Developing an Integrated Pest Management Solution for Pierce's Disease Spread By the Glassy Winged Sharpshooter in Temecula, California

A Final Report Submitted to the
American Vineyard Foundation

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Project Overview and Organization of the Document

Funding for this research was granted with the intent of "jump-starting" necessary research into the control and management of Pierce's disease and it major insect vector, the glassy-winged sharpshooter (GWSS), in Riverside county. The objectives of this research presented here fall along five independent major lines of inquiry, each of which is an integral component to an integrated pest management approach. Consequently, this document, rather than summarizing a single research project, will summarize the objectives and accomplishments of each of the major lines of inquiry. The major lines of inquiry on which this umbrella program was focused were

(1) Population monitoring of glassy-winged sharpshooters, sharpshooter parasitoids, and of the disease organism responsible for Pierce's disease, *Xylella fastidiosa.*

(2) The development of biological control for glassy-winged sharpshooter.

(3) The development and evaluation of insecticides to control sharpshooters and limit the spread of Pierce's disease.

(4) Develop a physical control technique (large screen barriers) for reducing movement of sharpshooters from citrus to vineyards.

(5) The development and evaluation of chemotherapeutic materials applied to diseased grapevines with the intent of curing them of Pierce's disease.

This document is organized along the above lines of inquiry. Below, the reader will find individual sections detailing the research summaries, objectives, methods, results and accomplishments of each line of inquiry. The research scientists associated with each line of inquiry are also indicated, as is their responsibility to the overall project.

Inquiry 1

Population Monitoring of Glassy-winged Sharpshooters, Sharpshooter Parasitoids, and the Disease Organism Responsible for Pierce's disease, *Xylella fastidiosa.*

Principle Investigators: R. A. Redak, M. J. Blua, and N. Toscano, M. S. Hoddle, H. C. Costa and D. Cooksey.

1.1 Summary

Sharpshooter Monitoring: Prior to the start of this portion of the project, CDFA initiated their own sharpshooter and monitoring program covering the entire Temecula valley emphasizing vineyards and citrus orchards. We consequently adjusted our monitoring efforts to focus upon those areas that the CDFA project was not addressing -- non-agricultural areas of the Temecula valley. Both the CDFA project and this one were tightly integrated to maximize the amount of information gathered. Here we report on the monitoring efforts in non-agricultural areas with references to the CDFA project. We have attached the CDFA project final report as Appendix I to this report.

Monitoring of non-agricultural areas demonstrated that glassy-winged sharpshooters occur at significant densities within the landscaped, riparian, and natural areas surrounding and interspersed within the agricultural areas of the Temecula valley. Although sharpshooter densities were not as high in non-agricultural areas as those found in citrus, non-agricultural areas support healthy and viable populations of sharpshooters. As it will be both politically and biologically impossible to eradicate sharpshooters from these non-agricultural areas associated with the Temecula vineyards, these areas will have to be considered to be permanent and constant sources of glassy-winged sharpshooters. Consequently, the management of vineyards in the Temecula valley must always take into consideration the potential for the rapid introduction and spread of Pierce's disease.

Parasitoid Monitoring: Insecticide treatments of citrus had a dramatic and significant effect on parasitoids of glassy-winged sharpshooters in the Temecula region. With insecticide treatments few sharpshooter eggs were found in treated areas and only a minute proportion of these were parasitized. In untreated organic citrus, sharpshooter populations were high during the first half of the year, resulting in a strong build up of natural enemies. At the beginning of April, 2000, glassy-winged sharpshooter parasitoids were recorded in 30% of all sharpshooter eggs collected. By June the parasitism rate had risen to 90%. Parasitism rates continued in excess of 90% for the remainder of the year. The primary parasitoid encountered was *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae). The remaining parasitism was accounted for by *G. morrilli*.

Disease Monitoring: Results from the first six months of sampling have yielded positive detection of *X. fastidiosa* in grapes at many sites throughout the valley. This was expected. *Xylella fastidiosa* was also consistently detected in almond and oleander in the Temecula region. We assume that the oleanders are infected with the oleander strain of the pathogen which reportedly does not infect grape. Three other plant species (coyote brush, mustard, and elderberry) showed weak positive results for the presence of *X. fastidiosa* when tested by ELISA; however, neither culturing or PCR techniques supported these results. This suggests the ELISA results may be false positives and these these species will be tested with improved methods in this coming season. We expect that more plants will yield positive results as we

begin to routinely use the more sensitive immunocapture PCR method. Thus far, no obvious source of inoculum of PD, other than grape and almond, was detected in the valley using these methods.

1.2 Objectives

a. Determine the distribution of the glassy winged sharpshooter within and among the major cropping systems in the Temecula Valley (with the development of the CDFA project this objective was modified and focused upon non-agricultural areas).

b. Determine the distribution of the sharpshooter egg parasitoids, (*Gonatocerus sp.),* within and among the major cropping systems of the Temecula Valley.

c. Determine the temporal and spatial occurrence of the Pierce's disease bacterium in the Temecula Valley in wild and cultivated hosts and in the GWSS**.**

1.3 Methods

Monitoring Glassy winged sharpshooters (Blua, Redak, and Toscano): Glassy-winged sharpshooters were monitored at 45 locations throughout the Temecula valley with yellow sticky cards (double-sided, 11.5cm x 17.5cm, Trécé Inc., Salinas CA) positioned within the following habitat types (1) ornamental landscapes (2) riparian areas and (3) areas of native vegetation. Insect populations were monitored semimonthly through the year. Differences between habitat types were analyzed with repeated measures analysis of variance with habitat type being considered as the main effect treatment. Sharpshooter densities in non-agricultural areas were compared subsequently with sharpshooter densities from agricultural areas (determined from the CDFA project) to determine the overall sources of these insects in the Temecula region.

Monitoring sharpshooter egg parasitoids (Hoddle)*:* Thirty sites were selected in Temecula so as to represent two cropping systems (citrus: n=20; grapevines: n=10; Figure 1). The citrus sites were selected to include untreated (organic) locations (n=6), locations treated with imidacloprid (n=6) and locations treated with imidacloprid and chlorpyrifos (n=7). All grapevine monitoring sites were located close to citrus.

Every week from April 1 until October 1, 2000, each site was visited. Plants were searched for a minimum of 10 man-minutes. All fresh egg masses were collected and placed in a zip-loc bag. The leaves were incubated at 26°C for two weeks then placed in a freezer to kill all material. The number of egg clutches, the number of eggs in each clutch and the proportion parasitized, unparasitized, and otherwise damaged were recorded. The parasitoids were identified to species and counted, and the number of nymphs in each bag was recorded.

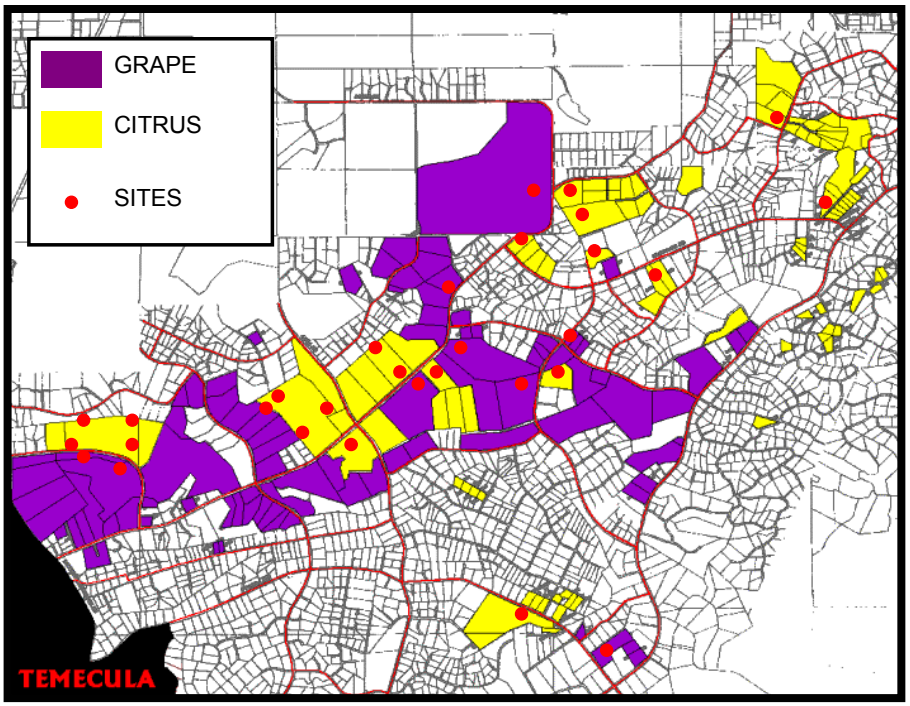

Figure 1. Monitoring sites for parasitoids of glassy-winged sharpshooter in the vineyards and citrus of the Temecula region.

Monitoring X. fastidiosa (Costa and Cooksey)*:* Ten sites in the Temecula area were selected for monitoring populations of *X. fastidiosa* in grapes, citrus, and many other cultivated and native plants (see Table 1). Sites were selected to represent a broad range of combinations of grapes and different plant types. In several sites, grape and citrus are in close proximity. Three sites also include almonds, a known host of the Pierce's disease bacterium. Other sites include a variety of other cultivated fruit tree crops, landscape ornamentals, and native plant species.

Weekly sampling was conducted from May through November 2000, and now continues through the winter at monthly intervals. Each week from May through November, five of the 10 sites were sampled, with the other five sites sampled in the alternate week. Approximately 125-150 samples were processed each week in our laboratories at UCR. Samples were tested in three ways: (1) Media Culture: Samples are macerated in buffer and plated on selective media for *X. fastidiosa*; (2) ELISA, a serological test that can detect the presence of *Xylella* in plant tissue; and (3) DNA-based (PCR) detection. All samples from plants that tested positive by culture or ELISA were also tested by PCR. Some samples that tested negative by culture and ELISA are still in the process of being tested by PCR and the results are not reported here.

1.4 Results and Accomplishments to Date

Monitoring Glassy winged sharpshooters (Blua, Redak, and Toscano):

Non-agricultural areas of the Temecula valley are and will be a significant source of glassy-winged sharpshooters. We found significant densities of sharpshooters in all three habitat types sampled: natural vegetation, ornamental landscapes, and riparian areas (arroyo washes, drainage

creeks, etc). In all three habitat types, the seasonal distribution of sharpshooter density was similar to that of previous year's (Blua et. al. 1999); adult densities peaked in July and in mid-fall (Figure 2). Among these three habitat types sampled, sharpshooter densities were highest within the natural vegetation and riparian areas. Ornamental landscapes supported the fewest sharpshooters and as a habitat type was significantly lower than the other two habitat types (which were not significantly different from each other).

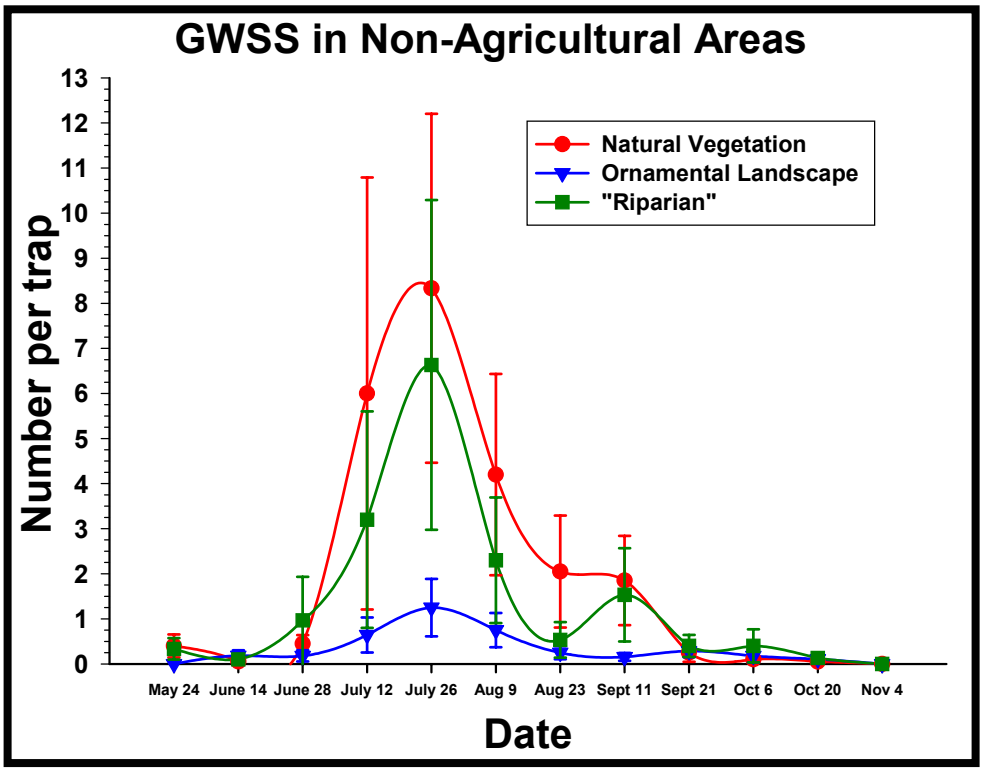

Figure 2. Glassy-winged sharpshooters occurring in non-agricultural areas of the Temecula valley. Data were taken from mid-May through early November. Values shown are mean densities (n=15); vertical bars represent 1 standard error.

Our data from non-agricultural areas were then transferred to the Temecula region-wide monitoring project to better assess the efficacy of the experimental treatments applied to citrus with regard to controlling sharpshooter. We have attached the Annual Report of that project as Appendix 1 and summarize the research highlights of that project below.

Highlights of the Temecula Region-wide Monitoring Program (Toscano and Redak):
The purpose of the Temecula Region-wide monitoring program was to (1) assess the efficacy of experimental pesticide treatments applied to citrus in an effort and (2) provide an estimate of the nature and extent of the sharpshooter distribution within the Temecula area. The reader is referred to Appendix 1 for detailed methodology results.

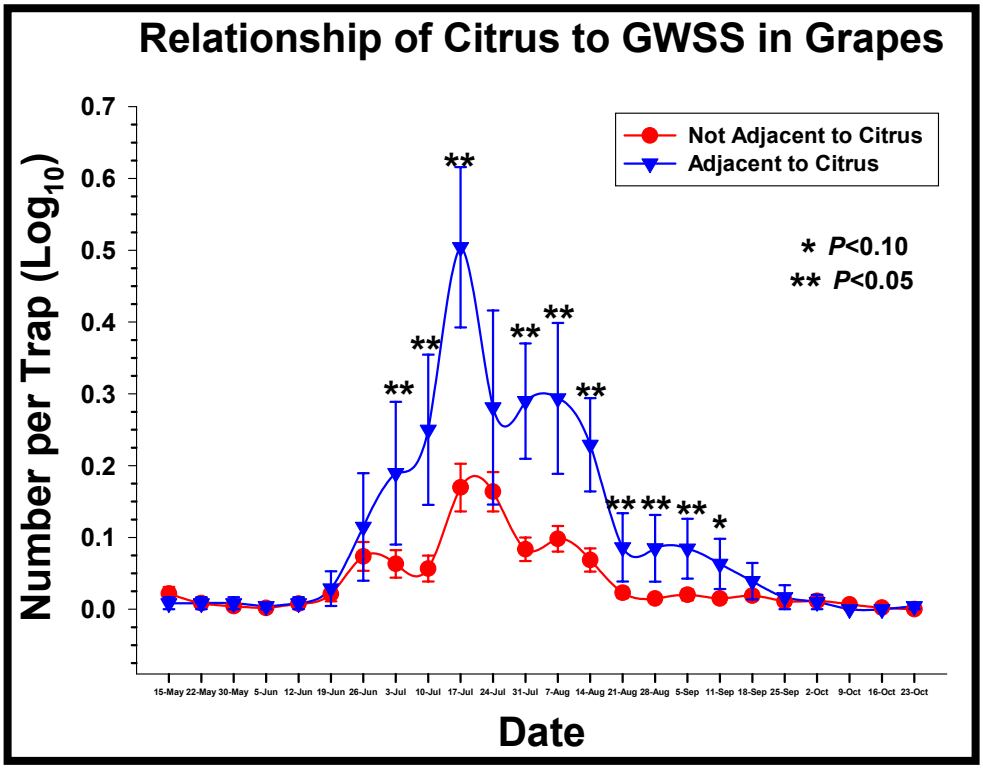

Figure 3. Glassy-winged sharpshooters occurring in grape vineyards adjacent to citrus or not adjacent to citrus in the Temecula valley. Data were taken from mid-May through early late October. Values shown are means; vertical bars represent 1 standard error.

The monitoring data for the year 2000 verified our results from last year; citrus is a major source of sharpshooters. Vineyards adjacent to citrus experiences significantly greater sharpshooter densities than vineyards with little physical association with vineyards (Figure 3). As with 1999, sharpshooter adult populations peak in mid summer.

Overall the application of pesticides had a moderate, but significant effect on the numbers of sharpshooters found in citrus (Figure 4). This effect was most pronounced in mid-summer for plots receiving both Admire and Admire + Lorsban. Overall, however, there were no differences in the insecticide treatments with respect to their level of control.

While the treatment of citrus had a minimal to moderate effect on sharpshooter densities within citrus, the insecticidal treatment of citrus had a strong and significant effect on sharpshooter densities found within vineyards adjacent to citrus. Vineyards adjacent to untreated citrus experienced significantly greater numbers of sharpshooters than vineyards adjacent to citrus that was treated with insecticides (Figure 5). Overall the treatment of citrus resulted in sharpshooter densities in adjacent vineyards to approximate densities found in vineyards with no association with citrus. It should be noted that while the treatment of citrus was a success in terms of reducing sharpshooter numbers in vineyards adjacent to citrus, the resulting numbers (which are similar to vineyards not associated with citrus) are still a significant vector source for Pierce's disease.

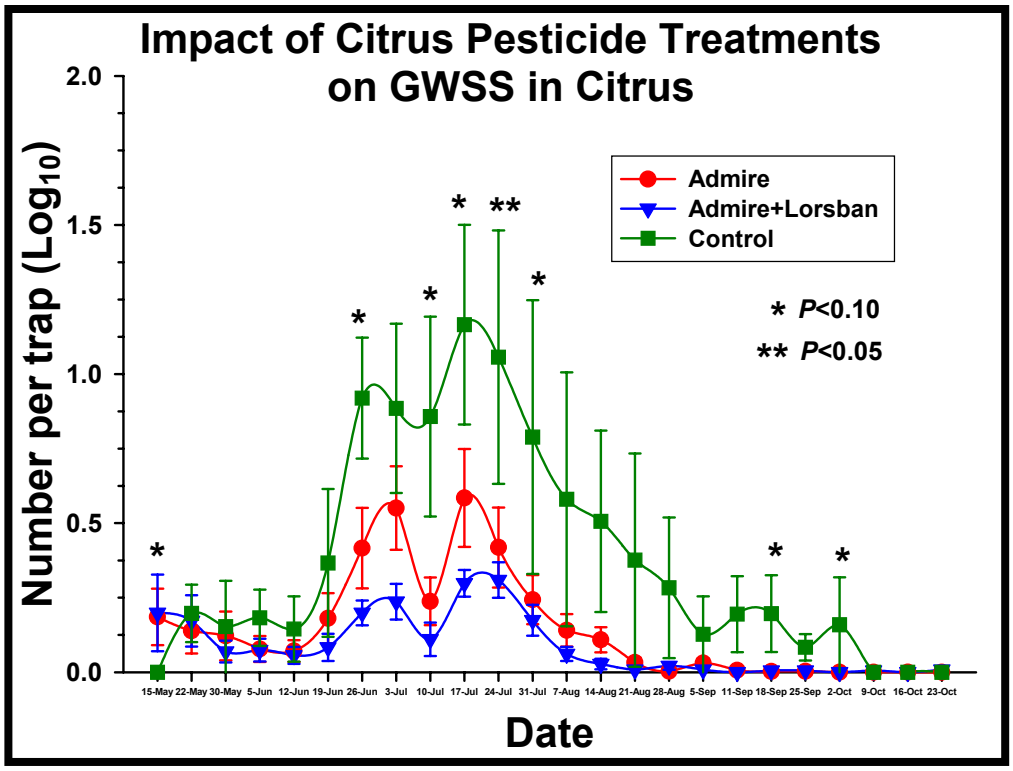

Figure 4. Glassy-winged sharpshooters occurring treated (either Admire or Lorsban) and un-treated citrus orchards in the Temecula valley. Data were taken from mid-May through early late October. Values shown are means; vertical bars represent 1 standard error.

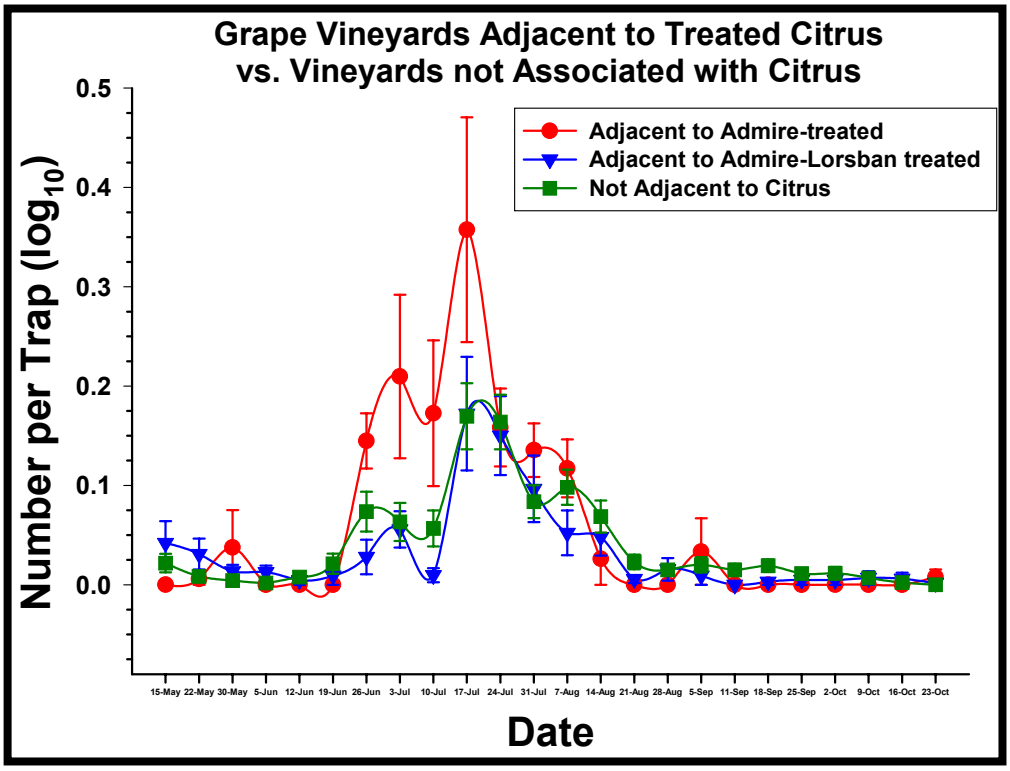

Figure 5. Glassy-winged sharpshooters occurring in vineyards either adjacent to treated- or untreated-citrus. Values shown are means; vertical bars represent 1 standard error.

Monitoring sharpshooter egg parasitoids (Hoddle)*:*

Dramatic differences in oviposition rates were observed when comparing citrus treated with insecticides vs. untreated citrus (Figure 6). In insecticide treated sites, oviposition rates declined soon after treatment and oviposition rates remained low throughout the growing season. No differences were found in egg production rates between the two different insecticide treatments.

Egg production came in two batches: March-May and July-August (Figure 6). By the time of the second egg production period, insecticide treatments had reduced adult population levels to such an extent that egg production in these areas was negligible after June. In untreated sites, the number of egg masses was significant, however the vast majority of egg masses failed to hatch as nymphs. Mortality of egg masses collected in insecticide treated areas was considerably lower (Figure 7).

When causes of sharpshooter egg mortality at organic sites were investigated further, the most important effect was parasitism (Figure 8). *Gonatocerus ashmeadi* parasitism was responsible for approximately 70% of egg mortality from May onwards while *Gonatocerus morrilli* was responsible for a further 13% over the same time period.

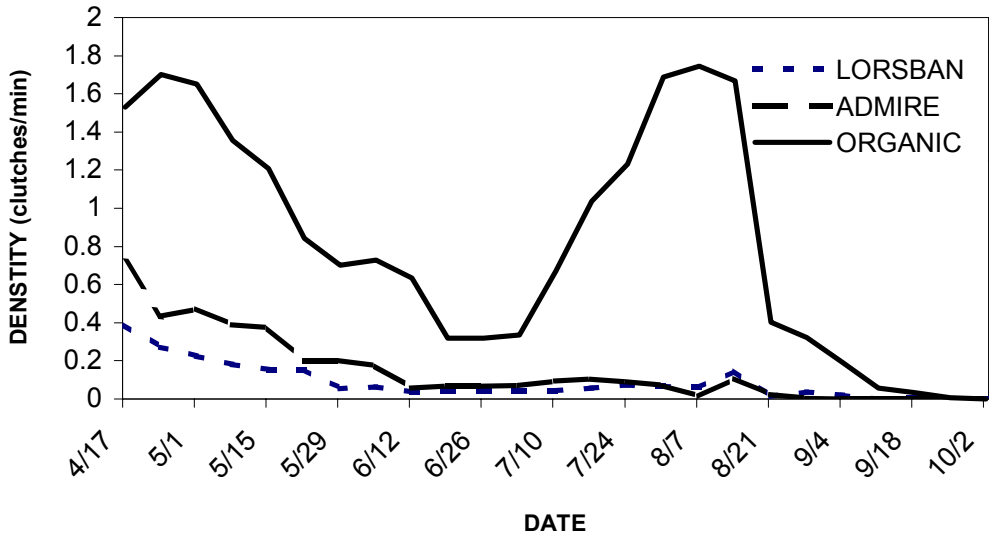

Figure 6. Egg mass density in untreated (="ORGANIC") sites, sites treated with Admire®, and sites treated with Admire® and Lorsban®.

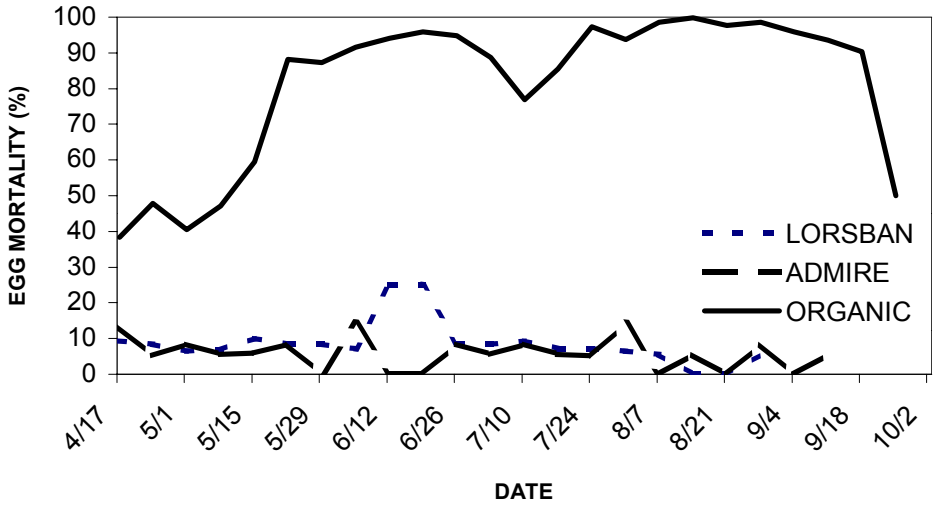

Figure 7. Egg mass mortality in untreated (="ORGANIC") sites, sites treated with Admire®, and sites treated with Admire® and Lorsban®.

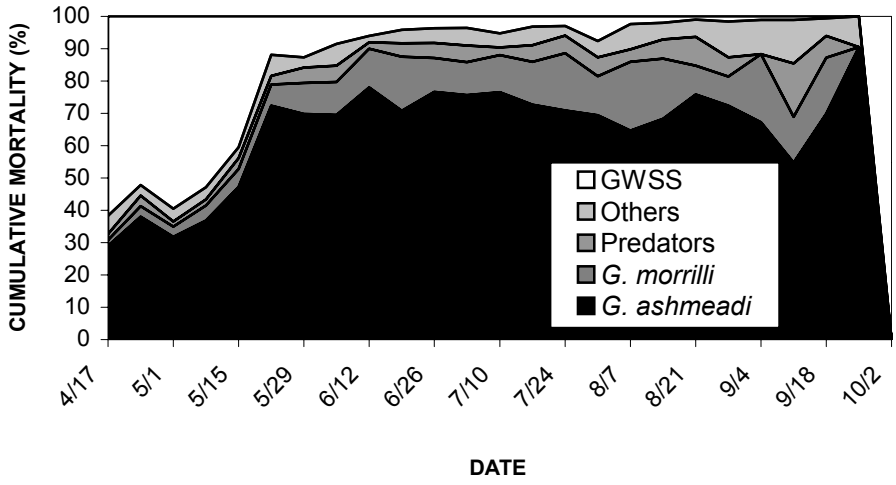

Figure 8. Egg mortality factors in untreated citrus sites.

Insecticide treatments reduced sharpshooter oviposition rates dramatically, however parasitism rates in these sites was also reduced (Figure 9). A more informative means of examining the data may be by looking at the density of eggs that eclose successfully as nymphs.

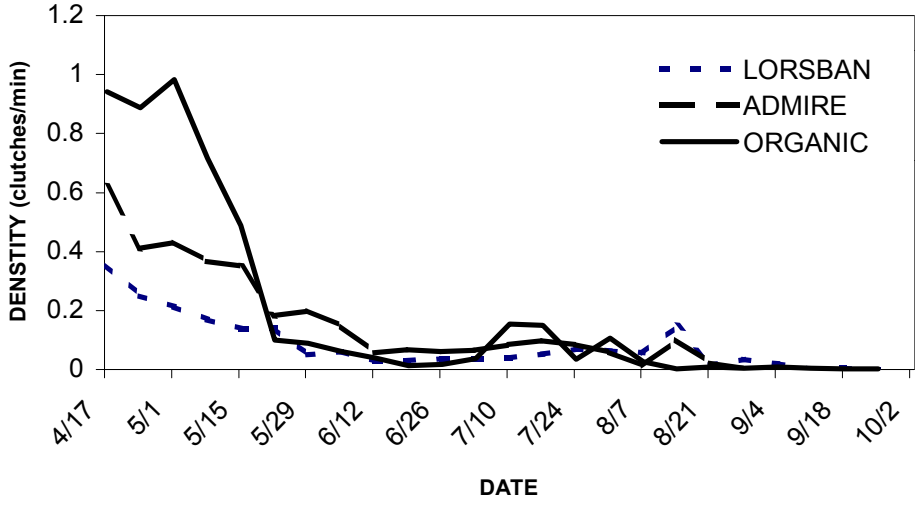

Figure 9. Density of eggs eclosing as nymphs in untreated (="ORGANIC") sites, sites treated with Admire®, and sites treated with Admire® and Lorsban®.

Over the period of time that sharpshooter eggs are available, parasitoids and natural enemies can assert enough pressure to result in similar mortality levels as those observed in insecticide treated areas. Unfortunately this is not consistent throughout the year. In the first half of the year, parasitism rates were not sufficiently high to control sharpshooter populations. If a means can be found for increasing parasitism levels early in the year, either by augmenting already present natural enemies or by introducing new natural enemies.

Monitoring X. fastidiosa (Costa and Cooksey)*:*

Results from our first six months of sampling have yielded positive detection of *X. fastidiosa* in grapes at many sites, as expected (Table 1). Additionally, we have consistently detected the pathogen in almond and oleander in Temecula. We assume that the oleanders are infected with the oleander strain of the pathogen which reportedly does not infect grape. Additional tests are underway to allow separation of these strains. A few samples of three other plant species (coyote brush, mustard, and elderberry) showed weak positive results when tested by ELISA. However, either culturing or PCR techniques did not support these results, which suggests that these results could be false positives. Additional samples of these species will be tested with improved methods in this coming season. We expect that more plants will yield positive results as we begin to routinely use the more sensitive immunocapture PCR method. Thus far, no obvious source of inoculum of PD, other than grape and almond, was detected in the valley using these methods.

We have initiated greenhouse studies testing the relative ability of *Xylella* to infect grape, citrus, almond, oleander, blackberry, bougainvillea, and vinca sp. These species have all previously been reported as hosts of *Xylella*. Our preliminary results suggest that grape-to-grape transmission by sharpshooters can occur as suspected. Six weeks after inoculation by insects, 6 out of 11 grape plants already tested positive for *Xylella* by ELISA. Thus far, three months after inoculation, none of the other inoculated host plants have tested positive for *Xylella*, however, it is common that infection may take more than 6 months after inoculation to be detectable in plants.

During our studies, we had difficulty developing a reliable method of extracting and detecting *Xylella* from insect samples. When sharpshooters were fed on known infected grape plants in the field for three days, 75% of the insects tested positive for the presence of *Xylella* using PCR. Additional studies will be conducted to determine why some insects tested negative. We recently have acquired materials to include an immunocapture step in the process to increase the sensitivity of the assay to more accurately detect the presence of the pathogen in individual insects.

We are directly comparing the sensitivity and specificity of various methods to screen plant and insect samples for the presence of PD. The comparison of detection techniques is ongoing, but this will help us to refine our studies as the project continues. We are comparing their sensitivity with a dilution series of suspensions of *X. fastidiosa*. Selectivity of the methods is being tested by comparing reactions with authentic *X. fastidiosa* to those with related *Xanthomonas* species, as well as other plant pathogenic genera and several genera of commonly observed saprophytic bacteria from plants. We are also evaluating a PCR-based diagnostic kit for Pierce's disease being developed by Chemicon International (Temecula, CA). Thus far, neither standard PCR nor the Chemicon kit has proven to be more reliable than ELISA for detecting the bacterium in grapes. We are now comparing immunocapture PCR, which should be more sensitive and reliable, since the bacteria are separated from plant material, including inhibitors of PCR reactions, in the initial step of the assay. We have also made progress in refining PCR methods to be able to differentiate PD strains from other strains of *X. fastidiosa*, particularly those from oleander.

Table 1. Plant Species Sampled in the Temecula Valley, 2000					
Host Plant		***# Samples***	***Testing Results***		
Scientific Name	***Common Name***	***Tested***	***ELISA***	***Culture***	***PCR***
Amaranthus retroflexus	Redroot pigweed	30	Negative	Negative	
Amsinkia intermedia	Fiddleneck	11		Negative	Negative
Anagallis arvensis	Scarlet pimpernel	20	Negative	Negative	
Artemisia spp.	Mugwort	11		Negative	Negative
Arundo donax	Giant reed	2	Negative	Negative	
Baccharis pilularis	Coyote brush	16	Positive (6/16)	Negative	Negative
Baccharis spp.	Mule-fat	10	Negative	Negative	
Brachychiton spp.	Bottle Tree	5	Negative	Negative	
Brassica spp.	Mustard	185	Positive (4/185)	Negative	Negative
Bromus rigidus	Ripgut brome	26		Negative	Negative
Citrus spp.	Citrus	205	Negative	Negative	Negative
Cucurbita foetidissima	Buffalo gourd	16	Negative	Negative	Negative
Cydonia oblonga	Quince	1	Negative	Negative	
Datura meteloides	Datura	31	Negative	Negative	
Diospyros kaki	Persimmon	2	Negative	Negative	
Encelia californica	Brittlebush	17		Negative	Negative
Erigeron spp.	Daisy	15	Negative		
Eriobotrya japonica	Loquat	6	Negative	Negative	
Erioganum spp.	Wild Buckwheat	62	Negative	Negative	Negative
Eucalyptus spp.	Eucalyptus	70	Negative	Negative	Negative
Ficus carica	Fig	2	Negative	Negative	
Fortunella margarita	Kumquat	3	Negative	Negative	
Hedera helix	Ivy	5	Negative		
Helianthus annuus	Sunflower	15	Negative		
Heterotheca grandiflora	Telegraphplant	11		Negative	Negative
Juglans californica	Walnut	11	Negative	Negative	
Liquidambar spp.	Sweet gum	5	Negative	Negative	
Malus spp.	Apple	4	Negative	Negative	
Malva parviflora	Cheeseweed	131	Negative	Negative	Negative
Morus spp.	Mulberry	25	Negative	Negative	
Nerium oleander	Oleander	86	Positive	Positive	Positive
Nicotiana glauca	Tree tobacco	35	Negative	Negative	Negative
Olea europaea	Olive	27	Negative	Negative	
Persea spp.	Avocado	15	Negative	Negative	Negative
Pistachia vera	Pistachio	10	Negative	Negative	
Platanus racemosa	Sycamore	9	Negative	Negative	
Populus spp.	Poplar	55	Negative	Negative	

Prunus amygdalus	Almond	102	Positive	Positive	Positive
Table 1 (continued) . Plant Species Sampled in the Temecula Valley, 2000					
Host Plant		***# Samples***	***Testing Results***		
Scientific Name	***Common Name***	***Tested***	***ELISA***	***Culture***	***PCR***
Prunus domestica	Plum	4	Negative	Negative	
Prunus persica (nectarine)	Nectarine	9	Negative	Negative	
Prunus persica (peach)	Peach	17	Negative	Negative	
Psathyrotes ramosissima	Velvet turtleback	10	Negative	Negative	
Punica granatum	Pomegranate	13	Negative	Negative	
Pyrus communis	Pear	3	Negative	Negative	
Quercus spp.	Oak	15	Negative	Negative	
Rhus laurina	Laurel sumac	10	Negative	Negative	
Rosa spp.	Rose	5	Negative	Negative	
Salix spp.	Willow	109	Negative	Negative	Negative
Salvia spp.	Sage	17		Negative	Negative
Sambucus canadensis	Elderberry	82	Positive (1/82)	Negative	Negative
Schinus molle	Pepper tree	35	Negative	Negative	
Vitis vinifera	Grape	578	Positive	Positive	Positive
	Total:	**2199**			

Inquiry 2

The Initiation and Development of Biological Control of Glassy-winged Sharpshooters.

Principle Investigators: M. S. Hoddle, S. Triapitysn, R. A. Redak, and M. J. Blua.

2.1 Summary

To date, five parasitoid species have been investigated as potential biological control agents of GWSS. Two were found to lack the host plant range required of an effective biological control agent and the remaining three were found suitable for further research. Rearing methodologies for sharpshooters and parasitoids have been significantly improved. We are now providing sharpshooter egg masses to two laboratories for biological control research, one laboratory for behavior research and three laboratories involved in insecticide research. The work on determining host plant preference of sharpshooters was largely abandoned as it was deemed no longer necessary with the successful development of rearing methodologies. The construction of the phenological degree-day model is continuing. To date we have taken the insect through a single generation at several temperatures; this research will require additional funding and at least another 18 months of effort before any significant conclusions are made.

2.2 Objectives

a. Determine the oviposition rate for the sharpshooter on a variety of host plants in a greenhouse and determine parasitism rates by egg parasitoids on selected host plants. Selection of the most suitable plant varieties for rearing GWSS and parasitoids will optimize rearing efficiency for GWSS and parasitoids.

b. Develop rearing methodologies for GWSS egg parasitoids, including determining the viability of both parasitized and unparasitized eggs after periods of long-term storage under refrigeration (required for mass rearing of parasitoids).

c. Construct a degree day model for development of both sharpshooters and parasitoids (required for mass rearing and release of parasitoids, also will allow predictions of periods of high and low population densities).

d. Conduct preliminary field release studies and evaluate parasitoid longevity, reproduction/parasitism rates, field persistence, dispersal, and impact on field populations of sharpshooter in both citrus and grapes.

e. Conduct exploration for other egg parasitoids in the GWSS's native range (southeastern U.S. to south Texas and northern Mexico).

2.3 Methods

Sharpshooter host plant preference (Redak, Blua): Within a greenhouse situation, sharpshooters were reared on a variety of host plant material to determine their oviposition preference. Chrysanthemum, citrus, sweet potato, lima bean, and grape, alone and in combination, were evaluated for greenhouse support of a sharpshooter colony.

Mass Rearing of Parasitoids (Hoddle and Redak)*:* Using the information derived from studies above. Mass rearing techniques were developed in the greenhouse and environmental chambers for producing high numbers of egg parasitoids. This work was largely technical and non-experimental in nature. It involved maintaining clean host plants, host plants infested with sharpshooters, host plants infested with sharpshooter eggs and egg parasitoids to produce parasitized egg masses, and storage facilities for maintaining unparasitized egg masses as well as parasitized egg masses. The effect of storage time in refrigeration is currently being determined for both sharpshooter eggs and developing parasitoids. The ability to store both eggs and parasitoids will be helpful for successful mass rearing and release of parasitoids. Already, we have made substantial progress on parasitoid rearing.

Phenological degree day model for development (Redak, Blua, and Hoddle)*:* A degree-day model for sharpshooter and parasitoid development is currently being created using environmental chambers and monitoring heat unit accumulation at several different temperatures. Currently this aspect of the project is focusing upon sharpshooters only. Populations of sharpshooters are reared at a variety of constant temperatures. Developmental times for each life stage is being calculated at each temperature. Using these data, degree-day models can then be developed. Data from this study will be compared with peak trap catches of sharpshooters in the field from prior years to determine the suitability of our degree-day model. This information will be used to make predictions of the optimum time for parasitoid releases in the field (i.e., when egg masses should be present and vulnerable to parasitism).

Biological Control and Releases (Hoddle and Triapitsyn)*:* Once mass rearing methodologies have been worked out, for all egg parasitoids of concern, experiments will be performed to evaluate the efficacy of parasitoids to limit sharpshooter populations. To date sharpshooter-infested citrus orchards are selected and utilized as either control (without releases) or treatment (with releases). Populations of both sharpshooters and parasitoids are closely monitored before and after releases. Monitoring includes includes assessments of parasitoid populations persistence, reproductive ability, and impact on sharpshooter egg mortality. Additionally, the efficacy of seasonal inoculative releases of sharpshooter parasitoids into orchards early in the season is ongoing by determining the distances over which parasitoids can disperse following point releases within orchards. Ultimately we intend to mark released parasitoids with molecular tags that change color when analyzed in the lab. Capture records will allow us to determine how far parasitoids can disperse within given periods of time. Parasitism data, sharpshooter density data, and parasitoid dispersal data when taken together will allow a rigorous evaluation of the effectiveness of augmentative releases of GWSS egg parasitoids as a viable control strategy.

Exploration for additional parasitoids (Hoddle and Triapitsyn)*:* A total of four trips were made out of state to search for natural enemies of glassy-winged sharpshooters. Trips covered Texas

(Mission and Weslaco), Louisiana (Baton Rouge and environs), Florida (Tampa), and Mexico (Nuevo Leon and Tamaulipas). In addition egg masses were sent from USDA facilities in Texas throughout the year. Full quarantine procedures were be utilized upon importation prior to release. Additional parasites will be evaluated should the need arise following additional foreign exploration efforts in Texas and Mexico.

2.4 Results and Accomplishments to Date

Sharpshooter host plant preference (Redak, Blua): These trials are still ongoing as we are constantly seeking to improve our rearing methodology. Our host plant preference summary data are shown in Table 2. Of the plants evaluated to date, citrus was the most preferred in terms of sharpshooter egg and egg mass production. Providing insects with a mixture of host plants are resulted in high egg and egg mass production. Rearing sharpshooters soley on crape myrtle, poinsettias, citrus or chrysanthemum proved to be inadequate as multiple generations of insects cannot be produced on a single host. We have been able to produce multiple generations on a mixture of host plants.

Table 2. Mean proportion (±SEM) of glassy-winged sharpshooter mortality, number of egg masses, total eggs, and eggs per mass and proportion of eggs hatched from sharpshooters placed in feeding/oviposition trials with citrus, crepe myrtle, chrysanthemum, and poinsettia and a mix of all four plant types. No eggs were laid on poinsettias. Means followed by the same letter within a column are not significantly different; means in columns with no letters following values indicate no means differ significantly (ANOVA; $P < 0.05$)

Treatment	Proportion adult mortality	# egg masses	# eggs	# eggs/mass	Proportion eggs hatched
Mums	0.401 ± 0.074	4.91 ± 1.09^{B}	31.36 ± 8.43^{B}	6.23 ± 0.55	0.69 ± 0.11
Points	0.436 ± 0.071	-	-	-	-
Crepe myrtle	0.391 ± 0.062	5.18 ± 1.38^{B}	36.64 ± 9.40^{AB}	7.44 ± 0.95	0.605 ± 0.041
Citrus	0.409 ± 0.065	11.09 ± 1.75^{A}	63.54 ± 14.1^{A}	5.22 ± 0.72	0.370 ± 0.061
Mixed cage	0.323 ± 0.057	6.00 ± 1.45^{B}	39.82 ± 12.2^{AB}	5.76 ± 0.60	0.665 ± 0.12

Mass Rearing of Parasitoids (Hoddle and Redak)*:*

The procedure for rearing sharpshooter egg parasitoids has been completely resolved. Leaves containing sharpshooter egg masses are exposed to wasps for 24 hours, then removed and incubated for 12-14 days at 25°C, 90% RH, 14:10 L:D. Wasps emerge and are either released or diverted to continuing the colony. Of greatest difficulty has been developing a method for producing a reliable and consistent supply of sharpshooter eggs for parasitism. A range of host plants and hosts were evaluated, and at present a mixture of sweet potato or potato, euonymus, and chrysanthemum are used to optimize egg production. It remains to be seen whether these host plants will be sufficient the induce oviposition throughout the whole year.

Presently, Foothill Agricultural Research (Corona) has been sub-contracted to CDFA to supply eggs for natural enemy production and are doing so with great efficiency (currently in excess of 100 egg masses per day). Mass rearing strategies are being taken over by the CDFA, based in Riverside, so that UCR can more efficiently compare and evaluate natural enemies for release.

We were able to determine the egg loads (i.e. an estimate the "power" of the parasitoid to limit sharpshooters) of the three predominant parasitoid species. None of three parasitoid species evaluated differed dramatically in the production of eggs (Table 3). Any difference in success of the parasitoids as natural enemies of sharpshooters will be unlikely to be due to differences in fecundity.

Table 3. Mean egg load data of the three dominant species of sharpshooter parasitoids.

Parasitoid Species	Egg load
Gonatocerus ashmeadi	35-64
Gonatocerus morrilli	44-72
Gonatocerus triguttatus	38-67

Phenological degree day model for development (Redak, Blua, and Hoddle):

The process of producing parasitoids and sharpshooters at the greatest rate has not allowed us to pause to compare temperatures for rearing these insects. Currently, we are performing these experiments as time allows. To date we have partially completed developmental studies at a single constant temperature for the sharpshooter only (data not shown). This work will continue forward and we will seek extramural funding to complete it. Once CDFA has fully taken over mass rearing responsibilities, greater care can be taken in developing a degree-day model, and so optimizing production.

Biological Control and Releases (Hoddle and Triapitsyn)*:*

Gonatocerus triguttatus has been released in four locations in California to date (Table 4). Each site has had a series of three preliminary releases after intensive pre-release monitoring.

Further monitoring has been made post-release at each location. To date, no recoveries of the released parasitoid have been made. This may be due to the population not successfully colonizing the sites, but it is unlikely that we would recover individuals at such an early date after release given the small number released.

Releases are continuing as mass rearing and release responsibilities are being handed over to the CDFA. UCR will continue to be involved in evaluation and monitoring of release sites and organisms.

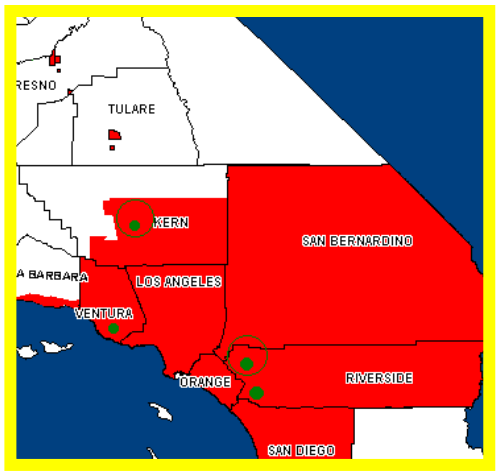

Figure 10. Biological Control Release Sites.

Table 3. Statewide release locations and dates of *Gonatocerus triguttatus*s. Numbers given are total number of individual wasps released to inoculate local areas with parasitioids.

Site	Late Aug.	Early Sept.	Late Sept.
Temecula (organic lemons)	100 ← Two Week Intervals →	100 ← Two Week Intervals →	100
Riverside (organic oranges, lemons, grapefruit)	180	180	180
Bakersfield (oranges)	100	100	100
Ventura (oranges)	100	100	100

Exploration for additional parasitoids (Hoddle and Triapitsyn)*:*

Five species of sharpshooter egg parasitoids were recovered from exploration. Of these, one species died without issue (*Gonatocerus atriclavus*). A further species died after only one generation (*Zagella sp.*, undescribed). Of the remaining species, only one was exotic to California, *Gonatocerus triguttatus*, the others being *G. ashmeadi* and *G. morrilli*. All three of these latter species were maintained for three generations, evaluated, and USDA and CDFA issued permits for their release. Only one species, *G. triguttatus*, was released in California (see above).

Inquiry 3

Use of Insecticides to Control Glassy-winged Sharpshooters and Limit Spread of Disease.

Principle Investigators: R. A. Redak, and M. J. Blua.

3.1 Summary

Field-trials conducted at U.C. Riverside in 2000 showed that two soil-applied and one foliar-applied neonicotinoid induced high sharpshooter mortality through an 8 week period. This was consistent with prior studies conducted in Temecula. We also examined the influence of neonicotinoids on sharpshooter feeding. Most striking is our observation that imidacloprid applied to grapevines in September 1999 had a substantial impact on sharpshooter feeding almost a year later. This may, in fact, be more important to protecting plants from *Xylella fastidiosa*-carrying sharpshooters then inducing mortality.

3.2 Objectives

a. Determine the degree to which neonicotinoids (e.g. imidacloprid) affect transmission of the PD organism to grapevine by pathogen-carrying sharpshooters through time after plants are treated.

b. Determine the optimal deployment of neonicotinoids on grapevines to reduce vector pressure and disrupt transmission of the PD organism.

c. Determine the impact of neonicotinoids in citrus on GWSS.

Our initial objectives were to examine the efficacy of neonicotinoids against the GWSS on grape and citrus, and to determine its impact on disease transmission in grapes. We altered these objectives to reduce overlap with the research endeavors of other investigators that was already in progress. Specifically, we delayed our objective of examining the efficacy of neonicotinoids on citrus against the sharpshooters to 2001, and are incorporating in this efficacy trial other insecticides in conjunction with the research of Dr. D.H. Akey (USDA ARS). We are aware other investigators are examining the impact of neonicotinoids on the sharpshooter, including University of California researchers Drs. B. Grafton-Cardwell and P. Phillips. In addition, we are involved in monitoring a recent CDFA abatement program is treating Temecula citrus with imidacloprid (Admire, Bayer Corp.) and other insecticides with Dr. N. Toscano.

3.3 Methods

We conducted an experiment on grapevines to examine the impact of three neonicotionods and oxydemeton-methyl, an organophosphate, on sharpshooter mortality, feeding, and transmission of *Xylella fastidiosa*. This experiment took place at U.C. Riverside where we maintain 100 plants each of Chardonnay and Cabernet Sauvignon. For each cultivar, we divided the vines into groups of 4 plants, each group representing a block in a randomized block experimental design. The experiment consisted of 6 treatments (Table 4).

Table 4. Details of insecticide treatments on grapevines.

Insecticide Treatment	Rate (lb active ingredient /acre)	Appliation Date	Application Type
control	-	-	-
Imidacloprid	0.50	Sept. 1999	Soil
Imidacloprid	0.50	Aug. 2000	Soil
Imidacloprid	0.25	Aug. 2000	Soil
Acetamiprid	0.05	Aug. 2000	Foliar
Thiamethoxam	0.26	Aug. 2000	Soil
Oxydemeton-methyl	0.09	Aug. 2000	Soil

Soil-applied materials were pre-mixed in water, and 1 liter of the solutions containing the appropriate concentration was poured into basins at both sides of each plant where drip irrigation was applied. Dead leaves and weeds were removed from the basin before application. Foliar-applied materials were applied with a back-pack sprayer to each grapevine in 2.5 l of water (300 gal/Ac assuming 452 grapevines/Ac). Before and after application of insecticides the vineyard was irrigated for ca. 6 hours.

To determine efficacy of each insecticide, 10 *H. coagulata* adults collected at U.C. Riverside were placed on grapevine canes in sleeve-cages 2, 3, 4, 6, and 8 weeks after applications of insecticides. Dead insects were counted at 24 hours after exposure to vines. Analysis of variance (ANOVA) was used to detect differences in the percentage of dead sharpshooters among treatments.

We also determined the impact of insecticide treatment on sharpshooter feeding. The importance of this parameter is that sharpshooters require hours of access time and must feed to acquire the PD bacterium from infected grapevines, or transmit it to healthy vines. Techniques that restrict feeding may be more important to reducing PD spread than inducing sharpshooter mortality. On a short length of vine of each experimental plant, we enclosed three sharpshooters in a pre-weighed plastic vial (85mm x 47mm diameter). After 2 hours the vials, without sharpshooters, were re-weighed to determine the volume of liquid excreta sharpshooters produce. Because the sharpshooter feeds from the xylem, the water-conducting tissue in plants that is nutrient dilute, they must process a large volume of fluid that can be measured to compare feeding among treatments. Volume of excreta generated by sharpshooters on experimental treatments was compared by ANOVA.

Finally, we are in the process of examining the degree to which grapevines were protected from inoculation with *X. fastidiosa* by glassy-winged sharpshooters 4 weeks after the Aug. 2000 treatments. In this experiment sharpshooters were allowed to feed on PD-infected grapevines in a greenhouse cage for 1 week. Afterwards, two sharpshooters were placed in a sleeve cage and left on a cane of experimental plants for four days before they were removed. The cane that infectious insects were allowed to feed on was not removed when vines were pruned in the winter of 2000/2001 so any *X. fastidiosa* inoculated into the canes would not be removed. Data from this experiment will not be available until August of 2001 when we expect to see symptoms of PD, and can conduct diagnostic analyses. Unfortunately, we detected symptoms in some of the grapevines in the early fall, long before we would expect symptoms to be produced from our inoculations. Obviously, natural spread of PD in

our vineyard exists, and we had not anticipated that. This may obscure results of this experiment. The grapevines were planted 14 months before the August 2000 insecticide applications, so any further experiments along this line of investigation will necessarily be conducted under screen covers.

3.4 Results and Accomplishments to Date

Efficacy of insecticides against the glassy-winged sharpshooter

The greatest degree of mortality was induced by neonicotinoids at the full rates applied to grapevines in August 2000 (Figure 11). Lower levels of mortality were induced by imidacloprid at full rate applied in September 1999 and at half rate applied in August 2000. Lower levels of mortality were also induced by oxydemeton-methyl applied in August 2000. It should be pointed out that we mistakenly applied a lower level of oxydemeton-methyl to grapevines. We applied 6 ounces of product per acre when we should have applied 6 pints per acre. This resulted in a 16 fold decrease in the rate applied. Still, at that low rate, mortality was frequently statistically greater than control mortality. Overall, high rates of mortality induced by neonicotinoids applied in August 2000 did not last as long after application as they did in experiments conducted in 1999 and 1998. In addition, control mortality was higher in 2000 than previous years. However, through three years of studies with neonicotinoids we conclude that they are efficacious against the sharpshooters on grapevines and have a long residual effect.

Effect of insecticides on GWSS feeding

Anti-feeding qualities are one of the important aspects of neonicotinoids that we have been aware of since our studies 1999. These studies revealed that sharpshooters caged on field-grown grapevines treated with imidacloprid did not feed enough to generate visible amounts of excreta, a trait for which sharpshooters are known. Yet, sharpshooters on untreated vines generated a significantly larger volume of excreta than did controls. In our most recent experiment, we showed this effect for two other neonicotinoids, including soil-applied thiamethoxam, and foliar-applied acetamiprid (Figure 12). This effect is shown for 2 and 6 weeks after the August 2000 application of insecticides. It is doubtful that GWSS could acquire *X. fastidiosa* from grapevines treated with these materials within at least 6 weeks, or that infective GWSS could inoculate treated vines. Also interesting is our finding of reduced feeding on plants treated with metasystox (Figure 12), even though the rate of oxydemeton-methyl used was well below that which we intended. Further experiments with oxydemeton-methyl are warranted. The most important obserservation made from this experiment is that imidacloprid applied to grapevines in September 1999 had a substantial impact on sharpshooter feeding 11 months later (Figure 12).

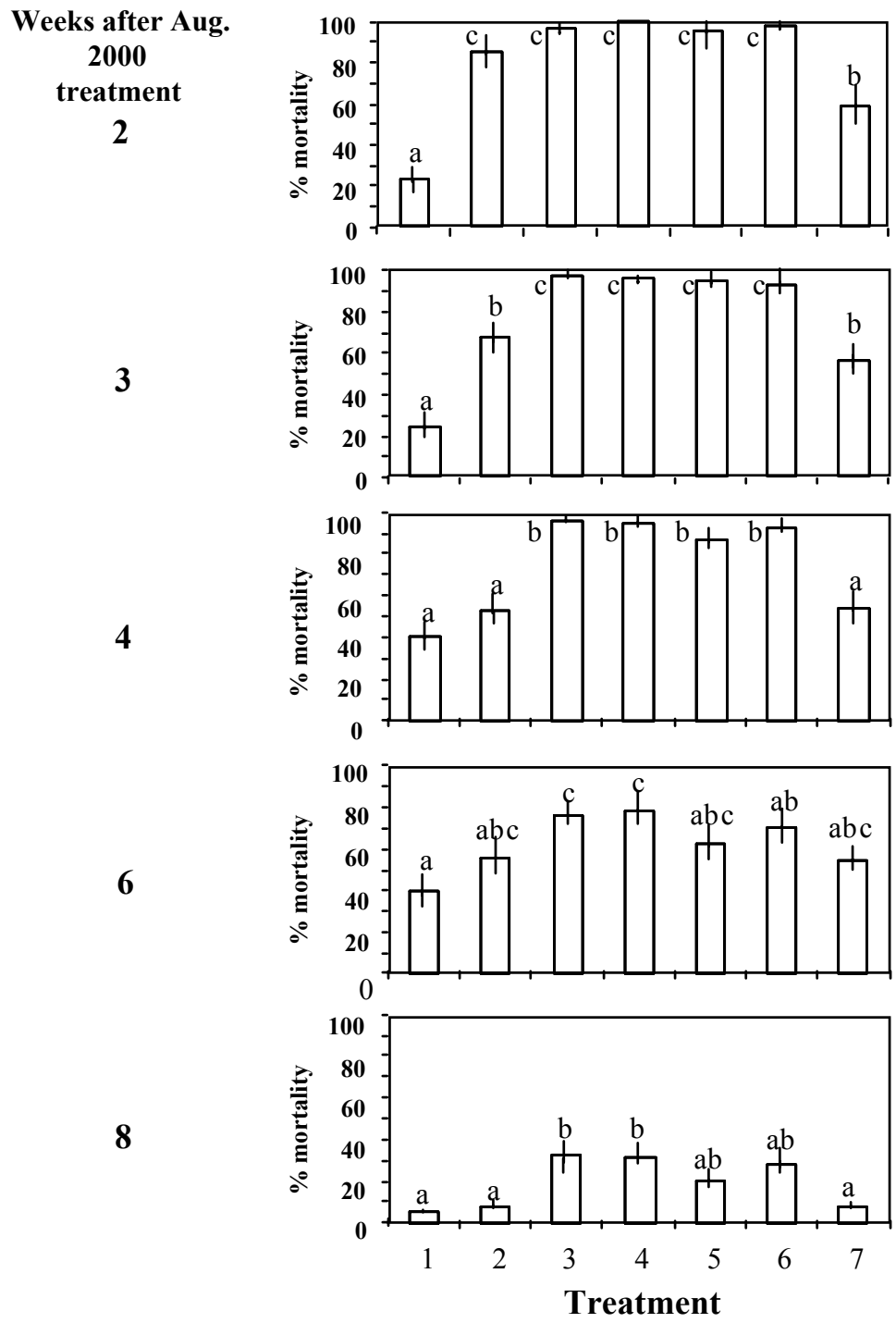

Figure 11. Impact of neonicotinoids and oxydementon-methyl R2 on insect mortality. Data are percent mortality of insects 24 hrs after caging on grapevines 2, 3, 4, 6, and 8 weeks after August 2000 treatments. Treatments are **1**: untreated controls, **2**: imidacloprid at 0.5 lb AI/ac in 9/1999, **3:** imidacloprid at 0.5 lb AI/ac in 8/2000, **4**: imidacloprid at 0.25 lb AI/ac in 8/2000, **5:** acetamiprid at 0.05 lb AI/acre in 8/2000, **6:** thiamethoxam at 0.26 lb AI/acre in 8/2000, and **7:** and oxydemeton- methyl R2 (MSR) at 0.09 lb AI/acre in 8/2000. Bars with different letters are significantly different at $P < 0.05$.

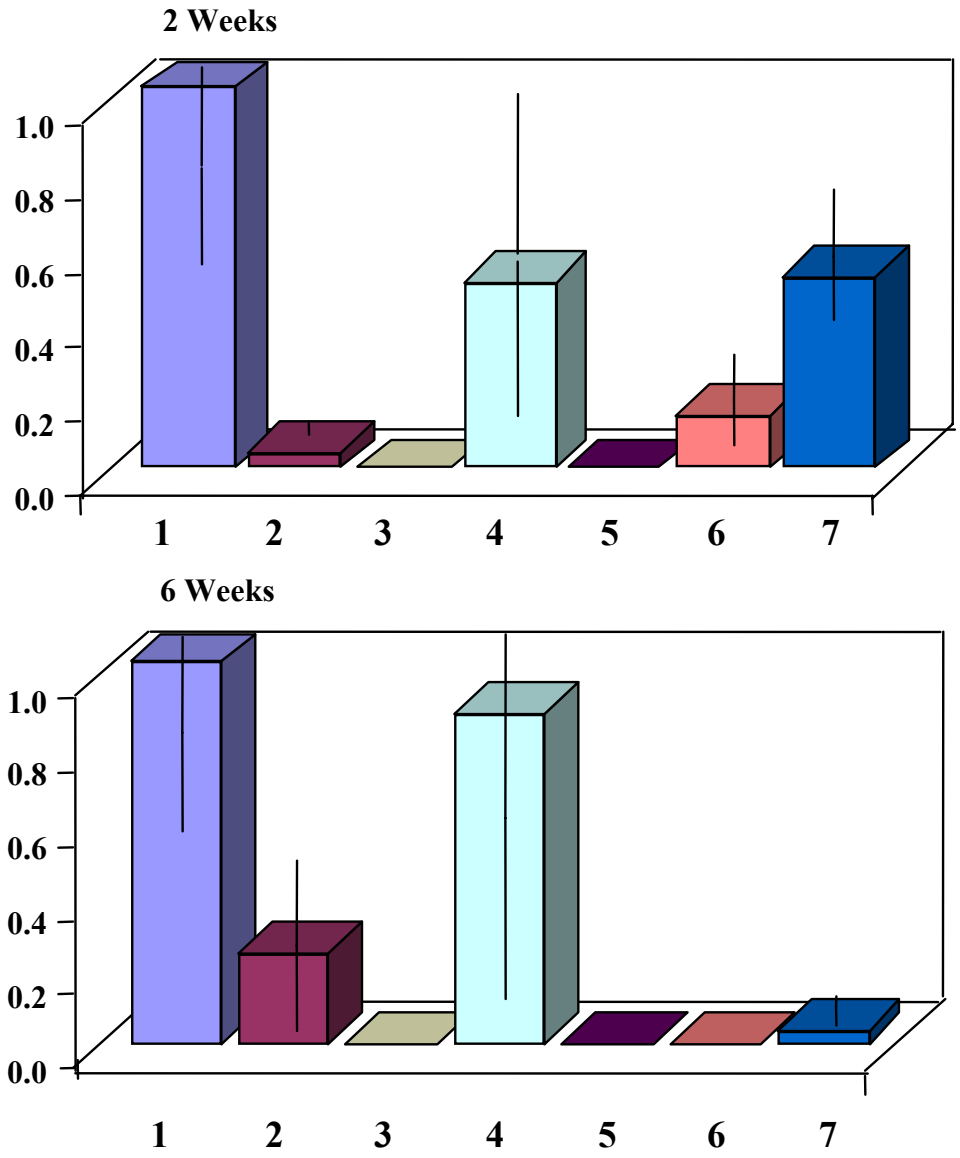

Figure 12. Impact of neonicotinoids on excretion by sharpshooters, 2 and 6 weeks after August 2000 treatments with insecticides. Data are ml of excretions produced by 3 individuals after 2 hours access time presented as a proportion of the untreated controls. Note the impact of imidacloprid applied to grapevines in September 1999, one year before the experiment. Treatments are **1:** untreated controls, **2:**imidacloprid at 0.5 lb AI/ac in 9/1999, **3:** imidacloprid at 0.5 lb AI/ac in 8/2000, **4:** imidacloprid at 0.025 lb AI/ac in 8/ 2000, **5:** acetamiprid at 0.05 lb AI/ac in 8/2000, **6:** thiamethoxam at 0.26 lb AI/ac in 8/2000, and **7:** oxydemeton-methyl R2 at 0.09 lb AI/acre in 8/2000

Inquiry 4

Use of Large Screen Barriers to Limit the Movement of Glassy-winged Sharpshooters from Citrus to Grapes

Principle Investigators: R. A. Redak, and M. J. Blua.

4.1 Summary

This aspect of the project ultimately was not possible to conduct. Funding for this aspect of the project was returned to AVF.

Inquiry 5

An Evaluation of Plant Micronutrients and Antibiotics as Potential Bactericides for Eliminating *Xylella fastidiosa* in Grapevines.

Principle Investigators: B. Kirkpatrick

5.1 Summary

Plant microelements such as zinc, copper, manganese and iron, as well as three antibiotics were tested for inhibition against *Xylella fastidiosa in vitro*. Tetracycline was the most effective antibiotic and zinc was the most toxic microelement. Both prophylactic and therapeutic field plots were established in Napa and Temecula vineyards during 1999 and 2000. Prophylactic materials being evaluated included 3 inducers of systemic acquired resistance (SAR) and 4 microelement formulations. Therapeutic materials include several formulations of microelements and 2 antibiotics. All prophylactic field plots were mapped for PD each fall, however no new infections were found in either the treated or control vines in 2000. Bactericides were applied as foliar sprays, as materials packed into hollow nylon (DP) screws or in drilled holes packed with bactericides that were suspended in agarose and the ends of the holes were sealed with DP screws. Several of the drilled through/DP screw treated vines did not show any PD symptoms following treatment, however these vines were also severely pruned following treatment. We know from Purcell's work that severe pruning can produce vigorous growth in the season following pruning but many of these vines later develop PD in the second year. Thus final assessment of the efficacy of these bactericide treated vines will be made in the summer of 2001. An injection machine that is widely used for injecting avocado trees was found to work well for injecting vines in the spring but less effective in the fall. Several potted plant experiments were performed using soil drenches of microelements as therapeutic or prophylactic bactericides. Although significant phytotoxicity occurred with some of the materials, managanese and zinc treatments may have some potential. A custom-made pressure bomb was purchased and used to express xylem sap from 1 meter long grapevine canes that were treated with various microelements. The expressed xylem sap was analyzed for microelement concentrations with the assistance of Peter Andersen. Surprisingly high concentrations of zinc and managanese were found in the xylem sap of grapevines treated with amino acid chelates of these elements. Additional experiments are now being done to determine whether the xylem sap is actually toxic to *Xylella fastidiosa* or if the ions are too tightly bound

4.2 Objectives

a. Determine *in vitro* growth inhibition of *Xylella fastidiosa* (*Xf*) by selected plant micronutrients and antibiotics

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

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

d. Determine the efficacy of using micronutrients and antibiotics to cure Xf-infection in field-grown grapevines.

e. Determine efficacy of using plant micronutrients prophylactically to prevent *Xf*-infection in established vineyards.

f. Determine whether foliar and/or other treatments of grapevines actually results in increased levels of micronutrients in grapevine xylem sap.

4.3 Methods

In vitro growth inhibition of Xylella fastidiosa (Xf) by selected plant micronutrients and antibiotics.

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.

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

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.

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

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.

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

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.

Determine efficacy of using plant micronutrients prophylactically to prevent Xf- infection in established vineyards.

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. No results were obtained or are presented.

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

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.

4.4 Results and Accomplishments to Date

In vitro growth inhibition of Xylella fastidiosa (Xf) by selected plant micronutrients and antibiotics.

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*.

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

All grapevines treated with zinc citrate died within 5 weeks of treatment (Table 5). 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.

Table 5. 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

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

All vines treated with zinc citrate died as well as the vines being treated with 16X Fe (Table 6). 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 6. 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%

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

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 7.

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 7. Results of therapeutic bactericide application one 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**

Determine whether foliar and/or other treatments of grapevines actually results in increased levels of micronutrients in grapevine xylem sap

Detailed results of this study are presented in Table 8. 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 (Objective a). 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.

Table 8. 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		

Outside Presentations of Research

Bethke, J. A., R. A. Redak, M. J. Blua. 2000. Evaluations of selected pesticides against egg, nymph, and adult stages of the glassy-winged sharpshooter, *Homalodisca coagulata* (Say). California Citrus Nursery Society Conference, Bakersfield, CA. 12-12-2000. (poster)

Bethke, J. A., M. S. Blua, & R. A. Redak. 2000. Potential management of oleander leaf scorch using selected pesticides to control the glassy-winged sharpshooter (Homoptera: Cicadellidae): A laboratory study. 16th Conference on Insect and Disease Management on Ornamentals. Society of American Florists, Hyatt San Jose, San Jose, CA. Feb. 19-21, 2000. (Poster)

Bethke, J. A., R. A. Redak, M. J. Blua. 2000. Evaluations of selected pesticides against egg, nymph, and adult stages of the glassy-winged sharpshooter, *Homalodisca coagulata* (Say). Joint Annual Metting of the society of Entomology of Quebec, the Entomological Society of Canada, and the Entomological Society of America, Montreal, Canada. Dec 3-6, 2000. (poster)

Bethke, J. A., R. A. Redak, M. J. Blua. 2000. Evaluations of selected pesticides against egg, nymph, and adult stages of the glassy-winged sharpshooter, *Homalodisca coagulata* (Say). California Citrus Nursery Society Conference, Bakersfield, CA. 12-12-2000. (poster)

Blua, M.J. 2000. Testimony before the U.S. House of Representatives Committee on Agriculture, Subcommittee on Livestock and Horticulture. February 22, 2000. Napa, CA.

Blua, M. J. 2000. Potential of the Glassy-Winged Sharpshooter to spread Plant Diseases in the San Joaquin Valley. April 4, 2000. U.C.Riverside Entomology/Nematology Conference cosponsored with Tulare/Kings CAPCA. Parlier, CA.

Blua, M. J. 2000. The Glassy-Winged Sharpshooter & Pierce's Disease. June 28, 2000. U.C. Riverside Citizens University Committee, Temecula, CA.

Blua, M. J. 2000. Impact of Imidacloprid on Glassy-Winged Sharpshooter Feeding and Transmission of *Xylella fastidiosa*. July 13, 2000. Bayer Corporation Workshop, Temecula, CA.

Blua, M. J. 2000. Developing a management strategy for PD spread by the GWSS. December 6, 2000. Coachella Valley Glassy-Winged Sharpshooter/Pierce's Disease Meeting. Indio, CA.

Blua, M. S., J. A. Bethke, & R. A. Redak. 2000. Potential impact of the glassy-winged sharpshooter on the nursery industry. 16th Conference on Insect and Disease Management on Ornamentals. Society of American Florists, Hyatt San Jose, San Jose, CA. Feb. 19-21, 2000. (Poster)

Blua, M. J., and R.A. Redak. 2000. Interactions Among Organisms Driving the Epidemic of Pierce's disease in Temecula Wine-grapes. June 26, 2000. Glassy-Winged Sharpshooter Symposium, Entomological Society of America, Pacific Branch, Costa Mesa, CA.

Blua, M.J., and Redak, R.A. 2000. Update on the Glassy-Winged Sharpshooter and Pierce's disease in Temecula. January 10, 2000. Integrated Pest Management of Glassy-Winged Sharpshooters and the Diseases they Vector, UC-DANR Workgroup Meeting, Riverside, CA.

Blua, M. J. and Redak, R.A. 2000 Glassy-winged sharpshooter biology and ecology of PD spread in Temecula, CA. January 26, 2000. Unified Wine and Grape Symposium. Sacramento, CA.

Blua, M. S., R. A. Redak, & J. A. Bethke. 1999. Development of a management strategy for Pierce's disease spread by *Homalodisca coagulata* in southern California. Annual Meeting of the Entomology Society of America. Atlanta, GA. 12-14-99

Blua, M. J., R. A. Redak, J. A. Bethke. 2000. Impact of Imidacloprid on the transmission of Xylella fastidiosa by the GWSS. Bayer Corporation. Embasy Suites, Temecula. July 13, 2000.

Blua, M. J., R. A. Redak, J. A. Bethke. 2000. Effects of Neonicotinoids on Glassy-Winged Sharpshooter Feeding and Transmission of *Xylella fastidiosa.* Glassy-Winged Sharpshooter / Pierce's Disease "Meeting the Challenge" Workshop. Riverside CA. November 16, 2000.

Blua, M. J., R. A. Redak, J. A. Bethke. 2000. Biology of the Glassy-Winged Sharpshooter, a vector of *Xylella fastidiosa* New to California. Southern District Plant Pathology Workshop, Riverside Agricultural Commissioner's Office. November 17, 2000

Costa, H. S. 2000. Glassy-winged sharpshooter in nursery crops. Ornamental Horticulture Education Coordinating Conference. Davis, CA, May 9, 2000.

Costa, H. S., D. Cooksey, C. Gispert. 2000. Identifying inoculum sources and transmission pathways of *Xylella fastidiosa*. In Symposium: Pierce's Disease and Glassy-winged Sharpshooters: The threat to California Agriculture. Pacific Branch Meeting of the Entomological Society of America, Costa Mesa, CA, June 26, 2000.

Costa, H. S. and C. Gispert. 2000. Glassy-winged sharpshooters and the pathogens they vector. Landscape Management Research Conference and Field day. Riverside, CA, Sept 13, 2000.

Costa, H. S. 2000. Insect Identification Exhibit. Too Hot to Handle: Ornamental Horticulture Conference. Riverside, CA. Sept. 14, 2000.

Gispert, C., H. Costa, and D. Cooksey. 2000. Survey of *Xylella* in plants and transmission with the GWSS. Workgroup meeting: Integrated pest management of the glassy-winged sharpshooter and the diseases it vectors. Nov 7, 2000.

Gispert, C., H. Costa and D. Cooksey. 2000 Monitoring *Xylella fastidiosa* in native and cultivated plants in Temecula. GWSS/PD Workshop: Meeting the Challenge. Riverside CA. Nov 16, 2000.

Gispert, C., H. S. Costa and D. Cooksey. 2000. Identifying inoculum sources of *Xylella fastidiosa* in southern California. National Meeting of the Entomological Society of America. Montreal, Canada, Dec. 3-6, 2000.

Hoddle, M. S. 2000. Biological control of glassy-winged sharpshooter. Kern County Agricultural Center, Bakersfield California November 7, 2000.

Hoddle, M. S. 2000. Biological control efforts for glassy-winged sharpshooter: current situation and future directions." Riverside Convention Center, Riverside CA. November 16, 2000.

Hoddle, M. S. 2000. Biological control of exotic pests in California." California Rare Fruit Growers Annual Meeting, California State University at Fullerton, Fullerton California. November 18, 2000.

Hoddle, M. S. 2000. Glassy-winged Sharpshooter Update." Butler's Mill Inc. 18th Annual Professional Horticultural Seminar, San Diego Paradise Point Resort. November 21, 2000.

Hoddle, M. S. 2000. Prospects and limitations of biological control for glassy-winged sharpshooter." Pierce's Disease Conference, University of California, Davis. December 13, 2000.

Hoddle, M. S. 2000. Biological control efforts for glassy-winged sharpshooter in California." 35th Annual AAIE Conference, Riverside Convention Center. February 5, 2001.

Hoddle, M. S. 2000. Biological control of glassy-winged sharpshooter: current situation and future prospects." Sonoma County Grape Day, Luther Burbank Center, Santa Rosa. February 16, 2001.

Hoddle, M. S. 2000. Ecologically based management of invasive species: biological control of the glassy-winged sharpshooter." Performing Arts Center Pavilion, California Polytechnic State University, San Luis Obispo, CA. March 12, 2001.

Redak, R. A. 2000. Research update on Glassy-winged sharpshooter in California. California Association of Nurserymen Annual Research Conference. University of California, Riverside, September 14, 2000.

Redak, R. A. 2000. Glassy-winged Sharpshooter in California. California Fertilizer Association Nutrient Conference. Sacramento, CA. October 10, 2000

Redak, R. A., 2000. Chemical Control of Glassy-winged sharpshooters for Commercial Nursery Production. UC-DANR Meeting of the IPM of GWSS and the Diseases it Vectors Workgroup. November 7-8, 2000.

Redak, R. A. 2000. Monitoring and Control of Glassy-winged Sharpshooter" . Glassy-winged sharpshooter/Pierce's Disease "Meeting the Challenge" Workshop. Riverside CA, November 16, 2000.

Redak, R. A. 2000. Expectations and Limitations of Chemical Control for Glassy-winged sharpshooters. Symposium on Pierce's Disease and the Glassy-winged Sharpshooter in California: Reviewing and Defining Critical Research Agenda. University of California, Davis. December 12-14, 2000

Research Success Statements

The funds for this research were provided by the City of Temecula and County of Riverside, California to initiate several lines of research that are required to ultimately implement a region-wide integrated pest management program for the glassy-winged sharpshooter and the bacterial disease it vectors, *Xylella fastidiosa*. At the time (late 1999), these funds were provided to "jump-start" the necessary research such that applied projects could begin immediately toward finding a solution to the sharpshooter "problem". Toward that end, this project was an overwhelming success. There is now a region-wide sharpshooter monitoring program established in Temecula (funds from this project were used to determine sharpshooter distributions in non-agricultural areas and link past data sets to more accurately reflect the distribution and occurrence of these insects). There are now projects aimed at determining the distribution and sources of *Xylella* in the Temecula valley and developing more sensitive and accurate detection methodology. These projects were originally funded by this grant and are now funded by CDFA or USDA; we were able to start a full year earlier because of this funding. There is now a state-wide biological control program which was initiated by funding from this grant and a grant from the California Association of Nurserymen. This project provided funding to determine the efficacy of several new-generation insecticides for disrupting Pierce's disease transmission and acquisition; the results are extremely encouraging and will be used to justify further research support along these lines. Finally, several therapeutic and prophylactic approaches using plant micronutrients and antibiotics were evaluated, and several were shown to be somewhat effective in "curing" diseased vines.

Fund Status

Funds for this project are exhausted (as far as the P.I.'s can determine from current budgeting documents). Approximately $30,000 was returned to AVF as the barrier portion of this project was not performed. For all areas of research, the associated P.I.'s have and are continuing to seek funding for project continuation.

APPENDIX 1

Temecula Glassy-Winged Sharpshooter (GWSS)/ Pierce's Disease (PD) Project Annual Report – 2000

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Temecula Glassy-Winged Sharpshooter (GWSS)/Pierce's Disease (PD) Project
Annual Report – 2000

Project Title: Glassy-Winged Sharpshooter (GWSS) Areawide Management Project

Project Location: Temecula, CA

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Period: March 1 – October 31, 2000

Status of Project: Ongoing

Objective

Project proposes to examine the impact of areawide GWSS management on GWSS populations (numbers) in citrus and wine grapes.

Immediate Objectives

1. Determine the impact of an areawide management program on GWSS populations in citrus and grapes.
2. Determine the impact of the areawide program on GWSS adult oviposition, and nymphal development.
3. Determine the impact of the GWSS program on beneficial citrus insects and pest upsets and GWSS egg parasitism.
4. Evaluate the biological and economic effectiveness of an areawide program for GWSS.

Summary

To manage the GWSS in 3500 acres of grapes in the 2000 season, 1300 acres of citrus in Temecula, CA were treated with the systemic insecticide Admire (imidacloprid) during April and May. Approximately 160 acres of 1300 citrus acres were also treated with Lorsban (chlorpyrifos) in March, June and July. The insecticide treatments in citrus significantly reduced the GWSS populations in adjacent vineyards. No adverse effects on beneficial insects or secondary pest outbreaks were found in citrus because of these treatments.

Introduction

GWSS is a particular threat to agriculture because of its ability to spread the bacterium *Xylella* (PD). One of the most encouraging developments is the introduction of neonicotinoids such as imidacloprid (Admire) for the control of sucking insects such as GWSS.

Because Admire is an systematic insecticide and is applied through mini-sprinklers and drip irrigation systems it has a much lower negative impression on the public's perception of contact sprays such as the organophosphates, carbamate and pyrethroid insecticides, and therefore it may be the best candidate for areawide suppression of GWSS.
The emergency treatment of 1300 acres of citrus in Temecula, CA with Admire (imidacloprid) during April and May 2000 represented a pivotal shift toward an area-wide management of the GWSS. Although table and wine grapes are the most vulnerable crops to GWSS as a vector of the bacterium *Xylella fastidiosa*, the causal agent of PD, other crops are being scrutinized for their contributions to GWSS population growth. Perhaps more than any other source, citrus is viewed as an important year long reproductive host of GWSS, but also one that concentrates GWSS populations over the winter months during the time that grapes and many ornamental hosts are dormant. In the 2000 season, the opportunity to treat the entire commercial plantings of citrus in the Temecula area with Admire was seized upon in an effort to destroy a substantial portion of the regional GWSS population.

Procedures
To manage GWSS and suppress the transmission of Pierce's Disease (PD) 1300 acres of citrus trees were treated with Admire 2F (imidacloprid) at the rate of 32 oz per acre delivered through drip lines and mini-sprinklers. The Admire treatment was initiated in April and finished the end of May. To test the efficacy of Lorsban 4 EC (chlorpyrifos) on adult GWSS approximately 160 acres of citrus within the 1300 acre were treated with these insecticides.
The Lorsban was applied at 6 pts per acre in 40 gals of water by air to approximately 160 acres of citrus on March 17-18 and June 17.
Approximately 300 acres of Temecula citrus were not treated with Admire because they were under a growers organic management practices or considered impractical to treat.

To determine the impact of an areawide treatments of Admire and/or Admire + Lorsban on GWSS and beneficial insects within the "Temecula Demonstration Area" were sampled/monitored weekly from February through October 2000. Information collected from the monitoring was faxed, e-mailed, and/or phoned to participating growers, pest control advisors, and vineyard managers. (See attachments).

Sampling for GWSS

1) Double-sided Yellow-sticky traps, (Pherocon AM traps purchased from TRECE inc.), 7 inches x 9 inches and/or 18 cm x 23 cm, were used to monitor for adults. The yellow sticky traps were replaced as needed, but at least once a month.
2) Citrus – yellow-sticky traps were placed at the edge of the groves across from vineyards and 50 meters (55 yards) within the groves.
3) Grapes – yellow-sticky traps were placed on the edge of the vineyards across from the citrus groves at 50 meter intervals throughout the vineyards, i.e. 50, 100, 150, 200 meters, etc.
4) The terrain determined the number and disposition of the traps. 456 traps were monitored weekly. Adult's trapped were counted and the information provided to the grower, PCA, managers, etc. (See attachments)
5) Visual, beats in citrus and A-vacing were conducted in vineyards where sticky traps indicate high numbers of GWSS. A total of 850 beats a week were performed in citrus and grapes. Thirteen A-vacs samples each week in grapes were dependent on GWSS population in the vicinity.

Yellow Sticky Traps. **In grapes:** One yellow sticky trap was placed approximately every 50 meters in the vineyards and around the perimeter, using large binder clips to attach them to the existing posts and wires in the field. Transects of traps running perpendicularly from the perimeter towards the center of the vineyard were employed whenever citrus trees surround the perimeter of the vineyard.
In citrus: Traps were attached with large binder clips to wooden stakes around the perimeter of the grove. In large groves, traps were placed in the interior as well. This assisted in determining adult dispersal out of the citrus into the vineyards. Traps were checked weekly. The GWSS found on the traps were counted and then removed from the trap. The traps were replaced when necessary. This data was then recorded in the laboratory and graphed weekly. 456 traps were placed in the Temecula citrus/grape ecosystem. Of these 456 traps, 90 were placed in citrus (76 in insecticide treated and 14 untreated), 364 in vineyards and 2 in oleander.

A-vacing of vineyards. A-vac suction samplers were used to sample pests and beneficials found in the grapes along the perimeter where they border citrus. Samples were taken weekly at predetermined locations. Insects were sampled by dragging the orifice of the A-vac through the vines, for a distance of 50 row-feet. A total of 13 sites in vineyards were A-vaced. Knee-high nylon socks were used to collect the insect samples; the samples are then frozen to kill the insects; the samples are counted in the laboratory. GWSS adults, nymphs, and all beneficial insects were recorded and graphed weekly. The beneficial insects recorded were green lacewing adults and nymphs, *Chrysopa* spp., minute pirate bugs, *Orius* spp. Big-eyed bugs, *Geocorius* spp., Ladybird beetle adults and immatures, mantids, wasps and spiders.

Insect Beats in citrus. With a sweep net and a heavy-duty stick, beatings were performed where citrus borders grapes. One beat consisted of two hits with the stick on one branch per tree with the sweep net being underneath the branch to catch the insects that are dislodged, then moving onto the next tree. Fifty trees were sampled randomly in each designated grove

and the each sample was placed into labeled zip-lock bags. The samples were then returned to the laboratory and placed in the freezer to kill the insects; then counted. All stages of GWSS and beneficial insects were recorded and graphed weekly. Beatings were also done in the field onto a sticky card on various hosts such as Oleander, Mulefat, and sickly looking citrus trees, as well as healthy trees; looking only for adult and immature GWSS. The data is recorded in the field and then graphed weekly in the laboratory.

Visual Inspections for GWSS egg masses. Weekly visual inspections were performed on citrus, grapes and other GWSS hosts for egg masses. Once found, a clip-cage was placed onto the egg mass and labeled with location name and data of finding. Each week this cage will be monitored for hatched, unhatched, parasitized and dead egg masses. The data was recorded and graphed weekly.

Statistical analysis of the GWSS trapping and monitoring data was done using repeated measures of variance.

To determine the impact of the GWSS program on beneficial citrus insects and pest insects.

1) *Aphytis* parasites of red scale, *Aonidiella aurantii*, were put into 2 x 3" vials containing leaves from each of the insecticide treatments. After 24 hours, the *Aphytis* were emptied onto black paper; the number of *Aphytis* that have died were counted. This procedure was conducted in the Admire and the Admire plus Lorsban plots that were treated. The tests were conducted three times during the season beginning at 2 and 10 days after application then at 3-week intervals. *Aphytis* parasitoids for the tests were donated by Foothill Agriculture Research, (FAR), Corona, CA.
2) Red scales, *Aonidiella aurantii,* were also monitored by visually inspecting green twigs and fruit for scale and by placing pheromone traps in the citrus groves to monitor adult male scale flights.
3) GWSS egg parasitism was monitored during the GWSS ovipositional periods (March-May and August-September) on untreated citrus and trees treated with Admire and Admire + Lorsban.

Results:
GWSS Trapping – GWSS captured per week on the 456 yellow sticky traps varied at the various placement sites. The GWSS trap catches peaked March 14 and again early and late July (Fig. 1). The highest average number of weekly GWSS catches was 16 at the end of July, with one individual trap from a grove under organic practices having 50 adults. Figure 1 shows that trap location does impact the number of GWSS caught per week, i.e., citrus having the highest numbers of GWSS catch and traps in vineyards catch the least number of GWSS. The second GWSS generation in July – August was greatly reduced with Admire and/or Admire + Lorsban citrus treatments when compared to untreated citrus areas. The untreated citrus had anywhere from 2 to 8 times more GWSS catches per trap during July - August (Fig. 2).

The significance of the relationship of citrus to GWSS in vineyards, the effect of citrus insecticide applications on GWSS in vineyards and the effect of citrus insecticide applications on GWSS in citrus is explained by logarithm of GWSS captures and are presented in Figs. 3, 4 and 5. The analysis of trap GWSS capture data indicates a positive consistency in the relationship between treating citrus with insecticides and the number of GWSS captured on yellow sticky traps in vineyards. Figure 3 shows that GWSS are significantly higher in vineyards adjacent to citrus groves. Figure 4 indicates the GWSS from July 10 through September 16 were significantly higher in vineyards adjacent to untreated citrus groves when compared to citrus groves treated with Admire or Admire + Lorsban. Figure 5 shows that GWSS populations are significantly higher in untreated citrus than in citrus treated with Admire or Admire + Lorsban. A-vacing in vineyards next to treated citrus indicated that numbers of GWSS stages ranged from an average high of 0.75 adults to 0.50 for immatures from June 12 through October 23 (Fig. 6).

Beneficial insects and secondary pest outbreak sampling
The total number of beneficial insects found in the beat samples were not very abundant in any of insecticide treated or untreated citrus groves. Total number of beneficial insects never exceeded 0.19 per beat throughout the sampling period. The data does show that greater numbers of beneficial insects were present in the Admire + Lorsban treatments between the period of July 3 through September 11 (Fig. 7).
Male Red Scale trapped on pheromone baited traps indicates that the numbers varied among 12 monitoring sites. Nine of the sites were located in Admire or Admire + Lorsban treated citrus and 3 in untreated citrus. The greatest numbers of red scales were trapped in citrus on September 4, reaching of peak 4,200 at site M-6 near GWSS sticky trap #137 (Fig. 8).

Aphytis melinus, a parasite of Red Scale, was tested for it's sensitivity to Admire and Admire + Lorsban treatments in citrus. Figure 9 indicates that 24% mortality to *A. melinus* occurred August 4, at site M-7 near GWSS sticky trap #358, almost 2.5 months after the application of Admire. Average mortality of *A. melinus* in our sensitivity tests to Admire and Admire + Lorsban was 4.6% (Fig. 9). An average of 4.6% was below the 6% that occurred in the control (see SR/CZ Fig. 9), therefore the insecticide treatments probably had no long term effect on the red scale parasites.
No egg parasites such as *Gonatocerus* spp. were observed or recorded in GWSS egg masses in our sampling/monitoring sites during this study.

Discussion:
Analysis of GWSS sticky trap data during the spring, summer and early fall season indicate that GWSS populations were reduced in citrus treated with Admire and Admire + Lorsban, this in turn significantly reduced the GWSS trap captures in adjacent vineyards. This data indicates that the insecticide treatments had a major impact on GWSS populations in the Temecula area.

The data does not show that the treatment of 1300 acres of citrus had a detrimental impact on beneficial insects or caused outbreaks of red scale.
Continuation of the Temecula project will provide more information on the impact of "areawide management" programs of GWSS/PD and beneficial insects and possible secondary pest outbreaks. Further data collection is also necessary for statistical analysis and formulation of guidelines for the management of GWSS/PD.

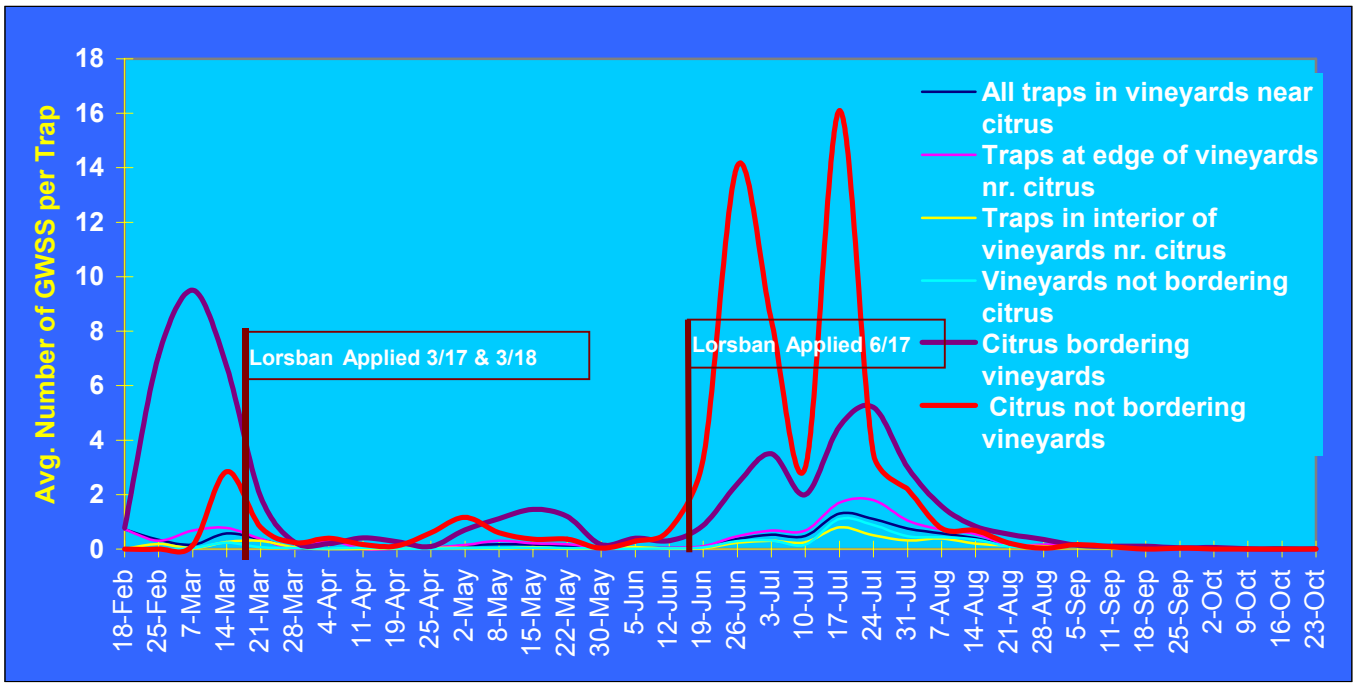

Figure 1. Weekly average number of Glassy-Winged Sharpshooters per yellow sticky traps in relation to citrus groves.

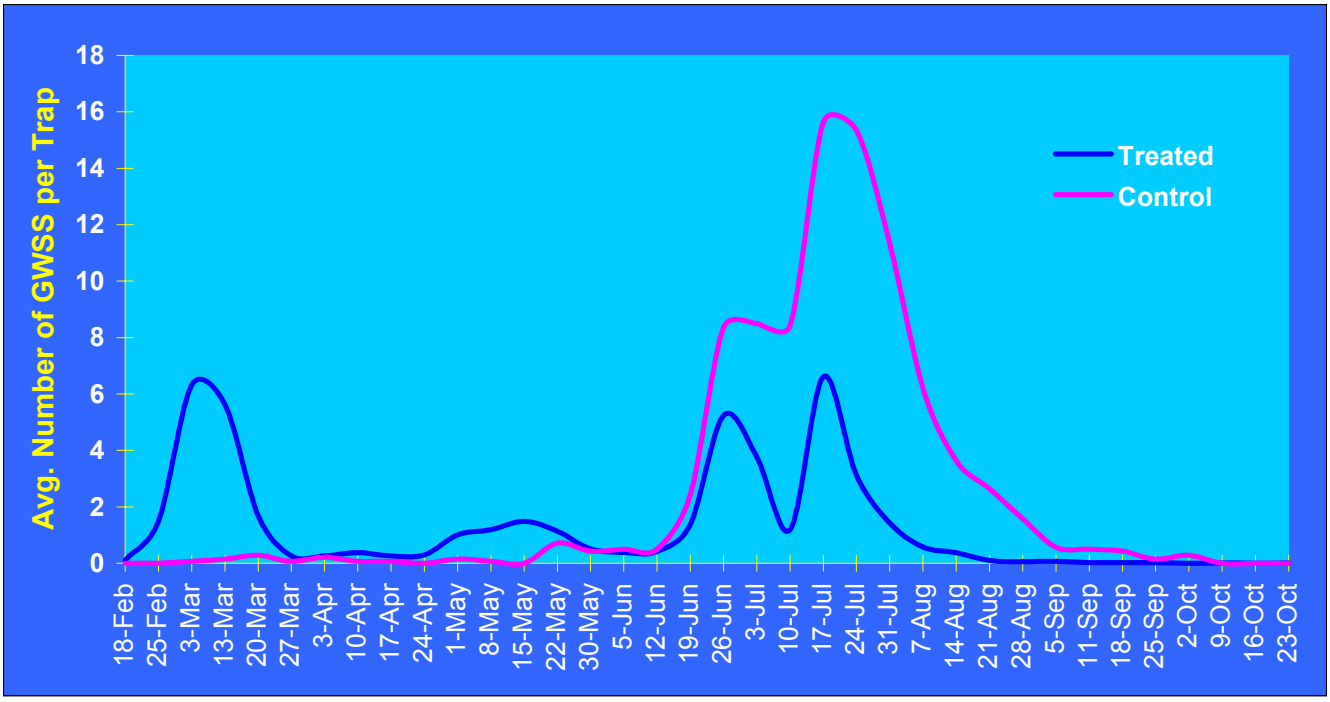

Figure 2. Weekly average number of Glassy-Winged Sharpshooters per yellow sticky trap in citrus grooves that were treated with Admire and/or Admire + Lorsban versus untreated citrus groves.

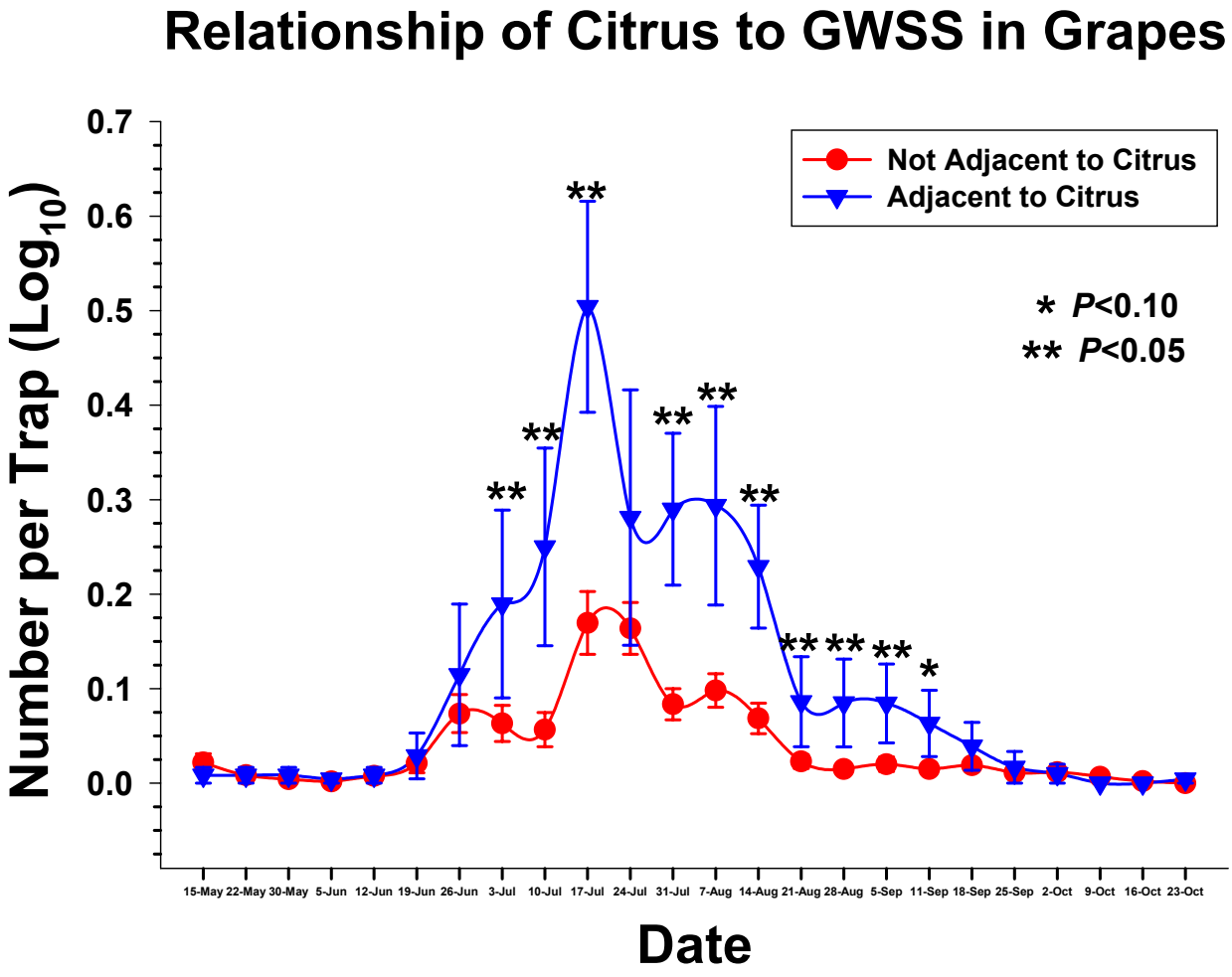

Figure 3. Numbers of Glassy-Winged Sharpshooters are significantly higher in vineyards adjacent to citrus groves.

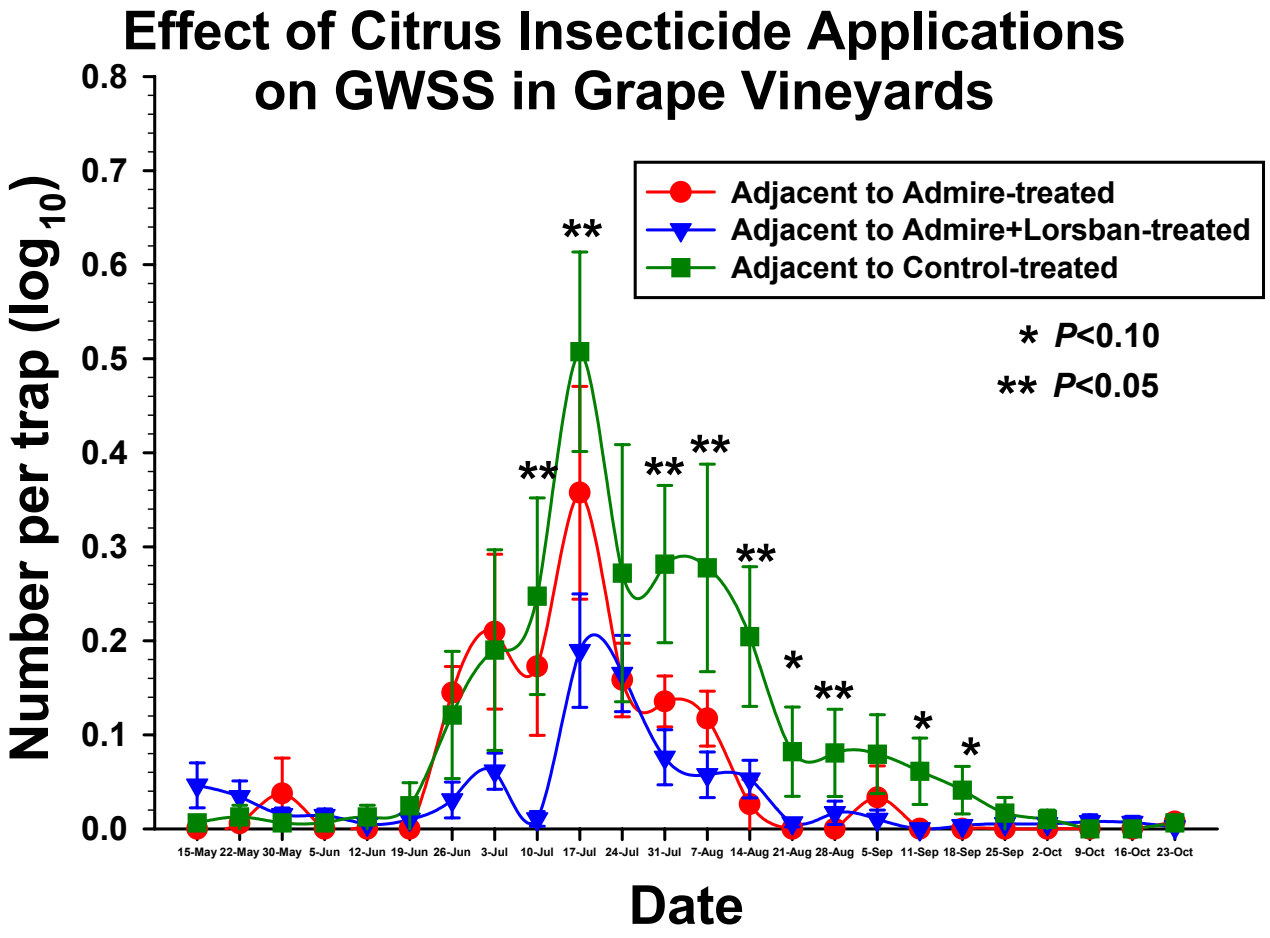

Figure 4. Number of Glassy-Winged Sharpshooters per yellow sticky trap were significantly higher in grape vineyards that were adjacent to untreated citrus groves in comparison to treated groves.

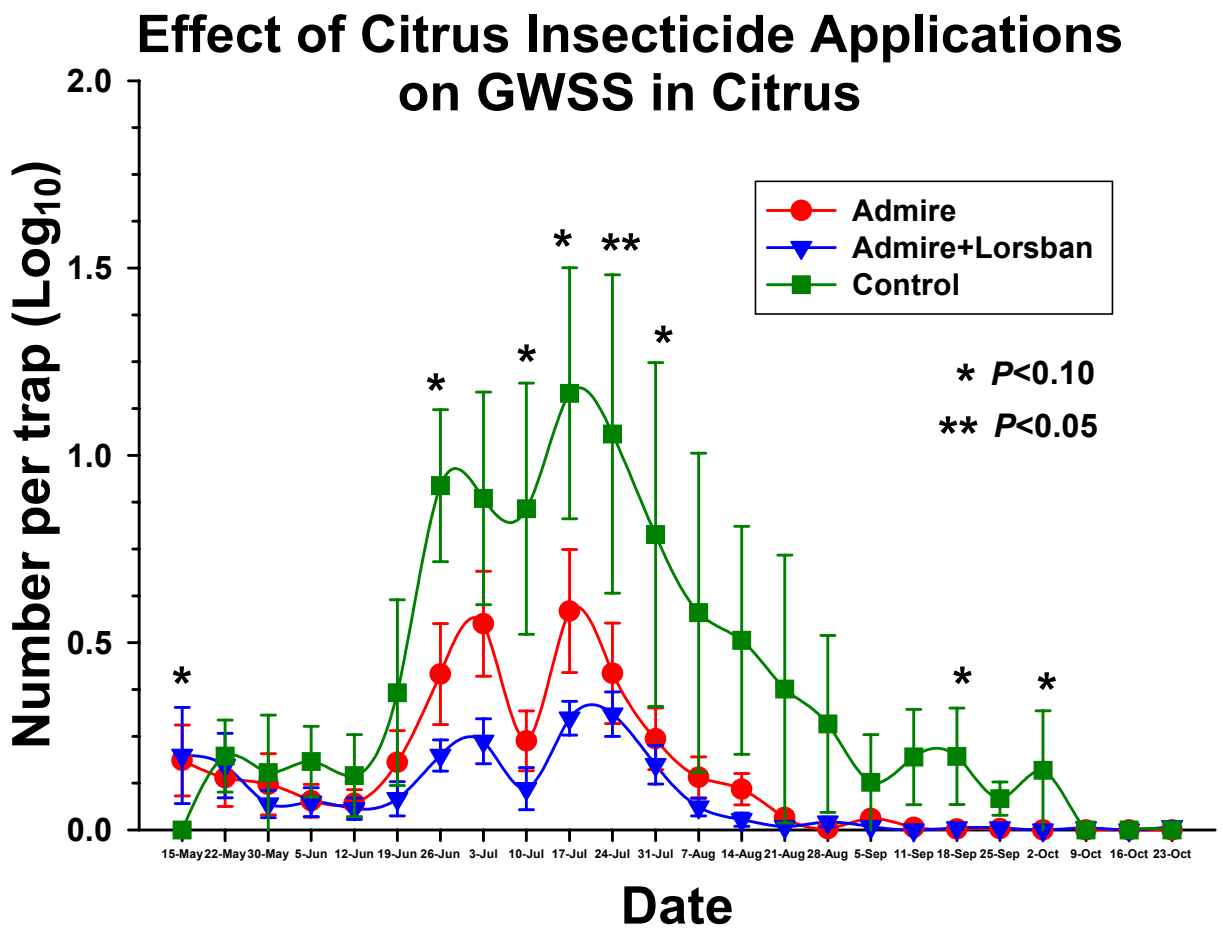

Figure 5. Number of Glassy-Winged Sharpshooters are significantly higher in untreated citrus than in citrus treated with Admire and/or Admire + Lorsban.

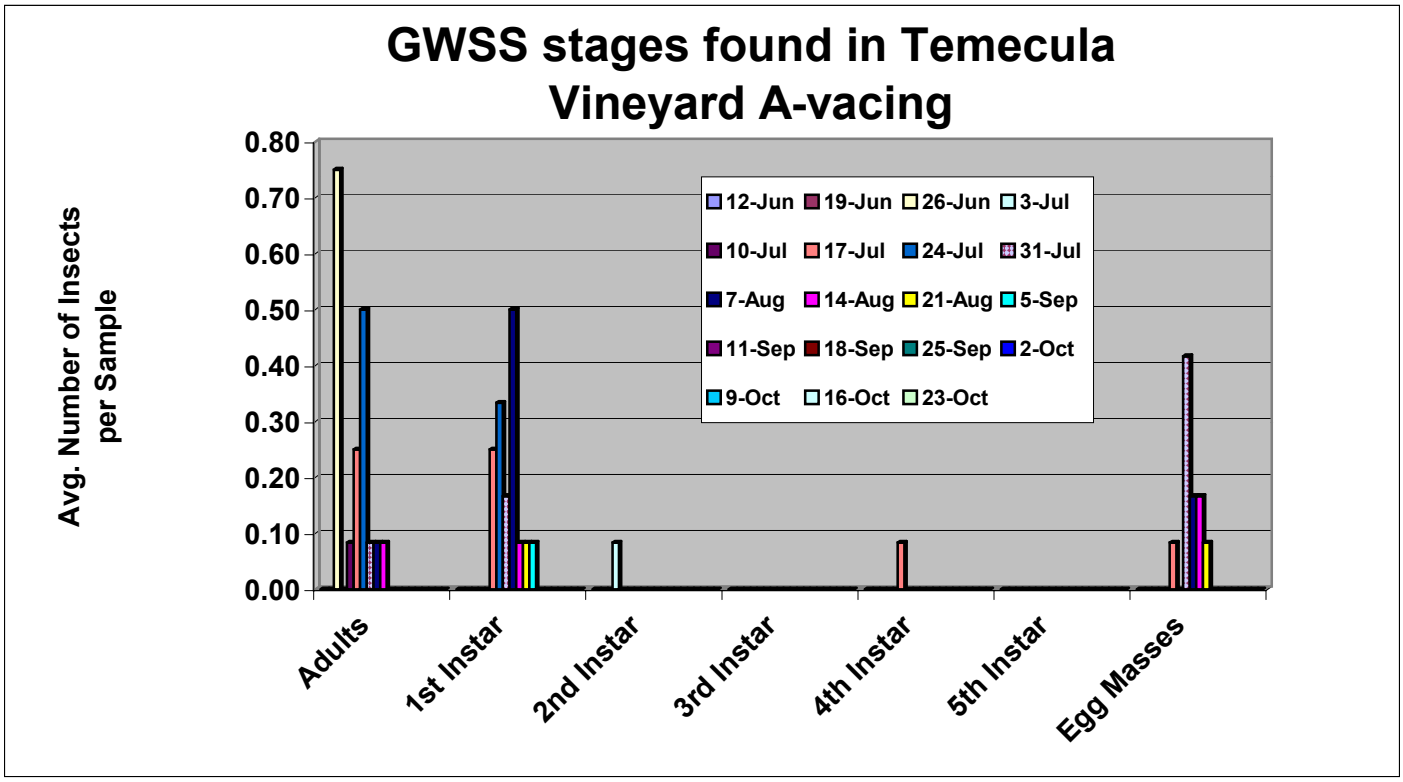

Figure 6. Average number of Glassy-Winged Sharpshooters life stages found in A-vac suction samples in vineyards next to citrus. Egg masses were recorded from visual leaf inspections.

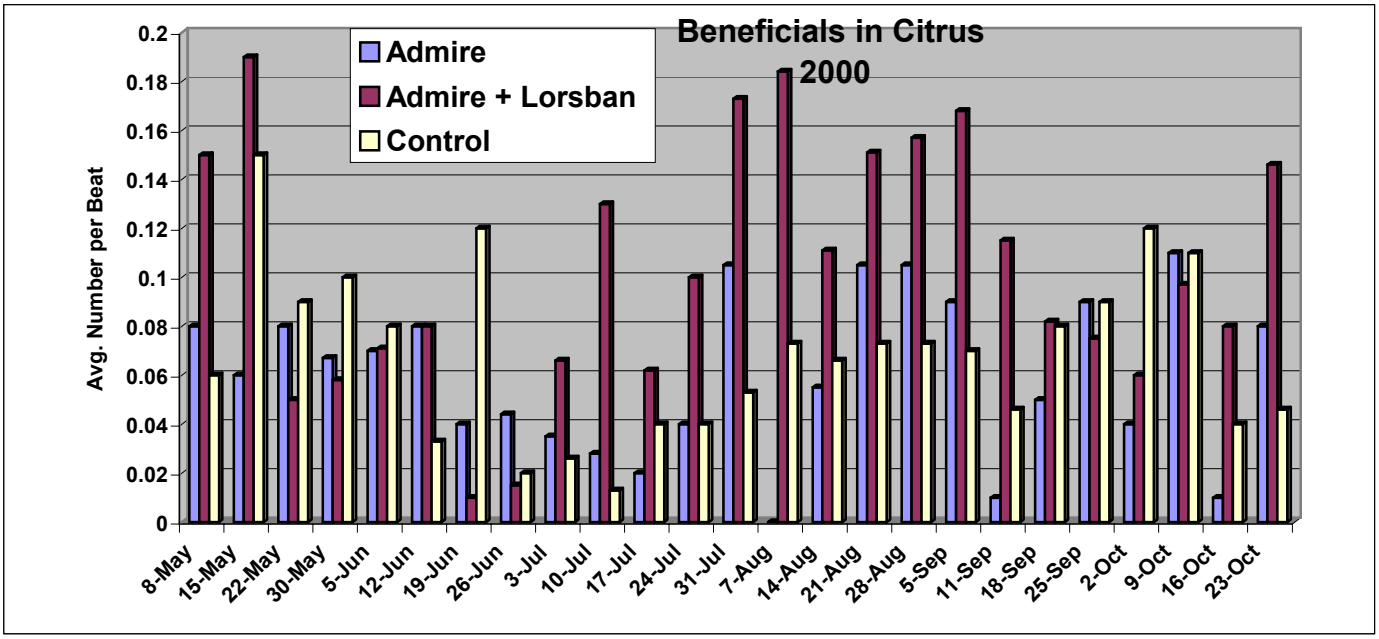

Figure 7. Number of beneficial insects found in weekly samples in Admire, Admire + Lorsban and untreated citrus groves.

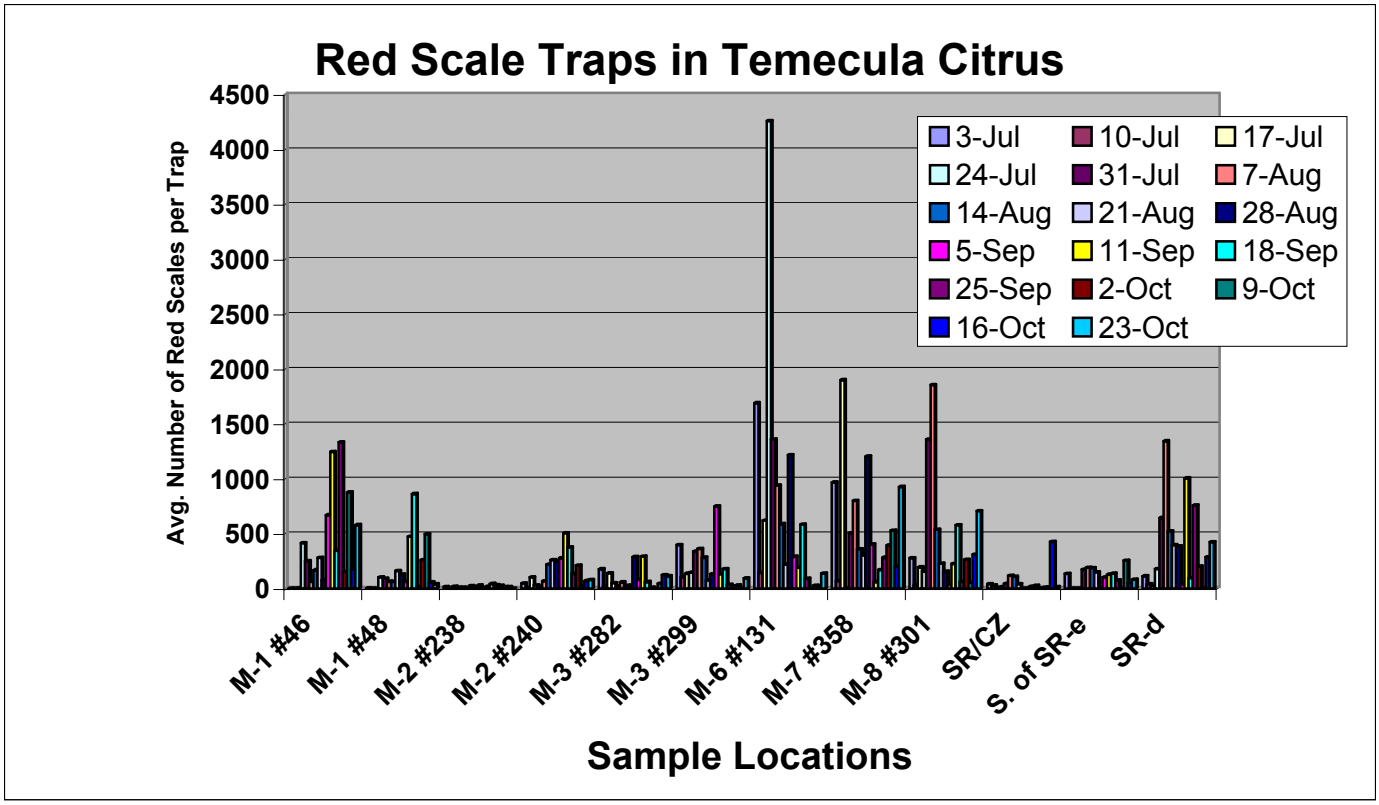

Figure 8. Average number of red scale found in citrus grooves in the Temecula "Areawide Demonstration Program".

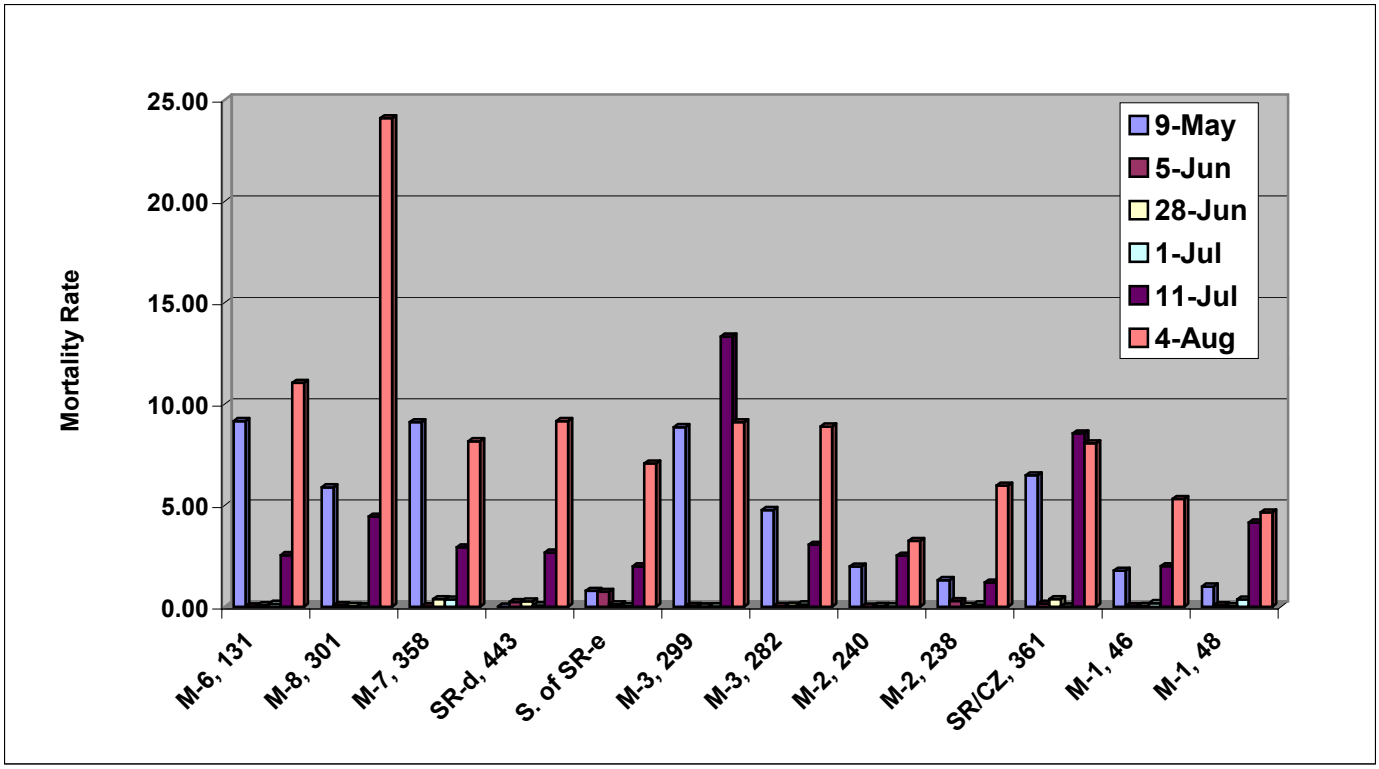

Figure 9. *Aphytis melinus* mortality recordings from placement on citrus leaves from groves that had been treated with Admire in April and May and Lorsban on March 17-18 and June 17.

Advisory Committee/Cooperators

Ben Drake, Drake Enterprises, Inc.
Craig Weaver, Callaway
Mike Rennie, Stage Ranch
Gary McMillan, McMillan Farm Management
Phil Baily, Baily Winery
Roberto Ponte, Temecula Valley Vineyards
Peter Poole, Mount Palomar Winery
John Snyder, Office of Agricultural Commissioner, Riverside co.
Michele Tracy, Office of Agricultural Commissioner, Riverside co.
Ed Ishida, Bayer Corp.
Edwin Civerolo, ARS-USDA