Etiology, Epidemiology, and Management of Lower Limb Dieback (LLDB) and Band Canker of Almond

Project No.: **07-PATH5-Michailides**

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Objectives:

1. To survey affected orchards early in the season to determine initial symptoms and putative pathogens involved.
2. To determine whether *B. dothidea* and *Phomopsis* spp. can cause LLDB symptoms in the field.
3. To determine when infections by putative fungi occur for LLDB and of *B. dothidea* for band canker.
4. To compare fungicide treatments against band canker and LLDB.

Interpretive Summary:

Lower limb dieback: In the last few years, growers in the Sacramento and San Joaquin Valleys have been noticing an increasing incidence of a dieback of lower limbs on almond trees. This dieback (named lower limb dieback (LLDB)) seems to be most pronounced on the Butte and Padre varieties but has also been found on other cultivars including Nonpareil and Sonora, and other varieties to a lesser degree. It is a general belief that symptoms on Butte/Padre begin when orchards reach about 7 to 8 years of age and continue to get worse as the orchard ages. Affected trees initially (during April) develop yellow leaves on lower limbs that die during summer. Some defoliation may occur, although some leaves will die and remain hanging on the limbs. Entire limbs die back, and significant death of lower canopy wood can occur by late summer. Scraping the bark of affected limbs reveals necrotic, brown lesions that sometimes are circular which appear to be initiated from around lenticels. We have observed a diverse range of

symptoms. Most of the time, no definite canker margins are obvious and the discolored wood usually is in the upper surface of the limb. Cross sections of affected limbs show discolored tissues which are sometimes V shaped. Sap may develop at the base of the limb at the junction of the main scaffold.

Isolations from multiple samples from these limbs collected, starting in September 2004 and continuing through 2005 and 2006 have indicated the presence of both *Botryosphaeria dothidea* and *Phomopsis* spp. in high frequency. Samples collected in April 2005 had higher levels of *Botryosphaeria* and *Phomopsis* than those collected in September. The most frequently isolated species of fungal pathogen was a *Phomopsis* species. Both *Botryosphaeria* and *Phomopsis* have been reported to cause canker diseases on almond in California and in Europe, Australia, and South America. In addition, a presumably *Phomopsis amygdali* has been reported causing fruit rot and shoot blight of almond in California, and *B. dothidea* causes band canker and fruit blight in almond. Although both these fungi have been isolated at high incidences, isolations from shoots affected by lower limb dieback have not revealed the same microorganisms from all the almond samples collected in several counties. For instance, isolations in 2005 from an orchard in Kern County with lower limb dieback had only very low levels of *Botryosphaeria* and *Phomopsis.* In addition, isolations from branches showing symptoms of lower limb dieback in 3 orchards in 2007 revealed mainly saprophytic fungi than *Botryosphaeria* or Phomopsis. Because of these inconsistencies in the isolated fungi and because of the frequent isolation of a number of saprophytic fungi in 2007, it is urgent to determine whether truly either Botryosphaeria and or *Phomopsis* are capable of causing lower limb dieback or are just secondary colonizers of affected lower limbs. Presently, it is not known what the effect of lower limb dieback would be on yield reduction or orchard productivity.

1. To survey affected orchards early in the season to determine initial symptoms and putative pathogens involved.

Surveys to identify the putative pathogens were initiated before the official approval of this project by the Almond Board of California. Samples were collected from a number of orchards in various counties where LLDB occurred. In addition a large number of samples were sent by farm advisors in 2005 and 2006. Isolations from all these samples were done following procedures routinely used in our laboratory and the results are summarized in Tables 1 and 2.

Table 1. Incidence of fungi isolated from shoots showing with lower limb dieback symptoms collected from 10 (Glenn, Madera, and Stanislaus Co.) and 18 (Butte, Colusa, Fresno, Glenn, and Kern Co.) almond orchards in 2005 and 2006, respectively.

Pathogen isolated	**Isolation frequency (%)**	
	2005	**2006**
Botryosphaeria dothidea	**44**	**37**
Botryosphaeria rhodina	12	11
Phomopsis sp.	**69**	**42**
Colletotrichum	12	21
Ceratocystis fimbriata	12	---
Aspergillus species	19	32
Alternaria sp.	19	21
Cladosporium	6	---
Penicillium sp.	12	---
Nattrassia mangiferae	---	11
Trichoderma sp.	---	6
Mucor sp.	---	6
Botrytis cinerea	---	5
Macrophomina phaseolina	---	11

Because *B. dothidea* and *Phomopsis* spp. were isolated more frequently than other fungi, we used these species in inoculation experiments in the field to determine whether these fungi can cause symptoms of lower limb dieback (see objective 2 below).

Table 2. Incidence of fungi isolated in 2006 from 18 almond orchards from Butte, Colusa, Fresno, Glenn, and Kern counties with symptoms of lower limb dieback separated by region.

Pathogen isolated	**Isolation frequency (%)**		
	Sacramento Valley[1] (14 orchards)	**Northern San Joaquin[2] (11 orchards)**	**Southern San Joaquin[3] (10 orchards)**
Botryosphaeria dothidea	**50**	**45**	**20**
Botryosphaeria rhodina	0	9	20
Phomopsis sp.	**64**	**73**	**30**
Colletotrichum	14	18	20
Ceratocystis fimbriata	7	9	0
Botrytis cinerea	7	0	10
Pseudomonas syringae	0	0	10
Aspergillus species	21	9	40
Nattrassia mangiferrae	7	9	10
Alternaria sp.	21	36	20
Fusarium sp	29	18	20
Trichoderma sp.	14	0	0
Mucor sp.	7	0	0
Macrophomina phaseoli	0	0	20

[1] Butte, Colusa, and Glenn counties; [2] Madera and Stanislaus counties; [3] Fresno and Kern counties.

2. To determine whether *B. dothidea* and *Phomopsis* spp. can cause LLDB symptoms in the field.

Inoculations were done with mycelial plugs (four sites per shoot) using three isolates each of *B. dothidea* and *Phomopsis* on June 5 and July 26 in an experimental orchard at Nickels Soil Laboratory in Arbuckle (Colusa County). Cankers that developed were recorded on August 29 and the results are presented in Table 3.

Table 3. Inoculation with *Phomopsis* and *Botryosphaeria* isolates and LLDB canker formation from the June 5, 2007 inoculation.

Isolate	Inoculation rating score[1]	
	Thrifty trees (good growth)	Unthrifty trees (poor growth)
Phomopsis #07019	1.2 bc	0.4 b
Phomopsis #07022	0.4 c	0.4 b
Phomopsis #3774	0.8 bc	0.8 b
Botryosphaeria #661	3.6 a	2.2 a
Botryosphaeria #809	2.8 ab	2.4 a
Botryosphaeria #3449	2.2 abc	0.6 b

[1] Limbs were inoculated at four sites per limb, measuring from the terminal to the basal sections, corresponding to 0.5, 1, 2, and 3-5 cm diameter sites on the limb. Rating scale: a rating of 1 means that only the terminal inoculation caused a canker (mildly virulent isolate), while a rating of 4 means that all four inoculation sites down the shoot caused a canker (virulent isolate).

Only results of the June 5 inoculation, which had more time to develop, are reported here. In general, the *Botryosphaeria* isolates were more virulent, especially isolates #661 and #809. The isolates were more virulent on the thrifty trees, although none of the isolates were significantly different on the thrifty vs. the unthrifty trees (statistics not shown in table). However, when the inoculation rating score of all the *Phomopsis* and *Botryosphaeria* isolates were averaged together, the average score of 1.8 on the thrifty trees was significantly different than the score of 1.1 on the unthrifty trees $P < 0.05$. This indicates that thrifty trees may be more susceptible to LLDB.

3. Determine when infections by putative fungi occur for LLDB and of *B. dothidea* for band canker.

For the lower limb dieback, once we have identified the causal agent(s) of the LLDB, then we will design experiments to determine **when** infections" take place and proceed with this objective. However, the etiology of the LLDB needs to be elucidated first.

Answering the question when infections of band canker pathogen occur is of major importance to be able to develop effective control measures against band canker. We have determined, based on 2 years of monthly inoculations that early spring seems to be the period when canker development is most rapid. It can be argued that, although this is when cankers appear to develop faster, this may or may not be the time when most of the infections occur in almond trees.

To determine when infections take place, trunks of trees killed by band canker and which we had both the airborne and water splashed inoculum were brought to Kearney Agricultural Center and placed on a wire roofed structure. Ten potted trees were placed every 2 weeks underneath "the source of inoculum". Five of the trees were wounded and five trees were not wounded. A Burkard spore trap was set among the potted trees to collect water-splashed and airborne spores of *B. dothidea* originating from the dead tree trunks. After exposure for 2 weeks, the trees were placed in the lath house and a new set of healthy trees was exposed underneath the infected trunks ("source of inoculum"). A misting system was installed to provide supplemental "precipitation" of 4 hours duration for the periods where no natural rain occurred during the 2-week period that the trees were exposed to the inoculum under the structure. This procedure started on May 9, 2007 and has continued until the current set (October 10).

We observed that gum had developed from 20 to 40% of the wounded trees from the December 6 and August 15 sets but the gum seemed currently inactive. We observed active gumming from the 20% of the wounded trees in the January 15th set. Evaluation of the trees will also be done after the completion of the experiment.

4. Compare fungicide treatments against band canker and eventually LLDB.
Field experiments: In cooperation with Joe Connell, on November 1, 2005, a randomized design experiment was set in an orchard with severe band canker in Butte County. The fungicides applied were Abound at 15.4 fl oz, Pristine at 14.5 oz, Captan 4L at 1.125 gallons, and Plant Shield at 2.5 lb. We had two controls: one with paint only and an unpainted control. The fungicides were added to white interior flat latex paint at a dilution of 1 part paint and 1 part water. Ten gallons of paint were sprayed with a Hudson-type sprayer using the coarse setting. The trunks were sprayed from the ground to the scaffolds. The purpose of the experiment was to determine whether fall fungicide applications will prevent infections and protect the trees. Although the Plant Shield showed significant reduction of the severity of the disease, in general the results were not very promising. This experiment will be evaluated once again in early spring to determine survival of the trees, cankers that have been cured and active cankers. The experiment will be terminated in May 2008.

In the Nonpareil orchard in Colusa Co. to prevent infections on the tree canopy by *Botryosphaeria*, trees were sprayed at petal fall and some of the sprays were also applied one month later (also have one treatment at 4 weeks after petal fall) with the fungicides listed in Table 4. Blighted almond fruit per tree was recorded on 29 Aug 2007. One more recording of the fruit remaining on the trees (mummies) will be done during winter.

Table 4. Fungicide efficacy of canopy sprays on Nonpareil almonds in Colusa County in 2007.

Treatment (fungicide(s))	Rate per acre (100 Gallons per acre)	Rate per 15 trees	Petal fall (March 5))	4 weeks after petal fall (April 4)	Blighted almond fruit per tree (August 29)
Topsin M 4.5 Ziram	20 fl oz 8 lbs	60 ml 363 g	X		21.3 a
Topsin M 4.5 Ziram	20 fl oz 8 lbs	60 ml 363 g	X	X	22.4 a
Topsin M 4.5 Ziram	30 fl oz 8 lbs	90 ml 363 g	X		10.9 a
Pristine	14.5 oz	41 g		X	31.6 a
Control	Untreated				25.7 a

Greenhouse/lath house experiments: In 2004 we developed a successful method of *B. dothidea* inoculation of young potted almond trees and were able to treat some of these trees at the time of inoculation or 4 days after inoculation. Since only the fungicide treatments that were applied at inoculation time were effective, suggesting that fungicide treatments are effective as protectants, in 2007 we have emphasized fungicide protective treatments. Treatments included inoculation with *B. dothidea* 0, 7, 14, 21, and 30 days after treating the trunks of trees with Abound®, Captan®; Pristine®, Plant Shield, Break®, and the untreated control. Five replicated trees were used per treatment. Only the spray with Pristine had some effect in restricting the canker and resulting in higher survival of trees when inoculations were done either immediately or after one week, suggesting that this fungicide will protect the tree trunks only for several days (Tables 5 and 6).

Table 5. Effect of inoculation date on band canker development after fungicide application.

Treatment	Canker length (cm)				
	Inoculation (days after treatment)				
	0	7	14	21	30
Abound	37 ab	26 c	28 ab	15 a	29 b
Pristine	11 b	26 c	14 b	18 a	22 b
Captan 4L	65 a	80 a	49 a	24 a	70 a
Plant Shield	29 ab	67 ab	33 ab	18 a	32 b
Break	25 ab	39 c	32 ab	24 a	33 b
Control	28 ab	45 abc	20 b	22 a	37 b

Table 6. Effect of inoculation date on band canker development after fungicide application.

Treatment	Survival rate (%) Inoculation on days after treatment)				
	0	7	14	21	30
Abound	60 ab	80 a	80 ab	100 a	100 a
Pristine	100 a	80 a	100 a	100 a	100 a
Captan 4L	20 b	0 c	40 b	80 a	40 b
Plant Shield	80 ab	20 bc	60 ab	100 a	80 a
Break	80 ab	60 ab	80 ab	100 a	100 a
Control	80 ab	60 ab	100 a	100 a	80 a

The completed report for this current 2007 – 2008 project will be available on CD distributed at the 2008 Conference.

Conclusions:

1. Isolations from limbs of trees with symptoms of lower limb dieback in 2007 did not confirm the high incidence of *Botryosphaeria* and *Phomopsis* species isolated from similar limbs collected from a large number of orchards in 2005 and 2006.
2. However, in inoculations with isolates of *Botryosphaeria* and *Phomopsis* species, only *Botryosphaeria* resulted in significantly larger cankers and typical lower limb dieback symptoms (yellowing of the leaves and eventually shoot death).
3. The experiment to determine when infections by *Botryosphaeria dothidea* occur is in progress. However, infections that occur during April to May caused the largest cankers.
4. Greenhouse experiments showed that efficacy of Pristine will last the most for one week on the trunk of the trees.