Bacterial Canker and Almond Leaf Scorch

Project No.: 01-BK-00

Project Leader: Bruce Kirkpatrick, Dept. of Plant Pathology, UC Davis
Ken Shackel, Ted DeJong, Dept. of Pomology, UCDavis

Cooperating Personnel: Mike McHenry and Roger Duncan

Objectives

1) Determine how ring nematode infestation predisposes Prunus species to develop bacterial canker disease.
2) Determine if supplements of nitrogen, calcium or IAA can decrease the incidence or severity of bacterial canker disease.
3) Determine the relative susceptibility of current almond cultivars to almond leaf scorch disease caused by the bacterium, *Xylella fastidiosa* (Xf).
4) Determine what time of year inoculations of Xf produce systemic infections that result in almond leaf scorch disease the following year.

A. Bacterial Canker Research:

Bacterial canker (BC) is an important disease of most *Prunus* species, including almond. It is caused by the bacterium *Pseudomonas syringae* pv syringae; however, BC only occurs on trees that are "stressed" by various biological and/or abiotic factors. Probably the most important biological agent that predisposes tress to BC is high populations of the ring nematode, *Criconemella xenoplax*, which occurs in many almond growing districts with sandy soils. With funding provided by the Almond, Prune and Cling Peach Boards have been studying BC for the past 5 years, primarily focusing the genetic make up of Pss strains that cause BC, determining where the pathogen resides on trees and the influence of various horticultural practices such as rootstock, budding height, nitrogen fertilization and copper based bactericides on the occurrence and/or severity of BC. Results from these studies can be found in previous Almond Board Project Reports.

Beginning in 2000 we have shifted our focus away from Pss to examine how various factors can predispose trees to develop BC. The primary factor we are examining, in cooperation with Mike McHenry at UC Kearney, is the effect that ring nematode infestations have on altering nitrogen and calcium uptake and possibly altered levels of IAA, a plant hormone. These are long-term experiments that have just been undertaken in the past year. This winter we will inoculate experimentally stressed trees with cultures of Pss to determine how various parameters such as nitrogen fertilization, supplemental calcium, and ring nematode populations affect the development of BC.

The following paragraphs summarize that experiments underway at Kearney and at a peach orchard located in Stanislaus County.

Kearney Ring Nematode Plots:

In April, 2001, 72 one-year old peach trees (cv Elegant Lady on Nemaguard rootstock) were planted in 18 concrete tanks filled with sandy soil at Kearney. Soil in the tanks was fumigated with methyl bromide 3 months prior to planting the trees. In May, 2000, 10,000 juvenile ring nematodes were inoculated in 9 of the tanks that contained 4 trees each. Each tree in the nematode infested and un-infested tanks were treated with one of the following treatments: 10 mM $CaS0_4$ injected into the trunk, 1.1% urea applied as a foliar spray, 10 ppm IAA applied as a foliar spray and one tree that was left as the untreated control. The various treatments were applied at 3 to 4 week intervals during the growing season throughout 2000 and 2001. Bark samples from the Ca and urea treated and control trees were collected during the winter of 2000 and analyzed by the DANR Analytical Laboratory at Davis for nitrogen and Ca levels. As expected we found no significant differences in cambial concentration of Ca and N. Cambial tissues are considered to be the most informative for our purposes because this is the tissue in which Pss multiplies and spreads throughout the tree in the Spring. Because the trees seemed to grow well the first year in all of the plots and the cambial analysis showed no major differences in treated versus non-treated trees we did not inoculate the trees with Pss during the winter of 2000. Nematode samples were taken from infested and non-infested tanks and high levels were still present in the infested tanks whereas no ring nematodes were found in the non-infested tanks. It is also important to note that BC rarely occurs in newly planted trees in the field, typical onset of disease occurs in the second through fifth year following planting.

Nitrogen and Ca analyses will be performed on dormant cambial tissues this winter. This winter individual limbs on all of the trees will be inoculated with cultures of Pss in mid-December and late January. In addition, small portions of limbs will be removed from the trees and inoculated with Pss using a laboratory system that has allowed the experimental determination of cambial susceptibility in the laboratory. We hope to determine whether supplemental treatments with N and Ca can compensate for the decreased uptake of these nutrients by roots that have been damaged by ring nematode feeding. We have hypothesized that trees treated with foliar application of N and Ca will have smaller BC cankers, in both the field and laboratory inoculations, than control trees, and that lesions produced on ring nematode stressed trees will be larger than lesions produced on non-infested trees.

Stanislaus County field experiments:

In cooperation with Roger Duncan we have examined the relative susceptibility of 4-year-old Riegel peach trees growing in BC-affected sandy soils in the Modesto area. The relative susceptibility of 18 trees growing in either fumigated or non-fumigated soils was evaluated. Trees were either inoculated in the field with cultures of Pss or small limbs were detached and evaluated in the laboratory using the *in vitro* assay we have previously developed. The following table summarizes the results of these inoculations:

Soil Treatment	BC lesion length (mm)		
	Field Inoculated Limbs*	Laboratory Inoculated Limbs**	
		Freeze/thaw (+)	Freeze/thaw (-)***
Fumigated	40.9 +/- 4.2	33.7 +/- 5.6	11.0 +/- 0.5
Non-fumigated	134.8 +/- 20.8	54.5 +/- 13.3	9.8 +/- 0.6

*Similar diameter limbs and amounts of Pss were inoculated in both the field and laboratory experiments. Following inoculation in the field the limbs allowed to incubate at ambient temperatures for 5 weeks (2/1/2001 to 3/8/2001).
** Following inoculation the limbs were incubated at 15 C for 2 weeks, then rated for lesion length.
***Limbs were either inoculated at room temperature or as they thawed after being exposed to –5C for 12 hours.

These results indicate that larger BC lesions appeared, in both field and laboratory inoculated trees that were growing in the non-fumigated, "stress" soil environment. The size of lesions obtained with the field-inoculated trees were greater than the laboratory inoculated limbs but a similar trend was observed with the laboratory inoculated trees subjected to the freeze/thaw inoculation.

During the 2001 growing season, 30 representative trees growing in the non-fumigated and fumigated BC hotspot were treated monthly with Ca nitrate foliar sprays to determine if exogenous foliar applications of Ca or nitrogen might decrease the incidence and/or severity of BC. Treated and untreated trees will be inoculated in the field and in the laboratory using methods similar to those used previously.

B. Almond Leaf Scorch Research:

There were two objectives that we wanted to pursue on the almond leaf scorch (ALS) project. The first was to determine the overall susceptibility of current almond cultivars to ALS, which is caused by the bacterium *Xylella fastidiosa* (Xf). Xf is transmitted from infected to healthy trees by several species of xylem-feeding sharpshooters, including the newly introduced glassy winged sharpshooter (GWSS). The second major objective was to determine what time of year an inoculation event is most likely to produce a systemic infection the following season. Upon designing the experimental plan we also thought it would be important to determine whether Xf strains isolated from Pierce's disease grapes are just as capable of causing ALS as Xf strains obtained from almond in the 9 almond cultivars that we are evaluating. Sandy Purcell's previous work indicated that grape strains can cause systemic infections in almond, but we wanted to verify this prior to starting a large-scale time course inoculation study. This is also a particularly important factor to verify because it is likely that most of the Xf inoculum that GWSS will be spreading when it becomes established in an almond/grape growing region will come infected grapes because the GWSS prefers this host over almond and concentrations of the bacterium are much greater in grape than in almond so it is easier for a healthy insect to pick up Xf from grape than almond.

Trees used in evaluating the cultivar's susceptibility and strain pathogenicity studies were 12 years old and growing in the Plant Pathology field area at UC Davis. The following nine cultivars were evaluated: Butte, Carmel, Non Pareil, Mission, Padre, Price, Solano, Sonora and Thompson. Peerless will also be evaluated in the time of inoculation study using newly planted trees, as explained below. Two Xf strains from grape and one almond strain were individually used to inoculate nine new green shoots on each of 3 trees of each cultivar, i.e. 27 shoots were inoculated with one of the three Xf strains per cultivar for a total of 243 shoots. Pin-prick inoculations of cultured Xf cells were performed on July 15, 2000. (Unfortunately these older trees have been pruned to allow easy tractor access by UCD personnel and all of the new shoots were located high in the tree and inoculations had to be done with the aid of a ladder. For this reason we felt it would be better to perform the time of inoculation studies on new trees, trees which we obtained courtesy of Duarte Nursery this past spring, 2001). The inoculated shoots were evaluated 3 months later and very few had developed symptoms. For this reason we waited to evaluate the inoculations until September 15, 2001, approximately 14 months later. There were considerable differences among the 9 cultivars in the percentage of inoculated shoots that produced systemic disease the next year (Table 2). However, there was no significant differences between the numbers of systemic infections produced by the grape strains compared to the almond strains as shown below:

Table 1. Number inoculated shoots that developed ALS symptoms 13 months following inoculation.

Strain	# of ALS (+) shoots/# inoculated (%)	Missing inoculated shoots*
Grape A	20/78 (26)	3
Almond	25/75 (32)	6
Grape B	28/69 (40)	12

* Some of the identification tags or the shoots themselves could not be positively identified 14 months following inoculation.

Because there was no significant difference between the ability of the grape and almond to cause ALS we combined all of the Xf strain inoculation data together to determine the relative susceptibility of the cultivars. The relative susceptibility of the 9 cultivars that were inoculated is shown in the following table:

Table 2. Incidence of ALS disease in 9 almond cultivars following inoculation with *X. fastidiosa*

Cultivar	# ALS shoots/# inoculated* (%)		Missing inoculated shoots**
Thompson	16/22	(73)	5
Sonora	13/23	(57)	4
Solano	11/25	(44)	2
Non Pareil	6/24	(25)	3
Padre	6/24	(25)	3
Butte	6/26	(23)	1
Price	6/26	(23)	1
Carmel	2/26	(8)	1
Texas Mission	1/26	(4)	1

*9 shoots on 3 trees of each cultivar were inoculated with one of 2 grape or 1 almond strain of *X. fastidiosa*. On July 15, 2000. All of the shoots were evaluated for any symptomatic leaves in September 15, 2001. Data for all X. fastidiosa strains were combined.
**some of tags on the inoculated shoots, or the shoots themselves could not be found or positively identified 14 months later.

These results show that the rate of survival of overwintering Xf inoculations was relatively low in all of the varieties except Thompson, Sonora and Solano. In comparison similar inoculation of grapevines in July would result in nearly 80% infection the follow year providing the inoculated cane was not pruned out.

Table 3. Severity of almond leaf scorch disease in 9 almond cultivars expressed as number of symptomatic leaves and spurs that developed on inoculated shoots after 14 months.*

# of ALS (+) spurs/ shoots	# of ALS (+) leaves/ # spurs on ALS (+)shoot	(%)	Number of total # leaves on ALS (+) Shoot	(%)	ALS (+)
Thompson	95/263	(36)	181/1407	(13)	16
Sonora					13
Solano	34/207	(16)	42/663	(6)	11
Non Pareil	45/147	(31)	75/588	(13)	6
Padre	11/90	(12)	11/381	(3)	
Butte	9/108	(8)	9/371	(2)	6
Price	7/65	(11)	7/223	(3)	6
Carmel	4/21	(19)	4/51	(8)	2
Texas Mission	1/21	(5)	1/116	(1)	1

*only spurs and leaves on any shoot that developed any symptom of almond leaf scorch (ALS) were counted, i.e. non-symptomatic shoots were not counted.

In general, the disease severity ratings correlated with the disease incidence in the 9 cultivars. These results suggest that once infection occurs, the bacteria moves more rapidly and probably reach higher concentrations in Thompson, Solano and Sonora, than in does in the other 6 varieties. We hope to compare the relative concentrations of Xf in these varieties next summer to determine if the more susceptible varieties do in fact have higher populations of Xf. Both the incidence and severity of disease following inoculation were lowest in Padre, Butte, Price, Texas Mission and Carmel, at least in these 12 year old trees.

Because these inoculated shoots are high up in the canopy of the tree we are going to let the infection continue to develop over the next few years and monitor the spread of Xf over time. When the symptoms become quite obvious to an observer on the ground, as they would be to a grower walking through an orchard, we will then prune out these strikes at various distances below the last symptomatic leaves. We suspect that pruning can be used to eliminate ALS infections because our experience with other experimentally inoculated almond trees suggests Xf moves much more slowly in almond than in grape. However, that work was done with only one cultivar.

Beginning next May we will begin our time of the year inoculations on the young trees we planted this fall. It will be much easier to inoculate these smaller trees than using ladders to inoculate the older trees used in this first part of the project. We also hope to evaluate some of the most promising bactericides that we are now evaluating for use in Pierce's diseased grapevines in Xf-infected almond. In this manner we hope to develop implementable guidelines that growers can use to manage almond leaf scorch, which will likely become more of a problem as the glassy winged sharpshooter become established in additional almond growing districts.