Studies on Bacterial Canker and Almond Leaf Scorch Diseases 2001 Project Progress Report

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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 (Pss); however, BC only occurs on trees that are "stressed" by various biological and/or abiotic factors. Probably the most important biological agent that predisposes trees to BC is high populations of the ring nematode, *Criconemella xenoplax*, which occur in many almond growing districts with sandy soils. With funding provided by the Almond, Prune and Cling Peach Boards we 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 have 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 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 Kearny, is the effect that ring nematode infestations have on altering nitrogen and calcium uptake and possibly alter levels of IAA, a plant hormone. These are long-term experiments that have just been undertaken in the past year. This winter we will again inoculate ring nematode stressed trees at Kearney with cultures of Pss to determine how various parameters such as supplemental treatments of nitrogen, calcium, and IAA, as well as ring nematode populations affect

the development of BC. The following paragraphs summarize the experiments that are underway at UC Kearney and at a peach orchard located in Stanislaus County and a prune orchard in Yuba County.

Kearney Ring Nematode Plots:

In April, 2000 72 one-year old peach trees (cv Elegant Lady on Nemaguard rootstock) were planted in 18 concrete tanks filled with sandy soil at UC 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 non-infested tanks was treated with one of the following: 10 mM $CaSO_4$ injected into the trunk (200mls/tree/injection, 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, i.e. a total of 8 treatment during the 200 and 2001 growing seasons. Cambial 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. Results from these assays are presented below:

Table 1. The effect of ring nematodes and nitrogen foliar sprays on leaf and cambial tissue nitrogen and phenolic content of cambial tissues after 4 applications in 2000.

Nematode (+/-)	Treatment	Leaf N%	Cambium N%	Total phenolics (mg/g fresh wt)	No. of trees
+	Control	2.82 +/- .32a	1.78 +/- .37a	32.6 +/- 10.8a	9
-	Control	2.86 +/- .38a	1.70 +/- .23a	24.8 +/- 7.5b	9
+	N foliar spray	3.07 +/- .40a	1.78 +/- .36a	27.9 +/- 4.1ab	9
-	N foliar spray	2.95 +/- .24a	1.81 +/- .31a	27.0 +/- 5.2a	9

Table 2. The effect of ring nematodes and calcium injection on leaf and cambium Ca concentrations after 4 applications in 2000.

Nematode (+/-)	Treatment	Leaf Ca%	Cambium Ca%	No.of trees
+	Control	1.43 +/- 0.20a	2.99 +/- 0.54a	9
-	Control	1.71 +/- 0.24b	2.57 +/- 0.36a	9
+	Ca foliar spray	1.50 +/- 0.32a	2.86 +/- 0.45a	9
-	Ca foliar spray	1.61 +/- 0.37ab	2.88 +/- 0.55a	9

There were no significant differences in N content of leaves or cambial tissues due to ring nematode infestation or foliar application of urea. There was no consistent difference in phenolic content due to nematode infestation or urea sprays. However, a significant difference in leaf, but not cambial tissue Ca content occurred in nematode infested trees. It should be emphasized that we really didn't expect to see significant differences occur in the treated trees in their first year of growth. The initial difference in Ca leaf content may translate into significant differences in the second and subsequent years of growth, which may affect their susceptibility to BC. 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.

Nematode samples were taken from infested and non-infested tanks and high levels (225 +/- 58/250 cc of soil) 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.

Field and laboratory inoculation experiments were performed on the Kearney peach trees. Trees were inoculated with Pss in the field on 1/10/01 and the inoculation site was covered with parafilm and allowed to incubate at ambient temperature for 8 weeks. At the time these trees were inoculated in the field, small limbs were removed from these same trees and inoculated with Pss in the laboratory using the non-frozen and freeze-thaw procedures described below. The following tables summarize the results of those experiments:

Table 3. The effect of ring nematode, calcium infiltration, urea and IAA foliar spray on bacterial canker lesion size in field-grown peach trees (cv. Elegant Lady/Nemaguard, planted in April 18, 2000) in KAC.

Treatment	Nematode (+/-)	Average bark canker size*		
		Length (mm)	Width (mm)	Area (mm^2)
Control	+	71.1±25.4 a	7.7±1.7 a	431.9±230.4 a
Control	-	21.9± 2.3 b	5.6±0.5 a	65.6± 12.3 b
Calcium	+	36.4± 6.4 b	5.9±0.9 a	124.2± 36.9 b
Calcium	-	30.1± 6.5 b	6.2±0.8 a	113.9± 38.6 b
IAA	+	32.0± 5.7 b	6.1±0.7 a	109.8± 27.5 b
IAA	-	33.4± 4.6 b	4.8±0.6 a	89.2± 19.6 b
Nitrogen	+	33.8± 7.2 b	5.7±0.6 a	99.8± 22.0 b
Nitrogen	-	26.1± 5.5 b	5.3±0.8 a	83.2± 29.7 b

* Average of 9 replicates ± standard error.
Trees were inoculated on 1/10/01 and the size of the lesions determined 8 weeks later on 3/13/01.

Even though we did not expect to see significant difference in these 1 year old, field inoculated trees, it was apparent that the large numbers of ring nematodes that were added to the tanks sufficiently stressed the untreated control trees to predispose them to develop BC. Compared to this untreated, positive control, all of the nematode-exposed trees that were treated with calcium, nitrogen or IAA produced significantly smaller size cankers. There were no significant differences in the magnitude of the lesion size reduction among the treatments. We anticipate that our planned December 2001 and January 2002 inoculations should produce even greater differences since the trees will have been exposed to the nematodes for another year and the trees will have received additional treatments.

Table 4. Effect of ring nematode, freezing/thawing, calcium infiltration, urea and IAA foliar spray on bacterial canker size in excised peach stems sampled from KAC.

Treatment	Nematode (+/-)	Average canker size*		
		Length (mm)	Width (mm)	Area (mm^2)
Inoculation during thawing process				
Control	+	20.5±3.2 ab	3.2±0.3 bc	35.0± 7.0 b
Control	-	15.1±2.7 b	2.7±0.3 c	24.2± 7.1 b
Calcium	+	23.7±2.4 ab	3.9±0.2 ab	46.9± 6.2 b
Calcium	-	20.7±3.4 ab	3.8±0.5 abc	43.2± 8.8 b
IAA	+	25.9±8.3 ab	3.7±0.3 abc	53.4±21.1 ab
IAA	-	20.6±4.5 ab	3.0±0.2 bc	33.1± 8.9 b
Nitrogen	+	27.8±4.8 ab	4.5±0.3 a	61.8± 9.1 ab
Nitrogen	-	37.5±8.7 a	4.3±0.4 a	91.2±28.4 a
Inoculation without freezing				
Control	+	9.0±1.0 a	1.8±0.2 a	8.8±1.5 a
Control	-	9.1±0.9 a	2.3±0.2 a	10.5±1.4 a
Calcium	+	10.3±0.6 a	1.9±0.1 a	9.7±0.6 a
Calcium	-	9.1±0.7 a	2.0±0.2 a	9.4±1.3 a
IAA	+	9.4±0.6 a	1.8±0.2 a	8.8±1.2 a
IAA	-	8.7±1.1 a	1.8±0.1 a	8.3±1.6 a
Nitrogen	+	9.7±0.6 a	1.9±0.1 a	9.2±1.0 a
Nitrogen	-	9.1±0.7 a	2.0±0.1 a	8.9±0.8 a

* Average of 9 replicates ± standard error.

Unfortunately, results obtained with the laboratory-inoculated limbs did not correlate with the field-inoculated limbs. The only significant differences occurred in limbs that were exposed to the freeze/thaw method of inoculation. The greatest size cankers were produced on limbs from trees that did not receive an additional supplement of nitrogen. No other treatment produced significant results. In this assay the limbs are evaluated 15 days after inoculation, where as the field inoculated limbs were evaluated 8 weeks post inoculation. Given the possibility that longer incubation of the laboratory inoculated limbs might give better results, we plan to incubate the laboratory inoculated, excised limbs for 3 or 4 weeks during 2001/02.

In summary, we are trying to determine whether supplemental treatments with N, Ca or IAA can compensate for the decreased uptake or production of these nutrients by roots that have been damaged by ring nematode feeding. We have hypothesized that trees treated with supplemental foliar applications of N, Ca and possibly IAA will produce smaller BC cankers, in both the field and laboratory inoculations, than ring nematode stressed control trees.

Stanislaus and Yuba County field experiments using peach and prune trees, respectively:

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 peach 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:

Table 5. Effect of soil fumigation and plastic mulching on bacterial canker lesions that developed on inoculated branches in field grown peach trees (cv. Riegel/Nemaguard) growing in Modesto[x]

Preplant soil treatment*	Plastic mulching	Ring nematodes[y]	Average BC lesion size[z]		
			Length (mm)	Width (mm)	Area (mm^2)
Fumigated	Mulched	7.7 ± 3.6 b	24.0± 2.1 b	3.5±0.3 b	46.6± 8.7 b
Fumigated	Non-mulched	0.5 ± 0.5 b	40.9± 4.2 b	4.6±0.3 b	97.1± 13.3 b
Non-fumigated	Non-mulched	188.7 ± 80.0 a	134.8±20.8 a	13.9±1.5 a	1150.0±240.4 a

[x] Incubation period following Pss inoculation was 5 weeks (02/01/2001-03/08/2001).

[y] Numbers of ring nematodes/250cc of soil. Means ± 1 standard error of six samples. Each sample was a combined sample of three adjacent trees.

[z] Average of 18 replicates ± 1 standard error. Means followed by the same letter are not statistically different.

*Preplant soil fumigation: DiTera 27.5 gallon/1.3 acre (04/24/96); 22.5 gallon/1.3 acre (10/22/96). Trees were replanted in March 1997

Results of these field-inoculated trees show the significant effect that ring nematode infestation has on stressing trees and predisposing them to develop bacterial canker.

Similar, although not as dramatic, results were also obtained with 8 year old prune trees that were growing in a BC hotspot area that was infested with ring nematodes, described below as "diseased area" in the following table, versus mildly diseased trees growing in an area of the orchard that had low numbers of ring nematodes, i.e. "healthy area" of the orchard.

Table 6. Effect of tree disease status and orchard location on the size of BC lesions that developed following inoculation of Pss into limbs of field-grown French prune trees growing in Yuba county.

Area sampled in orchard	Sampled tree disease status	Number of trees	Average canker size**		
			Length (mm)	Width (mm)	Area (mm^2)
Healthy	Healthy	15	15.7±1.6 b	3.6±0.2 b	28.7±3.5 b
Healthy	Diseased	15	19.1±4.4 ab	2.9±0.2 c	28.6±7.0 b
Diseased	Healthy	15	19.9±4.2 ab	2.9±0.3 c	29.3±6.4 b
Diseased	Diseased	13	28.3±4.1 a	4.2±0.2 a	59.5±9.6 a

* Incubation period (8 weeks): 01/20/2001-03/21/2001.

** Mean ± 1 standard error.

The largest lesions developed in diseased trees that were growing in the nematode infested area while the smallest lesions developed on healthy trees growing in a non-infested area. Healthy trees growing in an infested area and mildly diseased trees growing in a non-infested area produced intermediate size lesions. What is particularly interesting is that completely healthy appearing trees

growing in a hot spot area were still being predisposed as much as trees with mild disease symptoms that were growing in a non-infested area of the orchard.

We also wanted to see if our comparatively rapid laboratory assay correlated with the results of the field inoculation. Similar size branches were removed from the same field-grown trees and inoculated in the lab using one of two techniques. Pss bacteria were either injected into non-frozen limbs or into limbs that had been frozen overnight at –5C and then inoculated as the limbs came to room temperature (the freeze/thaw inoculation). Following inoculation, all excised stems were incubated at 15 C for 1 or 2 weeks, compared to the 5 week or 8 week incubation of field-inoculated trees. Results from this experiment are shown below.

Table 7. Effect on BC lesion size that developed on excised peach limbs that were inoculated with Pss under laboratory conditions.

Soil treatment	Plastic mulch-ing	Variety	One week*		Two weeks	
			Freeze (+)	Freeze (-)	Freeze (+)	Freeze (-)
Fumigated	+	Riegel	29.8±3.7a	11.0±0.4a	31.3± 2.9 a	11.2±0.7 a
Fumigated	-	Riegel	22.2±1.7a	10.4±0.4a	33.7± 5.6 a	11.0±0.5 a
Non-fumigated	-	Riegel	23.4±5.9a	10.9±0.9a	54.5±13.3 a	9.8±0.6 a

* Data are average of 18 replicates ± standard error

These results showed a much less dramatic difference than what was observed with the field-inoculated peach trees however there was a general trend that wood collected from nematode stressed trees produced larger size lesions than non-stressed trees using the freeze/thaw inoculation procedure and a 2 week incubation time.

As the following table shows there was a better correlation between the field and the laboratory inoculations using excised prune limbs taken from nematode infested, versus non-infested, areas of the orchard:

Table 8. Effect of tree status and orchard locations on the size of bacterial cankers in excised French prune stems inoculated and incubated under lab conditions (incubation for 2 weeks at 15°C).

Health status of the site	Tree disease status	+/- Freezing treatment	Average bark canker size*		
			Length (mm)	Width (mm)	Area (mm^2)
Healthy	Healthy	Freezing	16.0±2.2 b	3.9±0.3 ab	34.6± 7.6 b
Healthy	Diseased	Freezing	18.4±2.4 b	3.5±0.3 b	35.9± 7.0 b
Diseased	Healthy	Freezing	21.5±3.5 b	3.0±0.3 b	37.2± 9.0 b
Diseased	Diseased	Freezing	52.4±9.3 a	4.7±0.4 a	139.3±31.9 a
Healthy	Healthy	Nonfrozen	9.4±0.6 B	1.7±0.1 B	8.5± 1.0 B
Healthy	Diseased	Nonfrozen	11.1±0.7 B	1.9±0.1 AB	10.7± 1.0 B
Diseased	Healthy	Nonfrozen	15.3±2.3 A	1.7±0.1 B	13.6± 2.5 AB
Diseased	Diseased	Nonfrozen	16.0±1.1 A	2.2±0.2 A	18.0± 2.0 A

In these assays, the freeze/thaw inoculation always produces greater size lesions than material inoculated at room temperature. We plan on repeating these assays again this winter and hope to include almond materials in both field and laboratory inoculations.

In addition to the previously described studies, during the 2001 growing season, 30 representative peach and 30 prune trees growing in healthy and BC hotspots in these orchards 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. Limbs on 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 infected grape compared to infected 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 Pariel, 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 Pariel	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.*

Cultivar	# of ALS (+) spurs/ # spurs on ALS (+)shoot	(%)	# of ALS (+) leaves/ total # leaves on ALS (+) Shoot	(%)	Number of ALS (+) shoots
Thompson	95/263	(36)	181/1407	(13)	16
Sonora	42/180	(23)	42/466	(9)	13
Solano	34/207	(16)	42/663	(6)	11
Non Pariel	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 becomes established in additional almond growing districts.