and molecular-based identification and characterization of *Botryosphaeria* species and *Diaporthe* sp., the primary causes of Dothiorella canker on avocado in California, were performed and are ongoing.

Fig: 1. Dothiorella branch dieback, canker and stem-end-rot symptoms on avocado.

Summary of results to date

Thus far, multiple species of *Botryosphaeriaceae* and *Diaporthe* sp. have been found to cause the typical Dothiorella canker and stem-end rot on avocado in California. Percent recovery of *Botryosphaeria* spp. based on morphological characters ranged from 40-100% in Riverside county, 42-53% in Ventura county, 33% in Santa Barbara county, 60% in San Diego county and 32-60% in San Luis Obispo county (Fig. 3). A *Diaporthe* sp. was also isolated from 20% of branch canker tissue from a Riverside location and 38% of canker tissue from a San Luis Obispo location. *Diaporthe sp*. is known to cause stem-end rot on avocado and branch cankers on other woody plants (2). To our knowledge, this is the first report of *Diaporthe sp*. causing branch cankers on avocado in California

Molecular methods were used to identify species based on the analysis of the internal transcribed spacer region (ITS) of rDNA. Species identified, based on the nomenclature of Crous et al. (1), include *Neofusicoccum australe, B. dothidea, "Botryosphaeria" iberica, N. luteum, N. parvum, N. ribis, "B." rhodina, "B." stevensii,* and *N. vitifusiforme*. The *Diaporthe* sp. recovered from canker tissue is in a different fungal class from the *Botryosphaeria* and *Neofusicoccum* spp. Using rDNA analysis, the species was identified as *Diaporthe phaseolorum*. The symptoms caused by all these fungi are similar and include shoot blight and dieback, leaf scorch, fruit rot, and perennial cankers on branches and bark.

Koch's postulates were conducted in the greenhouse with six *Botryosphaeriaceae* isolates which included *B. dothidea, N. luteum, N. parvum, N. australe, N. mediterraneum and N. vitifusiforme*. In the greenhouse, four 1-year-old cv. Hass seedlings per isolate were stem-wound inoculated and four seedlings were inoculated with sterile PDA plugs as a control. This inoculation method

was also executed on mature avocado trees at the UCR field plot. After six months, symptoms including leaf scorch and thickening and bulging at the inoculation site were observed. At the inoculation site, wounds increased in size from 2 x 2 mm up to 15 x 13 mm. Sub-surface tissue displayed dark vascular lesions as much as 12.8 cm in length (Table 1). Koch's postulates were completed when each species was consistently re-isolated from inoculated seedlings and mature trees.

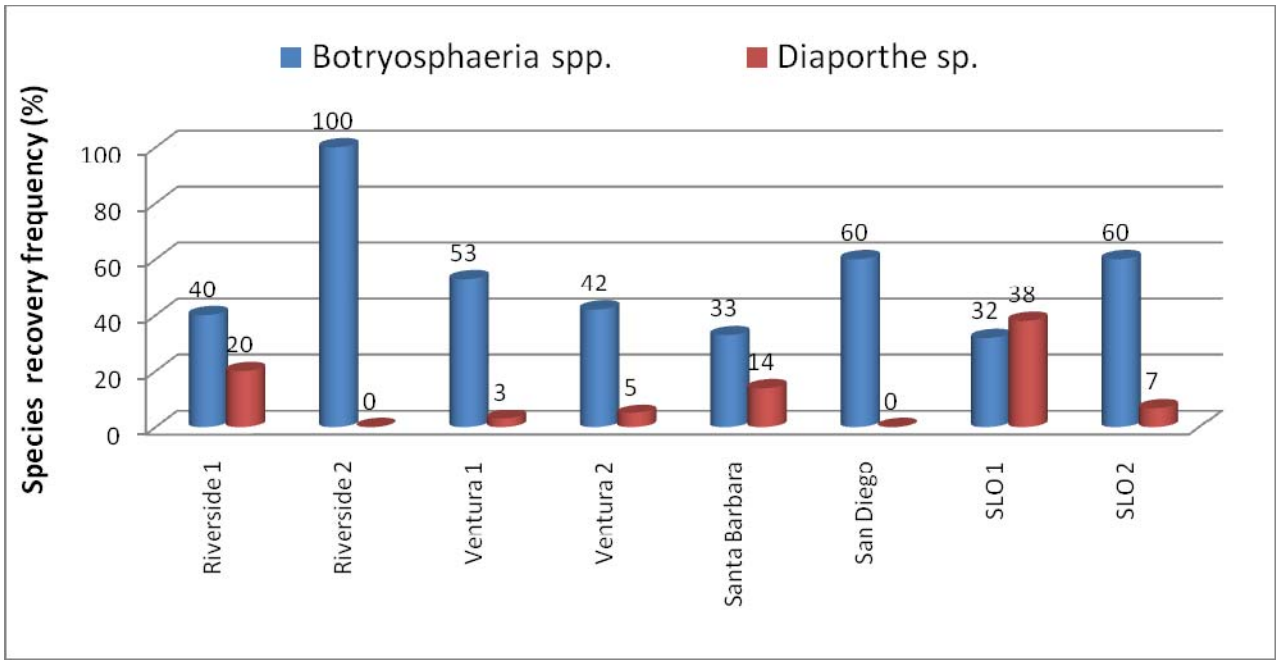
The isolation and identification of three additional *Botryosphaeriaceae* species from California avocado groves constituted a first report and was published in the September 2009 issue of Plant Disease (3).

A more comprehensive pathogenicity test is underway in the greenhouse for the following *Botryosphaeria* and *Neofusicoccum* species identified by analysis of the ITS region of rDNA: *N. australe, B. dothidea, "B." iberica, N. luteum, N. parvum, N. ribis, "B." rhodina, "B." stevensii,* and *N. vitifusiforme*. This pathogenicity test used the same inoculation method as the first test described above, except that there were ten 1-year-old cv. Hass seedlings inoculated per isolate instead of four. This test is currently being taken down and the results should be available soon.

To date, *Botryosphaeria* spp. were the most frequently isolated pathogens from branch and trunk cankers on avocado and *Diaporthe* sp. was isolated less frequently (Fig. 3). In pathogenicity tests, *Botryosphaeria* spp. showed some differences in aggressiveness in greenhouse studies, but were all similarly aggressive in the UCR field study (Table 1). The *Diaporthe* sp. was similarly aggressive as *Botryosphaeria* spp. in both the greenhouse and UCR field plot.

Dothiorella canker pathogens enter the avocado plant through fresh wounds (pruning, frost and mechanical). For the first time in California groves, fruiting bodies (pycnidia and perithecia) of *Botryosphaeria* and *Neofusicoccum* spp. were observed on old diseased avocado tree parts, branches and bark. Airborne spores of *Botryosphaeriaceae spp*. and *Diaporthe sp*. were frequently trapped on spore traps during or after rain events (Fig. 4-8). Based on this evidence, one can conclude that precipitation initiates the release of large numbers of conidia and ascospores from these fruiting bodies. This information can help growers schedule their pruning activities during the drier parts of the year, to avoid inoculation of pruning wounds by these airborne spores. Currently, we are also investigating the most efficacious fungicidal pruning wound protectants to help further reduce the incidence of canker development.

Fig. 3. Pathogen recovery frequency (%) from avocado trunk and branch cankers from eight groves in five California counties, 2008-2009.



SLO = San Luis Obispo

Fig: 2. Dothiorella perennial canker symptoms on avocado.

Table 1. Surface wound expansion and internal lesion length of inoculated avocado seedlings in the greenhouse and mature avocado trees in the field, after six months.[1,2]

	Greenhouse			UCR Field plot		
Pathogen	Surface wound length	Surface wound width	Internal lesion length	Surface wound length	Surface wound width	Internal lesion length
	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)
B. parva	13 a	12 b	65 a	15 a	13 a	101 a
B. australis	8 b	12 b	17 b	10 a	11 a	44 a
N. mediterraneum	11 ab	9 b	39 b	15 a	13 a	128 a
N. vitifusiforme	9 b	11 b	21 b	11 a	13 a	110 a
Diaporthe phaseolorum	12 ab	18 a	68 a	9 a	11 a	103 a

[1]Means followed by different letters within a vertical column are significantly different by Waller's k-ratio t test, *P*=0.05.
[2]Greenhouse and UCR Field data were analyzed separately.

Fig. 4. Air-borne spores trapped in Riverside County avocado grove, Sep. 2008-Sept. 2009.

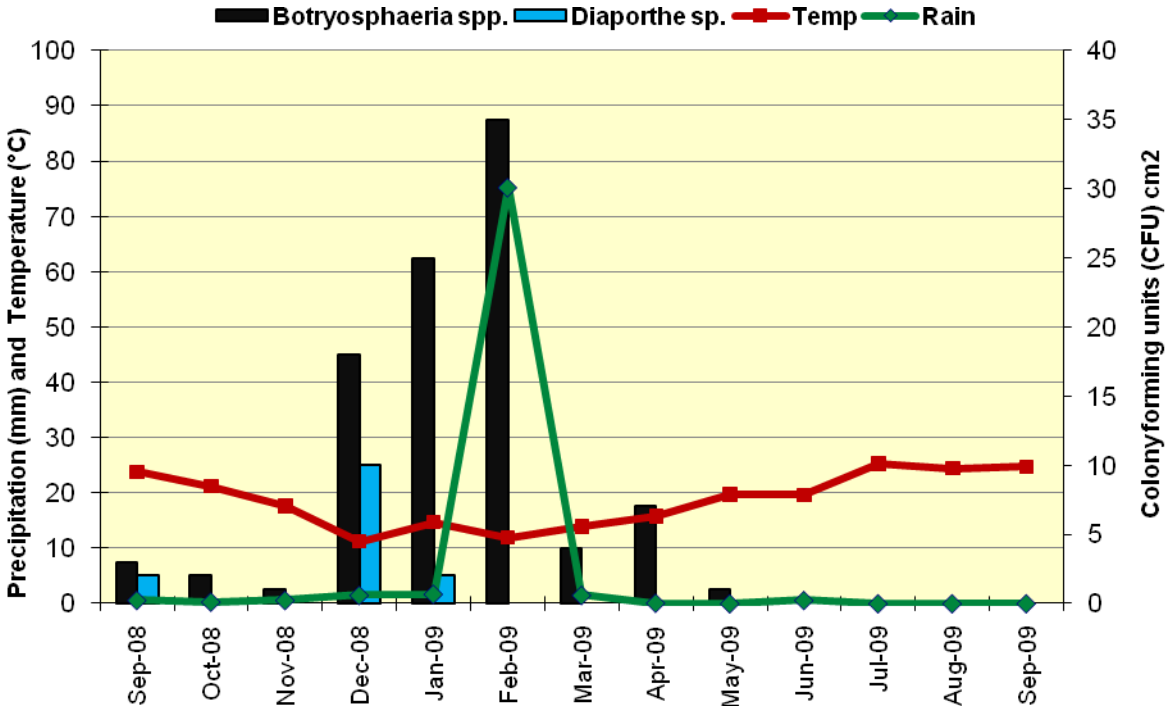


Fig. 5. Air-borne spores trapped in Ventura County avocado grove, Nov. 2008-Sept. 2009.

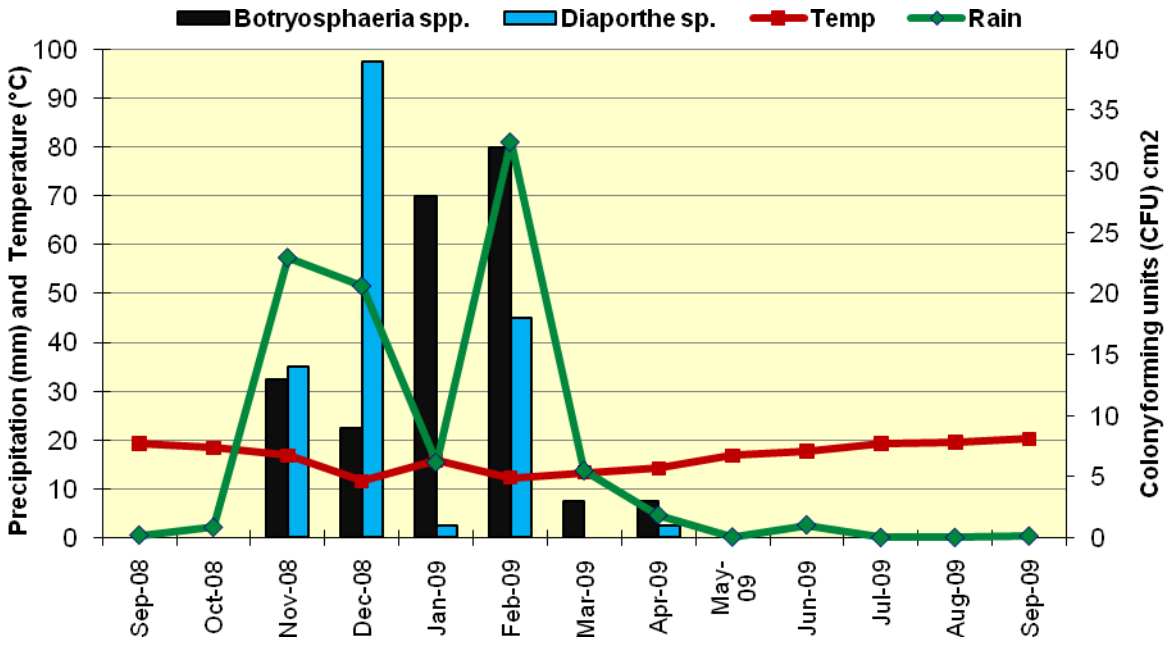


Fig. 6. Air-borne spores trapped in Santa Barbara County avocado grove, Nov. 2008-Sept. 2009.

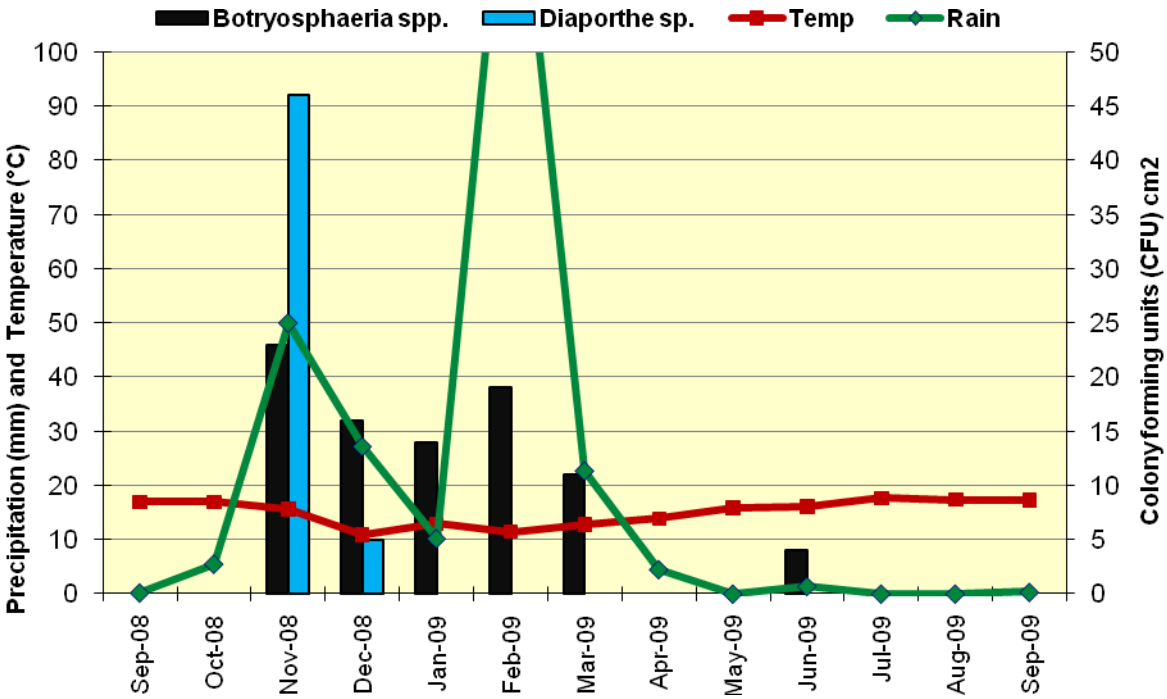


Fig. 7. Air-borne spores trapped in San Luis Obispo County avocado grove, Mar 2009-Sept. 2009.

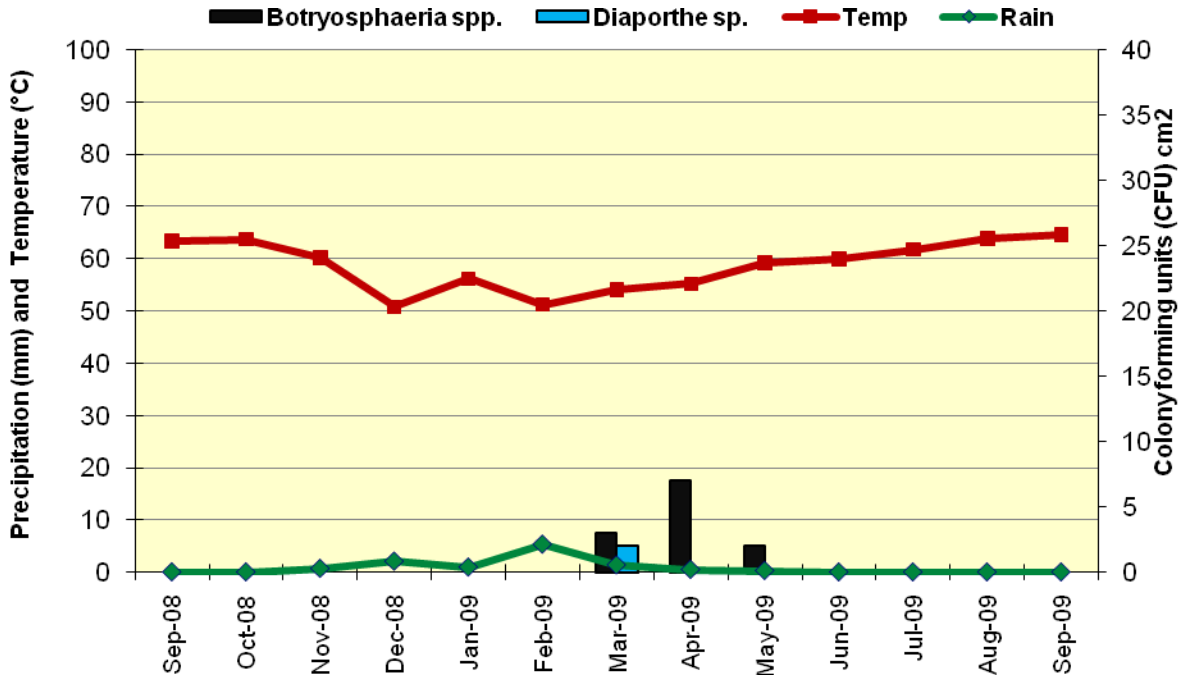
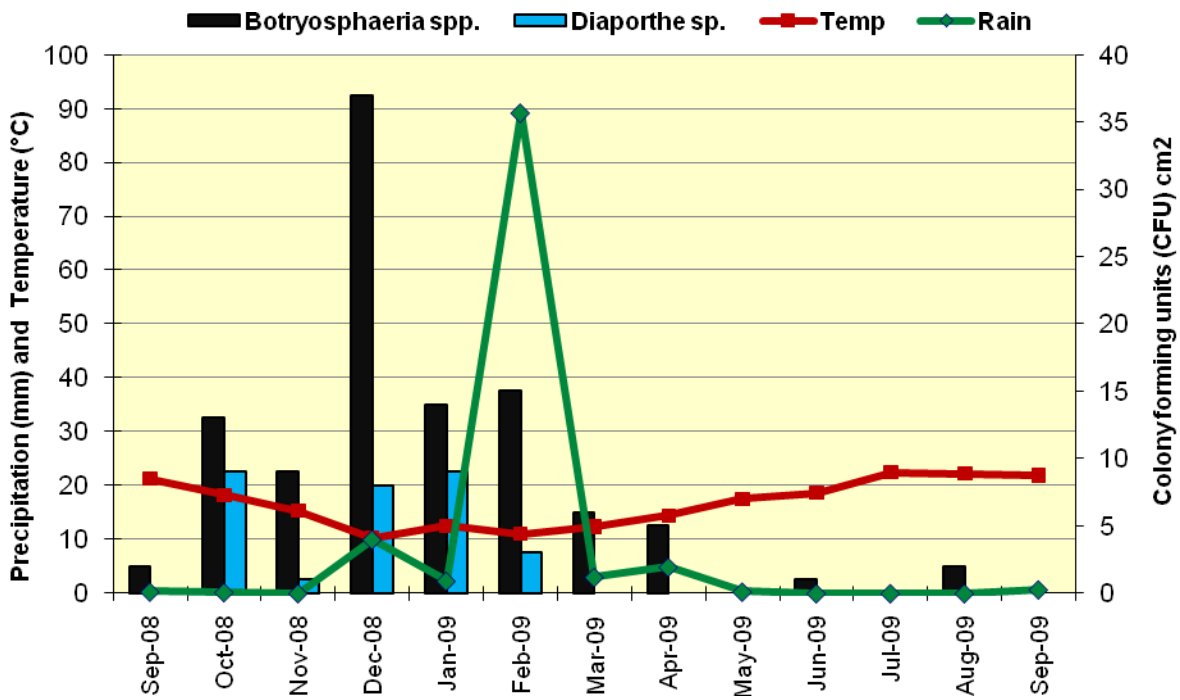


Fig. 8. Air-borne spores trapped in San Diego County avocado grove, Oct. 2008-Sept. 2009.



1. *XYLELLA FASTIDIOSA*: A new threat to the California avocado industry

Many factors contribute to the serious threat of *Xylella fastidiosa* on California avocado groves including its recovery from avocado in Costa Rica, its establishment on a wide range of hosts in California, and the presence of the Glassy Winged Sharp Shooter (GWSS) vector in this state. To address this threat, a series of pathogenicity tests were established and an intensive survey was initiated throughout avocado production areas in California. The highly probable presence of *X. fastidiosa* on California avocado can be mitigated through early detection so that aggressive control measures are implemented to prevent the establishment and spread of this disease.

Methods and Preliminary results

Collaboration

Collaborative efforts between researchers have played an integral role on the progress of this project. Dr. Caroline Roper's lab (UCR Plant Pathology) and Dr. Donald Cooksey's lab (UCR Plant Pathology) provide outreach on culture preparation and isolation techniques. Dr. Stouthamer's lab (UCR Entomology) is the primary collaborator for molecular detection.

Pathogenicity Tests

Because reports indicate that the Costa Rica strain is 99-100% similar to the Calif-Temecula 1 strain (Pierce's disease strain), Temecula isolates from grapevine were used to test *X. fastidiosa* virulence on avocado.

Fresh shoots of one-year-old avocado saplings cv. Hass were used to test three bacterial strains recovered from grapevine in Temecula, California (Riverside County). A 10^8 cfu/ml bacterial suspension provided by Dr. Roper was made for each strain. One 20 µl drop of suspension was placed onto five stems each of four replicate plants per strain. An 18 gauge syringe needle was then poked through the drop until it was sucked into the xylem tissue. This procedure was repeated on the other side of the stem to ensure a cross-sectional inoculation. Plants inoculated with water were used as negative controls. One grapevine plant per strain was inoculated to test the virulence of inoculum.

Plants continue to be monitored for symptoms caused by *X. fastidiosa*. Symptomatic and asymptomatic tissue will ultimately be screened for the bacterium utilizing detection strategies described below.

Grove Surveys

Riverside County was the first of several avocado growing counties to be surveyed in California thus far. Surveys were initiated in Riverside County because the GWSS is present within grapevine-growing areas of Temecula, where the probability of detecting *X. fastidiosa* is the highest. Tissue from five symptomatic trees in each of five groves representing Riverside County was collected for testing. Tissue from asymptomatic trees was collected from each grove for negative controls. Between ten and fifteen leaves were collected from each tree.

Pathogen Detection

A pathogen detection strategy was implemented that firstly includes screening samples for bacterium presence utilizing an enzyme-linked immunosorbent assay (ELISA). In the event that a sample is positive for *X. fastidiosa*, the pathogen is then cultured from remaining tissue following methods adopted from Hernandez-Martinez et al. In addition to culturing, samples will then be submitted to Dr. Stouthamer's lab for molecular detection.

ELISA detection

Eight leaves were selected from each sample for processing and washed in de-ionized water. Each leaf tip was cut off and mid-veins and petioles were cut out with a flame sterilized blade. All eight of the mid-veins/petioles were bulked, coarsely chopped, and weighed into a mesh bag. Samples were then tested for *X. fastidiosa* using the Agdia® DAS ELISA for *Xylella fastidiosa* kit (Agdia Incorporated, Indiana USA). Thus far 22 samples were processed from Riverside County. All samples tested using ELISA were negative for *Xylella.* Other avocado production counties in California are in the process of being surveyed for *X. fastidiosa* following the aforementioned methods.

2. LAUREL WILT DISEASE: A new threat to the California avocado industry

Laurel wilt disease, a new avocado wilting disease, was reported in Florida and is caused by *Raffaelea laurelensis*. Laurel wilt is a vascular disease on Redbay (*Persea borbonia*) and other plants in the family Lauraceae. The fungus is vectored by a non-native insect of Asian origin, the redbay ambrosia beetle (*Xyleborus glabratus*). Ambrosia beetles cause vascular disease by carrying the fungus to the wood and cultivating it there in a symbiotic relationship. Currently no effective control strategies have proven to reduce disease incidence. Avoiding transport of infested wood is the best short-term option to reduce (or at least delay) the impact of laurel wilt disease.

We have currently developed close working relationships with Laurel wilt researchers in the southeastern U.S. (specifically Georgia, Florida and South Carolina) to understand the state of our knowledge of this problem. As findings are shared by our collaborators, we will develop protocols to detect the disease in California and most importantly, minimize the risk of exposing the California avocado industry to these diseases. Should detection of these diseases within California occur, tentative treatment protocols will be developed. Currently, education and extension to stakeholders in the California Avocado Industry has been established through seminars in California.

References

1.Crous, P. W., Slippers, B., Wingfield, M. J., Rheeder, J., Marasas, W. F. O., Philips, A. J. L., Alves, A., Burgess, T., Barber, P., and Groenewald, J. Z. 2006. Phylogenetic lineages in the *Botryosphaeriaceae*. Studies in Mycology 55:235-253.

2.Margosan, D. A., and Smilanick, J. L. 2000. Fungi isolated after harvest from decayed California avocado fruit. http://www.avocadosource.com/.
3.McDonald, V., Lynch, S., and Eskalen, A. 2009. First report of *Neofusicoccum australe, N. luteum* and *N. parvum* associated with avocado branch canker in California. Plant Disease 93 (9):967.