BACTERIAL CANKER: MECHANISMS, PATHOGEN CHARACTERIZATION, AND CONTROL

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Abstract

Bacterial canker (BC), caused by *Pseudomonas syringae* pv. *syringae* (Pss), is a widespread and devastating disease of French prune and other stone fruits in California. Numerous predisposing factors, such as nutritional deficiencies, freezing temperatures, and orchard soil characteristics, can induce stresses that increase the incidence of BC. We are trying to understand how Pss causes BC, define BC risk factors and develop more effective management strategies for BC.

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 greatly increased the size of BC lesions. Contrary to widely held, but not experimentally substantiated belief, we found that prune leaf scars were only susceptible for 4 hours to infection by Pss. It is likely that other tree niches, such as bark lenticles, serve as more important sites for Pss infection. Experiments are now under way to test this hypothesis.

In order to understand the field conditions that affect the development of BC, we established several "stress" test plots at the Plant Pathology facility at UC Davis. A simulated hardpan was created in one of these sites and French prune trees were planted in 1995 and 1996. Although some cankering occurred on apricot trees in 1997, we saw no evidence of BC in the hardpan plot during 1998.. The effect of scaffold budding rootstocks in the field is being examined in six commercial prune orchards with BC histories. Although the least amount of BC developed on prune trees grown on Lovell peach rootstocks, high budded nursery trees, and field-grafted french prune scions on plum rootstocks developed less BC than low grafted nursery trees on plum rootstocks. We evaluated the effectiveness of fall defoliation treatments with zinc sulfate as well as dormant copper applications and fertilizer amendments for controlling BC.

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Canker-inducing Pss strains were not be recovered from dormant buds or wood washes of healthy prune trees. These results suggest that large populations of canker-inducing Pss do not routinely overwinter in the buds or on the surface of healthy prune trees. High bacterial populations were recovered from orchard weeds, however we were unable to recover canker-inducing Pss from orchard weeds. More isolations will be performed in 1999 to verify these initial observations. Soil analyses showed that high level of sand was the single greatest risk orchard site 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 in1999.
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.

Objectives 1: Experimental systems for reproducing bacterial canker.
Materials and Methods:

a. Laboratory experiments for reproducing bacterial canker disease:

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. Mr. Tiesen Cao, a visiting scientist from China, recently assessed several different techniques for reproducing bacterial canker under experimental conditions, initially in the laboratory of Dr. Ken Schakel, Dept. of Pomology, UC Davis and currently as a research associate in our laboratory.

1) Freezing increases the susceptibility of prune branches to BC:

Tiesen 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 (Figure 1). He found that 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 preliminary results support the conclusion that water content of dormant Prunus tissues significantly influences BC. It is interested 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 which may aid in the dispersion of Pss in water films as the cambium/twig thaws. Freezing may also damage cambial cells which 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 now allows 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. Similar Pss inoculation experiments, using wood collected from healthy and stressed prune trees growing at our orchard test sites, are being evaluated during January-March, 1999.

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 (Table 1). These result 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). We are now using similar procedures to determine whether dormant or emerging buds differ in their susceptibility to Pss infection.

FIGURE 1

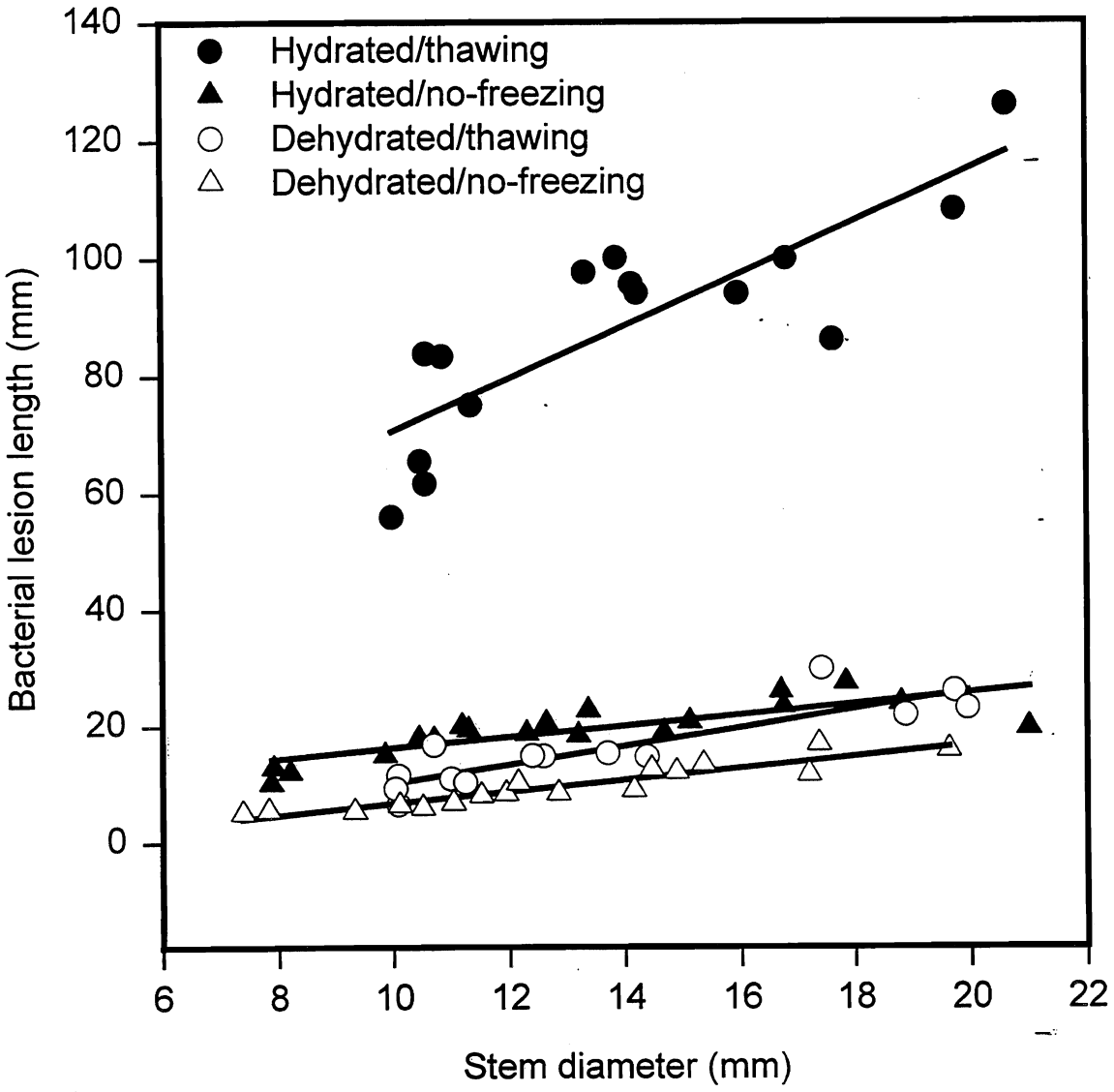
Hydrated/thawing
Hydrated/no-freezing
Dehydrated/thawing
Dehydrated/no-freezing
Bacterial lesion length (mm)
Stem diameter (mm)
0
20
40
60
80
100
120
140
6
8
10
12
14
16
18
20
22

TABLE 1

Table 4. Effect of exposure time of leaf scar on disease incidence and lesion length of leaf scar infection in prune, peach and cherry tree.

Species	Treatment	Disease incidence (% diseased scars)	Lesion length (mm)
Prune	0 hour	40.3 ± 5.0 a	2.9 ± 0.3 a
	2 hours	26.5 ± 2.3 b	3.0 ± 0.2 a
	4 hours	5.3 ± 2.7 c	2.0 ± 1.1 a
	12 hours	0.0 c	0.0 b
	1 day	0.0 c	0.0 b
	2 days	0.0 c	0.0 b
	5 days	0.0 c	0.0 b
Cherry	0 hour	85.0 ± 7.6 a	3.2 ± 0.3 ab
	2 hours	76.2 ± 3.1 a	3.4 ± 0.1 a
	4 hours	40.0 ± 5.8 b	3.2 ± 0.1 ab
	12 hours	17.4 ± 9.0 c	2.0 ± 1.0 ab
	1 day	40.0 ± 6.7 b	3.1 ± 0.1 ab
	2 days	5.9 ± 5.9 c	1.2 ± 1.2 bc
	5 days	0.0 c	0.0 c
Peach	0 hour	43.9 ± 2.5 a	2.8 ± 0.3 a
	2 hours	24.8 ± 5.6 b	2.5 ± 0.2 a
	4 hours	4.1 ± 2.1 c	1.6 ± 0.8 ab
	12 hours	2.2 ± 2.2 c	0.8 ± 0.8 bc
	1 day	0.0 c	0.0 c
	2 days	0.0 c	0.0 c
	5 days	0.0 c	0.0 c

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, 1997 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.

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. Initial results from these two isolation dates indicate that total bacterial numbers are increasing during the cooler, wetter months, thus we will continue to isolate and characterize the bacteria that occupy prune lenticles. In addition, we are now conducting Pss lenticle inoculation experiments, similar to those shown in Figure 1, to assess whether freezing and water content can alter the susceptibility of cambial tissues that lie beneath scaffold lenticles.

b. Field plots for reproducing bacterial canker/blast disease at UC Davis:

Two field plots were established at the Plant Pathology orchard facility to attempt to induce bacterial canker (BC) disease by growing trees on shallow (hardpan) soils. The artificial hardpan was created by removing the top 14" of soil from a 200' X 400' plot and lining the resulting hole with 3 to 4 " of kaolite, a clay material that is used to line irrigation ditches. The top soil was then filled in over the clay layer. Young French prune, as well as other BC susceptible varieties such as apricot and cherry, were planted in these plots in 1995 and 1996. All trees were watered by microsprinklers only after they showed some early signs of drought stress, a factor which is thought to predispose trees to BC. Subsamples of trees in the hardpan and control plots were given spring and fall nitrogen applications in 1996-98. Scaffolds of some trees were inoculated with virulent strains of Pss either in Fall and/ or in the Spring, 199-98. Although we saw some small limb cankers on inoculated apricot trees growing in the hardpan site in March, 1997 (see 1997 Progress Report) we did not observe any cankers on any Prunus species in either plot in March, 1998. Trees were again inoculated with Pss in early November, 1998 and a subset of trees will be inoculated again in February, 1999 just prior to bud break. Trees will be rated for the development of BC in April, 1999. It would obviously be desirable to

be able to reproduce BC using these plots however BC typically develops in 2 to 3 year old trees; thus it is possible that we did not made the artificial hardpan shallow enough to cause sufficient stress to induce BC.

Objective 2. 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 are 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 1998 and 1999 we are continuing 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.

Experimental Procedures:

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 which 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:

Over sixty *P. syringae* pv. *syringae* isolates from prune buds of both healthy and diseased trees and at least sixty isolates from French prune cankers were isolated in 1998. Preliminary genetic analysis revealed that of the five prune canker strains analyzed from Wolfskill, all were indistinguishable and identical to the rep-PCR profiles previously generated in Dr. Elizabeth Little's work. Three of the prune bud isolates from diseased trees were identical to the canker isolates. Of four isolates analyzed from healthy prune buds, two were somewhat similar to the canker strains and two were completely different (data not shown). The remaining *P. syringae* pv. *syringae* isolates from prune cankers will be analyzed this winter. Approximately 60 Pss isolates were obtained from buds of disease French prune trees and ten were obtained from buds of healthy trees from the Wolfskill experiment station. An equivalent number of buds were analyzed for both healthy and diseased trees. As in 1997, we found comparatively few Pss strains residing within prune buds.

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. Although we are continuing to sample weeds on a monthly basis during 1999, results to date suggest that orchard weeds are not important reservoirs of Pss strains that cause BC.

SubObjective 2b: Development of a rapid test for identifying canker-inducing Pss strains

One of the major difficulties in trying to identify major reservoirs of canker-inducing strains has been the inability to rapidly differentiate the plant pathogenic from nonpathogenic fluorescent pseudomonads. To date we have had to rely on the induction of a hypersensitive reaction in tobacco to differentiate pathogenic from nonpathogenic strains, a test which requires growing up the strain in question, harvesting the cells by centrifugation, resuspending the harvested cells to the correct concentration, injecting the suspension into tobacco leaves and waiting for 2 days to determine whether a hypersensitive reaction occurred. The amount of work this process requires has greatly limited the number of strains we would like to characterize. In order to expedite this process we have recently developed DNA hybridization and PCR tests which can detect the presence of the syringomycin synthesis gene which is present in nearly all plant pathogenic Pss strains. The hybridization assay allows large numbers of bacterial colonies to be transferred en mass to nylon membranes on which the cells are lysed and their DNA is bound to the membrane. Membrane bound bacterial DNA is then analyzed for the presence of the syringomycin gene by hybridization. Both the PCR and the hybridization test will allow us

to more rapidly characterize Pss strains from diverse plant species.

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 3 years and will continue at least one more season. 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:

Only two of the four orchards have developed disease to date so the results presented in **Tables 2** and **3** are from these orchards only. We expressed the results of this trial both as an average disease value/treatment (Tables 2a and 3a) and number of severely cankered or dead (Tables 2b and 3b) because we wanted to determine if any of the treatments could prevent tree death.

Table 2a

Average bacterial canker disease rating for a French prune orchard located south of Gridley, CA. (Values represent the mean of disease rating on a 0 to 5 scale; 20 trees per treatment)

TREATMENT	1997	1998
Copper hydroxide and fertilizer	0.48	1.42
Fertilizer only	1.10	1.05
Copper hydroxide only	0.55	1.32
Zinc sulfate and copper	1.35	1.94
Unsprayed control	*0.70*	*1.33*

Note: one tree each from the untreated and the zinc sulfate treatments died.

Table 2b

Number of French prune trees with a disease rating of 3 (heavy gumosis with old cankers reactivating) and 4 (heavy gumosis including scaffold death) from an orchard south of Gridley, CA. (Same orchard as in Table 2a).

TREATMENT	1997	1998
Copper hydroxide and fertilizer	0/20	1/20
Fertilizer only	0/20	4/20
Copper hydroxide only	0/20	3/20
Zinc sulfate and copper	0/20	3/20
Unsprayed control	*0/20*	*8/20*

Table 2c

Number of French prune trees with a disease rating of 2 (gummosis and small cankers on one side of the tree) from an orchard south of Gridley, CA. (Same orchard as in Table 2b).

TREATMENT	1997	1998
Copper hydroxide and fertilizer	3/20	6/20
Fertilizer only	7/20	3/20
Copper hydroxide only	3/20	8/20
Zinc sulfate and copper	6/20	4/20
Unsprayed control	*8/20*	*6/20*

Table 3a

Average bacterial canker disease rating for a French prune orchard located south of Marysville, CA. (Values represent the mean of disease rating on a 0 to 5 scale; 20 trees per treatment)

TREATMENT	1997	1998
Copper hydroxide and fertilizer	0.15	0.15
Fertilizer only	0.15	0.15
Copper hydroxide only	0.30	0.10
Zinc sulfate and copper	0.18	0.05
Unsprayed control	*0.15*	*0.05*

Note: There were **no** trees in Orchard 3 with disease ratings of 3 or 4.

Table 3b

Number of French prune trees with a disease rating of 2 (gummosis and small cankers on one side of the tree) from an orchard south of Marysville, CA. (Same orchard as in Table 3a).

TREATMENT	1997	1998
Copper hydroxide and fertilizer	3/20	6/20
Fertilizer only	7/20	3/20
Copper hydroxide only	3/20	8/20
Zinc sulfate and copper	6/20	4/20
Unsprayed control	*8/20*	*6/20*

Although there was a lot of variation in the amount of disease from year to year, the average ratings of disease in 1997 and 1998 for all treatments, in both BC orchards, suggests there is little benefit associated with any of the treatments. However, in Orchard #1 where disease pressure is high, 8 trees in the unsprayed controls sustained heavy damage compared to only 3 or 4 trees given some copper, fertilizer, or zinc treatments and only 1 sustained heavy damage in trees that were both fertilized and sprayed with copper (Table 2b). There appeared to be no value associated with any treatment for reducing mild stages of BC (Tables 2c and 3b).

During the past 3 years, the one thing that has been consistent with evaluating the efficacy of these bactericides for managing BC has been the variability of the results, both from orchard to orchard, tree reps within an orchard, and disease incidence over time. Some treatments that looked promising in 1997 did not surface in 1998. For these reasons additional time and replication in other orchards will be necessary before firm conclusions can be made regarding the potential benefit of bactericide applications. The evaluation of these products in one controlled orchard site at UC Davis would also decrease the effects of individual grower's orchard management practices that could influence the efficacy of these treatments.

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

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.

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 4.**

Table 4

Summary of tree mortality data of French prune scions grafted on various rootstock. Values are the averages of treatments conducted in four orchards located in Butte, Colusa and Yuba counties. Project was undertaken in collaboration with Dr. Steve Southwick of the Pomology Department at the University of California, Davis.

TREATMENT	% MORTALITY	# OF TREES
Low budded 29C	25%	76
Medium budded (20") on 29C	21%	87
High budded (36") on 29C	18%	80
Lovell peach rootstock	9%	80
Field grafted 29C	*18%*	*34*

Forty additional myrobolan rootstocks were field grafted in three locations south of Marysville, California in April 1998 from which data will be collected in 1999. From the results presented in Table 4 it is clear that Lovell peach rootstock provides the greatest protection from tree mortality due to bacterial canker as reported previously. The percentage of field-grafted trees which died was equal to that of the scion high budded onto myrobolan 29C suggesting that budding height has an effect on resistance to bacterial canker. If this approach is successful then growers would be able to use high grafted plum rootstocks, which are more resistant to soil borne disease than peach rootstocks, to impart some additional resistance to bacterial canker compared to low grafted nursery trees. However, a grower would have to be willing to invest significantly more time and cost in keeping rootstocks suckers under control if field-grafted rootstocks were to be used.

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

In May 1997, we planted ten M-40 rootstocks in an orchard south of Marysville, CA 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 will be evaluated for the incidence and severity of BC in Spring, 1999. 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

Progress on this objective was minimal during 1998 because we shifted our resources into other project objectives. However, extracts of prune were identified that both induced and suppressed the expression of the syringomycin gene in Pss. However, we found no obvious differences in the activities of extracts from healthy versus BC-affected prune trees. All mixtures obtained to date are very complex and considerable more work will be required to purify and identify the biologically active compounds in the extracts. We will continue to address this objective as time and resources permit.

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.

Numerous soil characteristics including mineral content, nutrient availability, particle composition, and water retention capacity were determined at the DANR laboratory at UC Davis. Ring nematode populations were determined by Becky Westerdahl's laboratory, Nematology, UC Davis. The results for one orchard, for which full data was available at the time this report was written, are presented in **Table 5**.

Table 5
Soil and ring nematode sampling results from an orchard south of Marysville, California.

Transect Tree # (current status)	Ring Nematodes (per Kg soil)	Sand %	Silt %	.3ATM %
1 Alive	900	44	41	18.3
2 Alive	900	39	45	18.2
3 Alive	2100	40	45	18.5
4 Dead	**100**	**62**	**28**	**13.2**
5 Dead	**2100**	**69**	**21**	**10.6**
6 Dead	**100**	**67**	**24**	**11.0**
7 Dead	**250**	**78**	**14**	**8.8**
8 Dead	**1500**	**56**	**29**	**14.5**
9 Alive	100	40	39	18.8
10 Alive	100	22	45	23.6

Some soil characteristics such as phosphorus, potassium, nitrates, clay content and pH levels were not correlated with disease at this particular site (data not shown). However, several other soil factors were clearly associated with BC-induced tree mortality. When the percentage of sand in the soil was above approximately 50% tree mortality occurred. Also, when the percentage of silt falls below approximately 34% and the water retention capacity, as measured by the parameter labelled ".3 ATM" in Table 5, falls below approximately 16%, trees succumb to bacterial canker. It is interesting to note that when the soils become more sandy and the water retention capacity decreases, the likelihood of tree mortality greatly increases. It has been previously documented by scientists in France that trees grown in sandy soil have lower water content in the summer than trees grown in loam soils. These moderately drought-stressed trees then tend to " rebound" in winter and absorb more water during dormancy than trees grown in loam soils. We recently confirmed that prune branches with higher water content produced greater size BC lesions when experimentally inoculated with Pss (see Objective #1 of this report). The increased water content of dormant, stressed trees may facilitate the ingress and spread of *Pseudomonas syringae* pv. *syringae* through the cambium. These results suggest an obvious connection between soil water retention capacity, water content or partioning within the plant and the subsequent development of bacterial canker.

No obvious trend was observed between ring nematode numbers and tree death due to bacterial canker in the transect taken at this particular site. However, one important factor that probably influenced nematode numbers was only one year replants were present in tree sites that were marked as "Dead" in Table 6. Because the ring nematode prefers and multiplies better on stone fruit roots compared to other hosts, insufficient root densities of the replanted trees may have reduced nematode numbers in these "Dead" tree spaces. Five year old healthy trees would likely support higher nematode numbers than one year replants or sites without any trees. Because three of

the healthy trees growing in silty soils also had high ring nematode numbers, ring nematode alone was not the only factor predisposing these French prune trees to bacterial canker.

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 4 years we made substantial progress on all of the original project objectives and we have initiated, or will initiate, additional field trials on the effect of silicone-based surfactants which may expedite the delivery of bactericides into hydrophobic tree spaces and develop 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. This results indicates it is likely that other tree openings, such as bark lenticles, serve as more important sites for Pss infection than leaf scars. Experiments are now under way to test this hypothesis.

In 1998 there was no difference in the average amount of BC that occurred in several bactericide and fertilizer treatments. However, in one orchard with high disease pressure, much less tree mortality occurred in the copper plus fertilizer treatment than in the unsprayed control. Additional field evaluations of bactericides, including the use of silicone-based surfactants in combination with bactericides, and fertilizer amendments, will help determine the efficacy of these treatments.

The establishment and 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 and it has provided us with the necessary reference isolates 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 wood surfaces indicate that canker-inducing Pss populations are very low in these tissues. Isolation performed in 1998 found no evidence for the systemic movement of Pss in cambial tissues of French prune trees. Future isolations will focus on other natural openings, such as bark lenticles, to determine if canker-inducing Pss strains reside within these trees spaces. 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 2 years. Although more orchard weed isolations will be performed in 1999 to further clarify the potential role of weeds for providing BC inoculum, our results to date suggest weeds do not provide significant amounts of Pss inoculum. A PCR/hybridization test was developed which should greatly expedite the identification of plant pathogenic Pss from environmental samples. 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. Biochemical analyses of phenolic compounds from prune leaves identified chemical fractions that can either induce of suppress the expression of syringomycin toxin genes in Pss. Further characterization of the compounds that are responsible for eliciting or suppressing Pss toxin production may explain how diverse biological and environmental factors can predispose prune trees to BC. 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. Sandy orchard soils cannot retain water as well as loam soils and during the summer these trees tend to become moderately dought stressed at some times. In response to this stress, these trees tend to accumulate more water during the dormant period compared to trees grown in loam soils. Our experimental inoculation of Pss into high and low water content prune branches showed significantly greater size BC lesions occurred in high water content branches compared to normal water content branches. These preliminary results suggest that careful water management during the growing season may be an important factor for reducing losses to BC.