Project Number: 99-BK-o0

Almond Board Research Report
1999-2000

Title: Almond bud drop and Bacterial Canker:

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The following research report is divided into 3 sections. Section I reports the results of field trial to control almond bud drop which was conducted by Roger Duncan during 1999/00. Section II reports the results of bacterial isolation work that was conducted during 1999/00 to determine the role that Pseudomonas syringae may or may not play in almond bud drop. Section III reports the results of basic and applied research on bacterial banker disease which was partially funded by the Almond Board in cooperation with the California Prune Board.

I. Results of Field Trials on Almond Bud Drop

Year-end Summary, 1999

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Ron Sayler, Graduate student, UCDavis
Cooperators: Albert Nydam & Robert Longstreth, growers

Dormant bud drop has become a serious problem in many young almond orchards growing in Northern San Joaquin Valley sandy soils. In severely affected orchards, 80-100% of the fruitful and lateral buds fail to swell and begin falling to the ground by early January. Terminal leaf buds grow normally which results in large areas of blind wood after a few seasons. Therefore, bud drop not only reduces the current season's yield, but also results in long term losses.

Bud drop is associated with young trees (generally 4th – 9th leaf) growing in sandy soil. Carmel appears to be most severely affected, but bud drop can be severe in other varieties including Wood Colony, Price, Butte, Mission, Nonpareil, and Fritz. The cause of bud drop is still unclear. Early surveys of affected orchards showed now clear trend of nutrient deficiencies. Many conditions in bud drop affected orchards are very similar to areas with bacterial canker (i.e. young trees, sandy soil, replanted orchards with high ring nematode populations.) Affected buds are colonized by *Pseudomonas syringae*, the bacterium associated with bacterial canker. It is unclear if bud drop is a previously unreported symptom of bacterial canker or if both problems coincidentally occur in similar orchard conditions.

Several field experiments have been conducted in Stanislaus County since 1997. Treatments include soil-applied fertilizers, foliar nutrient sprays, nematicides, microbiological soil inoculants, and organic and inorganic soil amendments. In the spring of 1999, trees were evaluated for bud drop by examining 250-300 bud positions of treated and untreated trees. At the end of the season, yields were recorded.

Keyes, CA Almond Bud Drop Trial.

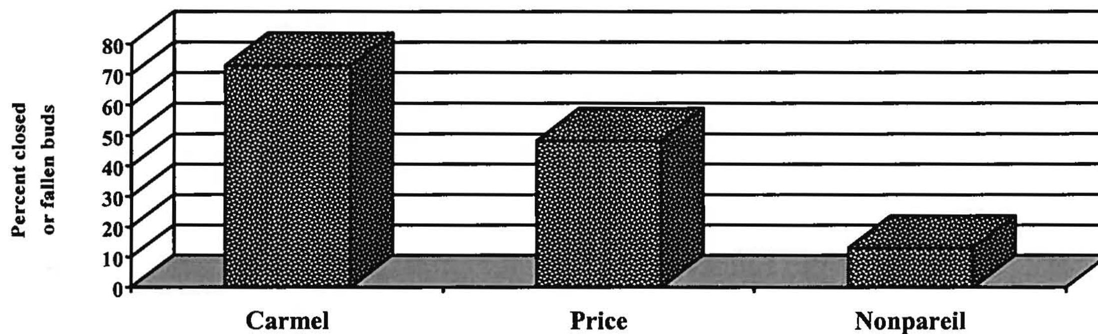


Fig. 1. Varietal Differences in bud drop expression. February 26, 1999.

Results of Albert Nydam Bud Drop Trial, Keyes

Monthly applications of CAN-17 (30 units of N) + foliar applications of calcium chloride (StopIt®) significantly reduced bud drop severity. It is unclear whether it was the nitrogen or calcium that improved bud retention. One spring application and two fall applications of Enzone drastically reduced ring nematode numbers and also reduced bud drop. However, the concentrated, multiple applications resulted in severe root damage and killed some trees. The best treatment included a combination of all treatments, including a fall and winter application of Bordeaux mix (copper sulfate and lime). Yield data were highly variable between trees and it was difficult to draw conclusions. Many of the trees with lowest levels of bud drop were the combination treatments that included Enzone. Unfortunately many of these trees declined during the season due to extensive root damage, resulting in drastically reduced yields.

Almond Bud Drop Trial, Keyes, CA.

February, 1999

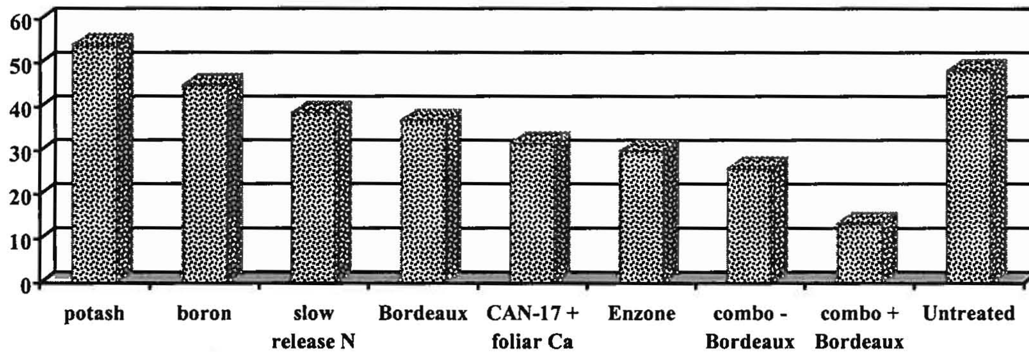


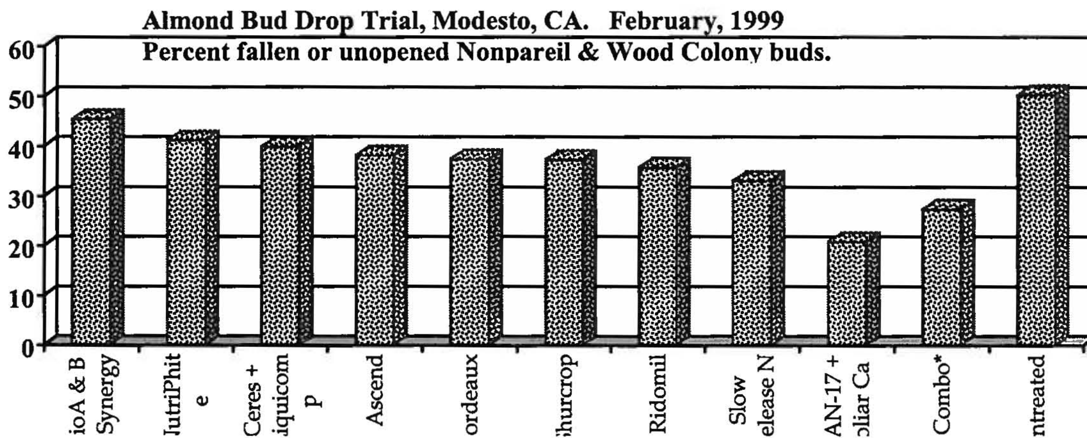
Fig. 2. Percent of fallen or unopened buds on Nonpareil, Carmel, and Price almond trees.

*Combination treatment includes potash, boron, slow release nitrogen, CAN-17, Enzone, and foliar calcium sprays.

Results of Longstreth Trials, Modesto

- Single Tree Plots

From our first year's results of the Nydam trial, we learned that Bordeaux mix (possibly any copper spray) was an important component of a bud drop management program. Therefore all treated trees were also sprayed with Bordeaux mix in the fall and winter in this trial. As in the Nydam trial, monthly applications of CAN-17 plus foliar calcium chloride was the single best treatment in reducing bud drop. An application of slow release N fertilizer + micronutrients also appeared to reduce bud drop, as did Ridomil. A



combination treatment of CAN-17 + calcium chloride, Ridomil, slow release nitrogen + micronutrients, and Liquicomp did not reduce bud drop any better than the CAN-17 + calcium chloride alone. Yield data were similar to the bud drop data. Trees not sprayed with Bordeaux had the lowest yields.

Yields of Nonpareil and Wood Colony Almond Trees Treated with Various Fertilizers and Amendments.
Longstreth Bud Drop Trial, Single Tree Plots, 1999 Harvest

Treatment[1]	**Meat Pounds per Tree**	**Significance**[2]	**Calculated Meat Pounds per Acre**
CAN-17 + $CaCl_2$, Slow release N, Ridomil, Liquicomp	22.4	A	2464
CAN-17 + $CaCl_2$	20.4	AB	2244
Slow release N + micronutrients	20.2	AB	2222
Ridomil	18.6	ABC	2046
Ceres & Liquicomp	18.0	ABC	1980
Ascend	18.0	ABC	1980
Bordeaux mix	16.2	BC	1782
Shurcrop	15.1	BCD	1661
NutriPhyte	14.1	CD	1551
Bio A + Bio B & Synergy	13.5	CD	1485
No Bordeaux	10.1	D	1111

[1]All treatments except where noted were also treated with Bordeaux mix in November and January.
[2]Values followed by the same letters are not statistically different ($P<0.05$)

Results of Longstreth Trials, Modesto
➢ Large Plot

In this trial, we looked at commercial scale applications of several soil amendments
- **Composted green waste at 10 tons per acre (partially donated by Grover Landscaping)**
- **Gypsum and lime to increase soil calcium and correct a low soil pH problem**
- **Ceres & Liquicomp microbiological soil amendments**
- **A combination of the above three treatments.**
- **20 tons of composted green waste per acre**
- **Ascend, a mycorrhizal soil inoculant.**

Treatments were first applied in April 1998. In February 1999, ten trees per plot (five Nonpareil, five Wood Colony) were examined for bud drop. At harvest, yields were collected for the same ten trees in each plot.
There were no significant differences in bud drop between treatments. Yields tended to be higher in the combination treatment for the Wood Colony, but differences were not statistically different. It seems logical that if changes in soil structure and microbiological activity may affect tree "health" and yield, affects may be delayed longer than one year. We will continue to treat and monitor this trial for two more seasons.

II. The Involvement of *Pseudomonas syringae* in Almond Bud Drop.

The role of *Pseudomonas syringae* pv. *syringae* (Pss) in the disease of almond bud drop has been a question for some time. To date we've discovered that Pss can be found in both healthy and dropped almond buds although the numbers have appeared to be some what higher in the buds of trees showing typical bud drop symptoms. *Pss* isolates from almond buds, both healthy and diseased, displayed the same genetic profile as *Pss* isolates that cause bacterial canker, suggesting that this pathogenic bacteria could be the cause of almond bud drop when almond trees are growing on sites with the same predisposing conditions as bacterial canker. The predisposing conditions being mainly young trees in sandy soils on replant sites.

To answer the question of whether *Pss* is the main culprit in almond bud drop or merely living as an epiphyte, we monitored bacterial populations over time from October through February every 3 weeks. Four healthy and four diseased Carmel almond trees were repeatedly sampled over time. Three sub-samples of ten pooled buds were analyzed per tree. Both internal and external microbial populations were analyzed. Individual buds were analyzed for both healthy and diseased trees in which *Pss* appeared all three sub-samples to determine the frequency at which individual buds are infected. Results are discussed.

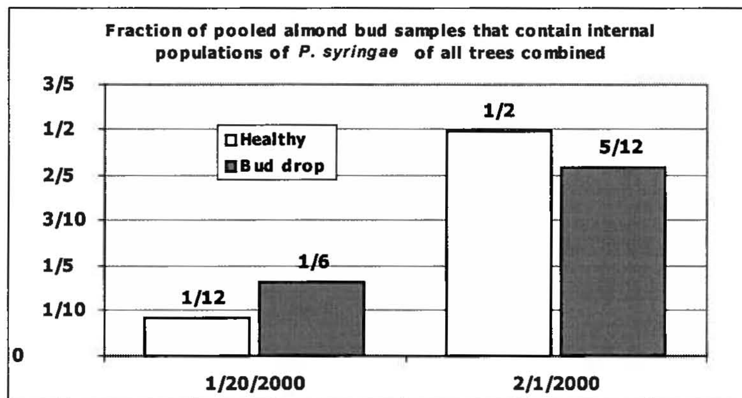


Figure 1. Fraction of pooled almond bud samples that contain internal populations *Pss*.

Pss did not appear in the almond buds until the 1-20-2000 sampling time. All fractions presented in figures 1 and 2 are internal populations only. External populations of Pss were found in only 1/12 healthy pooled bud samples and 3/12 diseased pooled bud drop samples at the 2-1-2000 time point.

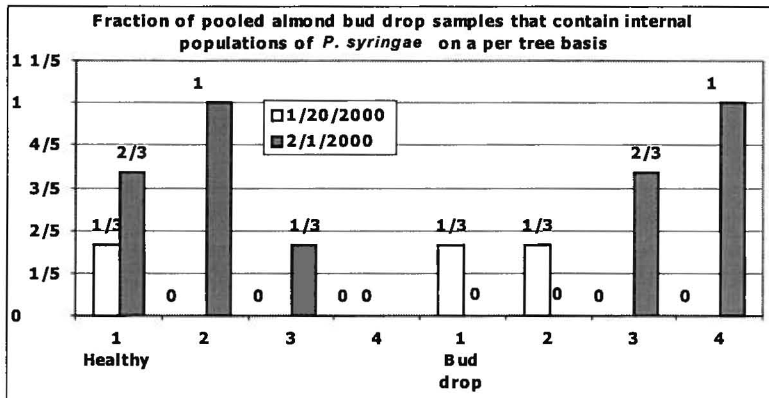


Figure 2. Fraction of pooled almond bud samples by tree that contain internal populations of *Pss*

Table 1. Number of individual buds containing internal putative populations of *Pss*.

	Healthy Camel	Diseased Carmel
Pss*	**1/20#**	**7/20**

*Bacterial identification has been confirmed only by HR, fluorescence and the oxidase test not by *syrB* colony hybridization.

Twenty buds were ground and plated individually. These buds came from a tree in which *Pss* was isolated from all three pooled sub-samples of ten buds each at the 2-1-2000 sampling time.

Results and discussion on the prevalence of *Pss* in almond buds.

Clearly *Pss* resides in almond buds during the winter months of January and February. The frequency of individual or pooled bud drop samples containing P. syringae was relatively infrequent and roughly equivalent between healthy and diseased trees. Most buds do not contain Pss (Table 1.) even when all three pooled sub-samples from that tree came up positive for the bacterium. In other words, even if a pooled sub-sample of ten buds contain at least one bud colonized by Pss, most of the buds in the sub-sample likely do not contain detectable numbers of Pss. Even most pooled bud samples come up negative for Pss (figure 2.) suggesting that most almond buds, both healthy and diseased, do not contain appreciable numbers of Pss even approximately 70% of the bud on diseased Carmel dropped in 2000 (Roger Duncan). These results imply the Pss is either not the cause of almond bud drop or is responsible for small fraction of the dead buds in this disease.

Environmental conditions most certainly play a role in the number of Pss present in almond buds. In the winter of 1997/1998 and el nino caused about 50% more rain to fall on the Central Valley than the yearly average and as a result, the populations of Pss were approximately 100 fold higher than in the winters of 1998/1999 and 1999/2000. The level of bud drop was also higher (95%) than this last year (70%) on Carmel. The fraction of individual buds containing putative Pss in 1997/1998 was 9/10 bud where as the values for this year were 7/20. In

1998/1999 the fraction of individual buds from bud drop trees harboring Pss was 7/80 (20 buds per tree) with two trees having no buds containing Pss. Rain dramatically increases both the fraction of buds containing Pss and the numbers of Pss in individual buds. This data supports the conclusion that most of the bud drop that occurs on Carmel almond trees is not due solely to the presence of Pss.

Microbial and Pss growth trends over time.

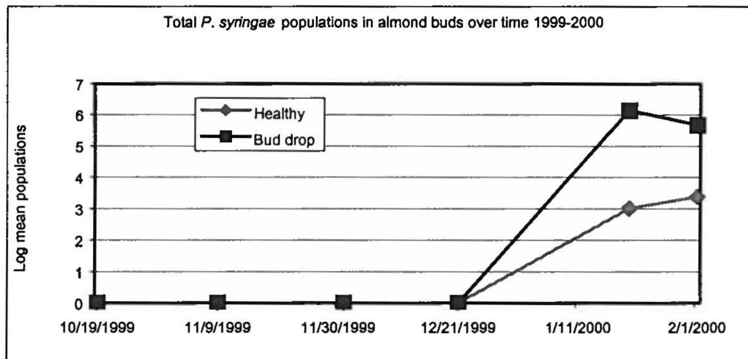


Figure 3. Log mean populations of Pss in healthy and diseased Carmel almond buds over time.

Populations of Pss did not appear in our pooled bud samples until the 1-19-2000 sampling time. No rainfall events over 0.1 inches occurred from 11/19/1999 to 1-11-2000, the 2 months of dry weather might have prohibited the growth of Pss. It is unclear whether bud drop samples have higher Pss numbers than the healthy because they are causing disease or whether Pss is growing saprophytically on already senescing buds. No Pss were found on the external surface of the buds until the last sampling time of 2-1-2000. Pss was detected in the external pooled buds at a fraction of 1/12 healthy and 3/12 diseased pooled bud samples on 2/1/2000 vs. internal ratios of 6/12 healthy and 5/12 diseased. The exact same buds were used to determine internal and external populations of Pss and other bacteria at all time points. This bacterium appears to colonize the internal tissues of almond buds more than the external surfaces, while other bacteria found in almond buds appear in the internal and external tissues with the same frequency. Pss may have a preferential ability to invade the internal tissues of plants compared to other types of epiphytic bacteria during wet conditions. Alternatively, the effects of desiccation and exposure to ultraviolet light maybe more severe on external populations of Pss than on other types of bacteria. The later scenario is a more likely because several other types of microorganisms can be found in both internal and external surfaces of both healthy and diseased almond buds throughout the year.

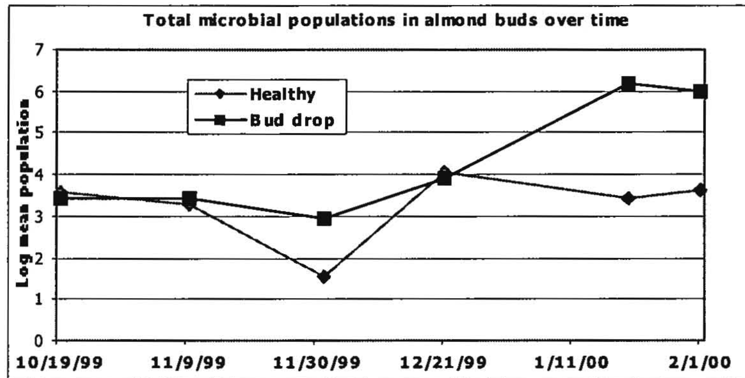


Figure 4. Total microbial populations in Carmel almond buds over time

Microbial populations are roughly equivalent between healthy and diseased trees until the 1-19-2000 sampling time when the bud drop microbial populations became 2 log units or 100 fold higher. Most of this increase is due to Pss numbers increasing.

Conclusions.

The overall microbial populations were relatively low through most of the year because rainfall was very infrequent until the middle of January and as a result Pss did not appear in our samples until after that time. Pss has appeared in our samples at least by the end of December in previous years due to earlier and heavier rainfall in those seasons. This season, more than the others, has helped elucidate the role of Pss in the disease of almond bud drop.

Pss appears to have a comparatively minor role in the disease of almond bud drop. Carmel experienced over 70% bud drop in 1999/2000, yet Pss was isolated from less than half of the pooled bud sub-samples (10 buds per pool) at the 2-1-2000 sampling time. In addition, when twenty buds from a tree in which all three pooled bud samples were positive (minimum of 1/10 per pool required) only 7/20 were positive. In other words, most buds in a sub-sample positive for Pss do not contain detectable Pss populations. With these numbers we can estimate that between 10-20% of the buds from diseased Carmel trees contain detectable numbers of Pss, far lower that the 70% dropped buds we would expect from Carmel. Pss is pathogenic in the cambial tissues of stone fruit and populations (internal) in infected pools range from $10 - 2 \times 10^6$ per bud. A marginally healthy bud may succumb to bud drop when colonized by several thousand Pss cells, thereby contributing to the overall disease problem. The effect of Pss would be greater in years of higher rainfall such the El Nino year of 1997/1998 in which populations of this pathogen often surpassed 1 x 106 per bud and nearly all buds, both healthy and diseased, were colonized.

This data supports the conclusion that Pss, especially in relatively dry years,year plays a minor role in the disease of almond bud drop. Both healthy and diseased trees support substantial populations of Pss and the frequency of isolation is nearly equal between the two. Although the diseased trees have higher numbers of Pss, it is difficult to ascertain whether the diseased buds

are being parasitized or whether the bacterium is simply multiplying in the already senescing tissues. It appears that some other factor, perhaps nutrient deficiency causing root stress, is responsible for the majority of the almond bud drop that occurs. In wet years, root stress in sandy soils would be acerbate by accelerated nutrient leaching. The concomitant build up of Pss populations could increase the percentage of buds the tree aborts. Overall, Pss is probably a minor component of almond bud drop with some other predisposing stress being the major cause.

III. BACTERIAL CANKER: MECHANISMS, PATHOGEN CHARACTERIZATION, AND CONTROL

Bruce Kirkpatrick, Ron Sayler, Tiesen Cao, and Richard Bostock
Cooperators: Doug Gubler, Steve Southwick, Becky Westerdahl, Bill Krueger, Bill Olson

Abstract

Bacterial canker (BC) is a complex disease whose expression is more a function of the vigor of the tree than it is the presence of the bacterial pathogen. The ability to identify implementable orchard management practices to minimize the risk of developing BC will need the collective expertise of plant pathologists, pomologists and nematologists. During the past 5 years we made substantial progress on all of the original project objectives. The recent involvement of Mr. Cao on the project has greatly expedited progress on Objective 1. We have initiated additional field trials on the effect of silicone-based surfactants which may expedite the delivery of bactericides into hydrophobic tree spaces and on the development of BC risk assessments for new orchard sites based on analysis of orchard soil characteristics.

In 1998 we developed experimental systems to create BC lesions under controlled conditions. We found that even briefly exposing prune branches to moderate freezing conditions (21 F) greatly increased the size of BC lesions. Contrary to widely held, but not experimentally substantiated beliefs, we found that prune leaf scars were only susceptible for 4 hours to infection by Pss. We found that a low percentage of prune lenticles could be inoculated using non-invasive inoculation techniques which were similar to those that could occur in nature. However, we did not isolate large number of pathogenic Pss from the lenticles of healthy or BC-affected prune trees. Numerous experiments are currently underway to investigate how microelements, drought, nitrogen, tissue water content and other factors can induce stresses that predisposes trees to developing BC.

In 1999 we found a significant difference in one test orchard between trees that were treated with copper and/or a time-released application of 16-16-16 fertilizer containing additional micronutrients. In addition, much less tree mortality occurred in the copper plus fertilizer treatment than in the unsprayed control. However, there was no effect on the incidence or severity in another low disease pressure orchard. Additional field evaluations of bactericides, including the use of silicone-based surfactants in combination with bactericides, and fertilizer amendments, are currently being evaluated will help determine the efficacy of these treatments.

The genetic characterization of a collection of Pss strains from cankers of french prune and other stone fruits, as well as other diverse plant hosts, is now complete. This information has provided us with the necessary tools s to critically examine the role and relationship of epiphytic populations of Pss to canker-inducing strains of Pss. Isolation and genetic characterization of Pss strains from prune buds and bark surfaces conducted from 1996 to 1999 indicates that canker-inducing Pss populations are low, but present, in these tissues. Large and diverse bacterial populations were isolated from the surfaces of orchard weeds, however no canker-inducing Pss strains were recovered from weeds during the past 3 years. These results suggest weeds do not provide significant amounts of Pss inoculum. Isolation performed in 1998 found no evidence for the systemic movement of Pss in cambial tissues of French prune trees. By better understanding the dynamics of Pss populations on tree surfaces, in buds and on developing tissues we hope to formulate implementable management practices to reduce losses to BC. In 1999, methods were developed to extract sap from prune xylem and cambial tissues. Some differences were found in the ability of these extracts to induce the expression of an important toxin gene in Pss. Analyses of soil collected in a BC orchard "hotspot" showed that a high sand and low silt level were the single greatest soil risk factor for developing BC in prunes.

PROJECT OBJECTIVES:

1. Develop a laboratory/growth chamber/ field model system which allows the consistent production of bacterial canker (BC) in prune trees at UC Davis.

2[a]. Determine the genetic variability of *Pseudomonas syringae* pv *syringae* (Pss) strains, the causal agent of BC, isolated from French prune, other stone fruits, and weeds.

3. Test the efficacy of dormant copper sprays for controlling overwintering populations of *Pseudomonas syringae pv. syringae* (Pss) on prunes and monitor the potential development of copper resistance in these populations.

4a. Determine if prematurely defoliating French prune trees and treating them with topical applications of copper can reduce the incidence of BC.

b[b]. Determine if silicon-based surfactants can facilitate the delivery of copper bactericides into tree spaces and reduce the incidence of BC.

5. Determine if high worked rootstocks afford better protection against BC than low budded trees purchased from the nursery.

6. Identify plant stress metabolites that can activate virulence determinants of Pss.

7. Determine if measurable soil quality parameters, such as mineral content, pH, texture, nematode populations, etc. can be used to identify potential bacterial canker sites.

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a. characterization of Pss stone fruit strains is complete, final characterization of orchard weed strains will be completed in 2000.
b. a new objective for 1999/00.

PROCEDURES:

Note: Many of the procedures described below are the same as those described in the previous progress reports, other have been updated to include new or expanded objectives.

<u>a. Laboratory experiments for reproducing bacterial canker disease:</u>

One of the major impediments to the study of bacterial canker (BC) has been the inability to reproduce BC under controlled, experimental conditions at UC Davis. This objective has become the central focus of Mr. Tiesen Cao's, Ph.D. research which is being undertaken with the guidance of myself and Dr. Ken Schakel, Dept. of Pomology, UC Davis. In 1998/99 Tiesen undertook the following experiments to determine the role of lenticles as possible avenues for bacterial ingress into prune tree cambium as well as several experiments which focused on the role of micronutrients in making trees susceptible to BC. The results of these studies are presented below.

Objective 1.. Develop a laboratory/growth chamber/ field model system which allows the consistent production of bacterial canker (BC) in prune trees at UC Davis.

1) Freezing increases the susceptibility of prune branches to BC, summary of 1998/99 results:

We found that bacterial canker lesions produced by injecting small amounts of *Pseudomonas syringae* pv. *syringae* (Pss) into branches of various Prunus species, including French prune, were much larger if the branches were subjected to a 2 hour exposure at -5 C (21 F) then thawed, compared to non-frozen tissues. Branch pieces that were completely hydrated by soaking in water for 2 hours developed much larger lesions than branches that were dehydrated. There was also a direct relationship between lesion size and stem diameter. These results support the conclusion that water content of dormant Prunus tissues significantly influences BC. It is interesting to note that some peach and almond growers in Central San Joaquin Valley report they experience more problems with BC if trees are given a late fall irrigation. Experiments with dyes suggest that cambial tissues separate slightly from the bark during the freezing process that may aid in the dispersion of Pss in water films as the cambium/twig thaws. Freezing may also damage cambial cells that would release nutrients for Pss growth. Freezing may also aid in the physical ingress of bacteria in water films through natural tree openings, such as lenticles. The ability of Pss cells to act as catalysts for the formation of ice crystals at temperatures where water does not normally freeze also suggests that even mild freezing temperatures could influence the development or severity of BC. This experimental system has allowed us to begin accessing whether trees growing under "stressed" conditions, which greatly predispose trees to developing BC, undergo physical or anatomical changes which permit Pss to move more readily and cause disease.

2) Leaf scars probably do not serve as significant sites for Pss infection in French prune:

It has been historically believed that Pss gains entry into the cambium by infecting leaf scars during early storms in the fall. This concept is largely based on experimental work on sweet cherry in England. We wanted to assess the relative susceptibility of leaf scars as avenues for Pss infection in French prune and other Prunus species growing in California. Leaves were physically removed from attached branches of various Prunus species in mid-November, 1998. Sections of the branch were then removed from the tree at various time points after stripping off the leaves and brought to the lab. Exposed leaf scars were then inoculated with suspensions of Pss and then incubated at 12 C for one week to allow infection to occur. The size of the resulting brown lesion below the leaf scar was then measured and isolations were performed from the margin of the lesion to show that Pss could be recovered from the lesion. Leaf scars from sweet cherry remained susceptible to infection for up to 2 days. In contrast, French prune leaf scars were immune to infection 4 hours after the leaves were stripped from the branch . These results suggest that prune leaf scars heal fairly quickly and they may not be as important in the overall infection process of Pss as compared to sweet cherry. This data may also help explain why we have not seen any significant benefit from prematurely defoliating prune trees with zinc sulfate, one of the chemical treatments we were evaluating (see Objective 3).

3) Pss does not systemically infect cambial tissues in French prune:

Even if Pss does gain some entry into some leaf scars, these infections cannot account for

the very large and often-lethal bacterial cankers that occur on major scaffolds and the trunk. In February and March 1998 and again in 1998 we attempted to isolate Pss from healthy cambial tissues, which were 3 or 4 inches away from either a dormant (February), or active (March) BC cankers on the scaffolds or trunks of six BC-affected prune trees growing at Wolfskill. No Pss and only a few yellow- colony bacteria were isolated on either occasion. These results indicate that it is unlikely that Pss enters through distant leaf scars and then systemically moves through the tree to cause the large cankers that are so typical in prune. If this assumption is correct then Pss must gain entry into major branch and trunk tissues through some other opening.

4. Do lenticles act as natural openings for Pss to cause bacterial cankers?

Other than natural growth cracks, the other major site where Pss could possibly come into contact with inner tree cambium is through bark lenticles. During the past 3 years we have often noticed small, red BC lesions directly below the lenticles near cankers on prune scaffolds and trunks. Sometimes small lesions were found directly below a group of lenticles that later coalesced and formed a large trunk canker. In order to determine if lenticles are important natural openings for Pss entry we have begun isolating from lenticles on healthy and BC-affected prune trees. Very few total bacteria, and no Pss, were isolated from lenticles of healthy and diseased trees in early November, results which are similar to the low number of Pss and other bacteria isolated from prune buds and bark surfaces. Total bacterial populations were 100 to 1,000 times greater in lenticles in December, but only a few of the bacteria were Pss. There was no significant difference in the numbers or types of bacteria isolated from healthy compared to BC-affected trees

In 1999 we conducted a number of experiments to determine whether lenticles could act as natural opening for Pss in french prune. The populations of green fluorescent pseudomonads on/in lenticels were investigated in prune, cherry, and peach. Theresults of these experiments are summarized below:

Materials and methods

These experiments were conducted between December 1998 and May 1999, in the Department of Plant Pathology of the University of California, Davis. The plant materials were about 5-year-old French prune, Fantasia nectarine and 8-year-old Bing cherry trees grown in the experiment farm in Armstrong. Some diseased and healthy French prune trees of 5-year-old were grown in the Wolfskill Experimental Orchard near Winters, California.

a. Lenticle inoculation experiment

The bacterial inoculum used in this experiment was B3A 206, a virulent strain of *Pseudomonas syringae* pv. *syringae*. The bacteria were grown in King's medium B (King et al., 1954) at 28 °C for two days with shaking at 180 rpm. The bacteria were harvested by centrifugation at 3000×g for 7 min at 4 °C., the bacterial pellet was suspended in sterile distilled water, and the bacterial concentration was adjusted to approximately 10^8 CFU/ml as estimated by measuring the optical density at 600 nm. Before inoculation a silicone-based surfactant (BreakThru^R) whose concentration was used between 0.05-0.5% was added to the bacterial suspension in order to increase the permeability of bacterial liquid into the lenticels.

French prune stems 4-5 cm in diameter were cut into pieces approximately 15-20 cm long and were brought into the laboratory. The two ends and all artificial fresh wounds of each stem piece were then sealed with hot wax to protect from undesired infection or contamination. The inoculations of lenticels were made either by spraying bacterial suspension combined with 0.05-0.5% surfactant to the surface of the sealed stems or by putting drops of bacterial suspension on specific, labeled lenticles on the stem. Four to six stems were used for each inoculation treatment. After the inoculation, the inoculated stems were covered with a plastic bag and incubated in an incubator at 15 °C for two weeks. The lesion size was measured with a digital caliper after removing the bark over the inoculated lenticles. The disease incidence of each treatment was also recorded by dividing the number of infected lenticels by total number of lenticels inoculated.

To evaluate the freezing effect on lenticel infection, one group of stems was put in a freezer at –5 °C over night, and the inoculation was made during the thawing process by spraying bacterial suspension to the surface of each stem. Another group was inoculated without freezing.

b. Isolation from field-collected Prunus lenticels

Lenticel isolations were started in December 1998. Bark samples with lenticels were harvested every three weeks by cutting off a piece of bark from the trunk or scaffold of the tree with a punch and a hamper. The bark pieces with several lenticels were then put into a clean plastic bag and brought back to the laboratory. The lenticels were then cut off from the bark with a scalpel and put in 4 ml of 0.01 M phosphate buffer (pH=7.2). Five lenticels and five pieces of bark that were approximately the same size and weight were cut off from each stem and ground in phosphate buffer with a polytron grinder at 15,000 rpm for 10 seconds. Between samples, the polytron grinder was rinsed with large amount of sterilized water. A dilution series of each sample was plated in duplicate onto King's medium B. The dilution plates were incubated at 28 C for 2 or 3 days, then all kinds of bacterial colonies were counted. Green fluorescent colonies were then tested for oxidase and HR (hypersensitive response) on tobacco leaf. Blue fluorescent, oxidase negative and HR positive colonies were considered to be *P. syringae* pv. *syringae*. Six replicates were used for the comparison between diseased and healthy trees. Four replicates were used for the Prunus species comparison. Fluorescent pseudomonad populations on bark surfaces and in lenticels were compared.

Results

1. Infection induced by lenticel inoculation with freezing and without freezing in 'French' prune.

Data shown in Tables 1-4 indicates that lenticels could be an avenue for bacterial infection although except for the direct injection method (Tables 2 and 4) of inoculation, the infection rate was only 1 to 4% (Tables 1 and 3). Inoculation during thawing appeared to increase the disease incidence and severity (Table 2).

Table 1. Bacterial infection caused by artificial lenticel inoculation

Treatment 1	Treatment 2	Infection %	Lesion length (mm)	Infected lenticels	Inoculated lenticels
Freezing/thawing	Bacteria	1.8	26.7	1	57
Freezing/thawing	Water	0	0	0	68
Nonfreezing	Bacteria	2.8	8.8	2	71
Nonfreezing	Water	0	0	0	67

Table 2. Effect of freezing temperature on lenticel infection in 'French' prune

Freezing/thawing	Infection %	Average lesion length (mm)	Lenticels inoculated	Infected Lenticels
Inoculation during thawing	15.7 a	24.6 ± 12.8 a	83	13
Inoculation without freezing	11.9 a	17.9 ± 7.5 a	84	10

1.Means followed by the same letters are not significantly different at p=0.05 by t-test.
2. 0.1 % surfactant was added to the bacterial suspension.

2. Effect of inoculation method on disease incidence and severity in 'French' prune

Bacterial infection could be initiated by either spraying or putting drops of bacterial suspension on the surface of lenticels (Table 3). Direct injection of the lenticles with the bacterial inoculum injection appeared to increase the disease incidence and severity (Table 4).

Table 3. Bacterial infection in "French" prune stems induced by artificial inoculation through lenticels.

Inoculation method	Infection %	Lesion length (mm)	Infected lenticels	Lenticels inoculated
Dropping	1.9	11.5	1	54
Spraying	0.8	18.5	3	400
Vacuum infiltration	2.4	8.7	1	41

1. 0.05% surfactant was added to bacterial suspension except for vacuum infiltration.
2. Vacuum infiltration was applied by keeping stem piece with drops of bacterial suspension on the lenticels under –80 Kpa for 2 minutes.

Table 4. Bacterial canker incidence and severity induced by lenticel injection of bacterial suspension (10^8 CFU/ml)

Inoculation	Infection %	Lesion length (mm)	Infected lenticels	Lenticels inoculated	Number of stems
Bacteria	22.2	25.7	8	36	4
Bacteria + 0.1% surfactant	13.3	23.1	8	60	6
Water	0.0	0.0	0	32	4

3. Seasonal change of susceptibility of lenticel to bacterial infection

During the early spring as the trees broke dormancy there was no obvious change in susceptibility of lenticels to bacterial infection. However, once the trees were growing vigorously and the temperature increased, the lenticels were no longer susceptible to bacterial infection (Table 5).

Table 5. Season variation in susceptibility of lenticel to bacterial infection

Treatment	Date of inoculation	Infection %	Lesion length (mm)	Infection per stem	Lenticel inoculated per stem	Number of stems
Freezing/thawing	Mar. 8	0.7	40.6	0.6	92	5
Nonfreezing	Mar. 8	0.4	5.8	0.4	105	5
Freezing/thawing	Apr. 2	0.6	19.3	0.5	92	6
Nonfreezing	Apr. 2	0.2	4.0	0.2	93.3	6
Freezing/thawing	Apr. 17	0.0	0.0	0.0	133	5
Nonfreezing	Apr. 17	0.0	0.0	0.0	123.8	6
Freezing/thawing	May 1	Freezing injury	--	--	99.7	6
Nonfreezing	May 1	0.0	0.0	0.0	103.3	6

4. Influence of tree status (healthy versus diseased trees) on lenticel susceptibility to bacterial infection

On two of the three inoculation dates it appeared that smaller lesions developed on stwm taken from diseased trees compared to healthy trees. This may be due to the fact that diseased tree cambium was less hydrated due to impairment of the xylem that healthy trees (Table 6).

Table 6. Influence of stems from healthy and diseased 'French' prune trees on lesion length induced by pin-prick-inoculation

Stems from	Lesion length (mm)	Inoculations	Optical density of bacterial suspension	Sample date
Healthy tree	76.7 a	30	0.795	Mar. 18
Diseased tree	40.8 b	30	0.795	Mar. 18
Healthy tree	72.7 a	35	0.795	Apr. 04
Diseased tree	72.8 a	35	0.795	Apr. 04
Healthy tree	95.6 a	30	0.790	Apr. 26
Diseased tree	53.6 b	30	0.790	Apr. 26

Means followed by the same letters are not significantly different by t-test.
Diseased trees were trees where bacterial canker occurred before. Cankers on these trees were not active when stem samples were harvested.

5. Tissue suitability to bacterial infection in 'French' prune stems

Much greater size lesions developed in cambial, compared to cortical, tissues (Table 7). Since cortical tissues separate the bottom of the lenticle from the cambium, the relatively insensitive cortical tissue may provide a protective barrier generally prevent Pss within the lenticle from contacting the cambium. This might be one reason why natural lenticel infection is comparatively low. If this cortical barrier was compromised through mechanical or freezing injury one would expect greater incidence/severity of lesions to occur beneath lenticles.

Table 7. Influence of tissue type on bacterial canker lesion length in 'French' prune

Tissue type	Lesion length (mm)	Lesions measured
Cambium	66.6 ± 19.3 a	25
Cortex	2.2 ± 1.5 b	25

Inoculations were made by shallow injection of bacterial suspensions into cortical tissues or deeper injection into the
cambial tissues.

6. Effect of surfactant on bacterial viability and lesion length in prune stems

Bacterial viability indicated by colony forming unit per milliliter decreased somewhat with increasing surfactant concentration (Table 8). However, it appeared the decrease in viability had no significant influence on bacterial pathogenicity as determined by artificial inoculation of apricot, peach and prune stems (Table 9).

Table 8. Effect of surfactant concentration on bacterial viability

Surfactant concentration	CFU/ml	Log (CFU/ml)
0.00% (control)	5.3×10^{10}	12.7 a
0.01%	1.5×10^{9}	10.2 b
0.05%	7.9×10^{9}	9.9 cd
0.10%	1.0×10^{9}	10.0 c
0.50%	6.1×10^{9}	9.8 d

Surfactant was mixed with bacterial suspension for one hour.
Means followed with the same letter are not significantly different.

Table 9. Influence of 0.1% surfactant, bacterial suspension and water on lesion length in apricot, peach and prune stems

Species	Treatment	Lesion length (mm) ± 1 SD	Number of inoculation
Apricot	Bacteria + 0.1% surfactant	79.5 ± 19.4 a	30
	Bacteria	76.0 ± 20.6 ab	30
	0.1% surfactant	1.9 ± 0.7 d	30
	Sterilized water	1.7 ± 0.5 d	30
Peach	Bacteria + 0.1% surfactant	69.4 ± 17.6 b	30
	Bacteria	69.9 ± 18.2 b	30
	0.1% surfactant	2.9 ± 1.7 d	30
	Sterilized water	2.6 ± 1.3 d	30
Prune	Bacteria + 0.1% surfactant	34.8 ± 16.9 c	20
	Bacteria	41.3 ± 18.2 c	18
	0.1% surfactant	1.7 ± 1.2 d	30
	Sterilized water	1.3 ± 0.4 d	30

Means followed by the same letters are not significantly different at P=0.05.

7. **Isolation of fluorescent pseudomonad populations in and on lenticels in cherry, peach and prune during in winter and spring**

Our 1999 isolation results indicated that there was very low numbers of pathogenic pseudomonads in healthy cherry, peach and prune lenticles or diseased prune trees. by isolating from the lenticels under the conditions of this year. However, high populations of nonpathogenic, fluorescent pseudomonads were found in both lenticels and bark surfaces of healthy cherry, peach and prune trees (Figure 1, Figure 2). There was no clear difference in number of fluorescent pseudomonads between lenticel and bark in all species. The populations increased with increasing air temperature and disappeared in late May. The same situation was also found in diseased prune trees (Figure 3, Figure 4).

Conclusions

Under laboratory conditions, bacterial infection could be induced by lenticel inoculation. However, the disease incidence via lenticel was very low, except when bacterial inoculum was directly injected into the lenticle. Freezing temperature could not significantly increase the disease incidence and severity induced by lenticel inoculation. Surfactants reduced the viability of bacteria, but had no significant influence on bacterial pathogenicity and stem injury. Pathogenic *P. syringae* pv. *syringae* isolates were isolated from of lenticels or bark surfaces during the period of this experiment. High populations of green fluorescent, non-pathogenic pseudomonads were found on the bark and in lenticels of all Prunus species. These data suggested that lenticels may serve as a natural infection opening, but the overall incidence of lenticle infection would be comparatively low. Obviously, there are a very great number of lenticle openings on a tree so that even a low percentage of lenticle infections could have a significant effect on bacterial canker. Because of the difficulty of sacrificing a whole tree, we had to restrict our experiments to large lenticles on scaffold branches. It may be possible that lenticles on trunks may differ in their susceptibility or they may harbor a different population of bacteria than branches.

Experiment II
Water relations involved in bacterial canker complex in 'French' prune

Objective

This experiment was to study bacterial canker disease of prune from a standpoint of water relations under field conditions with an attempt to understand how tree water status is involved in this disease.

Materials and methods

This experiment was conducted in June and July 1999 with 6-year-old 'French' prune trees growing in an orchard near Live Oak, California. The midday stem water potential, which reflects the closest conditions of tree water status, was measured with a pressure chamber in diseased and healthy prune trees. The xylem water potential was estimated by measuring water potential of leaves which were close to the trunk of the tree or a main scaffold. The leaf was covered with a mylar bag for water equilibration for one hour prior to measurement. The xylem water potential would be equal to the leaf water potential owing to the lack of leaf transpiration resulting from the closure of stomata.

In this experiment, diseased trees were considered as those on which gummosis or cankers were found on the branches or trunk. Otherwise, trees were considered as healthy.
Disease severity was evaluated by rating all trees based on levels from 0 (healthy) to 5 (dead).

Results

Measurements indicated that diseased trees had a significantly lower midday xylem water potential than healthy trees (Table 1). It appeared that midday xylem water potential decreased with increasing disease severity (Table 2).

Table 1. Comparison between healthy and diseased trees in midday water potential (June11-12, 1999)

Diseased/healthy	Water potential (Mpa)	Number of trees
Diseased tree	-0.86 ± 0.43 **	41
Healthy tree	-0.70 ± 0.09	46

** Significant at P=0.01 by T-test.

Table 2. Relationship between water potential and disease severity (June 11-12)

Water potential (Mpa)	Disease rating (7/20/99)	Number of trees
-0.70 ± 0.09 a	0.0	46
-0.64 ± 0.05 a	1.0	6
-0.75 ± 0.16 a	2.0	7
-0.73 ± 0.13 a	3.0	15
-0.79 ± 0.07 a	3.5	3
-1.11 ± 0.38 b	4.0	3
-0.79 ± 0.21 a	4.5	3
-1.79 ± 0.88 c	5.0	4

Means with the same letters are not significantly different.

Within the tree canopy, midday xylem water potential was significantly lower in diseased branches than in healthy ones (Table 3). The measurements of midday xylem water potentials above and below canker lesions (approximately 1 meter in distance) suggested that existing bacterial cankers played significant roles in blocking xylem water transportation (Table 4).

Table 3. Comparison between healthy and diseased main branches in midday water potential within one tree. (July 3)

Diseased/healthy	Water potential (Mpa)	Number of trees
Diseased branch	-1.40 ± 0.36 **	15
Healthy branch	-1.12 ± 0.27	15

** Significant at P=0.01 by T-test.

Table 4. Effect of bacterial canker lesion on water transportation within the xylem of main branch[1]. (July 22)

Diseased/healthy	Above/below Lesion	Water potential (Mpa)	Difference (Mpa)	Number of trees
Diseased tree	Above	-1.66 ± 0.53	0.52 ± 0.47 **	15
	Below	-1.15 ± 0.29		

Healthy tree	Above	-1.38 ± 0.27	0.12 ± 0.05	15
	Below	-1.27 ± 0.26		

** Significant at P=0.01 by T-test.

1. The distance between leaves above lesion and below lesion is approximate 1 meter.

Conclusions

1. Diseased trees with bacterial canker had a lower midday xylem water potential and daily xylem water potential than healthy trees.
2. Bacterial canker lesion had a pronounced impediment effect on xylem water transportation within main branch or scaffold.
3. The midday xylem water potential decreased with increasing disease severity (disease rating).

Experiment III. Influence of chilling temperatures on the susceptibility of fresh leaf scars to bacterial canker in peach

Introduction

Early experiments have shown that leaf scar is an avenue of bacterial infection in cherry (Crosse, 1951; 1957; 1966), but the maximum lesion length of the canker developed is limited within several millimeters (Crosse, 1957). Chilling temperature (generally below 10 °C) alone can induce cankers on young peach seedlings (Davis and English, 1969). Chilling temperatures are very common in late fall during peach leaf defoliation in California. So, the question is if chilling temperature in late fall increases the susceptibility of fresh leaf scar to bacterial canker in peach.

Materials and methods

Peach green wood cuttings (cv. Angelus) were propagated with the method introduced by Couvillon (Couvillon et al., 1975). Self-rooted cuttings were transplanted into one-gallon plastic pots filled with sand and vermiculite (3:1) when greenwood cuttings produced roots. After two weeks of transplanting, the transplanted cuttings began to show some yellowish leaves and were ready to be dormant, since the depletion of nutrients in the stem caused by producing roots. At this time the cuttings were ready for chilling treatment. The chilling treatments were made by exposing cuttings to 2.2 °C temperature for 1 day, 2 days, 4 days and 7 days. After the completion of chilling treatments, fresh leaf scars created by forcible defoliation were instantly inoculated by putting a drop of bacterial suspension on the surface of the fresh leaf scar. The bacteria used in this experiment was B3A 206, a virulent of *P. syringae* pv. *syringae.* The bacteria were harvested with methods described before (Cao et al., 1999). Non-chilling treatment was used as control. Each treatment was replicated 10 times. Two additional pots for each treatment were inoculated with sterile distilled water for negative control. Two to six leaf scars were inoculated for each cuttings. After the inoculation, the inoculated cuttings were covered with plastic bags for two days and were put in two growth chambers for symptom expression. The two growth chambers were set up for 11 hours for daytime and 13 hours for night time.

During the daytime, the temperature inside the chamber was between 14 and 15 °C, during the night the temperature dropped to 8-9 °C for one chamber and 11-12 °C for the other. Equal number of cuttings was incubated in each of the two growth chambers. After three weeks, the disease infection rate was investigated and lesion length measured with a digital caliper by cutting off the surface of the bark with a razor blade. The data were statistically analyzed.

Results

Since there was a little different in night temperature inside the two growth chambers, the data were carefully collected separately for each chamber. The data were analyzed and there were significant differences between the two chambers. So the data were then combined and analyzed together. The results indicated that as the increase of exposure to chilling temperature, the leaf scar infection rate appeared to be decreased and lesion length reduced remarkably (Table 1). These data suggested that chilling temperature increase the resistance of leaf scar to bacterial canker.

Table 1. Effect of chilling temperatures (2.2 °C) on canker lesion length and infection rate through leaf scar inoculation in Angelus peach

Treatment	Inoculation	Lesion length (mm)	Infection rate (%)	Replicates
No chilling (CK)	Bacteria	4.5 ± 1.2 A	89.7 ± 13.7 A	10
1 day of chilling	Bacteria	3.3 ± 0.6 B	75.8 ± 29.0 A	10
2 days of chilling	Bacteria	3.1 ± 0.6 B	72.5 ± 23.5 A	10
4 days of chilling	Bacteria	2.2 ± 0.8 C	68.2 ± 33.7 A	10
7 days of chilling	Bacteria	2.6 ± 0.6 BC	65.8 ± 26.8 A	10
No chilling (CK)	Water	0	0	2
1 day of chilling	Water	0	0	2
2 days of chilling	Water	0	0	2
4 days of chilling	Water	0	0	2
7 days of chilling	Water	0	0	2

Means with the same letters are not significantly different.

Conclusion

Our data suggested that chilling temperature appeared to increase the resistance of fresh leaf scars to bacterial canker.

ADDITONAL EXPERIMENTS THAT ARE CURRENTLY BEING EVALUATED

Experiment IV

Effects of element deficiency and freezing temperature on the susceptibility of peach and prune trees to bacterial canker

Objective

1. To evaluate the influence of different element combinations and freezing temperatures and the combination of the two factors on the susceptibility of peach and prune trees to Bacterial canker.

2. To determine the relationship between element content of plant tissue and bacterial lesion length.

Background facts

1. There is no evidence that nitrogen increase the resistance of trees to bacterial canker (Wilson, 1939)
2. Nitrogen, phosphorus, potassium and each of the combination do not significantly influence the severity plum bacterial canker (Wormald and Garner, 1938)
3. Peach trees in low-nitrogen plots in sandy soil in California have significantly more disease than those given adequate nitrogen (English et al., 1961)
4. Highly significant relationships were found between low nitrogen status measured in leaves and increased incidence of bacterial canker in 'French' prune trees (Southwick et al., 1997)
5. Iron deficiency increases the susceptibility of peach trees to bacterial canker (Daniell and chandler, 1976)
6. Calcium content negatively correlated with tree susceptibility to bacterial cankers in apricot and peach (Vigouroux and Bussi, 1989; 1995)
7. Application of lime tended to induce susceptibility to bacterial canker in plum trees (Wormald and Garner, 1938)
8. Magnesium content in peach is correlated with bacterial canker lesion length (Vigouroux and Bussi, 1989)
9. Low phosphate treatment has a pronounced effect in reducing susceptibility of plum trees to bacterial canker (Beard and Wormald, 1936)
10. Pear trees irrigated with phosphonate derivatives and inoculated with P. syringae pv. syringae had significantly less disease than non-treated controls (Moragrega et al., 1998)
11. Induced infection is correlated with high levels of potassium and low levels of calcium in the branch cortex of young peach trees (Vigourox et al., 1987)
12. Potassium:iron ratio of less than 150:1 in the leaves and stems was associated with peach tree short life (Sharpe and Reilly, 1986)
13. No consistent relation between nutrition and canker development in plum trees (Crosses, 1966)

Questions

How does nutrient status in plant tissue influence tree susceptibility? Is the nutrition balance within the plant tissue responsible for the susceptibility to bacterial canker?

Procedures

Three-year-old peach and prune seedlings growing from seeds and two-year-old peach grafted seedlings (cv. Fairtime on Nemaguard) were transplanted from pot mixture into pure sand in 2-gallon-pot on 3 June, 29 June and 14 July 1999, respectively. Each pot contains approximate 7-kg water washed sand that was autoclaved for three hours before use. The potted trees were irrigated with deionized water for two weeks after transplanting and then 300 ml half-strength

Hoagland's solution (Table 1) with different element combinations (solutions lacking of nitrogen, phosphorus, potassium, calcium, magnesium or iron, respectively) were applied to each pot of corresponding treatment. The nutrition solution was applied twice per week. Depending on the weather conditions, each pot was irrigated with 1000-1500 ml deionized water once per week. Inoculation will be made with and without -5 C freezing treatment over night when trees are dormant in winter. Treatment combinations, replicated 7 times, were arranged in a randomized complete block design on a bench in screen house. Three to six inoculations will be made by injection of 5 ul virulent bacterial suspension to the cambium of the stem of each plant. Additional one plant from each treatment combination will be inoculated with sterile water as check. The puncture for inoculation will be covered with a piece of paraffin film after inoculation for the protection of water loss during incubation. The disease severity will be evaluated by measuring the canker lesion length after incubation. The element content of the leaf, bark or root in each treatment combination will be analyzed. The fresh and dry weight of the roots will be recorded.

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Table 1. Full strength Hoagland's solution with full nutrient and element deficiency (ppm)

		N	P	K	Ca	Mg	S	Fe	B	Mn	Zn	Cu	Mo
Full nutrient (ck)													
1 M KH_2PO_4	1 ml		31	39									
1 M KNO_3	5 ml	70		196									
1 M $Ca(NO_3)_2$	5 ml	140			200								
1 M $MgSO_4$	2 ml					49	64						
Total		**210**	**31**	**235**	**200**	**49**	**64**	**1.4**	**0.5**	**0.5**	**0.05**	**0.02**	**0.01**
Fe-deficiency													
1 M KH_2PO_4	1 ml		31	39									
1 M KNO_3	5 ml	70		196									
1 M $Ca(NO_3)_2$	5 ml	140			200								
1 M $MgSO_4$	2 ml					49	64						
Total		**210**	**31**	**235**	**200**	**49**	**64**	**--**	**0.5**	**0.5**	**0.05**	**0.02**	**0.01**
N-deficiency													
0.5 M K_2SO_4	5 ml			196			80						
1 M $MgSO_4$	2 ml					49	64						
0.05 M $Ca(H_2PO_4)_2$	10 ml		31		20								
0.01 M $CaSO_4$	200 ml				80		64						
Total		**---**	**31**	**196**	**100**	**49**	**208**	**1.4**	**0.5**	**0.5**	**0.05**	**0.02**	**0.01**
K-deficiency													
1 M $Ca(NO_3)_2$	5 ml	140			200								
1 M $MgSO_4$	2 ml					49	64						
0.05 M $Ca(H_2PO_4)_2$	10 ml		31		20								
Total		**140**	**31**	**---**	**220**	**49**	**64**	**1.4**	**0.5**	**0.5**	**0.05**	**0.02**	**0.01**
P-deficiency													
1 M $Ca(NO_3)_2$	4 ml	112			160								
1 M KNO_3	6 ml	84		235									
1 M $MgSO_4$	2 ml					49	64						
Total		**196**	**---**	**235**	**160**	**49**	**64**	**1.4**	**0.5**	**0.5**	**0.05**	**0.02**	**0.01**
Ca-deficiency													
1 M KNO_3	5 ml	70		196									
1 M $MgSO_4$	2 ml					49	64						
1 M KH_2PO_4	1 ml		31	39									
Total		**70**	**31**	**235**	**---**	**49**	**64**	**1.4**	**0.5**	**0.5**	**0.05**	**0.02**	**0.01**
Mg-deficiency													
1 M $Ca(NO_3)_2$	4 ml	112			160								
1 M KNO_3	6 ml	84		235									
1 M KH_2PO_4	1 ml		31	39									
0.5 M K_2SO_4	3 ml			117			48						
Total		**196**	**31**	**391**	**160**	**---**	**48**	**1.4**	**0.5**	**0.5**	**0.05**	**0.02**	**0.01**

Experiment V
Influence of ring nematode infestation on calcium nutrition and corresponding susceptibility of peach tree to bacterial canker

Introduction
Bacterial canker, caused by *Pseudomonas syringae* pv. *syringae*, is a devastating and widespread disease of stone fruit in California (English et al., 1980). Ring nematode parasitism on roots increases the susceptibility to bacterial canker in peach and plum (Mojtahedi et al., 1975; Lownsbery et al., 1977; English et al., 1982). Ring nematodes reduce root volume and feeder roots and induce water stress in peach and plum (Mojtahedi et al., 1975; Nyczepir et al., 1987). However, it has not been determined that how ring nematodes induce stresses to increase the risk of developing bacterial canker in stone fruit (Ogawa and English, 1991).

Vigouroux and Bussi (1989, 1995) inoculated peach and apricot with bacteria during dormant season and found that the length of cankers that developed in the stem was negatively correlated with calcium concentration in the bark. Calcium is an essential component in regulating membrane permeability and cell wall synthesis (McLanghlin & Wimmer, 1999) and is taken up by feeder roots (Clarkson & Hanson, 1980). Calcium has been proposed as a resistance agent in plant diseases (Shear, 1975; Volpin & Elad, 1991; Yamazaki & Hoshina, 1995; Biggs et al., 1997; Kostandi et al., 1998). Since calcium is known as a relatively immobile cation in the plant (Bangerth, 1979), water flow in the xylem is a major factor in its transport into the different parts of the plants (Kirkby, 1979). Irrigation increases the calcium content in the bark of the stem and improves resistance to bacterial canker in peach (Vigouroux & Bussi, 1989). Therefore, we hypothesize that stem injection of calcium salts will compensate for the purported reduction in calcium uptake by ring nematode-treated peach trees and reduce the severity of bacterial canker.

Objectives
The purpose of this proposal is to study: 1. the influence of ring nematodes on calcium nutrition status in the stem of peach and 2. the effect of calcium nutrition status in the stem on the susceptibility to bacterial canker in peach trees stressed by ring nematodes.

Materials and methods
Ring nematode susceptible peach seedlings on Nemaguard rootstocks (Lownsbery et al., 1977) and some adult peach trees will be used as plant materials for this experiment. B3A 206, a virulent strain of *P. syringae* pv. *syringae* (Cao et al., 1999) will be used to inoculate trees and soils infested with the ring nematode, *Criconemella xenoplax*, will be used to induce peach tree stresses (Nyczepir et al., 1987).

The peach seedlings will be planted in pots filled with autoclaved sandy soil, which favors ring nematode growth in early spring. The soil around the roots in each pot will be inoculated with a liquid extract containing 2000 juvenile nematodes at planting, The same amount of nematode-free liquid extract will be poured around the roots of each pot in the control. Treatment and control will be arranged in 32 randomized blocks (2 trees per block, trees with and without nematodes) in a lathhouse with pots sunk in a bed of wood shavings to protect nematodes and

roots from adverse environmental effects. The trees will be fertilized monthly with one-half strength Hoagland's solution (Hoagland and Arnon, 1938) and watered in a uniform manner. During the growing season, 16 blocks of trees that are randomly selected will receive injection of calcium chloride solution (100 ppm) in the stem. When trees become dormant, they will be inoculated by injecting bacterial suspension into the cambium zone of the stem. Each plant will receive three or four inoculations. The inoculation sites will be wrapped with parafilm to maintain moisture, which favors canker development. Before bacterial inoculation, stem samples will be collected for calcium analysis and soil samples will be used to determine nematode population. The inoculated plants will be incubated in a growth chamber at 15°C for three to four weeks and canker lesion size developed during incubation will be measured with a caliper by cutting the bark with a razor blade. The susceptibilities of trees to bacterial cankers among all the treatments and controls will be evaluated by analyzing the average lesion length of each plant statistically. The relationship of calcium content in the bark of the stem and canker length will be analyzed.

During the dormant season, the calcium concentration in the bark of the stem and the susceptibilities of excised stems from adult peach trees that suffer nematode infection and nematode-free trees of the same ages will be investigated. Populations of ring nematodes for these two types of trees will also be determined. The susceptibility to bacterial canker will be evaluated by injecting bacterial suspension to the cambium zone of the excised stems.

Experiment VI
Influence of soil water stress and freezing temperature on the susceptibility to bacterial canker in young peach

Objective

Determine the effect of soil water stress and freezing temperature on the susceptibility to bacterial canker in young peach.

Background facts

1. Late fall irrigation enhances the incidence and severity of bacterial canker disease (English et al., 1980)
2. Stem water content of dormant wood has been considered to play an important role in spreading bacteria within cambium of plant tissue in peach (Vigouroux, 1989; Vigouroux, 1997)
3. French prune trees severely stressed for moisture during the growing season were no more susceptible to canker development by artificial inoculation during the subsequent dormant season than were unstressed trees (Ogawa and English, 1991)
4. Bacterial canker lesion length increase or decrease with dormant stem hydration or dehydration, respectively (Cao et al., 1999)
5. Containerized trees whose roots were immersed in water for two months during the dormant season developed slightly smaller cankers from dormant season inoculations than did trees whose roots were not immersed (Ogawa and English, 1991)
6. Increasing irrigation levels resulted in a clearcut decrease in tree susceptibility with or without soil amendment (Vigouroux and Bussi, 1995)
7. Variations in soil moisture above the permanent wilting point did not affect progress of

the disease in plum trees (Wilson, 1939)

8. Freezing temperature increases disease severity of bacterial canker in peach (Weaver, 1978)

The effect of soil water stress on bacterial canker needs to be further clarified. Does the combination of water stress and freezing temperature increase or decrease peach susceptibility?

Procedures

Three-year-old peach trees growing from seeds were transplanted from 1-gallon-pot into 2-gallon pots filled with UC mixture on 27 July 1999. Each potted tree received about 10 gram of N-P-K 15-15-15 fertilizer with half-instant nutrient release and half time-release. Additional 300 ml of half strength Hoagland's solution was applied to each pot two or three times a week for two weeks in order to promote satisfactory extension shoot growth. Tap water was provided on a daily basis.

On August 31, 1999, four pots were fully irrigated and then remained unirrigated until leaf wilting appeared. The weight of each of the four pots was weighed daily within this drying period in order to measure how much water lose from the pot. After the completion of pot weighing, trees in wet treatment received 400 ml water every day. The dry treatment received 100-300 ml water every 2-3 days depending on the weather conditions. Pots of wet and dry treatments, replicated 7 times, were arranged in a randomized complete design on a bench in Armstrong screen house. The midday water potential of both treatments were measured once a week. All pots were sheltered with plastic film when it is raining. Inoculation will be made during dormant season by injecting approximate 5-10 μl virulent bacterial suspension to the cambium of the stem. The puncture will be sealed with paraffin to maintain moisture during incubation in a growth chamber. Each tree will receive five or six inoculations during the following dormant season with/without freezing. Additional one plant from each treatment combination will be inoculated with sterile water as a negative check. Disease severity will be evaluated by measuring the lesion length after the completion of incubation. Stem samples will be harvested for mineral element analysis before inoculation is made.

Partial results

During the entire period of water stress treatment, the stem midday water potential was determined on a weekly base. The water stress effect of each treatment combination was determined.

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<u>Objective 2.</u> Determine the genetic variability of *Pseudomonas syringae* pv *syringae* (Pss) strains isolated from French prune, other stone fruit trees, and orchard weeds.

The source of inoculum is an important facet of any disease cycle and in the case of BC the niche where canker-inducing strains of Pss reside has not been conclusively determined. For example, English and Davis (1960) [2] reported that *Pseudomonas* isolates that were pathogenic to peach were isolated from a large number of healthy woody plant species in California. Bacterial identification in all these studies was based on biochemical determinants that can be subject to environmental influences and not on genetic determinants specific for canker-inducing strains of *P. syringae* pv. *syringae.* Furthermore, previous work on this project by Dr. Elizabeth Little showed that genetically unrelated Pss strains isolated from tomato, rice, weeds and other non-Prunus hosts could induce bacterial cankers in peach twigs following injection into the cambium (see previous Progress reports for details). Thus biochemical tests using special media and pathogenicity tests using artificial means to introduce the pathogen into cambial tissues cannot differentiate between Pss strains from various sources. It must be emphasized that we have clearly demonstrated that a very specific subset (genotype) of Pss strains is ALWAYS recovered from bacterial cankers. Thus it is imperative that the specific niche of this specific canker-inducing genotype be identified in order to develop management strategies that target the niche(s) that harbor canker-inducing strains of Pss. We have found in previous years that only very low populations of canker-inducing Pss strains, as determined by a newly developed DNA fingerprinting technique called rep-PCR, are found in buds or on tree surfaces (see previous Progress Reports). During 1999 we continued to evaluate the importance of orchard weeds and stone fruit buds in providing *P. syringae* pv. *syringae* inoculum by comparing the genetic fingerprints of *P. syringae* pv. *syringae* strains recovered from cankers to the DNA profiles of *P. syringae* pv. *syringae* isolates from orchard weeds and French prune buds and lenticles.

<u>Experimental Procedures:</u>

French prune bud and weed samples were taken monthly from January through April from prune orchards located in the Wolfskill experiment station in Winters, California and from an orchard south of Gridley, California. *P. syringae* pv. *syringae* were isolated and biochemically identified from prune buds by the process of Schick et al. 1997. Orchard weed leaves were washed in 50mls of phosphate buffer and aliquots of the wash were spread on KBC plates. Subsets of each colony morphology type that grew on LBD4 were plated on Kings B medium, a medium which causes fluorescent pseudomonads, including Pss, to produce a fluorescent, water soluble blue- to green-colored pigment. Subsamples of those strains which produced fluorescent pigments were grown in liquid culture, harvested by centrifugation, and suspended in water to a concentration of approximately 10^7 cells/ml. These suspensions were then infiltrated into a tobacco leaf using a plastic hypodermic syringe to determine whether the strain could induce a hypersensitive reaction (HR), a plant reaction which indicates that the strain was plant pathogenic, i.e. HR (+) strains are plant pathogenic while HR(-) strains, such *Pseudomonas fluorescens*, are not pathogenic. A representative number of isolates identified as *P. syringae* pv. *syringae* were stored at –80° C. The genetic fingerprints of representative HR(+) strains were determined using PCR and repetitive element (ERIC) primers as previously described in the 1997/98 Progress report. DNA fingerprints of bud and weed strains were compared with the fingerprints of strains isolated from cankers that were previously determined

in 1995 through 1998. The genetic relatedness of the isolates was assessed by visually comparing DNA banding profiles on agarose gels.

Results and Discussion:

The prune bud, bark and orchard weed isolation date from 1996 to 2000 is shown in the following tables:

Table 1. Weed isolations of Pss from 1996 through 2000.

	Weed samples tested	Colonies/gram fresh wt.	Number of putative Pss	Number of Pss by *syr*B hybridization
1996/97	40	10^4 - 10^6	39	1 (HR test only)
1997/98	8	10^4 - 10^6	8	4
1998/99	4	10^4 - 10^6	240	2
1999/00	2	10^4 - 10^6	0	0

Table 2. Prune bud isolations of Pss from 1996 to 2000.

	Number of buds tested		Putative Pss		Confirmed Pss (*syr*B+)	
	Healthy	BC	Healthy	BC	Healthy	BC
1996/97	240	240	206[a]	638	nd	1/5 (HR+)
1997/98	120	120	18	60	7/18[b]	50/60
1998/99	360	360	490	424	20/33[c]	2/55
1999/00	240	240	0	0	0	0

Represents the total number of P. syringae isolates obtained in a year by visual identification.

1. Represents the fraction of visually identified *P. syringae* that contain the syrB gene by colony hybridization.
2. The fraction of P. syringae isolates identified by syrB colony hybridization may skewed by the number of positive or negative identifications of P. syringae in a single sample. The potential for misrepresentation is higher in years with low populations *P. syringae* such as 1998/99.

Table 3 Prune bud isolations of Pss over months in 1998/1999.

	Number of buds tested	% of buds containing Pss	
		Healthy	Diseased
December	180	4.4%	0
January	240	0	0
February	240	0	8%

During 1998 *P. syringae* pv. *syringae* proved to be very difficult to isolate from orchards weeds. Only two Pss strains, one from alfalfa and one from oat weeds growing at Wolfskill, were obtained last season. Although weed leaf surfaces support large numbers of fluorescent pseudomonads, only a very small percentage of them were determined to be *P. syringae* pv. *syringae*, i.e. most were not plant pathogenic as determined by the production of a hypersensitive reaction on tobacco. Genetic analysis, using rep-PCR, of these 2 Pss strains showed they were completely different from Pss strains associated with BC. These results to date suggest that orchard weeds are not important reservoirs of Pss strains that cause BC. In contrast prune buds of both healthy and diseased trees contained Pss isolates that were genetically identical to the canker-inducing strains.

Objective 3 and 4. Determine if prematurely defoliating prune trees with zinc sulfate and/or dormant application of copper and fertilizer amendments can reduce the incidence and/or severity of bacterial canker disease.

Bacterial canker of stone fruit is an extremely complex disease, which occurs only when trees are stressed by one or more predisposing factors. The causal agent of this disease, *P. syringae* pv. *syringae,* is a relatively weak pathogen that requires these predisposing conditions be present before infection can occur. Abiotic predisposing factors include sandy soils, shallow soils above a hardpan, inadequate nitrogen fertilization and fall irrigation. Ring nematode (*Criconemella xenoplex*) and possibly *Pythium* infestation, tree age and rootstock selection are some biotic factors that predispose stone fruit trees to bacterial canker disease. Of the biotic factors, parasitism by ring nematode seems to be the most important factor. Preplant backhoe and soil fumigation treatments with telone or methyl bromide significantly increased tree vigor as measured by trunk circumference, while reducing tree mortality. In one study, 87.5% of peach trees grown in sandy soils in a low nitrogen treatment died after three years, while those fertilized with NPK or high nitrogen had a 12.5% and 26.6% mortality, respectively. Similar results were observed in French prune fertigation trials conducted by Steve Southwick's group at Wolfskill station.

Environmental concerns, as well as the economic costs of soil fumigants, have made their use less practical than in years past. The use of methyl bromide for fumigating orchard sites will probably be banned after the year 2005, and so alternative control strategies will be needed. Many growers in California currently use copper sprays in an attempt to control bacterial canker without any knowledge of its efficacy. Frequent sprays with copper were reported to control epiphytic populations of *P. syringae* pv. *syringae* and significantly reduced the incidence of bacterial canker in apricot and cherry in northern Victoria province of Australia. In California, English and Hanson (1954) reported that spraying Bordeaux mixture on plum and French prune produced erratic results. The efficacy of copper sprays for controlling epiphytic populations of *P. syringae* pv. *syringae* has been recently questioned. Schick et al. 1996 reported that 48% of *P. syringae* pv. *syringae* isolated from woody species in 1992 in Oregon were resistant to 0.32mM $CuSO_4 \cdot 5H_2O$. Of forty strains isolated from stone fruit trees in California, twenty-one were resistant to 0.25mM copper and sixteen were resistant to 0.5mM copper (see 1996 Progress Report).. Because copper is still used commercially to control diseases caused by *P. syringae* pv. *syringae,* we are currently investigating its efficacy for reducing BC-losses in French prune. .

Experimental Procedures:

Trials have been conducted in four orchards near Marysville and Gridley, California for the past 4 years and we will evaluate the efficacy of our 1999/00 treatemnts one final time in Spring, 2000. Each orchard contains ten to twelve blocks with trees that received one of the following five treatments: 1. copper hydroxide (Kocide[R], (8lbs/100gallons) sprays with a combination of rapid and slow release fertilizer; 2. rapid and slow release fertilizer only; 3. copper hydroxide only; 4. zinc sulfate (10lbs/100gallons) just before leaf drop followed by copper for remaining sprays; and 5. unsprayed control. The scheduled times for copper only spray include one just before leaf drop in mid-October followed by one spray in December and January , and a final spray at bloom time using 2lbs/100gallons. In treatment four, zinc sulfate is applied only in the fall to accelerate leaf drop with Kocide being applied for the two remaining sprays. Our initial reason for evaluating zinc to induce premature defoliation was to cause leaf scars to heal in late fall before wet weather set in. However, as previously discussed, the apparent rapid healing of prune leaf scars may have reduced the potential importance of leaf scars as avenues for infection by Pss. A combination of 1 part slow release fertilizer (Meister[R] 17-6-12, Helena Chemical Co.), 1 part regular formulation of 16-16-16 and 1 part ammonium nitrate fertilizer was applied in the fall, at the rate of 1 lbs for 1-2 leaf tree and 2 lbs for 3-5 leaf trees. Disease evaluations were taken in early and late spring when cankers are active using a visual rating system of 0-5 with 0 representing no disease and 5 equaling tree death.

Results and Discussion:

In 199, no disease developed in 2 of the 4 orchards we have been treating and we discontinued treatments of therse two orchards in 1999/00. For the first time we found a significant effect of both fertilizer and copper applications in 1 of the orchards that had high to moderate ring nematode infestations. (Table 1).

Table 1. Mean disease ratings at a Riviera Dr. prune orchard on 4-15-1999 and 7-20-1999.

	4-15-1999	7-20-1999
Copper + NPK	0.65	0.95 A
NPK only	0.72	1.15 A
Copper only	0.92	1.67AB
Zinc followed by copper	1.75	2.10 AB
Control	1.60	2.42 B

Table 2. Percent mortality of Riviera Dr. prune trees on 10-21-1999 after grower removal of severely diseased trees.

	Percent Mortality
Copper + NPK	0%
NPK only	15%
Copper only	20%
Zinc followed by copper	25%
Control	45%

Table 3. Leaf nitrogen analyses of fertilized and control treatments in the Riviera Dr. orchard.

	Leaf nitrogen
NPK only	2.527 A
Copper	2.408 A
Control	2.336 A

Table 4. Feather River Blvd. prune orchard, mean disease ratings in 1999.

	4-20-1999
Copper + NPK	0.50 A
NPK only	0.25 A
Copper only	0.50 A
Zinc followed by copper	0.45 A
Control	0.15 A

The Riviera orchard has been losing trees to bacterial canker for many years. For the first time in 3 years, we observed a significant positive effect of both the additional fertization treatment and the copper application. Interestingly, fertilization alone was as good as the copper application and less costly. It is interesting to note that in New Zealand, where copper applications dramatically decreased the incidence of BC in apricot, it is common knowledge that the treatments need to be repeated annually for several years before any beneficial effect is noted. This is also what we found at this prune site. The fact that NO trees were lost in the copper plus nitrogen treatment is very noteworthy compared to the 45% tree losses in the control. Thus it would appear that is certain orchards with particular risk/stress factors there appears to be a definite benefit to nitrogen/microelement supplementation as well as topical applications of copper bactericides. We hope to identify what those risk factors are by correlating soil condition and nemtatode numbers with the incidence and severity of BC (see below).

<u>SubObjective 3b: Evaluation of surfactant/copper applications for BC control, beginning 1999:</u>

Recent work by Lindow, Olson and Buchner on walnut blight, caused by the plant pathogenic bacterium *Xanthamonas campestris pv juglandis*, suggests that new silicon-based surfactant can facilitate the penetration and efficacy of copper bactericides into hydrophobic tree tissues such as buds. If low numbers of canker-inducing Pss strains reside in buds or lenticles and then multiply to cause disease in the spring in stressed trees, then it is possible there might be some benefit to using surfactants in combination with bactericides for controlling BC. In early January, 1999 we will establish 2 reps of 20 trees each for a surfactant + copper hydroxide treatment as well as an unsprayed control treatment in Orchard #1 south of Gridley. We decided to establish 2 plots in Orchard #1 because of the increasing severity of BC at this site. Trees will be sprayed with a handgun sprayer to runoff using the same Kocide concentration that is used in the other treatments but containing 0.5% BreakThru. One more treatment will be applied in early February, approximately 2 weeks before bloom. Trees will be rated for incidence and severity of BC as previously described.

There was no significant disease development in either the treated or non-treated controls in 1999. Additional application of surfactant + Kocide/Manex are being made in 1999/00,

Objective 5. Determine if high worked rootstocks afford better protection against BC than low budded trees purchased from the nursery. In collaboration with pomologists at UC Davis, evaluate alternative prune rootstocks for relative BC-resistance

Stone fruit varieties are currently budded onto plum or peach rootstocks that impart added vigor and disease resistance compared to self-rooted trees. Peach rootstocks impart vigor and confer some resistance to bacterial canker compared to most plum rootstocks, but the latter are more resistant to soilborne diseases such as Phytophthora root and crown rots, which are common in heavy soils. In Georgia, Lovell and Guardian peach rootstocks provided the best control of peach tree short life, a disease complex that is caused by a combination of bacterial canker and cold injury. Vigorous rootstock growth has been repeatedly correlated with resistance to bacterial canker. One potential cultural control involves field-grafting the scion variety, French prune, onto rootstocks grown as rootstocks in the field for one year. The rootstocks that grew without the scion for one year appear to grow more vigorously than those rootstocks that were cut back during the scion budding process. Such field-grafted trees probably possess root systems that are more established than those grafted with a scion when planted. Thus, the rootstocks of field budded trees may provide more vigor to the scion and possibly reduce tree mortality due to bacterial canker.

Experimental Procedures:

In the spring of 1995 and 1996 myrobolan 29C rootstocks were planted in five orchards in the Sacramento valley known to have bacterial canker disease pressure and allowed grow for one year. The rootstock scaffolds were then budded with French prune at least 36 inches above the ground. Disease incidence and severity data were taken in the spring of 1998 and will continue to be recorded for the next two years.

Results and Discussion:

This objective, testing the value of field budding French prune scions onto one-year field grown rootstocks, has been running concurrently with a field experiment designed by Dr. Steve Southwick, UCD Pomology. Dr. Southwick's treatments include three nursery-grafted myrobolan 29C rootstocks of various budding heights and one low-budded nursery grafted Lovell peach rootstock. Results are presented in Table 1 thru 3 below:.

Table 1. Rootstock effects on the control of Bacterial canker in the French prune scion.

	Overall	Nevis	Chandon Av	Boitano	5th&Cutler
Low bud	2.76 A n=80	3.93 A n=16	3.37 A n=27	2.27 A n=18	1.83 A n=19
Medium bud	2.46 AB n=85	3.78 A n=19	2.88 A n=30	2.55 A n=18	1.60 AB n=18
High bud	2.37 AB n=87	3.77 A n=20	2.40 AB n=32	2.72 A n=18	0.70 AB n=17
Fieldgraft	2.01 B n=54	2.95 A n=20	1.37 B n=16	1.16 AB n=6	0.66 AB n=12
Lovell	0.28 C n=79	0.84 B n=13	0.03 C n=31	0.30 B n=20	0.33 B n=15

Rating Scheme
0 = healthy.
1 = 1 or 2 gumballs or points of infection.
2 = infection sites covering less than half of the tree.
3 = infection sites on more that half of the tree.
4 = tree scaffold death.
5 = death of entire scion.

Table 2. Overall level of disease in orchard rootstocks.

Orchard	Rating
Nevis	3.00 A
Chandon Av.	1.78 B
Boitano	1.66 B
5th&Cutler	0.91 C

Table 3. Percent mortality in French prune scions grown on different rootstocks rated 1999.

Mortality	Overall	Nevis	Chandon Av.	Boitano	5th &Cutler
Lowbud	42.50% n=80	75.00% n=16	44.44% n=27	38.88% n=18	15.78% n=19
Medbud	41.18% n=85	68.42% n=19	43.33% n=30	44.44% n=18	5.55% n=18
Highbud	32.18% n=87	65.00% n=20	21.87% n=32	38.88% n=18	5.88% n=17
Fieldgraft	31.48% n=54	50.00% n=20	12.50% n=16	16.66% n=6	33.33% n=12
Lovell	5.06% n=79	15.38% n=13	00.00% n=31	5.00% n=20	6.66% n=16

These data clearly show that the lowest incidence or severity of BC occurred with trees grafted on peach rootstocks. The greatest losses in these "hot spot" orchards occurred on standard low budded nursery trees and the incidence on field-grated and high budded trees were similar. One distint disadvantage associate with the field-grafted trees was the proliferation of rottstock suckers. However, it needs to be emphasized that these field-graftee trees were T-budded with buds rather than whip grafted with large pieces of prune scion. We observed virtually no rootstock proliferation with Jim Doyle's trees (see below) that he whip-grafted with french scions. Thus if a grower was to try to use field-grafted rootstocks in a particular site we would recommend that the rootstock be grafted with scion sticks rather than budded.

SubObjective 5b: New prune rootstocks being evaluated for BC-resistance in 1999/00:

In May 1997, we planted ten M-40 rootstocks in an orchard south of Marysville, where the soil is very sandy and tree mortality from bacterial canker was 100% in the previous 3 years. In April, 1997 Jim Doyle, UC Davis Pomology, field -grafted these rootstocks with three French prune scions using the whip-grafting technique and graft establishment was 100%. All of the trees grew very well during 1998 and they were pruned to produce major scaffolds in November, 1998. The trees were evaluated for the incidence and severity of BC in Spring, 1999. NO

disease was found on any of the trees and all trees grew vigorously.

Additional M-40 rootstocks could be planted in other sites during 1999. We believe that the identification or new development of a BC-resistant rootstock would probably provide the most powerful, cost-effective and environmentally friendly tool for reducing losses to BC. In the future, we will be happy to provide whatever plant pathological assistance is needed for assessing the relative resistance of other newly developed prune rootstocks.

Objective 6: Identify plant stress metabolites that activate virulence determinants of Pss

Considerable progress was made on this objective during 1999 because we developed methods to extract xylem and cambial sap from excised prune twigs. These extracts were much cleaner than the leaf extracts that were used previously.

The following three figures show the effect of these extracts on eliciting the expression of a toxin gene (syr B) within Pss. In xylem sap extracted from healthy cherry, apricot and prune twigs we found little difference in the ability of extracts from frozen versus non-frozen twigs to induce syr B expression (Figure 1). Extracts from apricot and cherry were similar in their inducing capability and these induced higher levels of syr B that did french prune extracts. There was little difference between the inducing ability of frozen versus non-frozen prune extracts analyzed in March, 1999 (Table 2) however there was a significant increase in the ability of extracts from frozen prune twigs collected in November (Table 3). The nature of this difference is unknown and these experiments are being replicated through 2000.

Objective 7. Analysis of soil factors associated with bacterial canker in French Prune.

Bacterial canker is complex disease occurring only where trees are grown under stressful conditions caused by various soil factors. Stone fruit trees grown on sandy soils and soils infested with ring nematode often succumb to the disease, however other soil-related factors can also contribute to tree stress. To further investigate the relationships between bacterial canker and various soil parameters in 1997 we initiated a project to study soil composition, nutrition and ring nematode populations in relationship to the occurrence of BC. The ultimate goal of this objective is to develop BC risk assessment criteria that would alert growers to potential problems associated with a potential orchard site,

Experimental Procedures:

On July 9, 1998, one kilogram soil samples were taken in a straight transect from around the base of every 5th tree through a BC-disease "hotspot" in a French prune orchard located south of Marysville California. Soil from the perimeter of healthy trees located on either end of the BC site were also sampled. Additional Bc-affected prune orchards along the Feather River were sampled on and September 24, 1999.

Numerous soil characteristics including mineral content, nutrient availability, particle composition, and water retention capacity are being determined at the DANR laboratory at UC Davis. Ring nematode populations were determined by Becky Westerdahl's laboratory, Nematology, UC Davis.

CONCLUSIONS:

Bacterial canker (BC) is a complex disease whose expression is more a function of the vigor of the tree than it is the presence of the bacterial pathogen. The ability to identify implementable orchard management practices to minimize the risk of developing BC will need the collective expertise of plant pathologists, pomologists and nematologists. During the past 5 years we made substantial progress on all of the original project objectives. The recent involvement of Mr. Cao on the project has greatly expedited progress on Objective 1. We have initiated additional field trials on the effect of silicone-based surfactants which may expedite the delivery of bactericides into hydrophobic tree spaces and on the development of BC risk assessments for new orchard sites based on analysis of orchard soil characteristics.

In 1998 we developed experimental systems to create BC lesions under controlled conditions. We found that even briefly exposing prune branches to moderate freezing conditions (21 F) greatly increased the size of BC lesions. Contrary to widely held, but not experimentally substantiated beliefs, we found that prune leaf scars were only susceptible for 4 hours to infection by Pss. We found that a low percentage of prune lenticles could be inoculated using non-invasive inoculation techniques which were similar to those that could occur in nature. However, we did not isolate large number of pathogenic Pss from the lenticles of healthy or BC-affected prune trees. Numerous experiments are currently underway to investigate how microelements, drought, nitrogen, tissue water content and other factors can induce stresses that predisposes trees to developing BC.

In 1999 we found a significant difference in one test orchard between trees that were treated with copper and/or a time-released application of 16-16-16 fertilizer containing additional micronutrients. In addition, much less tree mortality occurred in the copper plus fertilizer treatment than in the unsprayed control. However, there was no effect on the incidence or severity in another low disease pressure orchard. Additional field evaluations of bactericides, including the use of silicone-based surfactants in combination with bactericides, and fertilizer amendments, are currently being evaluated will help determine the efficacy of these treatments.

The genetic characterization of a collection of Pss strains from cankers of french prune and other stone fruits, as well as other diverse plant hosts, is now complete. This information has provided us with the necessary tools s to critically examine the role and relationship of epiphytic populations of Pss to canker-inducing strains of Pss. Isolation and genetic characterization of Pss strains from prune buds and bark surfaces conducted from 1996 to 1999 indicates that canker-inducing Pss populations are low, but present, in these tissues. Large and diverse bacterial populations were isolated from the surfaces of orchard weeds, however no canker-inducing Pss strains were recovered from weeds during the past 3 years. These results suggest weeds do not provide significant amounts of Pss inoculum. Isolation performed in 1998 found no evidence for the systemic movement of Pss in cambial tissues of French prune trees. By better understanding the dynamics of Pss populations on tree surfaces, in buds and on developing tissues we hope to formulate implementable management practices to reduce losses to BC. In 1999, methods were developed to extract sap from prune xylem and cambial tissues. Some differences were found in the ability of these extracts to induce the expression of an important toxin gene in Pss. Analyses of soil collected in a BC orchard "hotspot" showed that a high sand and low silt level were the single greatest soil risk factor for developing BC in prunes.