Etiology, Epidemiology, and Management of Lower Limb Dieback and Band Canker of Almonds

Project No.: **09-PATH5-Michailides**

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Objectives:

Lower limb dieback:

1. Survey orchards in Stanislaus, Butte, Glenn, and Kern Counties for lower limb dieback (LLDB) and determine common characteristics.
2. Select four orchards and arrange with the growers to measure herbicide drift on the tree canopy.

Band canker:

3. Initiate a statewide survey of canker diseases resulting from pruning wounds made to select primary scaffolds and wounds from tree shakers.
4. Determine susceptibility of pruning wounds to band canker caused by Botryosphaeriaceae fungi.
5. Perform band canker control experiment in the field.

Interpretive Summary:

In 2009 - 2010, we continued monitoring lower limb dieback (LLDB) and band canker throughout almond orchards in California. Although in previous years we isolated more *Botryosphaeria* than *Phomopsis* from limbs with LLDB cankers, samples collected in June 2009 from three orchards each in Butte and Stanislaus counties showed more *Phomopsis* than *Botryosphaeria* (**Table 1**). Interestingly, this season the incidences of *Phomopsis* and *Botryosphaeria* in branches without LLDB symptoms were very low. These findings are in contrast to previous seasons' samplings when approximately the same levels of *Phomopsis* and *Botryosphaeria* were recovered. The reasons for these differences are not clear. The results suggest that the frequency of isolation of these fungi is not consistent and may depend on various yet unknown factors, and this is another reason why the results thus far suggest that these fungi are not directly involved in LLDB. Although both *Botryosphaeria* and *Phomopsis* spp. were isolated from woody tissues of limbs, with the exception of one sample, these fungi were not isolated from the bark of the limbs, suggesting that these fungi are truly found inside the woody tissues and they are not just contaminants of the bark. However, the question remains whether these fungi enter the woody tissues as primary invaders or secondary colonizers of tissues that have been stressed due to other factors (abnormally wet soils, herbicide drift, shading and weakening of limbs, etc.). In samples collected in October 2009, there was no increase of isolation frequency of *Botryosphaeria* neither of *Phomopsis* spp. in plated tissues from either symptomatic or asymptomatic tissues. However, generally isolation of *Phomopsis* was higher from LLDB symptomatic tissues than from asymptomatic tissues (**Table 1**). In previous years, inoculations of almond shoots with either *Botryosphaeria* or *Phomopsis* isolates did not reproduce the typical cankers that are found in limbs with LLDB symptoms. Therefore the search continues.

In early 2010, in Stanislaus County, we backhoed 4 trees that had LLDB symptoms in 2009. To our surprise some of the roots of these trees had stained cortex tissues and extensive stain in the center of their trunks (**Figure 1**). In some cases, the stain was continuous from the roots to the trunk, and the "cankered" areas of limbs and branches. Isolations from the stained areas of the roots produced fungi such as *Acremonium, Phaeoacremonium,* and *Cylindrocarpon* while isolations from the dark stained areas ("cankered") tissues of the limbs produced *Phomopsis* (**Table 2**).These findings suggest that perhaps these fungi infect the roots first and predispose the limbs to infection (colonization) by other fungi (i.e., *Phomopsis* in this case or *Botryosphaeria* in other cases) leading to what we call LLDB syndrome. We will need to backhoe more trees with LLDB in addition to a few trees without LLDB in order to confirm these results and also make direct comparisons to determine the cause of the LLDB problem. Therefore, we ask growers who have had LLDB and have trees that are blown over by strong winds when they are ready to remove these trees to notify the farm advisors of their counties and the farm advisors us so that we sample more trees with LLDB and also healthy trees. Only after doing extensive sampling and comparisons of healthy vs. "sick (LLDB) trees we will be able to can make strong conclusions.

In 2008, we performed four herbicide experiments to test the hypothesis whether herbicide drift could cause symptoms resembling LLDB. In three commercial orchards where the grower was ready to apply herbicide spray, squash plants were set up at different heights to measure herbicide drift. Although symptoms of LLDB have not developed in these orchards, we are hesitant to reject this hypothesis just in case tree symptoms may take longer to develop in these experiments. The squash plants that were placed on the soil and definitely received herbicide drift were killed, suggesting that these plants can be used as good indicators of herbicide drift.

We continued diagnosing more and more samples submitted to our laboratory by farm advisors, pest control advisors, and growers. In a large number of these samples, we noticed that band cankers and other Botryosphaeria cankers were initiated from pruning cuts. For instance, in 2009, an orchard with blocks of 3- and 4-year trees in Mendota, Fresno County where the grower had lost 7% of trees due to infection by *Botryosphaeria,* we noticed that cankers on the trunk of these young trees were initiated from major pruning cuts (**Figure 2**) made to develop the canopy and from split trunks at the crotch of the trees. These cankers grew very fast from the pruning wound and killed the trees. A similar type of canker was also observed and reported in Merced Co. (David Doll, Farm Advisor, UC Coop. Extension, Merced Co., personal communication). In the orchard in Mendota, when sampled in 2010 the 4th leaf trees had a higher incidence of *Botryosphaeria* than the 5th leaf trees. However, the older trees had more *Eutypa* (**Table 3**). These results are in agreement with the fact that older trees seem to overcome the band canker infections. The presence of *Eutypa* on the older trees that had split trunks and/or broken scaffolds suggests that this fungus can colonize the wounded tissues of almond. Another fungus that was also found colonizing the split trunk due to band canker in an orchard in Glenn County was *Schizophyllum commune*, a wood decay fungus.

Six species and one newly-described species of *Botryosphaeriaceae* fungi were identified on almond. Species identified from band cankers in the tree trunk included *Botryosphaeria dothidea, Neofusicoccum parvum, Neof. mediterraneum, Neof. nonquaesitum, Diplodia seriata,* and *Macrophomina phaseolina*e while cankers in the tree canopy included *B. dothidea, Neof. mediterraneum, Neof. parvum,* and *Dothiorella sarmentorum* (**Table 4**). Among these the most virulent were *Neof. nonquaesitum, Neof. parvum,* followed by *M. phaseolina.* Thus, the disease that was known as band canker whose causal agent was reported as *B. dothidea* for years is now considered to be a very complex disease with multiple causal agents, new phases, and new infection sites. Deciduous nut crops in California share some of the species of Botryosphaeriaceae and when these crops grow next to each other can serve as donors and receivers of spore inoculum for Botryosphaeria blight and canker diseases. Cankers and blights in the riparian area trees (east of the Mendota almond blocks had willow, cottonwood, volunteer figs, and blackberries) were found to bear pycnidia and pseudothecia of *Botryosphaeria* spp. Disease declined with distance from the riparian area from east to west of the field (**Figure 3**), suggesting that almond trees closer to inoculum have a higher risk of infection than those further from the source of inoculum. As in previous years, these findings suggest that riparian sources of *Botryosphaeria* spp. can serve as inoculum for almond infection.

To control band canker, an experiment was set in Glenn County where symptoms of band canker were light with the thinking that perhaps management of the disease may be more successful when disease symptoms were not severe. The fungicides azoxystrobin, Pristine (pyraclostrobin+boscalid), Captan, and the biopesticide Plant Shield mixed with latex paint were applied on to the trunks of trees. In addition a set of healthy appearing trees were treated similarly in order to protect them from infection. Thirteen months after treatment, trees that were healthy when they were treated and those that were not treated were still healthy (**Table 5**). Additionally, when the less severe cankers were not treated, still there were no significant differences between treated and non-treated trees (**Table 6**). Pristine applied on trees with severe cankers was only marginally effective (**Table 7**). Naturally, as trees age, their resistance to band canker disease also increases. Three trees with severe band canker symptoms out of all used in this experiment died by June 2010.

Materials and Methods:

Lower limb dieback:

1. Survey orchards for LLDB to determine common characteristics.

Three orchards with symptoms of LLDB each in Butte and Stanislaus counties were sampled in 2009. Isolations were made immediately or the day after bringing the samples to the laboratory by plating small pieces of the cankered areas on Petri dishes with acidified PDA and malt extract agar. The plates were incubated at 25°C (77°F) and recorded one week later. More orchards were sampled in 2010; in addition, we diagnosed a number of samples sent to us by consultants and farm advisors.

Isolations from roots of trees with LLDB symptoms.

In spring 2010, we visited three orchards along with farm advisors where the grower arranged to have a backhoe available. Two of the orchards were in Stanislaus County and had severe symptoms of lower limb dieback (Padre and Butte almonds). The third orchard was in Fresno County with dying Nonpareil trees. The trees were removed with the backhoe after we arrived. Isolations were made from roots, trunks, and limbs that we collected. From the two Stanislaus orchards, an unusually dark staining of the interior of the roots was observed in some of the trees. This staining seemed to continue from the roots through the trunk up to the lower scaffolds.

*Inoculation experiment with fungi isolated from almond roots in 2010***.**

Small Padre/Nemagard and Nonpareil/Nemagard almond trees donated by an almond nursery were inoculated with three fungi frequently isolated from decayed tissues of roots of trees bearing LLDB symptoms. The fungi used for these inoculations were species of *Phaeoacremonium* and *Cylindrocarpon,* and *Paecilomyces variotii.* Six trees were inoculated using the methods of H. Scheck et al. (1998). To summarize briefly, on May 21, 2010, root balls were washed, clipped to injure roots, and the roots were dipped in a $1x10^6$ spores/ml suspension of each fungal species for 30 minutes. Trees treated similarly but without inoculation served as non-inoculated control. Inoculated and control trees were placed in the greenhouse. Disease will be recorded 3, 6, and 12 months after inoculation.

2. Select orchards for herbicide drift experiments.

Methodology for this objective was given in the previous year's final report. Briefly, glyphosate at label recommended levels was applied in 2 commercial orchards with the premise that natural drift will occur to test the hypothesis whether herbicide drift would result in LLDB symptoms. Applications and rates of herbicides used were done according the label's guidelines. Almond trees were checked 1 and 3 months later and evaluated for symptoms; trees will be evaluated again, 2 years after the herbicide application.

Band canker studies:

3. Initiate a statewide survey of canker diseases resulting from pruning wounds made to select primary scaffolds and wounds from tree shakers.

Survey.

In 2009 we visited an almond orchard in Mendota where 14% of the Padre almond trees within 800 feet of a riparian area harboring the perfect stage (airborne spores) of *Botryosphaeria dothidea*, had either died or were flagged for removal. Examination of and isolations from these trees showed that cankers infected with *B. dothidea* appeared to start from pruning wounds made in November 2008 when the trees were pruned to select the primary scaffolds (**Figure 2**). Once these cankers enlarged, either the scaffolds died or were weakened and in danger of breaking off.

Intensive isolations were also made in the summer of 2010 from different types of cankers on Padre almonds in the orchard in Mendota. The trees in Mendota showed an unusual way of canker development; these cankers usually initiated in the crotch area and resulted in the splitting of the tree in two parts or in the breaking of one or two scaffolds. Sampling was done by cutting pieces of bark and wood tissues from the margins of the cankers occurring around the split areas in the trunk. Because the orchard had blocks of trees of two ages, now 4^{th} and 5^{th} leaf, sampling was done separately for each age trees.

Distribution of band canker in the Mendota orchard.

The above orchard in Mendota had shown different levels of band canker in the east and west side. To determine if there was an influence on the development of the disease from the riparian area in the east of the orchard, the incidence of the disease was recorded in 8 entire rows of 93-105 trees and mapped.

Characterization of Botryosphaeriaceae isolates.

Molecular procedures were used to characterize all 45 isolates of Botryosphaeriaceae collected from almonds from both band canker symptoms and cankers in the canopy of the trees (cankers initiating from pruning wounds, blighted fruit, cankers from cracks at the base of branches and lenticels. The isolates from almond were also compared genetically with a number of isolates of Botryosphaeriaceae species recovered from pistachio and walnut, sometimes grown next to almonds. We have now identified the species involved in the band canker disease and those that cause canopy cankers as well as the species that can attack all three types of nuts grown in California.

4. Determine susceptibility of pruning wounds to band canker caused by Botryosphaeriaceae fungi.

Because we found that cankers initiated from major cuts made to select the main scaffolds of young almond trees and to determine the length of time pruning wounds are susceptible to *Botryosphaeria* fungi; we designed an experiment in an orchard at the Kearney Agric Center. Pruning cuts were made on limbs 1 to 2 inches in diameter in several Padre trees. The first pruning cuts/wounds were made on December 21, 2009. Ten cuts/wounds were inoculated with a 7-mm agar disc with either *Botryosphaeria dothidea* or *Neofusicoccum mediterraneum* which were placed in the center of the pruning cut and covered with Parafilm. For subsequent pruning cuts on 8, 15, and 22 January 2010, we sprayed a 5×10^4 spore suspension with about 5 ml per shoot and covered them with Parafilm. The goal of this experiment here was to determine whether infections of pruning wounds occur during a month when there is plentiful of moisture but temperatures are low and possible airborne inoculum (ascospores) of *B. dothidea*. For both dates of inoculation, Padre almonds were used since this cultivar seems to have more problems with band canker than other almond cultivars. Cankers will be evaluated 1 year after inoculation.

Another larger pruning experiment was initiated in early February 2010 and also will be repeated in October or November 2010. Because we noticed that a number of almond trees with band canker symptoms had major pruning cuts with extensive cankers initiated from them, this experiment is designed to determine the duration of time a pruning cut/wound is susceptible to *Botryosphaeria* and *Neofusicoccum* infection and whether a chemical application will protect the wound from infection. A total of 180 replicated limbs were pruned and 10 wounds (five each on Padre and Carmel) were inoculated immediately (0 days), and 3, 7, 14, and 21 days, and 1 month after pruning with a 50,000 spore suspension of a virulent *B. dothidea* and *N. mediterraneum* isolates from almond by spraying about 5 ml of spore suspension on the surface of the pruning cut.

5. Perform band canker control experiment in the field.

Fungicide trial.

To control band canker, an experiment was established in May 2009 in Glenn Co. where symptoms of band canker were light with the thinking that perhaps management of the disease may be more successful when disease symptoms were not severe. The fungicides azoxystrobin, Pristine (pyraclostrobin+boscalid), Captan, and the biopesticide Plant Shield mixed with latex paint were applied onto the trunks of trees. In addition a set of healthy appearing trees were treated similarly in order to protect them from infection. Furthermore, the trunks of 10 trees with severe band canker symptoms were treated (painted) with Pristine mixed in latex paint and the trunks of 10 more trees treated with the latex paint only. This trial was recorded in June 2010 and results are presented in this report.

A trial was also established in the experimental almond orchard at Kearney Agricultural Center. Ten each pruning cuts were treated by painting with a mixture of Pristine or Abound mixed in latex paint and immediately inoculated as above (objective #4). The fungicides used were incorporated at label rates into a 50% strength paint (interior latex paint diluted 1:1 with water) and painted shortly after the pruning cuts were made. Two fungicide treatments were used, a mixture of pyraclostrobin +boscalid (pristine at 14.5 oz/acre and azoxystrobin at 15 fl oz/acre and incorporated in a volume of paint estimated to be needed to cover the pruning cuts on an acre of almond trees (5 gallons). Incidence of infection will be compared among all the treatments. To avoid desiccation after inoculation, all the inoculated pruning cuts were covered with a plastic bag overnight. Ten pruning cuts were not inoculated and used as non-inoculated control. Canker development will be recorded regularly and depending on the growth of cankers, 5 each of these inoculated pruning cuts will be removed and the internal extent of canker by *Botryosphaeria* and *Neofusicoccum* will be determined. The remaining 5 inoculated pruning cuts from each inoculation date will be removed 1 – 1.5 years after inoculation to determine how far the cankers have moved and whether the pathogens have produced any spore producing structures.

Results and Discussion

Lower limb dieback:

1. Survey orchards for LLDB to determine common characteristics.

2009 survey.

Isolations from limbs of trees with symptoms of LLDB and without symptoms in March, June, and October 2009 again revealed both *Botryosphaeria* and *Phomopsis* species present in these limbs. Frequency of isolation was greater for *Botryosphaeria* than *Phomopsis* in 2008. The opposite was true in 2009 sampling (**Table 1**). In general the frequency of isolation of *Phomopsis* spp. was higher in the limbs with symptoms than those with no symptoms for all the isolation dates and it was higher than the frequency of isolation of *Botryosphaeria* species, which was very low in 2009 (**Tables 1 & 2**). The results suggest that the frequency of isolation of these fungi depends on the year, and this is another reason why the results thus far suggest that these fungi are not directly involved in LLDB. Although both *Botryosphaeria* and *Phomopsis* spp. were isolated from woody tissues of limbs, with the exception of one sample, these fungi were not isolated from the bark of the limbs, suggesting that these fungi are truly found inside the woody tissues and they are not just contaminants of the bark. However, the question remains whether these fungi enter the woody tissues as primary invaders or secondary colonizers of tissues that have been stressed due to other factors (abnormally wet soils, infected roots, herbicide drift, shading and weakening of limbs, etc.).

In samples collected in October 2009, there was no increase of isolation frequency of *Botryosphaeria* neither of *Phomopsis* spp. in plated tissues from either symptomatic or asymptomatic tissues. However, generally isolation of *Phomopsis* was higher from LLDB symptomatic tissues than from asymptomatic tissues. Inoculations of almond shoots with either *Botryosphaeria* or *Phomopsis* isolates did not reproduce the typical cankers that are found in limbs with LLDB symptoms.

2010 survey.
Isolations from the various samples sent by consultants and cooperative extension personnel (with putative LLDB symptoms according to their opinion) did not reveal any *Botryosphaeria* or *Phomopsis.* Instead secondary fungi such as *Paecilomyces variotii, Aspergillus niger,* and species of *Alternaria, Cladosporium, Fusarium,* and *Penicillium* were isolated. Similarly, samples of trees collected from Fresno and Merced showed only 16% (1 out of 5 samples) with *Botryosphaeria* and 5, 30, and 35% of the plated tissues from three Padre trees *Eutypa. Eutypa* spp. can cause cankers on various crops, and *Eutypa lata* has been reported to cause cankers on almond in Greece (Roumbos, 1997). Again other fungi isolated from the samples of these trees included the above mentioned secondary fungi (*Paecilomyces variotii, Aspergillus niger,* and species of *Alternaria, Cladosporium, Fusarium,* and *Penicillium.*). Again, isolations of *Botryosphaeria* or *Phomopsis* were not consistent from branches killed. However, these samples in 2010 from Fresno and Merced Counties did not fit well the description of the LLDB symptoms and this may explain why *Botryosphaeria* and *Phomopsis* were not isolated at this time.

Table 1. Frequency of isolations of fungi from almond limbs[1] with or without lower limb dieback (LLDB) symptoms collected in June 2009.

Orchard	Cultivar	With LLDB symptoms		Without LLDB symptoms	
		***Botryosphaeria* species (%)**	***Phomopsis* species (%)**	***Botryosphaeria* species (%)**	***Phomopsis* species (%)**
Butte 1	Nonpareil	0	1	0	0
	Aldrich	0	36	1	0
Butte 2	Nonpareil	0	5	5	1
	Carmel	0	14	0	1
Butte 3	Butte	0	2	0	0
	Aldrich	0	12	1	0
Stanislaus 1	Butte	2	1	0	0
	Padre	1	25	2	1
Stanislaus 2	Butte	0	5	0	1
	Padre	6	1	2	0
Stanislaus 3	Butte	0	18	1	0
	Padre	0	17	0	0

[1] Samples were collected from 10 trees per orchard.

Six orchards were sampled in Colusa County. These orchards had trees with severe Ceratocystis cankers which were surgically treated and most of the trees survived. We sampled from these trees to determine if other fungi were involved. The trees did not show any typical LLDB or band canker symptoms. *Botryosphaeria* was isolated from one orchard and *B. rhodina* and *Phomopsis* from two of the six orchards sampled (results are not shown). *Ceratocystis fimbriata* was isolated from 3 orchards, confirming that the major problem in these orchards was Ceratocystis canker as well as wood decay fungi (Basidiomycetes species) in two of the orchards. In one of the orchards one sample also had a *Cytospora* species. A more systematic survey is planned for the spring of 2011.

Isolations from roots of trees with LLDB.
From the two Stanislaus orchards, an unusually dark staining of the interior of the roots was observed (**Figure 1**). This staining seemed to continue from the roots through the trunk (**Figure 1**) up to the lower scaffolds/limbs and branches leading to LLDB cankers. It was surprising to find out that trees with typical LLDB symptoms on the top, in most cases had the cortex of some of their roots stained dark brown. Isolations from the roots with dark brown stained revealed species of *Acremonium, Phaeoacremonium, Cylindrocarpon,* and *Paecilomyces variotii* (**Table 2**). Also longitudinal cankers extended from the scaffolds to main branches to shoots of smaller than 1 inch, typical of the LLDB symptoms. It is well established that *Acremonium*, *Phaeoacremonium,* and *Cylindrocarpon* can cause decline of trees and vines. *Phomopsis* was isolated from the cankers of branches of these trees showing LLDB symptoms (**Table 2**).

Figure 1. Roots of a Padre almond tree with brown discoloration in the cortex area of root **(upper row and middle left**) and dark brown staining in the trunk (**middle right**), in the limb (**bottom left**), and in branches leading to LLDB cankers (**lower right**).

Table 2. Isolations from almond trees from Stanislaus County with severe LLDB symptoms which were sampled in the spring of 2010 after removal with a backhoe. Interior of lower scaffolds and roots had dark staining (**Figure 1**).

Location	**Tissue**	**Stain**	**Incidence (%)**		
			Phomopsis	***Acremonium***[a]	***Cylindrocarpon***
Orchard 1-Tree 1	Roots	+	0	53	20
Orchard 2-Tree 1	Shoots	-	5	0	0
	Limb	-	100	0	0
	Limb	+	29	44	0
	Roots	+	0	42	0
Orchard 2-Tree 2	Limb	+	0	0	0
	Roots	+	0	47	0
Orchard 2-Tree 3	Limb	+	0	0	0
	Roots	+	0	49	0

[a] Some of the isolates were *Phaeoacremonium* sp.

*Inoculation experiment with fungi isolated from almond roots in 2010***.**
No apparent symptoms have been observed in the inoculated almond trees kept in the greenhouse for 3 months after inoculation. Although some yellowing was present in all of the young plants, this could be attributed to the shock of cutting the roots for inoculation and transplanting the trees in large size pots. These plants will be examined periodically for any symptom development.

2. Select orchards for herbicide drift experiments.
We performed four herbicide experiments to test the hypothesis whether herbicide drift could cause symptoms resembling LLDB. In three commercial orchards where the grower was ready to apply a herbicide spray, squash plants were set up at different heights to measure herbicide drift. Although symptoms of LLDB have not developed in these orchards, we are hesitant to reject this hypothesis just in case tree symptoms may take longer to develop. The squash plants that were placed on the soil and definitely received herbicide drift were killed.

Band canker studies:

3. Initiate a statewide survey of canker diseases resulting from pruning wounds made to select primary scaffolds and wounds from tree shakers.
Surveys.
In a large number of these samples, we noticed that band cankers and other Botryosphaeria cankers were initiated from pruning cuts. For instance, in a 4-year-old orchard in Fresno County where the grower had lost 7% of trees due to infection by *Botryosphaeria,* we noticed that cankers on the trunk of these young trees were initiated from major pruning cuts (**Figure 2**) made to develop the canopy and from split trunks at the crotch of the trees. These cankers grew very fast surrounding the pruning wound and killed the trees. Of particular interest is the possibility that the record setting and

relatively warm rain in October/November 2009, or other rains, may have provided an opportunity for infection of young almond trees. In addition, at this time because of the increased acreage of almond planting, a number of young almond orchards are at the stage when the primary scaffolds are being selected. A similar type of infections was also reported in Merced Co. (David Doll, Farm Advisor, and UCCE - Merced County personal communication). Similarly in one orchard in Glenn County, a tree with a canker at the base of scaffold branching split in two and *Schizophyllum* commune (a wood decay Basidiomycete fungus) was growing on both the split surfaces of the tree.

Figure 2. A third-leaf orchard with severe band canker whose infections were initiated mainly from pruning cuts made to develop the tree's main scaffolds. (Note the running of canker above and below the pruning cut in both cases.)

In the orchard in Mendota, Fresno County, the 4^{th} leaf trees had a higher incidence of *Botryopshaeria* than the 5^{th} leaf trees. However, the older trees had more *Eutypa* (**Table 3**). These results are in agreement with the fact that older trees seem to overcome the band canker infections. The presence of *Eutypa* on the older trees that had split trunks and/or broken scaffolds due to band canker suggests that *Eutypa* can colonize the wounded tissues of almond afterwards as a secondary. Another fungus that was also found colonizing the split trunk was *Schizophyllum commune*, a wood decay fungus.

Table 3. Isolations from Padre almond trees near Mendota with cankers resulting from infected pruning wounds made while selecting primary scaffolds (collected on July 26, 2010).

Location	Incidence (%)		
	Botryosphaeria	***Phomopsis***	***Eutypa***
South block 4^{th} year	55.3	0.3	0.0
North block 5^{th} year	11.2	0.0	4.0

Distribution of band canker disease.
When the distribution of the band canker was recorded in the orchard in Fresno County more disease was present closer to the riparian area (east) where willow, cottonwood, volunteer figs, and blackberries were found to bear pycnidia and pseudothecia of species of *Botryosphaeriaceae* than the disease in the west side. Disease incidence decreased with distance from the riparian area from east to west of the field (**Figure 3**). This finding is in agreement with the band canker distribution in another almond orchard next to a walnut orchard with a lot of *Botryosphaeria* inoculum where disease was higher next to walnuts. As in experiments in previous years, these findings suggest that riparian sources of *Botryosphaeria* spp. can serve as inoculum for almond infection.

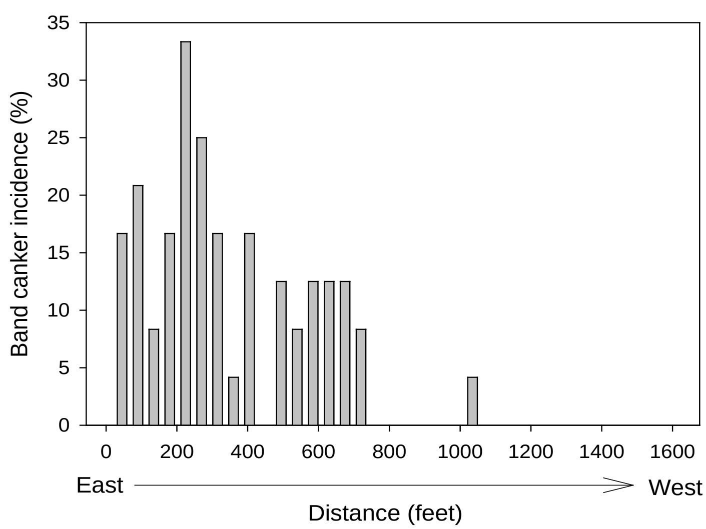

Figure 3. Distribution of almond band canker in a third-leaf almond orchard (average of eight rows) in Fresno County (East side had the riparian area; Padre and Butte cvs. in a 1:1 ratio, with the Padre severely infected).

Characterization of Botryosphaeriaceae isolates.
The study to characterize genetically isolates of Botryosphaeriaceae has been completed and now we have identified six different species and one newly described species that can attack almonds in California (Inderbitzin et al., 2010). Thus, the species identified from band cankers include *Botryosphaeria dothidea, Neofusicoccum parvum, Neof. mediterraneum, Neof. nonquaesitum, Diplodia seriata,* and *Macrophomina phaseolina*. The species isolated from cankers in the tree canopy include *B. dothidea, Neof. mediterraneum, Neof. parvum,* and *Dothiorella sarmentorum* (**Table 4**). Among the seven species of *Botryosphaeria* isolated from band canker, canopy cankers, and cankers of trees with LLDB symptoms, *Botryosphaeria nonquaesitum* and *Neof. Parvum* were the most virulent species followed by *Macrophomina phaseolina, Neof. Mediterraneum*, and *B. dothidea.* Cankers produced by these pathogens were typical Botryosphaeria cankers but differed from cankers associated with LLDB symptoms. Thus, the disease that was known as band canker whose causal agent was reported as *B. dothidea* for years is now considered to be a very complex disease with multiple causal agents, new phases, and new infection sites. Tree nuts in California share some of the species of Botryosphaeriaceae and when these crops grow next to each other

can serve as donors and receivers of spore inoculum for Botryosphaeria blight and canker disease.

Table 4. Species of Botryosphaeriaceae that cause cankers and blights in tree nuts grown in California.

Species of Botryosphaeriaceae	Almond	Pistachio	Walnut
Neofusicoccum nonquaesitum	+	---	---
Neofusicoccum parvum	+	+[a]	+[b]
Macrophomina phaseolina	+	+	---
Neofusicoccum mediterraneum	+	+	+
Botryosphaeria dothidea	+	+[a]	+
Diplodia seriata	+	+[c]	+
Dothiorella sarmentorum	+	---	---
Lasiodiplodia theobromae	+	+	+

[a] These species have been reported on pistachio in Greece.
[b] Also reported from walnuts in Spain and Greece.
[c] Also reported on pistachio in South Africa.

4. Determine susceptibility of pruning wounds to band canker caused by Botryosphaeriaceae fungi.

It should be noted that about 2-year-old cankers killed vigorously growing trees at the third to fourth leaf. Infections occurred and cankers started developing in some of the treatments. However, these experiments will be recorded in the fall of 2010 and again in spring/summer of 2011. Since inoculations of pruning wounds were done at different dates after the pruning, an expected outcome from this experiment will be determining of the duration pruning wounds remain susceptible to infection by these Botryosphaeriaceae fungi. Another expected outcome will be whether a fungicide application directly on the pruning wound as soon as the cut is made could prevent infection.

5. Perform band canker control experiment in the field.

Fungicide trial.

All the treated and non treated trees that initially did not show any band canker symptoms in 2009, still were free of cankers longer than a year later (**Table 5**). Additionally, there were no significant differences between treated and non treated trees when the trees had small cankers at the time they were treated in May 2009 (**Table 6**). Pristine applied on trees with severe cankers was only marginally effective (**Table 7**). In other words, it seemed that cankers were small as an average but the differences were not significant. Naturally, as trees become older, their resistance to band canker disease also increases. Only 3 trees from all used in this experiment died.

Ten each pruning cuts were treated by painting with a mixture of Pristine or Abound mixed in latex paint and immediately inoculated as above (objective #4). The experiments involving pruning wounds treated with fungicides are not ready to be recorded yet. Efficacy of fungicides in protecting pruning wounds will be recorded in the the fall 2010 and again in spring/summer 2011. Depending on the results from these experiments, other fungicides can then be tested.

Table 5. Effect of fungicides mixed with white latex paint and applied on the trunks of **initially symptomless** Nonpareil almond trees to control band canker in Glenn County.

Treatments	**Rate per acre**	**Canker rating[2]**	**Number of gumming sites[3]**
Abound	15.4 fl oz	0.0 a[4]	0.0 a
Pristine	14.5 oz	0.0 a	0.0 a
Captan 4L	1.125 Gallon	0.0 a	0.0 a
Plant Shield	2.5 lb	0.0 a	0.0 a
Control with paint only	---	0.0 a	0.0 a
Unpainted control	---	0.0 a	0.0 a

[1] Fungicides were added to a white latex paint and painted at 10 gallons/acre on May 14, 2009 and recorded on June 16, 2010.
[2] Cankers from each of five replicated trees were rated on a scale of 0 to 3 where 0 = no canker, 1=canker < 25% of trunk, 2 = canker < 50% of trunk, and 3= canker > 50% of trunk.
[3] The number of gumming sites was counted per tree.
[4] Numbers followed by different letters were significantly different according to the LDS test at $P = 0.05$.

Table 6. Effect of fungicides mixed with white latex paint and applied on the trunks of Nonpareil almond trees showing **moderate sized initial cankers** to control band canker in Glenn County.
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Treatments	**Rate per acre**	**Canker rating[2]**	**Number of gumming sites[3]**
Abound	15.4 fl oz	0.2 a[4]	1.4 a
Pristine	14.5 oz	0.4 a	0.4 a
Captan 4L	1.125 Gallon	0.8 a	0.2 a
Plant Shield	2.5 lb	0.0 a	0.2 a
Control with paint only	---	0.2 a	0.4 a
Unpainted control	---	0.0 a	0.0 a

[1] Fungicides were added to a white latex paint and painted at 10 gallons/acre on May 14, 2009 and recorded on June 16, 2010.
[2] Cankers from each of five replicated trees were rated on a scale of 0 to 3 where 0 = no canker, 1=canker < 25% of trunk, 2 = canker < 50% of trunk, and 3= canker > 50% of trunk.
[3] The number of gumming sites was counted per tree.
[4] Numbers followed by different letters were significantly different according to the LDS test at 0.05.

Table 7. Effect of Pristine fungicide mixed with white latex paint and applied on the trunks of Nonpareil almonds trees showing **severe initial cankers** to control band canker in Glenn County.

Treatments	Canker rating[2]	Number of gumming sites[3]
Pristine Treatment – Painted on severe band cankers(see below)	1.9 a	2.4 a
Unpainted control: trees with severe band cankers	2.1 a	2.7 a

[1] Pristine was added at a rate of 14.5 oz/acre to a white latex paint and painted at 10 gallons mixture per acre on May 14, 2009; disease was recorded on June 16, 2010.
[2] Cankers from each of five replicated trees were rated on a scale of 0 to 3 where 0 = no canker, 1=canker < 25% of trunk, 2 = canker < 50% of trunk, 3= canker > 50% of trunk, and 4 = dead.
[3] The number of gumming sites was counted per tree.
[4] Numbers followed by different letters were significantly different according to the LDS test at 0.05.

Research Efforts Recent Publications:

Inderbitzin, P., Bostock, R. M., Trouillas, F. P., and Michailides, T. J. 2010. A six locus phylogeny reveals high species diversity in Botryosphaeriaceae from California almond. Mycologia 102(6); doi:10.3852/10-006.

Michailides, T. J., and Morgan, D. P. 2010. Diseases of tree nut crops caused by Botryosphaeriaceae fungi. CAPCA Newsletter, October 2010, pp. 34-35 & 38 & 40.

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Inderbitzin, P., Bostock, R. M., Trouillas, F. P., and Michailides, T. J. 2010. A six locus phylogeny reveals high species diversity in Botryosphaeriaceae from California almond. Mycologia 102(6); doi:10.3852/10-006.

Michailides, T. J., and Morgan, D. P. 2010. Diseases of tree nut crops caused by Botryosphaeriaceae fungi. CAPCA Newsletter, October 2010, pp. 34-35 & 38 & 40.

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Scheck, H., Vasquez, S., Fogle, D., and Gubler, W. D. 1998. Grape growers report losses to black-foot and grapevine decline. California Agriculture 52(4):19-23.