Etiology, Epidemiology, and Management of Lower Limb Dieback and Band Canker in Almond

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

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Interpretive Summary

Isolations from multiple samples from limbs with lower limb dieback (LLDB) collected starting in September 2004 and continuing through 2005 and 2006 have indicated the presence of both *Botryosphaeria* and *Phomopsis* species in high frequency. 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 frequencies, 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 LLDB in 3 orchards in 2007 revealed mainly saprophytic fungi and very little *Botryosphaeria* or Phomopsis. Additionally, isolations in 2008 from samples with early symptoms of LLDB showed very low levels of *Phomopsis* and *Botryosphaeria,* with *Phomopsis* isolated more frequently than Botryosphaeria. We will continue these isolations over the season to determine whether there is a built up of these fungi on limbs with LLDB. Inoculations with isolates of *Botryosphaeria* and *Phomopsis* species showed that *Botryosphaeria* is more aggressive pathogen causing major cankers on almond and resulting in yellowing of the leaves and eventually death, typical of LLDB. However, because of inconsistencies in the isolated fungi and because of the frequent isolation of a number of saprophytic fungi in 2007 and in early 2008, it is urgent to determine whether truly either Botryosphaeria and or *Phomopsis* are capable of causing

LLDB. In 2008, we initiated an experiment to determine whether herbicide drift can reproduce the symptoms of lower limb dieback.

A detailed study of the pathogens causing band canker, shoot cankers, and fruit blight showed that there are more than one species of *Botryosphaeria* pathogen that are involved in causing band canker and canopy canker diseases of almond. Although infections that occur during April to May caused the largest cankers, it is still unclear when most of the infections by *Botryosphaeria dothidea* occur to cause band canker.

Objectives:

1. Refine the etiology of band canker and the pathogens isolated from branches with LLDB.
2. To survey affected orchards early in the season to determine initial symptoms and putative pathogens involved.
3. To determine whether *Botryosphaeria dothidea* and *Phomopsis* spp. can cause LLDB symptoms in the field.
4. To determine when infections by putative fungi occur for LLDB and of *B. dothidea* for band canker.

Materials and Methods:

<u>Band Canker</u>

1. Refine the etiology of band canker and canopy cankers

<u>Revision of band canker etiology</u> **-** For a successful management of a disease, it is essential to identify the exact species of the pathogen(s) involved. Although back in the 1960s *Botryosphaeria dothidea* was reported as the pathogen causing band canker, in more recent research using molecular approaches, we determined that *Botryosphaeria* isolates from almond identified as *B. dothidea* represent at least five different species. In addition, we recovered a number of isolates of *Botryosphaeria* from the canopy of the trees either from cankers on shoots, peduncles of blighted fruit, and isolates from limbs showing symptoms of LLDB. These unnamed species not only had different DNA fingerprints, they also showed differences in their virulence to almond. Different species may show differences in biology and sensitivity to various fungicides, thus requiring different management methods. The threat to almond plantations posed by *Botryosphaeria* might thus depend on the species being present, requiring efficient species identification. For this reason, we have been investigating how to differentiate the *Botryosphaeria* species from almond expeditiously and reliably. We have also undertaken literature research to find names for all these species.

Botryosphaeria isolates collected from both the bark of band-cankered trees and those from limbs in trees with lower limb dieback were maintained in storage on potato dextrose agar (PDA) plates in the refrigerator and as conidia suspensions in potato dextrose broth with 20% glycerol in an ultralow freezer at -80°C. For morphological studies, isolates were plated on pistachio leaf agar plates, and incubated under continuous fluorescent light at room temperature for at least one week. Microscopic examinations were made with a Nikon Microphot-SA light microscope at up to 1000 times magnification using differential interference contrast optics or phase contrast. Measurements were made with image analyses software SpotAdvance. Pycnidia were sectioned at 14 µm thickness with a Zeiss freezing microtome, and placed in 50% glycerol for microscopic examination. Permanent slides were prepared using Permount mounting medium.

All species were examined microscopically. Main morphological characters assessed were detailed characters of pycnidium morphology, sporogenesis, and conidia dimensions, as well as colony morphology. The easiest characters for species differentiation were conidia dimensions, supplemented by colony morphology. The other characters were relatively complicated to obtain, but are still required to describe new species.

<u>Lower Limb Dieback</u>

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

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. For more information on LLDB see also Dr. Lampinen's annual report (07-Path6-Lampinen, Lower Limb Dieback in Almonds).

Surveys to identify the putative pathogens were initiated before the official approval of the project on LLDB 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.

<u>Serial isolations from limbs with LLDB in several orchards</u> **-** After the official approval of the project, 6 orchards with trees showing LLDB symptoms were selected in early 2008 (3 each in Stanislaus and Butte counties) and trees with early symptoms (yellowing of the leaves), of LLDB were selected in April. Five of these orchards were the same orchards where Dr. Lampinen is doing measurements of the water/moisture content in the soil of these orchards (see Dr. Lampinen's report, 07-Path6-Lampinen). Samples of limbs and branches were collected to isolate putative pathogens, and more samples will be collected two to three more times from the same trees with LLDB until October/November. In this way, we will determine the succession of colonization of these limbs by putative fungal pathogens. Samples were initially collected in June 2008 (after the appearance of the symptoms), brought to the laboratory, and isolations were made within 1 to 2 days after collection. For isolation, media included acidified potato dextrose agar (APDA) to isolate the fast growing fungi. The goal of these isolations will

be to determine if there is a succession and/or an increase of colonization of the LLDB limbs with *Botryosphaeria* and *Phomopsis* species.

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

Inoculations of Butte almond trees were done with mycelial plugs (four sites per shoot), using three isolates each of *Botryosphaeria dothidea* and *Phomopsis* species on June 5 and July 26 2007 in an experimental orchard at Nickels Soil Laboratory in Arbuckle (Colusa County). Cankers that developed from these inoculations were recorded on August 29 and November 16, 2007 and the results are presented in Tables 5, 6, and 7.

A second set of inoculations were performed on July 2, 2008 in the Butte almond cultivar at the Nickels Soil Lab Estates orchard. Five trees in a vigorous block and five trees in a poorly growing block were selected and inoculated with 3 different *Botryosphaeria* and 3 *Phomopsis* isolates. Five limbs in each tree were inoculated. We inoculated with mycelial plugs various ages of wood; thus the inoculations were done in one year old wood and wood formed in other years as well. Inoculated sites were wrapped with Parafilm to prevent desiccation of the inoculum and disease symptoms (yellowing of leaves, development of canker, killing of limb, etc.) will be monitored throughout the summer 2008. At the end of the season infected shoots will be collected and canker morphology will be compared with that of cankers characteristic of LLDB. This second year inoculations will complete the inoculation studies with *Botryosphaeria* and *Phomopsis* species.

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

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, which provide the source of spore inoculum. A misting system was installed to provide supplemental “precipitation” for 4 hours when 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 October 10, 2007.

Results:

1. Refine the etiology of band canker and canopy cankers

Differentiation of *Botryosphaeria* species **-** *Botryosphaeria dothidea* had the longest and narrowest conidia, with mean conidia dimensions of 26.5 × 6.1 µm, and a mean length to width (l/w) ratio of 4.5. *Fusicoccum cf arbuti* conidia were shorter and wider at 24.0 × 7.3 µm (l/w: 3.3), *B. cf parva* conidia were 19.0 × 6.2 µm (l/w: 3.1), *Neofusicoccum mediterraneum* conidia were 20.8 × 7.0 µm (l/w: 3.0) and *B. parva* conidia were shortest and widest, at 14.6 × 6.8 µm (l/w: 2.2). Thus, all the *Botryosphaeria* species encountered on almond in our studies can be differentiated by conidia dimensions, except *B. cf parva* and *N. mediterraneum*. However, these differ in their appearance on pistachio leaf agar. *Botryosphaeria cf parva* colonies are light grey to white at the edges, darker in the center, with abundant aerial mycelium giving a cottony appearance. *Neofusicoccum mediterraneum* colonies are more uniform and darker in color, greenish gray to dark green, and the mycelium is closer to the agar surface and not fluffy.

Botryosphaeria cf parva and *Neofusicoccum cf arbuti*, two unnamed species from almond **-** (note: *cf* = confer = compare, it is a mycological term used for comparing fungi.). Two groups of *Botryosphaeria* isolates from almond differed from all other species based on DNA analyses. The two groups have preliminarily been named *B. cf parva* and *F. cf arbuti*, based on their close respective relatives (see phylogenetic tree below in Figure 1). The separation of the two species is supported by morphology. *Botryosphaeria cf parva* conidia differ in morphology from *B. parva*. Whereas *B. parva* conidia are on average shorter than 15 µm with a length to width ratio of 2.2, the conidia of *Botryosphaeria* resembling *parva* (*B. cf parva*) were longer and narrower, on average almost 19 µm long, and more than 3 times as long as wide. Whether there is a difference in virulence is being investigated.

The other unnamed species, *Fusicoccum cf arbuti*, is closest related to *Fusicoccum arbuti* from Pacific Madrones from the Pacific Northwest, as well as *Fusicoccum andinum*, from eucalyptus in the mountains of Venezuela. *Fusicoccum andinum* has not been found outside Venezuela, its conidia are narrower, with a length to width ratio of 4.8, as compared to *F. cf arbuti* (length to width ratio of 3.3). *Fusicoccum arbuti*, only known from the Pacific Northwest, has conidia that are smaller, less than 20 µm in length, than in *F. cf arbuti* where conidia are on average 24 µm in length. How *F. cf arbuti* compares to *F. arbuti* and *F. andinum* in terms of virulence is unknown, but *F. cf arbuti* isolated from almond was the most virulent species among almond and no almond isolates tested in pathogenicity assays on Padre almond (Figure 2).

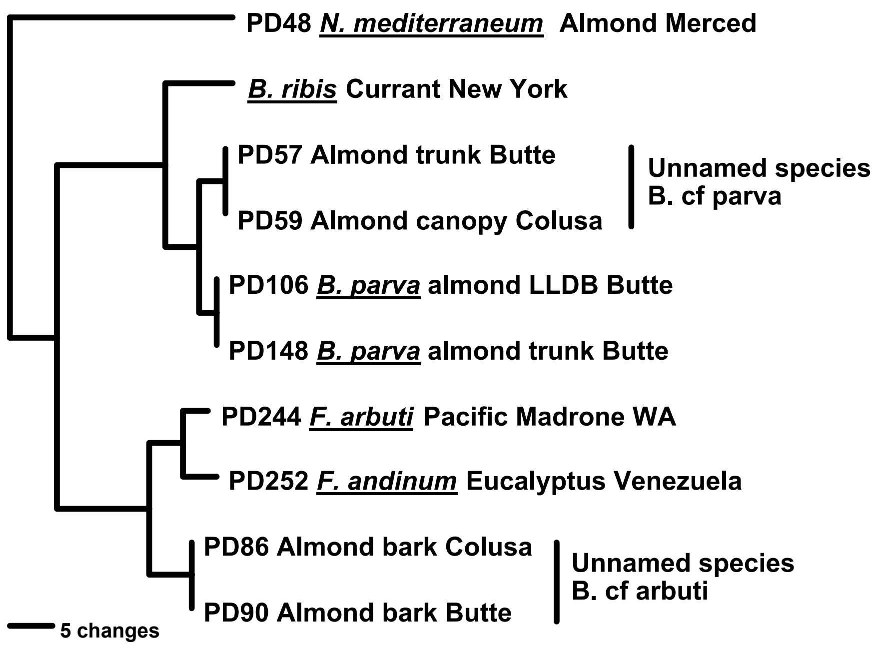

Figure 1. Phylogenetic tree showing the relationship of the unnamed species *Botryosphaeria cf parva* and *Botryosphaeria cf arbuti* to their closest relatives. Labels include identifiers and/or species names followed by the substrates and the geographic origins. Note that the DNA substitutions between unnamed and named species correspond approximately to the differences between the named species *F. arbuti* and *F. andinum*. Literature research is underway to find names for these species.

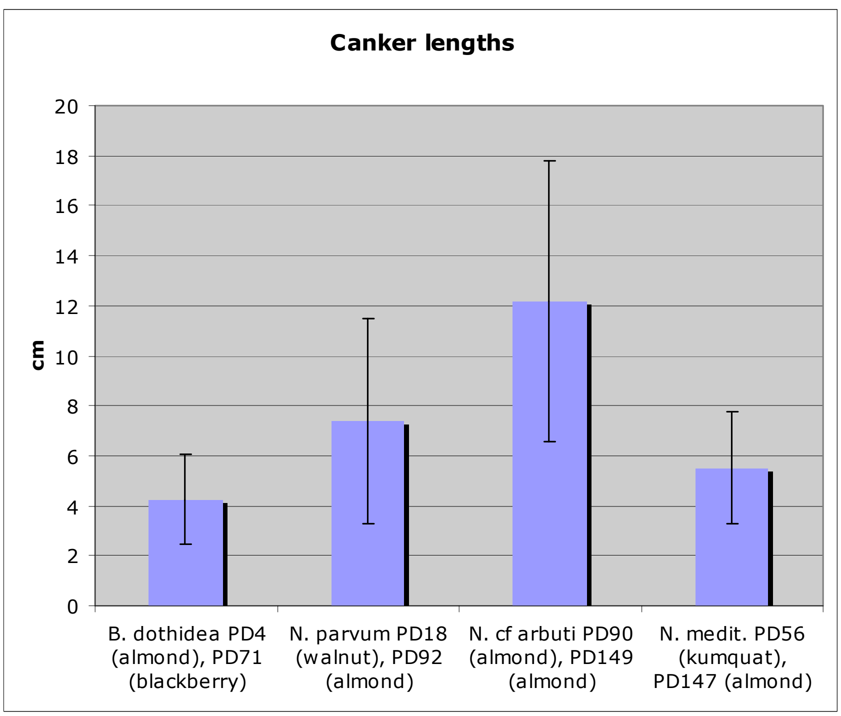

Figure 2. Canker length as affected by isolate of *Botryosphaeria* species 55 days after inoculation of Carmel almond trees at Kearney Agric Center in 2007.

Finding names for *Fusicoccum cf arbuti* and *Botryosphaeria cf parva* **-** Based on combined evidence from DNA sequence data and morphology, *Fusicoccum cf arbuti* and *Fusicoccum cf parvum* are new species isolated from almond cankers. However, only a small proportion of all *Fusicoccum* species ever described have been fingerprinted (sequenced). A total of 191 *Fusicoccum* species have been described since 1829, of which less than 10% have DNA sequences available for comparison. There is no book available on all *Fusicoccum* species, the information is spread out in numerous journals published all over the world, and comparing the two species from almond to all other already published species is a challenge. However, finding a name is easier for *F. cf arbuti*. *Fusicoccum cf arbuti* is characterized relatively large conidia, measuring nearly 25 µm in length. For the 104 of the 199 species for which conidia size could be found thus far in the literature, 23 have similar conidia lengths, defined here arbitrarily as falling between 20 and 30 µm. Only two of the 23 species have been sequenced, and thus 21 species have been compared to *F. cf arbuti* before this species can be declared as new. For this purpose, herbarium specimens are being ordered.

The situation for *B. cf parva* is more complex, its conidia are smaller, 19 µm in length, and thus less distinct. Only 35 of the 104 species for which conidia dimensions have so far been found have conidia of similar average lengths in the arbitrarily defined length range of 14 to 24 µm. To complicate things further, the neighboring genus *Dothiorella* comprises many species that should be transferred to *Fusicoccum*. *Dothiorella* currently contains 356 species that have to be investigated. These unknown, "new" species of *Botryosphaeria* from almond will be described in the near future. A phylogenetic tree (a diagram showing how different the almond species/isolates are from other known and described species).

Lower Limb Dieback:

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

Surveys to identify the putative pathogens shown in Tables 1 and 2 indicated that the most frequerntly isolated fungi from samples collected from various locations were *Botryosphaeria* and *Phomopsis* species. In both years of isolation, *Phomopsis* was more frequently isolated than *Botryosphaeria* species (Table 1). In addition, there was a trend of higher levels of isolation of these fungi from almond orchards of northern and central valleys than the southern parts of the State (Table 2).

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 results of objective 3 below).

Table 1. Incidence of fungi isolated from shoots 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[1]	**44**	**37**
Botryosphaeria rhodina	12	11
***Phomopsis* species**	**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

1 Under the name "*Botryosphaeria dothidea"* we included here all the *Botryosphaeria* species.

Table 2. Incidence of fungi isolated from almond orchards with trees suffering from lower limb dieback separated by region in 2006.

Pathogen isolated	**Isolation frequency (%)**		
	Sacramento Valley[1] (14 orchards)	**Northern San Joaquin[2] (11 orchards)**	**Southern San Joaquin[3] (10 orchards)**
Botryosphaeria dothidea[4]	**50**	**45**	**20**
Botryosphaeria rhodina	0	9	20
***Phomopsis* species**	**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 mangiferae	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.
4 Under the name "*Botryosphaeria dothidea"* we included here all the *Botryosphaeria* species.

<u>Serial isolations from limbs with LLDB in several orchards</u> **-** Results of the June 2008 isolations are reported in Tables 3 and 4. Generally, Phomopsis was more frequently isolated from samples than Botryosphaeria (Table 3) and isolated at higher levels in those trees showing symptoms than those without symptoms (Tables 3 and 4).

Table 3. Frequency of fungal isolations from almond limbs showing lower limb dieback symptoms collected in June 2008.

Orchard	Cultivar	Isolation results per 10 isolations per each of 10 limbs				
		***Botryosphaeria* species (%)**	***Phomopsis* species (%)**	***Nattrassia mangiferae* (%)**	***Aspergillus* species**	***Alternaria* species (%)**
Butte 1	Nonpareil	1	6	0	3	37
	Carmel	0	4	0	0	13
Butte 2	Aldrich	0	2	0	2	8
	Butte	0	0	0	4	7
Butte 3	Aldrich	2	18	0	0	23
	Butte	0	17	0	6	33
Stanislaus 1	Butte	0	4	0	4	47
	Padre	0	0	0	2	15
Stanislaus 2	Butte	1	3	0	2	51
	Padre	1	4	0	3	50
Stanislaus 3	Butte	0	1	0	0	49
	Padre	1	3	0	1	55

Table 4. Frequency of fungal isolations from symptomless (healthy) almond limbs collected in June 2008 from orchards wgere lower limb dieback was present as in Table 3.

Orchard	Cultivar	Isolation results per 10 isolations per each of 10 limbs				
		***Botryosphaeria* species (%)**	***Phomopsis* species (%)**	***Nattrassia mangiferae* (%)**	***Aspergillus* species**	***Alternaria* species (%)**
Butte 1	Nonpareil	1	1	0	3	43
	Carmel	0	0	0	0	18
Butte 2	Aldrich	0	0	0	6	4
	Butte	1	0	0	0	17
Butte 3	Aldrich	1	4	5	0	26
	Butte	1	0	0	1	16
Stanislaus 1	Butte	0	1	0	0	32
	Padre	3	0	0	6	28
Stanislaus 2	Butte	1	2	0	1	42
	Padre	1	0	0	0	31
Stanislaus 3	Butte	1	0	0	0	30
	Padre	1	0	0	0	29

It has been documented in published research that “stressed” plants are predisposed to infection by *Botryosphaeria* species. Although the results are from different years and different orchards, a comparison of the high levels of frequency of *Phomopsis* and *Botryosphaeria* in samples collected in September vs samples collected in June might explain partially this difference and may suggest that there is a continuous colonization of the plant tissues, particularly after major stresses (herbicide damage, frost, drought, etc.). Alternatively, it may also be that infections that may be successful in the early season progress further during summer and fall, resulting in higher frequencies of isolation of these fungi from the bark of limbs with LLDB.

3. To determine whether *Botryosphaeria dothidea* and *Phomopsis* species can cause LLDB symptoms in the field.

Results of canker development suggest that the *Botryosphaeria* isolates were more aggressive than those of Phomopsis, and among the *Botryosphaeria* isolates the #661 and #809 resulted in larger cankers (Tables 5 and 6). These 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.

Table 5. Canker formation from inoculations of Butte almond with *Phomopsis* and *Botryosphaeria dothidea* on **5 June 2007.**

Fungus/Ilsolate	**Inoculation rating score[1]**			
	Thrifty trees (good growth)		**Unthrifty trees (poor growth)**	
	Evaluated Aug. 29	**Evaluated Nov. 16**	**Evaluated Aug. 29**	**Evaluated Nov. 16[2]**
Phomopsis #07019	1.2 bc	1.2 ab	0.4 b	1.0 a
Phomopsis #07022	0.4 c	0.5 b	0.4 b	1.0 a
Phomopsis #3774	0.8 bc	1.2 ab	0.8 b	1.5 a
Botryosphaeria #661	3.6 a	Missing[2]	2.2 a	2.0 a
Botryosphaeria #809	2.8 ab	Missing	2.4 a	2.0 a
Botryosphaeria #3449	2.2 abc	2.0 a	0.6 b	1.0 a

[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).

[2] Some of the inoculated shoots were pruned off before the evaluation in November 2007.

Table 6. Canker formation from inoculations of Butte almond with *Phomopsis* and *Botryosphaeria dothidea* on **26 July 2007.**

Isolate	Inoculation rating score[1]			
	Thrifty trees (good growth)		Unthrifty trees (poor growth)	
	Evaluated Aug. 29	Evaluated Nov. 16[2]	Evaluated Aug. 29	Evaluated Nov. 16[2]
Phomopsis #07019	0.0 b	0.6 bc	0.0 b	0.8 a
Phomopsis #07022	0.8 b	1.0 abc	0.0 b	1.0 a
Phomopsis #3774	0.0 b	0.0 c	0.0 b	0.4 a
Botryosphaeria #661	2.0 a	2.2 a	1.2 a	0.3 a
Botryosphaeria #809	0.4 b	1.5 ab	0.2 b	0.2 a
Botryosphaeria #3449	0.4 b	1.2 abc	0.4 b	1.3 a
Combination of *Phomopsis* # 07019 and *B. dothidea* #809	0.0 b	0.4 bc	0.3 b	1.0 a

[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).

[2] Some of the inoculated shoots were pruned off before we performed our evaluation.

However, evaluations in November showed no differences. The inoculations in July started to be less aggressive, but by November there were no differences between the thrifty and non thrifty trees (Table 7).

Table 7. Overall effect of inoculations with *Phomopsis* and *Botryosphaeria dothidea* isolates and LLDB canker formation between thrifty and unthrifty Butte almond trees.

Condition of inoculated trees:	Inoculation rating score[1]			
	Inoculation on June 5		Inoculation on July 26	
	Evaluated Aug. 29	Evaluated Nov. 16[2]	Evaluated Aug. 29	Evaluated Nov. 16[2]
Thrifty trees (good growth)	1.8 a	1.1 a	0.5 a	1.2 a
Unthrifty trees (poor growth)	1.1 b	1.3 a	0.3 a	1.3 a

[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) which were evaluated on November 16.

[2] Missing data because some of the inoculated shoots were pruned off before the November evaluation of the cankers.

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

We observed that gum had developed in wounded trees set under the trunks with band cankers (spore inoculum source), as described in the Materials and Methods section, but infections by *B. dothidea* with typical canker symptoms have not developed yet. We will wait until the winter 2008 to do a second evaluation of these trees and determine whether any infections had occurred.

For the lower limb dieback, once we have identified the causal agent(s), 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.

Conclusions:

1. More than one species of *Botryosphaeria* pathogen are involved as the causes of band canker and canopy canker diseases of almond.
2. 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.
3. Isolations from limb samples showing early symptoms of LLDB revealed again low levels of *Phomopsis* and *Botryosphaeria,* with *Phomopsis* isolated more frequently than Botryosphaeria. More isolation will be done periodically to determine whether there is an increased incidence of these fungi with time on almond limbs.
4. Inoculations with isolates of *Botryosphaeria* and *Phomopsis* species showed that *Botryosphaeria* is more aggressive pathogen causing major cankers on almond and resulting in yellowing of the leaves and eventually death, a symptom that is characteristic of the LLDB before the limbs die.
5. Although infections by *Botryosphaeria dothidea* that occur during April to May caused the largest cankers on almond, it is still unclear when most of the infections by this pathogen occur to cause band canker.
6. In 2008, we initiated an experiment to determine whether herbicide drift can reproduce the symptoms of lower limb dieback.