Lower Limb Dieback in Almonds

Project No.: **07-PATH6-Lampinen**

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Interpretive Summary:

Three orchards in Stanislaus County with a history of lower limb dieback were monitored over the 2007 season. The lower limb dieback affected orchards tended to be wetter than the normal range of water potentials we would expect. Auger holes were dug in all three orchards in mid-June. Orchards #1 and #2 had uniformly wet soil down to 5 feet. Lower limb dieback symptoms increased until mid-June and then began to decrease as midday stem water potentials fell into the normal range. Canopy light interception levels were also not high enough to suggest that this was the cause of lower limb dieback.

These results suggest that excessively wet conditions early in the season could potentially have played a role in lower limb dieback. It is unusual to see orchards in the -6 to -7 bar midday stem water potentials that we observed in Orchards #1 and #2 in this study. It should also be noted that these are both flood irrigated orchards and water potential was measured at some random point in between irrigation events. The initial measurement in Orchard #1 was done about 10 days after the first irrigation had occurred suggesting conditions would have been much wetter immediately following irrigation. The symptoms also tended to get less severe as the season progressed and midday stem water potentials dropped into, and eventually below, the normal expected range (Fig. 1a, 1b). Field inoculation tests suggested that trees that were healthier were more resistant than less healthy trees to *B. dothidea* and *Phomopsis* spp. It is possible

that the excessively wet conditions that occurred in many of the affected orchards might lead to better conditions for infection by these organisms.

Objectives:

Growers in the Sacramento and San Joaquin Valleys have been noticing increasing dieback of lower limbs on almond trees. Beginning in late April, lower canopy leaves on affected trees begin to yellow and eventually turn brown and some drop off while others dry and remain hanging on the affected shoots. Eventually, entire limbs dieback and by late summer significant death of lower canopy wood can occur. Necrotic brown lesions can occur under the bark, primarily on the tops of the limbs around lenticels. Sometimes wedge shaped cankers are also visible on cross sections of affected limbs. Isolations for samples collected for two years (2005 and 2006), from these limbs have indicated the presence of both *Botryosphaeria dothidea* and *Phomopsis* spp. Both these fungi have been reported to cause canker diseases on almond in California and in Europe, Australia, and South America. Although both these fungi have been isolated at incidences up to 50 to 70%, isolations from lower limb dieback-affected shoots were not consistent from all of the almond samples collected in several counties.

Lower limb dieback (LLDB) seems to be most pronounced on the Butte and Padre varieties but has also been observed on Aldrich, Fritz, Neplus, Nonpareil, Sonora and other varieties to a lesser degree. Growers in Stanislaus County suggest the problem usually starts when Butte/Padre orchards reach about 7 to 8 years of age and continues to get worse as the orchard ages. Several growers have indicated the problem is worse in heavily shaded blocks although this does not always seem to be the case, particularly in Butte County orchards.

The objectives of the current study are to investigate the phenomena of lower limb dieback, determine the causative factors and develop methods to overcome the problem.

Plans and Procedures:

Three orchards in Stanislaus County with a history of lower limb dieback were chosen for detailed study in 2007. Orchard #1 and #2 are both flood irrigated Butte/Padre plantings. Orchard #3 is a microsprinkler-irrigated Butte/Padre/Ruby planting. Initial observations and limb sampling were done in early May 2007 in all three orchards.

Samples for disease isolations were taken from symptomatic limbs on Butte and Padre trees from all three orchards on 9 May 2007. Isolations included the following categories of media for putative pathogens:

1. Semi-selective PARP medium for species of *Phytophthora.*
2. Acidified PDA for other fungi.
3. Other specific media for *Phaeoacremonium* and *Phaeomoniella* spp.
4. Media for isolating *Verticillium dahliae.*

After collection, each sample was split into sub-samples and one sub-sample was sent to Themis Michailides' laboratory at the Kearney Agricultural Center (for isolations listed under media b-d above), another to Jim Adaskaveg's lab at UC Riverside (for isolations under media b-d above), and the third sub-sample was used for *Phytophthora* isolations by Greg Browne's laboratory at UC Davis.

Mid-day stem water potential was measured on bagged-lower-canopy-shaded leaves between 1 and 3:30pm. Overall orchard midday light interception and light interception under the tree canopies were measured with an Accupar light bar (Decagon Devices, Pullman, WA 99163 USA), within one hour of the time the sun was directly overhead. Overall, taking 100 measurements with the Accupar light bar around the target tree and comparing these values to full sun readings taken outside the orchard estimated orchard mid-day light interception. Mid-day light interception under the tree canopy was measured by taking 25 measurements in a circular pattern within the drip-line of the target tree and comparing these measurements to full sun readings taken outside the orchard. Mid-day stem water potential and light measurements were done approximately weekly early in season when symptoms were increasing and approximately monthly later in the season when symptoms generally were subsiding.

All trees were rated for lower limb dieback symptoms on each day water potential and midday light interceptions were measured. A rating of zero indicated no lower limb dieback was observed, a one indicated one limb of approximately one inch in diameter was exhibiting symptoms (or a number of smaller limbs that added up to the same leaf area as a one inch diameter limb), a two indicated two limbs were impacted, etc.

Fungicide treatments were applied in a LLDB orchard in Stanislaus County throughout the spring. The treatments included an eradicant and a protective treatment. Non-sprayed trees served as untreated controls. The first treatments were sprayed with a broad-spectrum fungicide such as Difolitan or Captan; and treatment 2 were the non-sprayed trees to serve as the non-treated controls. The Difolatan treatment was done twice per month during the season or before any large rainfall event. In April/ May, May/June, and July/August, trees in the plots were examined for new symptoms of LLDB and samples of shoots were collected.

Results:

Results from isolations taken from symptomatic Butte and Padre branches from the three orchards on May 9, 2007 (Table 1), did not produce as much *Botryosphaeria* or *Phomopsis* as the isolations done in 2005 and 2006 when up to 50 to 70% of isolations were positive. No *Phytophthora* species were isolated (Table 1). Because the symptoms during the 2007 season were mild and decreased over the summer, further isolations were not done during the season.

Table 1. Isolation Results From Almond Samples Collected on 9 May 2007:

Orchard	Cultivar	Number of diseased limbs yielding pathogen / number of limbs sampled		
		Botryosphaeria spp.	***Phomopsis***	***Phytophthora***
1	Padre, Butte	0/5	2/6	0/5
2	Padre, Butte	0/5	1/6	0/7
3	Padre	1/3	4/4	0/2

Midday Stem Water Potential and Light Interception

Midday stem water potentials in Orchard #1 and #2 tended to be wetter than the normal range of water potentials we would expect (shown as crosshatched area on Fig. 1a). Auger holes were dug in all three orchards in mid-June. Orchards #1 and #2 had uniformly wet soil down to 5 feet. This information is consistent with the water potential data (Fig. 1a). Orchard #3 had uniformly wet soil to about 4 feet and standing water filled hole at about 5 feet. These data are not consistent since the wet soil would suggest that water potentials should be in the wet range, but the trees were actually mildly water stressed (Fig. 1a). This suggests that the roots may be damaged due to excessively wet conditions, although we would have to monitor soil and plant water potential earlier in the season next year to confirm this. Lower limb dieback symptoms increased until mid-June and then began to decrease as mid-day stem water potentials fell into the normal range (Fig. 1a, 1b).

Overall canopy light interception was generally not above the 70% level (indicated by shaded area in Fig. 1c), where past experience tells us shading related dieback is likely to occur. Orchard #1 is approaching this level and some parts of the orchard were actually intercepting above 80% of the incoming light (data not shown). Orchards #2 and #3 were well below the light levels where extensive shading related dieback might be expected to occur. In order to check if the tall stature of Butte and Padre were exacerbating shading related dieback, light interception underneath the tree canopy itself was also measured (Fig. 1d). Only the Butte variety in Orchard # 2 was approaching the 92% under canopy light interception level where we have observed shading related dieback in previous work (Fig. 1d).

These results suggest that excessively wet conditions early in the season could potentially have played a role in lower limb dieback. It is unusual to see orchards in the -5 to -7 bar midday stem water potentials that we observed in Orchards #1 and #2 in this study. It should also be noted that these are both flood irrigated orchards and water potential was measured at some point in between irrigation events. The initial measurement in Orchard #1 was done about 10 days after the first irrigation had occurred suggesting conditions would have been much wetter immediately following irrigation. The symptoms also tended to get less severe as the season progressed and midday stem water potentials dropped into, and eventually below, the normal expected range (Fig. 1a, 1b).

Fungicide Trials

Fungicide treatments were applied in two LLDB orchards in Stanislaus County. The treatments included eradicant and protective treatments. In one orchard, foliar sprays of Pristine or Captan fungicides with or without the addition of a bark penetrating surfactant (Pentra-bark) were applied to the lower canopy in early May, just prior to the expected onset of symptom expression. In this orchard, very few LLDB symptoms developed throughout the summer and there were no differences among treatments. It was interesting to note that the application of Captan plus the bark penetrating surfactant caused fairly severe leaf necrosis, while very few symptoms were observed in the surfactant plus Pristine treatment or Captan with no surfactant.

In a second experiment, Pristine, Captan or Agri-fos (mono and di-potassium salts of phosphorous acid), plus a bark penetrating surfactant were applied to the trunks and lower scaffolds (not the foliage), in a Butte & Padre orchard with fairly severe LLDB symptoms. Treatments were applied 22 June. Prior to application of the treatments, all symptomatic limbs were pruned off. Trees were rated for severity of LLDB symptoms on 17 August on a scale from 0 (no LLDB symptoms), to 4 (severe symptoms, including limbs over one inch in diameter affected). Limb dieback symptoms were severe and significantly higher than untreated trees in the Agri-fos + surfactant treatment (Table 2). Other fungicide treatments were similar to untreated trees.

Table 2. Lower Limb Dieback Symptoms on Almond Trees Treated with a Fungicide Plus a Bark Penetrating Surfactant

Treatment	LLDB Symptoms (Rating 0-4)
Agri-fos @ 1.5 qt. in 1 gal. solution + 3 oz penetrant	3.6 a
Captan 80 WDG @ 5.66 lb + 3 oz penetrant	2.3 b
Untreated	2.0 b
Pristine @ 14.5 oz + 3 oz penetrant	1.6 b

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. Isolation from all these samples were done following procedures routinely used by Themis Michailides laboratory and detailed results are presented in his report. Two fungi, *Botryosphaeria dothidea* and *Phomopsis* spp. predominated in all these isolations.

Field inoculations

To determine whether *B. dothidea* and *Phomopsis* spp. can cause LLDB symptoms in the field, inoculations were made 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 Co.). Cankers that developed were recorded on August 29.

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. Details of these results are available in the report by Themis Michailides et.al. for project 07-PATH5-Michailides.

2008 Plans

Three orchards in Stanislaus County and two orchards in Butte County with a history of lower limb dieback problems were outfitted with soil moisture dataloggers in the spring of 2008 and are also being monitored for midday stem water potential, canopy light interception and lower limb dieback symptoms. Preliminary results suggest all five orchards were wetter than the fully watered baseline through May and all five orchards showed symptoms of lower limb dieback to varying degrees. Results from isolations from affected trees in these orchards will be presented at the Almond Board Meeting in Modesto in December 2008.

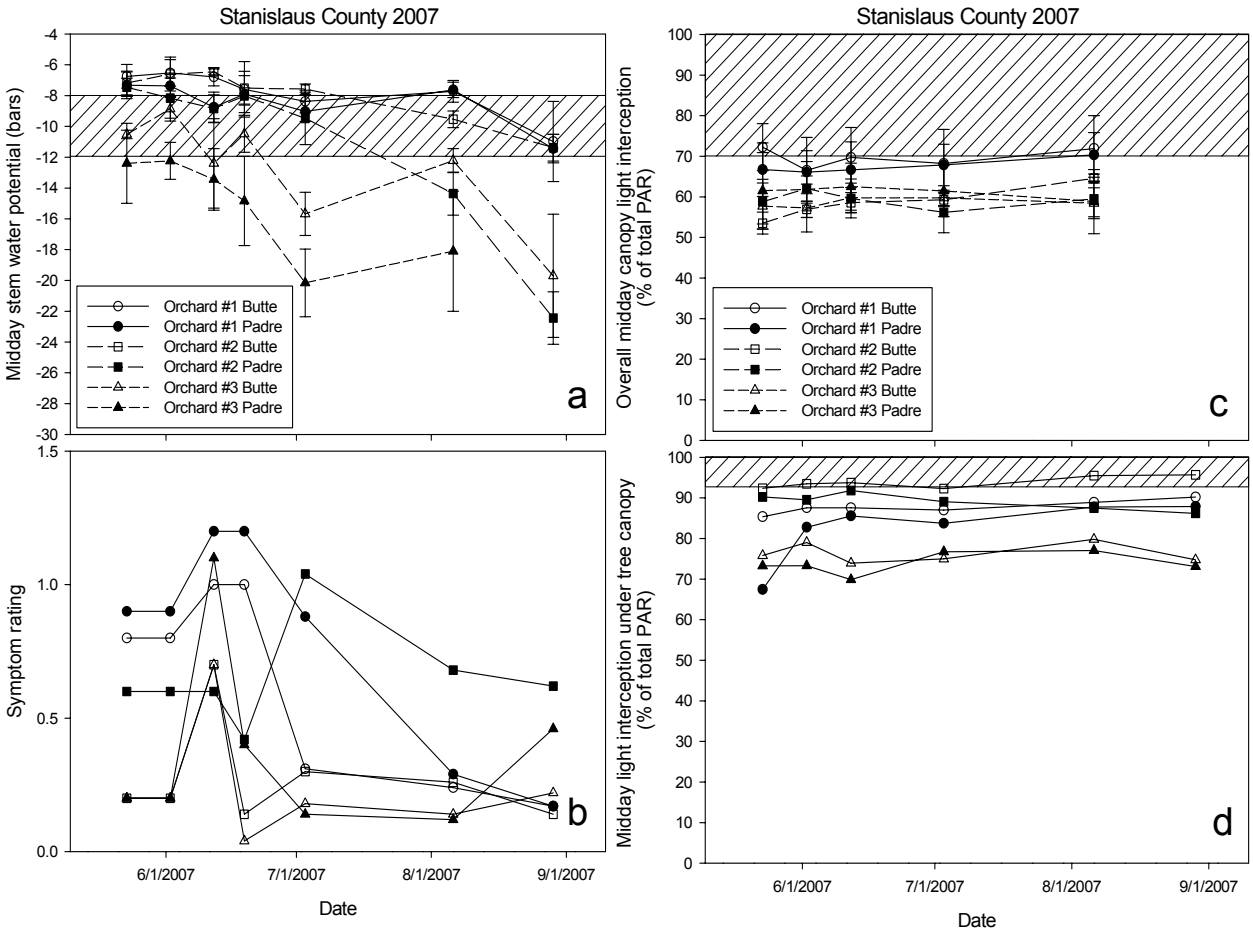

Fig. 1. Midday stem water potential (a) symptom rating, (b) overall canopy light interception (c) and midday light interception under tree canopy (d) by orchard and variety for Stanislaus County sites.