** Percentages are number with behavior per number surviving test.

CDFA Objective 2 (BCK): Evaluate plant micronutrients and antibiotics as potential bactericides for eliminating *Xylella fastidiosa* in grapevines.

Sub-Objective 2.1.
Determine *in vitro* growth inhibition of *Xylella fastidiosa* (*Xf*) by selected plant micronutrients and antibiotics.

MATERIALS AND METHODS

Reagent grades of metal salts were used to prepare 5 mM stock solutions, which were filter-sterilized and added to PD3 medium which is composed of tryptone, 4 g/L; soytone, 2 g/L; trisodium citrate, 1 g/L; disodium succinate, 1.0 g/L; hemin chloride, 0.01 g/L; potato starch (soluble), 2 g/L; $MgSO_4$ 7H2O, 1.0 g/L; K_2HPO_4, 1.5 g/L; KH_2PO_4, 1.0 g/L; and Noble Agar, 15 g/L (Davis et. al 1980). Stock solutions of 10 µg/ml were prepared for each antibiotic. The range of concentrations used was 0.0001 to 1 mM for zinc sulfate and cupric sulfate, 0.0006 to 5 mM for sodium tetraborate, ferric sulfate and manganese chloride. The concentrations of the antibiotics, tetracycline and streptomycin ranged from 0.01 to 256 µg/ml. The PD3 medium was supplemented with serial two-fold dilutions of each metal salt or antibiotic.

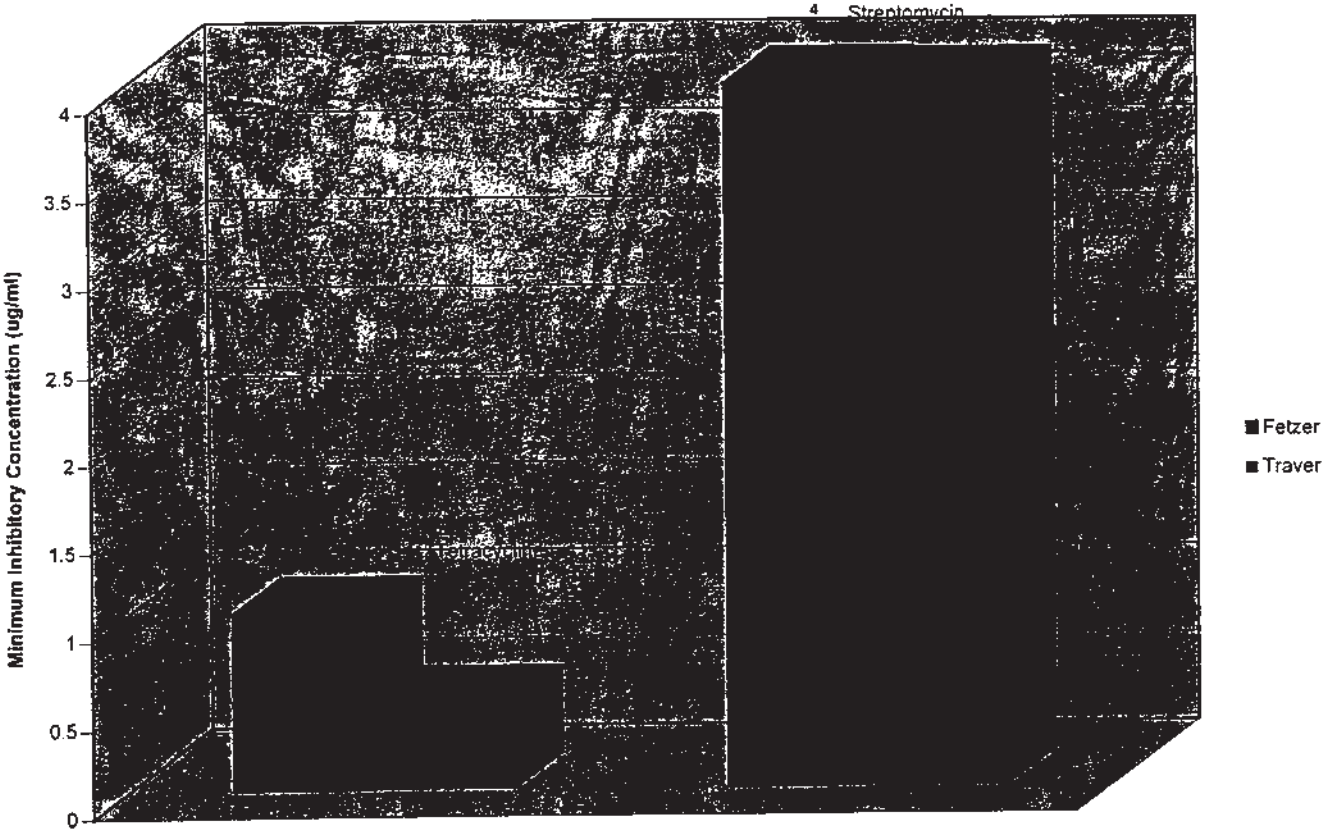
Two *Xf* strains isolated from PD-infected grapevines in CA were used in this study. These were the Fetzer strain (containing no plasmids) and the Traver strain (containing plasmids), kindly provided by Dr. A. H. Purcell, University of California, Berkeley. All bacterial cultures were grown in PD3 broth for 10 days on a shaker at 28°C. Each culture was diluted to A_{600} nm = 0.25 (ca. 10^8 colony forming units [cfu] per milliliter). The inocula were diluted to 10^5 cfu/ml before being applied (10, 10 µl droplets per plate) onto the metal salt or antibiotic supplemented plates. *Xf* inoculum was applied similarly to control plates containing no metal or antibiotic supplemented medium. Duplicate plates of each strain were incubated at 28°C for 10 days before growth was scored and the MIC determined. In a second experiment, each microelement and antibiotic was re-evaluated at two levels above and two levels below its previously determined MIC value. Final MICs were reported as the lowest concentration of each material that completely inhibited growth of both strains of *Xf* in the two experiments.

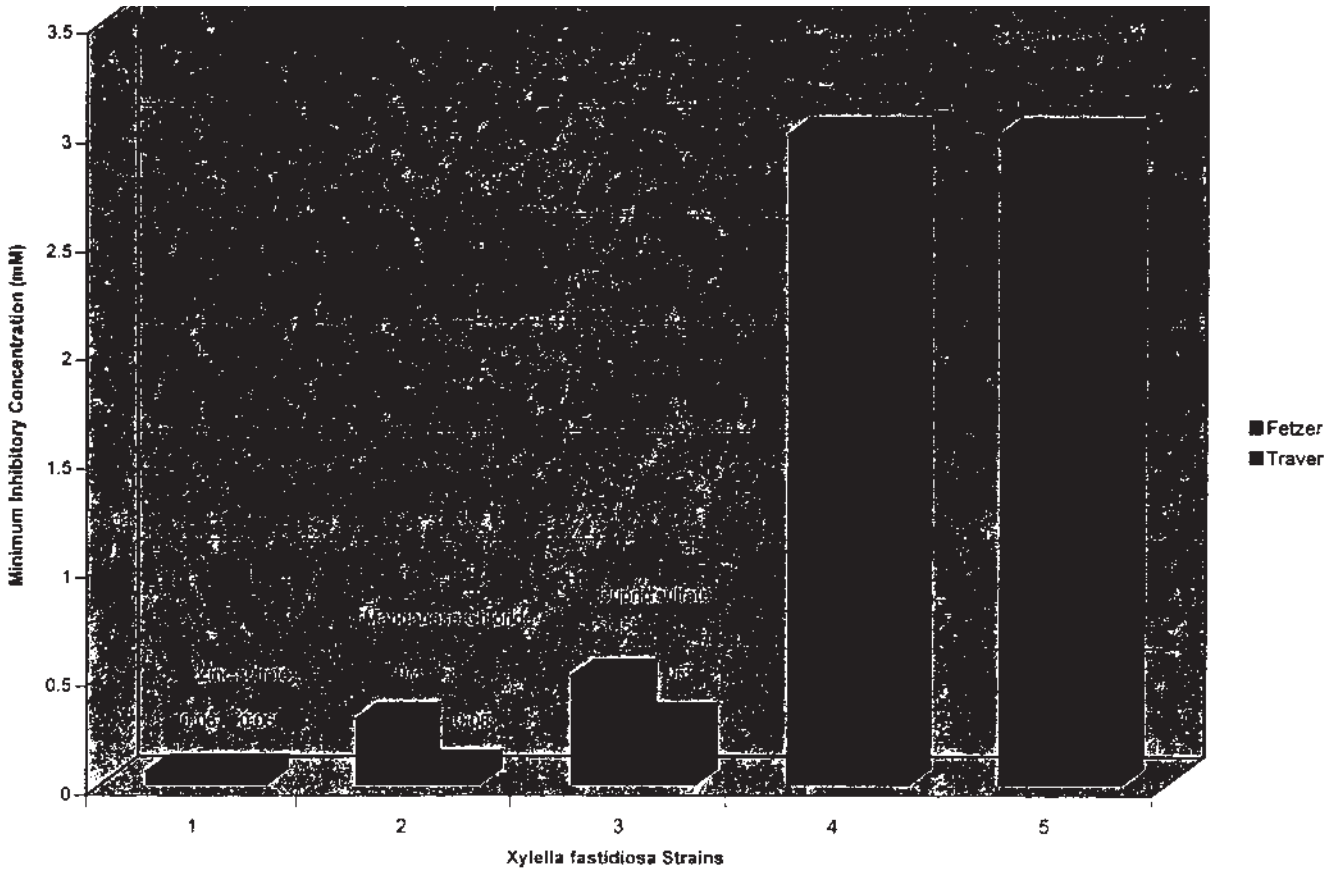
RESULTS

MICs that were determined following growth on PD3 medium supplemented with the 5 metallic plant micronutrients and the 2 antibiotics screened are presented in Figures 2.1 and 2.2. Both strains tested gave similar MIC results for zinc sulfate, ferric sulfate, sodium tetraborate and streptomycin. The MIC for cupric sulfate and tetracycline was two-fold lower for the Traver strain compared to the Fetzer strain and similarly, the MIC was four-fold lower for manganese chloride. Of all materials tested, tetracycline at 1 µg/ml and streptomycin at 4 µg/ml exhibited the lowest MIC values. Zinc sulfate had the lowest MIC value (0.06 mM) of the metal salts evaluated. Ferric sulfate and sodium tetraborate had the highest MIC values, both 3 mM. All materials evaluated had the same MIC values when re-tested, except for cupric sulfate which

exhibited an MIC value two-fold higher for the Fetzer strain than the Traver strain. In the first evaluation, cupric sulfate had an MIC value of 0.3 mM, and 0.5 mM when re-evaluated.

Using increased levels of plant micronutrients to reduce or prevent PD-symptoms would provide an alternative to the use of antibiotics. In this study, zinc sulfate was the most inhibitory material against *Xf* growth *in vitro*. The normal level of zinc, the most promising micronutrient examined in this study, in xylem exudate from grape is approximately 0.5 µm (Anderson and Brodbeck 1991). If this concentration could be increased by approximately 40-fold, this would provide an environment within the xylem that should be inhibitory to *Xf in vitro*. Agricultural grades of the plant micronutrients that were tested have already been approved for use on grapes in California and we are currently evaluating them as potential *Xf* bactericides *in planta*.





Sub-Objective 2:
Determine the efficacy of using plant micronutrients as soil drenches to cure established *Xf*- infections in potted Grapevines.

Rationale: It may be possible that soil applied micronutrients could be delivered through drip lines and be taken up by the roots of vines. If successful, this method of delivery could be easily implemented by most growers and would be the most economical method to deliver bactericides.

Materials and Methods:

Fifteen potted PD-infected 'Pinot noir' grapevines of similar size and disease severity were utilized in this experiment. Six treatments with 3 replications per treatment were evaluated. The treatments were zinc citrate (60.8 ml/ L), Nutra-Spray Zn 50 (9.6 g/L), Nutra-Spray Zn 25-Mn25 (19.2 g/L), Mantrac 500 (7.6 ml/ L) and Nutra-Phos Fe (14.4 g/L). All treatments were 4 times the recommended application rate for a micronutrient deficiency in grape (with the exception of zinc citrate). Potted vines were watered once per week for 9 weeks (9/99- 11/99) with 1 L of micronutrient solution. After 9 weeks of treatment, the vines were moved from the greenhouse into a screenhouse to allow the vines to progress into dormancy.

Results

All grapevines treated with zinc citrate died within 5 weeks of treatment. Two Zn 50 treated vines died and 1 vine of each of the Fe and Zn25-Mn25 treatments died, as well as one of the untreated controls. None of the Zn 50 or Zn25-Mn25 vines that survived treatment, tested positive for Xf by IC-PCR. One of the Fe treated vines was negative for Xf as well as one of the untreated controls. One of the Fe treated vines was positive for Xf and one of the untreated controls was also Xf positive. There was no obvious remission of symptoms in any of the grapevines however it was difficult to determine whether the foliar symptoms were due to Xf infection or excess mineral toxicity. One would further expect that old growth that had plugged and disfunctional xylem elements would still be incapable of supplying water to leaves and the previous PD-symptomatic leaves would remain symptomatic. The surviving test plants were cut back and placed in the screenhouse in order to undergo dormancy. We will test the new growth for Xf and observe these treated vines for Pd symptoms during the next spring and summer.

Results for Soil Drench Treatment of PD-Infected Pinot Noir

Treatment		**(+) for *Xf***
Zn citrate 4X		all died
Zn 50 4X	2 dead	0/1
Mn 4X		all died
Fe 4X	1 dead	1/2
ZnMn 4X	1 dead	0/2
Control	1 dead	1/2

Sub-Objective 3:

Determine the efficacy of using plant micronutrients as prophylactic soil drenches on healthy grapevines to prevent *Xf*- infection.

Rationale:

It may be possible that metallic microelements could be used prophylactically in healthy vines to prevent Xf infection following inoculation by infectious sharpshooter vectors. The numbers of Xf cells that are introduced by an infections vector are far less than the 10^8 cfu/gm of tissue in an established infections. It should also be noted that normal xylem transport functions are significantly reduced in diseased vines thus there may be greater difficulty in using bactericides to cure established infections than using them prophylactically to prevent infection.

Materials and Methods:

Sixty healthy potted 'Chardonnay' grapevines of similar size and appearance were utilized in this experiment. Fifteen treatments with four replications of each treatment were evaluated. The treatments were as follows:

	Application Rate		
	4X	8X	16X
Micronutrient			
Zn citrate	60.8 ml/ L	121.6 ml/ L	243.2 ml/ L

Nutra-Spray Zn25 Mn25	19.2 g/ L	38.4 g/ L	76.8 g/ L
Nutra- Spray Zn50	9.6 g/ L	19.2 g/ L	38.4 g/ L
Mantrac 500 (Mn)	7.6 ml/ L	15.2 ml/ L	30.4 ml/ L
Nutra-Phos Fe	14.4 g/ L	28.8 g/ L	57.6 g/ L

Treatments were 4, 8, and 16 times the recommended application rate for a micronutrient deficiency in grape (with the exception of zinc citrate). Potted vines were watered once per week for 6 weeks with 1 L of micronutrient solution. Following treatment the vines were placed in a screen house and allowed to progress into dormancy. In May, all vines were inoculated with *Xylella fastidiosa* strain 'Temecula' by a pin- prick method. Inoculum was suspended in SCP buffer and adjusted to 10^5 cfu/ml prior to inoculation. Vines were inoculated at 2 nodes with 20ul of inoculum. Vines were observed for symptom development and tested for the presence of *Xf* by IC-PCR.

Results:

All vines treated with zinc citrate died as well as the vines being treated with 16X Fe. Two vines treated with 16X Mn and 8X Mn also died. IC- PCR results are noted in Table 3.1.

Observation of the development or progression of disease symptoms was difficult for treated vines. Most vines showed some phytotoxicity due to the treatment which made it difficult to determine whether leaves were scorched because of PD or as a result of the high level of chemical accumulated in the pots of the grapevines. These type of experiments will be refined and repeated in the next year and we need to increase our percentage of vines that become infected following inoculation.

Table 3.1
Results of Chardonnay vines that were prophylactically treated with micronutrients then pin-prick inoculated with *Xylella fastidiosa*

Treatment		**(+) for *Xf***
Zn citrate 16X		all died
Zn citrate 8X		all died
Zn citrate 4X		all died
Manganese 16X	2 dead	0/2
Manganese 8X	2 dead	1/2
Manganese 4X		1/4
Zn50 16X		1/4
Zn50 8X		1/4
Zn 50 4X		1/4
ZnMn 16X		0/4
ZnMn 8X		3/4
ZnMn 4X		0/4
Fe 16X		all died
Fe 8X		1/4

Fe 4X		2/4
Control (No treatment)		1/4

Total Inoculated= 44
% infected~27%

Sub-Objective 4: Determine the efficacy of using micronutrients and antibiotics to cure Xf-infection in field-grown grapevines.

Background:

In the original proposal we were going to evaluate all of our bactericides and methods of delivery using potted or UC Davis grown vines before setting up plots in the field. However, because of the PD epidemic in Temecula we felt it was imperative to immediately begin field evaluations in order to access whether any of these materials and delivery mechanisms could be used by growers. This has resulted in our testing many more materials and various delivery technologies than we had originally proposed.

Materials and Methods:

Vineyard trials were established in the fall of 1999 in one site in Napa and three sites in Temecula to evaluate the effect of micronutrient treatments on *Xf*- infected grapevines. Prior to applying the treatments, all vines in the four vineyards were mapped for location and level of disease using the 0 (no disease) to 4 (near dead) scale that Sandy Purcell has used for years. Several methods of delivering micronutrient and antibiotics are being evaluated in the field trials. The methods under evaluation for 1999-00 include foliar sprays, plastic "DP" screws (developed and provided by Dick Peterson, Napa, CA), a "drilling/ injection" method which utilizes DP screws to seal the ends of a hole drilled through the grapevine, and soil application of zinc sulfate. Foliar spray treatments were applied in two concentrations, 4 and 8 times the recommended application rate for a micronutrient deficiency in grape and tetracycline foliar spray rates were 4X the concentration used for fireblight control. Soil treatments were also applied in two concentrations, 2lbs/ vine and 4lbs/ vine. The DP screw and drilling/injection treatments were applied at one application rate. In Napa, all treatments were applied in one 8 year old Merlot vineyard. A 6 year old Merlot vineyard in Temecula received all treatments as well. Because the variation in age and size of Chardonnay vines in Temecula did not allow for the application of all treatments in one vineyard, a second vineyard of 6-8 year old Chardonnay was used for the drill/injection treatment. Recently infected, two-year old Chardonnay vines in another vineyard received plastic DP screws, foliar sprays and soil treatments (the small diameter of the trunk did not allow for injection of materials). Non-treated vines of similar age and disease severity were flagged and mapped at all locations. A new Merlot therapy plot was also established in Napa in the fall of 2000 with the assistance of Ed Weber, Farm Advisor, Napa County.

Preparation and Application of Plastic DP Screws.

For all solid materials, 4g of metallic micronutrient was added to 4 g of sterile distilled water and mixed well. Eighty small cotton pellets were placed into the solution and allowed to soak up the micronutrient solution resulting in approx. 0.05 g micronutrient/ cotton pellet. Using forceps, 2 cotton pellets were inserted into the hollow plastic screw, resulting in approx. 0.1 g micronutrient/ screw. For liquid materials, 80 cotton pellets were allowed to absorb 4ml of micronutrient solution. Using forceps, 3 cotton pellets were inserted into the hollow plastic screw, resulting in approx. 0.15ml micronutrient/ screw. For the screw treatments, an 11/64 drill was used to make 2 shallow holes in each side of the trunk approximately 3 inches above the graft union. The screws were then fitted into the drilled holes.

Preparation and Application of Injected Micronutrient

The plunger was removed from a 10 ml syringe and the dispensing end of the barrel was covered with parafilm. Ten grams of each micronutrient was mixed with 15 ml of sterile distilled water. 15 ml of 2% agarose was added to the mixture and quickly poured into the syringe barrel. The mixture was allowed to gel before removing the parafilm from the end of the barrel and replacing the plunger. For tetracycline and streptomycin the agarose gel was cooled to 50 C before adding. For the injection/drilling treatment, an 11/64 hole was drilled all the way through the trunk of the vine. The resulting hole was immediately filled with the bactericide/agarose mixture using the syringe. Plastic DP screws were used to close up the holes on either side of the vine.

In February 2000 all vines were severely pruned. If only half of the vine was diseased then only that cordon was removed. If both sides of the vine were diseased, then the entire main trunk of the vine was cut off 2 or 3" above the graft union. A second application of all materials was made in May 2000 and a third application in September/October of 2000. The vines were evaluated visually for disease development and severity.

Results

Average disease ratings in each vineyard of treated vines as well as the number of vines that were symptomless and dead are presented in Table 4.1.

Of the treated 2 year old Chardonnay the lowest average disease rating of the foliar sprays was 1.6 compared to the untreated control at 2.3. The tetracycline spray treatment was the only foliar spray treatment that resulted in an average rating lower than that of the untreated control vines. In the same vineyard of young Chardonnay vines the lowest average disease rating using the DP screws was 0.9 for the Cu hydroxide treated vines, compared to 2.3 for the untreated control vines. It is likely that established *Xf* infections in young Chardonnay vineyards (characterized as a highly susceptible variety) cannot be treated using antibiotics and micronutrients. No further data will be collected in this vineyard, as it was discovered that 80% of the treated vines were removed by the vineyard manager this past fall.

In the 6 year old Merlot vineyard in Napa, three of the foliar spray treatments, tetracycline, Zn amino acid and Zn25Mn25 4X, resulted in average disease ratings of 0.25 when compared to the untreated controls at 1.4. In the same Napa vineyard, using DP screws, Zn 50 treated vines resulted in the lowest average disease rating, 0.6 compared to the untreated controls at 2.3. Using the drill through/ DP screw method average disease ratings in Napa Merlot were

relatively low compared to the other methods evaluated. The lowest average disease rating was for vines treated with streptomycin at 0.6. Vines treated with Mn carbonate and Cu hydroxide were also relatively low, 0.8, when compared to untreated controls at 2.3. In 6 year old Chardonnay vines in Temecula treated using only the drill through/ DP screw method, Cu hydroxide treated vines had the lowest disease rating at 1.3 compared to untreated controls at 2.8 (very severe). These initial results suggest that it may be possible to cause remission of PD-symptoms in more established vineyards (6-8 years of age) and more likely in a vineyard of an intermediately susceptible variety, like Merlot, rather than one that is highly susceptible, like Chardonnay. It is important to note that the effect of severe pruning can yield promising results one year after pruning (A.H. Purcell, personal communication); so the efficacy of the treatments that combine bactericides with severe pruning will need to be fully evaluated in Summer, 2001.

Table 4.1 Results of therapeutic bactericide application <u>one</u> year after application.

2 year old Chardonnay vines – Temecula
I. FOLIAR SPRAYS

Treatment	Number of vines	Average Disease Rating	Number vines Symptom-less	Number vines Dead
Tetracycline	8	**1.6**	0	0
Zn - amino acid - 4X	4	3.0	0	2
Zn - amino acid - 8X	4	2.5	0	1
Mn carbonate - 4X	4	2.8	0	0
Mn carbonate - 8X	4	2.8	0	0
Zn25Mn25 - 4X	4	3.0	0	0
Zn25Mn25 - 8X	4	2.3	0	1
Mn - amino acid -4X	4	3.0	2	0
Mn - amino acid -8X	4	2.0	1	1
Untreated Control	**8**	**2.3**	**2**	**3**

II. Dick Petersen's Nylon (DP) Screws:

Tetracycline	8	2.6	1	3
Streptomycin	8	2.4	1	2
Zn50	8	2.0	1	2
Manganese carbonate	8	2.4	2	3

Cu hydroxide/Kocide	**8**	**0.9**	**4**	**0**
Fe sulfate	8	3.1	0	3

NAPA, 6 year old Merlot
I. FOLIAR SPRAYS

Treatment	Number of vines	Average Disease Rating	Number vines Symptom-less	Number vines Dead
Tetracycline	**8**	**0.25**	**7**	**0**
Zn50 - 4X	4	1.0	3	1
Zn50 - 8X	4	1.8	1	0
Zn amino acid - 4X	4	1.3	2	1
Zn amino acid - 8X	**4**	**0.25**	**3**	**0**
Mn carbonate - 4X	4	0.6	3	0
Mn carbonate - 8X	4	1.1	1	0
Zn25Mn25 - 4X	**4**	**0.25**	**3**	**0**
Zn25Mn25 - 8X	4	1.4	0	0
Fe sulfate - 4X	4	1.0	2	0
Fe sulfate - 8X	4	1.3	2	0
Untreated Controls	**8**	**1.4**	**3**	**1**

II. DP screws

Tetracycline	8	1.1	5	1
Streptomycin	8	1.7	2	1
Zn50	**8**	**0.6**	**7**	**0**
Manganese carbonate	8	1.4	3	1

Cu hydroxid/Kocide	8	1.0	3	0
Fe sulfate	8	0.9	4	1
Untreated Control	**8**	**2.3**	**2**	**3**

Napa Valley, 6 year old Merlot
Drill through, inject and seal with DP screws

Treatment	Number of vines	Average Disease Rating	Number vines Symptom-less	Number vines Dead
Tetracycline	8	1.9	0	0
Streptomycin	**8**	**0.6**	**5**	**0**
Zn50	8	1.1	5	1
Manganese carbonate	8	0.8	4	0
Cu hydroxid/Kocide	8	0.8	5	1
Fe sulfate	8	1.1	3	1
Untreated Control	**8**	**2.3**	**2**	**3**

Temecula, 6 year old Chardonnay vines
Drill through + PD screws

Treatment	Number of vines	Average Disease Rating	Number vines Symptom-less	Number vines Dead
Tetracycline	4	2.3	0	2
Streptomycin	4	2.8	1	2
Zn50	4	2.3	1	2
Manganese carbonate	4	2.8	0	3
Cu hydroxid/Kocide	**4**	**1.3**	**2**	**1**
Fe sulfate	4	2.8	0	3
Untreated Control	**4**	**2.8**	**1**	**3**

Sub-Objective 5: Determine efficacy of using plant micronutrients prophylactically to prevent *Xf*- infection in established vineyards.

Materials and Methods:

Trials were established in 5 vineyard sites in Napa in high disease pressure areas (located next to riparian areas with the assistance of Ed Weber, Farm Advisor, Napa County) in the Spring of 2000. The study includes 4-6 year old vines of Chardonnay, Merlot and White Reisling. Six foliar spray treatments and 1 soil treatment (2lbs/vine) with between 10-25 replications per vineyard were included in the study. Materials being evaluated included, systemic acquired resistance (SAR) inducuers: i. ActiGuard (DuPont) ii. Resist (Stoller Chemical), Messenger (harpin protein, Eden BioSciences). Plant micronutrient elements included Zn50 (Leffingwell), ZnMn (Leffingwell) and Mn carbonate (Leffingwell) and Mn-, Zn, and Cu-amino acid cheleates (Albion Labs, Orem, Utah). Trials were also established in Temecula in a 3-4 year old Cabernet vineyard in a high disease pressure area (located next to a citrus grove). Foliar spray treatments and number of replications were the same at each site. Vines received a second application of materials in the Fall of 2000. In the Fall of 2000, an additional prophylactic trial was established in a 2 year old Syrah vineyard. Vines were evaluated in the fall for disease development. Unfortunately, in the fall of 2000, the majority of the Temecula Cabernet prophylactic plot had been removed by the vineyard manager, making it impossible to gathered useful data from this plot.

Sub-Objective 6: Determine whether foliar and/or other treatments of grapevines actually results in increased levels of micronutrients in grapevine xylem sap.

Materials and Methods:

In September, 2000, two healthy Cabernet and 2 Thompson Seedless grapevines in Davis were treated with each of the prophylactic and therapeutic microelement treatments and delivery methods that were currently being used in established vineyards in Napa and Temecula to cure or prevent Xf infections. Foliar sprays, DP screws, and the drill through/ DP screws were used to deliver treatments to the vines. Grapevines were sampled at 24 and 48 hrs. after treatment as well as 11 days after treatment. Xylem sap was expressed from 2-3, two foot canes from each of the treated vines as well as from untreated controls using a custom made pressure bomb (PMS Instruments). Xylem sap was frozen at –20 degrees until analyzed for microelement concentrations by Dr. Peter Anderson, Quincy, FL.

Results:

Detailed results of this study are presented in Table 6.1. The manganese amino acid treatment had the highest increase in the manganese-treated vines at both 24 and 48 hrs. At 24 hrs., the manganese levels in the treated vines were an average of 680 uM when compared to 11.3uM in the untreated controls. At 48 hrs after treatment, the average levels of manganese were 507 uM in the treated vines and 22.2 uM in the untreated vines. This foliar spray raises the level of manganese in excess of 200-300 uM higher than the minimum inhibitory concentration (MIC) for Xf manganese toxicity as determined by *in vitro* studies (see Sub-Objective 1). Eleven days following treatment the manganese level dropped dramatically for manganese amino acid treated vines, whereas the levels for other manganese treatments (Manganese carbonate 4X and 8X) increased between the time the vines were sampled at 48 hrs and then

again at 11 days. Although the levels of manganese for these other, non-amino acid chelated, treatments at the chosen sampling times does not reach the level necessary to inhibit the growth of Xf *in vitro* it may be possible that the manganese in these treatments becomes increasingly available in the vine over a longer period of time. In the zinc treatments, several of the treatments at different intervals resulted in concentrations of zinc that far exceeded the *in vitro* MIC of zinc, 60 uM. At 24 hrs. and still at 48 hrs. the zinc amino acid treatments were more than 4 times the MIC of zinc. With the zinc sulfate 4X and 8X treatments the concentrations of zinc increased dramatically from the time the vines were sampled at 48 hrs to the time the vines were sampled at 11 days following application of treatments (zinc sulfate 4X concentration 140; zinc sulfate 8X concentration 98.6, which were above the MIC of zinc).
None of the foliar zinc/manganese microelement treatment or the copper amino acid cheleate treatments resulted in levels of microelements that exceeded the MIC of that microelement *in vitro*, but compared to untreated vines, the copper amino acid treatment still resulted in a significant increase in levels of copper.

With all of the DP screw and Drill/Injection treatments levels of microelement in the xylem sap did not increase nearly as significantly as in some of the foliar treatments. It is possible, however, that these treatments take longer than 11 days to become available for uptake in the xylem.

Future Direction:

A similar study will be done in the Spring of 2001, to compare the uptake and retention of the microelements studied in this experiment. The major question that remains is whether the Zn- or Mn-amino cheleates actually provide enough free Mn or Zn to be toxic to Xf within xylelem elelements or whehther the ions are too tightly bound by the amino acid chelate. This would be very important in determining whether new infections during the spring could potentially be eliminated soon after inoculation of the vine by a sharpshooter vector. Sampling times will also be extended beyond 11 days to possibly 20 or more days following treatment.

Average Plant Microelement Concentrations in Xylem Sap Following Foliar Application Cabernet Sauvignon and Thompson Seedless

				MIC, *in vitro*	
	uM			**uM**	
	24hrs.	**48hrs.**	**11 days**		
Manganese amino acid	**680**	**507**	6.2	**300**	
Manganese carbonate 4X	6.2	9.6	11.3		
Manganese carbonate 8X	18.3	13	21.9		
Mn concentration untreated control	11.3	22.2	13.3		
Zinc amino acid	**327**	**322**	17	**60**	
Zinc sulfate 4X	16	18.6	140		
Zinc sulfate 8X	42.7	21.8	98.6		
Zn concentration untreated control	17.4	7.6	7		
Cu amino acid	35.1	24.1	2.7	**500**	
Cu concentration untreated	2	3.7	0.67		
Zinc/Mn 4X (Zinc concentration)	18.7	30.8	15.3	**60**	

Zinc/Mn 8X (Zinc Concentration)	19.6	20.7	16.2		
Zinc/Mn Untreated Control (Zn Concentration)	17.4	7.6	7		
Zinc/Mn 4X (Mn concentration)	7.6	18	15.8	60	
Zinc/Mn 8X (Mn Concentration)	10.6	10.4	8.8		
Zinc/Mn Untreated Control (Mn Concentration)	11.3	22.2	13.3		

Plant Microelement Concentrations in Xylem Sap Follwing DP Screw and Drill/Injection Cabernet Sauvignon and Thompson Seedless

	uM			MIC, in vitro uM
	24hrs	48hrs	11 days	
Manganese carbonate DP Screw	6.5	3.3	3.9	300
Mn concentration untreated	3	3.2	3.6	
Zinc sulfate DP Screw	8.6	3.9	3.7	60
Zinc sulfate Drill/Injection	3.5	2.6	2.4	
Zn concentration untreated	3.3	3.1	2.4	
Copper hydroxide DP Screw	0.67	0.59	0.59	500
Copper hydroxide Drill/Injection	0.94	1	0.9	
Cu concentration untreated	0.51	0.55	0.47	
Zn/Mn Drill/Injection (Zn concentration	16.2	2.4	1.53	60
Zn concentration untreated	3.3	3.1	2	
Zn/Mn Drill/Injection (Mn concentration	8.4	3.6	2.9	300
Mn concentration untreated	3	3.2	3.3	