PIERCE'S DISEASE CONTROL PROGRAM

Symposium Proceedings

Pierce's Disease Research Symposium
December 5-7, 2001
Coronado Island Marriott Resort

CALIFORNIA DEPARTMENT OF FOOD & AGRICULTURE

Proceedings of the

Pierce's Disease Research Symposium

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California Department of Food & Agriculture

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California Taste the Sunshine

Dear Attendees,

Welcome to the Pierce's Disease Control Program's Research Symposium. The California Department of Food and Agriculture (CDFA), the agriculture community and the many other stakeholders in this program have made significant progress in the statewide effort to control the glassy-winged sharpshooter and find a cure for Pierce's disease. Our long-term success in this program depends, to a very large degree, on research. CDFA is hosting this symposium to give all interested parties an opportunity to hear firsthand from the researchers who have conducted the "first wave" of projects for this program. These progress reports will help provide the information we need to assess our current roster of projects and to focus our future efforts on the most promising areas.

Thank you for attending this event. Our statewide program has enjoyed strong support and early success. Your continued dedication to this effort will help us stay on the right track.

Sincerely,

Bill Lyons J.

William (Bill) J. Lyons, Jr.

IDENTIFICATION OF MOLECULAR MARKERS IN THE GRAPEVINE'S RESPONSE TO INFECTION BY *XYLELLA FASTIDIOSA*

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INTRODUCTION

For the past three years we have had a project aimed at developing a functional genomics approach to grape berry ripening and defense. During the 1999 season we identified numerous genes that will be important in physiological and biochemical approaches to understanding ripening, but we also found many genes related to plant defense. Although the defense-related genes were isolated from fruit tissue, it is likely that some of them could prove useful for characterizing the grapevine's response to infection by *Xylella fastidiosa*.

Many of the ripening-related expressed sequence tags (ESTs) we found were associated with cellular housekeeping functions such as protein synthesis, protein processing and turnover, and enzymes of primary and secondary metabolism. Another distinct set of genes is expressed under water stress conditions in other plants, or related to abscisic acid (ABA) responses and water transport (aquaporins). The ones of interest in this proposal are antimicrobial proteins (AMPs) that include pathogenesis related (PR) proteins and non-specific lipid transfer proteins (nsLTP). We propose to focus on the AMPs and determine if any of them are expressed in grapevine stems infected with *X. fastidiosa*. By comparing susceptible and resistant grape varieties we hope to identify molecular markers that will be useful in characterizing the grapevine's response to infection by the pathogen that causes Pierce's disease.

OBJECTIVES

1. Complete the sequencing of genes from our EST collection that code for AMPs (PR proteins and nsLTP) so that these can be used as probes for characterizing the grapevine's response to Pierce's disease.

2. Determine if genes that code for grape PR proteins and nsLTP are expressed in grapevine stems inoculated with *Xylella fastidiosa*.

3. Determine if expression of PR protein and nsLTP genes differ in grape varieties that show different susceptibility to Pierce's disease.

RESULTS AND CONCLUSIONS

We have completed the sequencing of several genes from our EST collection that code for antimicrobial proteins. They include a pathogenesis-related (PR) protein (EST 433) that is highly homologous to a PR protein (PR3) from rice (*Oryza sativa*) and a lipid transfer protein (LTP, EST 429) that is homologous to LTP GH3 from cotton (*Gossypium hirsutum*). The full-length sequence of EST 433 is 47% identical and 62% similar to the PR3 from rice and EST 429 is 75% identical and 86% similar to a lipid transfer protein from cotton.

Primers for PCR have been designed for each of the genes. These primers will be suitable for determining if the genes are expressed in grapevine stems inoculated with *X. fastidiosa*.

In order to evaluate the expression of the genes we have selected, it is necessary to obtain RNA that can be reverse transcribed successfully. We have conducted a series of RNA isolations using grape tissue. We tried several different published protocols, and the best RNA preparations are being tested to determine if we can get good reverse transcription. We surveyed numerous methods for isolating RNA from 'difficult' tissues – tissues and species seen as containing high amounts of contaminating coprecipitating materials such as polysaccharides and polyphenols–including hot borate, perchlorate, Loulekakis et al. (grape), TRIzol, GITC, CTAB, and compilations of several. We found that most methods produced some RNA yield, however UV ratios indicated that the RNA contained contaminants. Some examples of typical ratios seen can be found in Table 1.

Table 1. Examples of typical RNA isolation ratios.

Method	*260/280*	*260/230*
Hot borate	1.1	0.3
Loulekakis et al	1.5	1.1
Perchlorate	1.0	1.2
Compilation A	1.3	0.7
Compilation B	1.5	1.4
Compilation C	1.8	1.0
GITC	1.7	not measured

These ratios are fairly low. Ideal 260/280 ratios are in the 1.8 to 2.0 range, while 260/230s are good above about 2.3. Some of the protocols we tried gave low yields or resulted in degraded RNA. We are currently using the GITC and CTAB protocol (Compilation C) for RNA isolation. Testing of the RNA is currently underway.

In addition to running RNA gels, we will test the best RNA preparations with a reverse transcription kit, using a simple one-step RT-PCR and gene-specific primers. Once a PCR product of the correct size is observed, we will use the SYBR Green two-step RT-PCR kit to compare a control gene with expression of the PR3 and the LTP gene in inoculated and uninoculated plant material. Taqman RT-PCR will be contrasted with the less sensitive SYBR Green system, in the hope of using the Taqman system for each of the genes we proposed.

When the tests to determine the quality of RNA are complete we will isolate RNA from grapevine stems inoculated with *Xylella*. The inoculated plant material is being provided by the cooperator (M. Andrew Walker) and inoculation of the plants is currently underway. These plants will be available in the next few weeks for RNA isolation and evaluation of gene expression. By comparing unwounded stem tissue with wounded (uninoculated) and inoculated stems we will be able to differentiate between genes that are simply induced by wounding and those that are actually up-regulated when the plant is inoculated with *X. fastidiosa*. The experiments to determine if expression of PR protein and nsLTP genes differ in grape varieties that show different susceptibility to PD will be carried out after we have had the opportunity to evaluate the results from the second objective.

SHARPSHOOTER FEEDING BEHAVIOR IN RELATION TO TRANSMISSION OF PIERCE'S DISEASE BACTERIUM

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INTRODUCTION

The establishment in California of the non-native glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*, a vector of Pierce's disease (PD) bacterium, *Xylella fastidiosa*, has transformed the previously low-level problem of PD into a potential disaster for California agriculture. Almost nothing is known of the exact feeding behaviors of GWSS and how they interact with the behavior of *X. fastidiosa*, (within the sharpshooter's foregut) to facilitate transmission to grapevine. The Backus project will combine all three of the most important and successful methods of studying leafhopper-feeding behavior (i.e. histology of fed-upon plant tissues, videotaping of feeding on transparent diets, and electro-penetration graph [EPG] monitoring) to definitively identify all details of feeding. Both AC and DC EPG monitoring will be performed. All recorded waveforms will be thoroughly correlated with stylet activities, cell types within the host plant in which activities occur, and presence or movement of *X. fastidiosa* in and out of the stylets. Thus, our research also will provide crucial baseline information for the present projects of collaborators.

Understanding the role of feeding behavior in the mechanism and efficiency of *X. fastidiosa* transmission will allow the development of a rapid, EPG-based screening tool for behaviors associated with inoculation (an "inoculation-behavior detection method"). Such a tool would have a direct impact on the grape industry by making many future, applied studies possible. For example, the detection method could be used to screen for differences in vector feeding or inoculation behavior among grape varieties, or formulations of artificial diet, or between infected vs. uninfected plants, or infective vs. uninfective vectors.

OBJECTIVES

1. To identify and quantify all feeding behaviors of GWSS on grapevine, and correlate them with location of mouthparts (stylets) in the plant and presence/population size of *X. fastidiosa* in the foregut.
2. To identify the role of specific stylet activities in *X. fastidiosa* transmission, including both the mechanisms of acquisition and inoculation, and their efficiency.
3. To develop a simple, rapid method to assess feeding, or detect the likelihood of *X. fastidiosa* transmission (an "inoculation-behavior detection method"), for future studies.

RESULTS AND CONCLUSIONS

In the four months since this project began, we have made the following significant achievements in training personnel, upgrading facilities and purchasing equipment, to prepare for performing the research in earnest.

A. Backus organized and taught an intensive, hands-on, five-day workshop on EPG monitoring (28 July – 2 August 2001), which was attended by 13 researchers from all over the world. Two of these researchers were Rodrigo Almeida and Keiko Okano, doctoral graduate students from Alex Purcell's lab at UC Berkeley, who will apply EPG technology to parts of their dissertation research. Okano returned to Berkeley directly after the course, and will use her new skills at UC Riverside in spring 2002 to perform most of the research for the Blua & Walker collaborative project. Almeida continued working in the Backus lab for three more weeks after the short course, studying the feeding behavior of the blue-green sharpshooter (BGSS; USDA importation permit no. 53604). During this time, we began and completed data collection of AC waveforms using 20-hour access periods, fine-tuned procedures for *in situ* staining of salivary sheaths in grape tissues, and used that technique to perform preliminary tissue correlations of putative ingestion waveforms (Figure 1). Almeida is measuring and analyzing his waveforms in Berkeley, with advice from Backus via email. We suspect that AC waveforms of GWSS will be similar to those of BGSS. Preliminary results support unique appearances for xylem- and mesophyll-ingesting waveforms (the latter not shown in Figure 1).

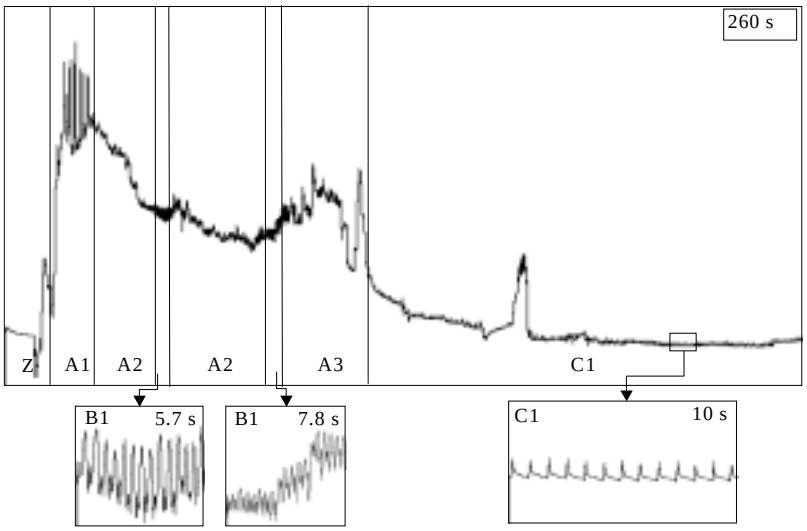

Figure 1. AC waveforms for the beginning of a representative stylet probe by BGSS on 'Cabernet Sauvignon' grapevine. Z = non-probing, A1 = high amplitude pathway activities, probably secretion of most of the salivary sheath (esp. spikes), A2 = descending-voltage pathway activities, B1 = high-frequency pre-ingestion activities, perhaps sap-sampling, A3 = variable amplitude pathway activities, C1 = xylem ingestion (correlated with watery excretion). (Provided by R. Almeida.)

B. Backus worked with Campus Facilities electrical engineers to install, test and ground a new, copper-screen Faraday cage for total elimination of in-line and external electrical noise. The cage is wired to an exterior earth ground and all EPG equipment is powered through a trans-former-cleaned, dedicated electrical main. Construction was completed before Almeida's research. Waveforms from his project were cleaner than any previously seen, making possible the detection of fine-structure waveforms (i.e. B1 in Figure 1) that will be crucial for the GWSS project.

C. The position of the post-doc who will perform the bulk of the research was advertised. An offer was made to an outstanding candidate and accepted, but hiring will depend upon receipt of an appropriate visa. Advertising continues, in case the visa is unavailable. The target start-date is 1 January 2002.

D. Backus applied for and received the USDA-APHIS-PPQ permit for importation of GWSS into Missouri (permit no. 54181). Also, while Almeida was in the lab, he instructed lab personnel in care and rearing of grapevine test plants, as well as the suggested GWSS rearing hosts, mugwort, basil and okra. We also acquired and fine-tuned the rearing of glabrous soybean (per the suggestion of R. Mizell, University of Florida), and rented extra greenhouse space.

E. We are modifying our existing insectary to handle the special needs of GWSS colonies, according to advice provided by Almeida and collaborator Matthew Blua. We are designing and building new rearing cages, and working with engineers at Campus Facilities to plan a renovation of two under-utilized, walk-in rearing rooms in the department. Renovation will provide better lighting, temperature and humidity control, drip-irrigation system for plant watering, as well as air locks and airflow requirements for quarantined insects. Backus wrote a campus grant proposal for $37,000 to match $30,000 in department and unit funds for this renovation.

F. We have purchased (or are in the final stages of purchasing) most of the supplies and equipment budgeted in the proposal (e.g. new optical fiber lamps, plant and insect rearing supplies, Windaq and Observer hardware and software, and computer). We have tested demonstration models of microtomes in our lab, and are about to order one. We expect to purchase other pieces of equipment in October and November. Backus is also working with her consulting electronics technician, Bill Bennett, to design and build the new AC-DC EPG monitor. Preliminary prototypes have been tested, and a final design is nearing completion. Construction will begin in November.

In conclusion, Backus and her co-workers are successfully implementing sweeping changes to her lab personnel, equipment and infrastructure, in preparation for the GWSS research. This includes purchasing supplies and equipment, designing and building EPG monitors, renovating electrical wiring and rearing rooms, applying for and receiving permits and visas, and hiring a post-doc. We are on target to begin the main research effort in January 2002.

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SAMPLING, SEASONAL ABUNDANCE, AND COMPARATIVE DISPERSAL OF GLASSY-WINGED SHARPSHOOTERS IN CITRUS AND GRAPES

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INTRODUCTION

The glassy-winged sharpshooter (GWSS) was first detected in California in 1990 (Sorenson and Gill 1996), and has since spread throughout southern California and into parts of Kern County (Blua et al. 1999). This insect feeds on a variety of ornamental and crop plants, some of which are susceptible to the bacterium, *Xylella fastidiosa.* Management of this pest will require a robust sampling plan for estimating population densities, as well as a better understanding of the factors that influence its dispersal and subsequent spread of Pierce's disease (PD).

OBJECTIVES

1. Develop, test and deliver a statistically-sound sampling plan for estimating density and inoculum potential of GWSS for research and management application.
2. Compare rates of movement between GWSS and the native smoke-tree sharpshooter (STSS) to better understand changes in the spread of PD.
3. Correlate the effects of crowding, sex ratio, reproductive status, host-plant quality and environmental variables with population dynamics and movement of GWSS as an aid to predicting insect and disease spread.

RESULTS AND CONCLUSIONS

An effective sampling method maximizes precision while minimizing time and costs. Four sampling tools are currently under evaluation in citrus orchards. The tools include bucket (5 gallon plastic bucket attached to an extendable pole), beat net (sweep net to collect dislodged insects), and A-Vac and D-Vac samplers (gasoline-powered suction devices). Although data is still being gathered that will ultimately support a recommendation for an optimal sampling tool, a number of preliminary observations can be made concerning the practical aspects of each candidate device. Both the bucket sampler and the beat net are easy to use as simple hand-held devices. Each one effectively captures GWSS nymphs and adults. The bucket sampler, however, is more efficient at funneling the captured insects into the collecting jar while leaving leaves and other debris behind in the bucket, whereas all contents in the beat net end up in the sample container and lead to more sorting time. The A-Vac and D-Vac require considerably more effort and preparation to acquire samples from citrus trees. The D-Vac is more sensitive than the A-Vac at detecting GWSS at lower densities by virtue of its much larger diameter suction tube.

During the past 6 months, the bucket sampler has been used to monitor population densities of GWSS in Riverside citrus orchards. It has proven sensitive to changes in population densities as nymphal stages during the spring months transformed into adults beginning in June (Figure 1A). The coefficient of variation for these data has remained relatively constant throughout the sampling period, ranging between 28 and 70 (Figure1B).

We also have established the validity of using an immunoglobulin protein marker (IgG) (Hagler et al. 1992, Hagler 1997, Hagler and Jackson 2001) for GWSS dispersal studies. Field trials indicated that the marker remained effective for a minimum of 19 days, with no significant effect on mortality relative to controls. Additionally, in our mark-release-recapture (MRR) studies, four concentrations (0.04, 0.2, 1 and 5 mg/mL) and two different IgG markers (chicken and rabbit) were found to be equally effective for marking sharpshooters. Our MRR studies were conducted from late July through the end of August, with releases ranging from approximately 10,000 to 20,000 insects (Table 1). Approximately 95% of the marked insects took flight within 3 hr after release. Takeoff rates were influenced by temperature and windspeed. Temperatures

above 17° C were correlated with an increased number of take offs, and windspeeds more than 5 mph were correlated with a decline in take-off activity. Recapture rates were determined after 3 to 24 hr, and it was determined that a 5-6 hr release was sufficient for the setting in which these trials were conducted (abandoned alfalfa field). Trials run over a 24-hr period only led to a 1.2% increase in the number of sharpshooters recaptured. The majority of sharpshooters (72-78% for GWSS and 55-57% for STSS) were recaptured 10 m from the release site on the three lowest traps (0, 1.4 and 2.8 m). However, a smaller percentage of both species were recaptured at the furthest traps (90 m), and up to 7 m in height (Figure 2A). As a percentage of those released, STSS were more likely to be recaptured at 50, 70 and 90 m than GWSS. At the 70 and 90 m annuli, the vertical distribution of both species was more evenly distributed than in the 10 to 50 m annuli (Figure 2B). Based on sex ratios before and after the releases, females were initiating flights more often than males. Females that remained on the host plants weighed significantly more than females that initiated flight, and although these studies have not been completed, we suspect that these females may have a higher egg load.

These findings suggest that the increased incidence of PD that has been attributed to GWSS, cannot be directly attributed to an increased capacity to disperse relative to the native STSS. It is more likely that their expanded host range, and higher densities are more important in explaining the increased incidence of PD. These studies have provided some evidence that gender, reproductive status, and species, as well as environmental parameters, are important in understanding the movement of sharpshooters.

Figure 1. Mean densities (± SEM) of GWSS in Riverside, CA Valencia orange groves (A) using a bucket sampler. Also presented are the corresponding coefficients of variations for the mean densities of GWSS for each date (B).

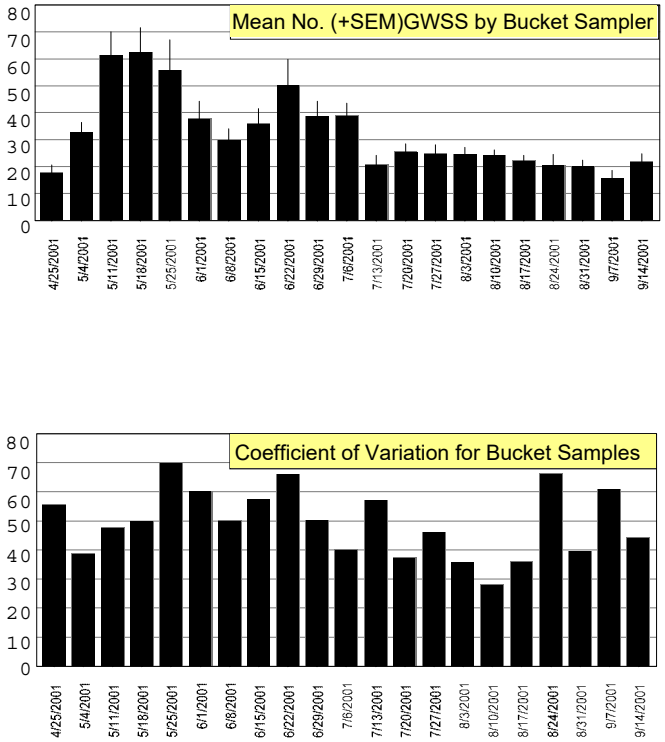

Figure 2. Percentage of male and female glassy-winged (GW) and smoke tree (ST) sharpshooters recaptured relative to A) distance and B) trap height and distance.

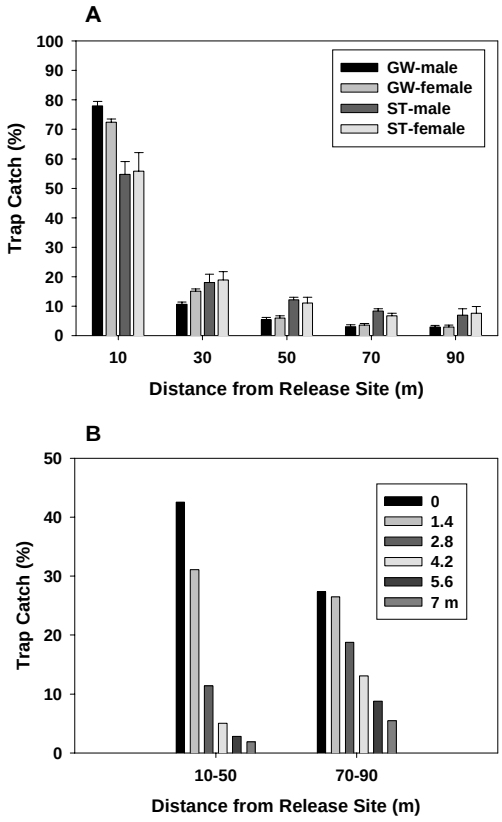

Table 1. Parameters for mark-release-recapture trials, Moreno Valley, CA, 2001

Trial	Date	Released (#)	Recaptured (%)	Remained on Host (%)
1	7-26	13800 GW/ 5000 ST	11.5 GW/ 8.4 ST	4.3 GW/ 0.5 ST
2	8-10	13300 GW/ 7700 ST	14.8 GW/ 5.5 ST	3.9 GW/ 1.7 ST
3	8-17	11600 GW/ 4500 ST	5.2 GW/ 6.1 ST	10.2 GW/ 6.9 ST
4	8-30	8500 GW/ 1200 ST	14.2 GW/ 32.4 ST	2.7 GW/ 4.6 ST

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IMPACT OF SUB-LETHAL DOSES OF NEONICOTINOIDS ON GLASSY-WINGED SHARPSHOOTER FEEDING AND TRANSMISSION OF PIERCE'S DISEASE

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INTRODUCTION

The current epidemic of Pierce's disease (PD) of grapevines spread by the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*, has characteristics that are remarkably different from outbreaks previously known in California since the 1880s (Gardner and Hewitt 1974). At the crux of this difference is the remarkable speed of these epidemics, and the distance of disease spread from the edge of the vineyard (Perring et al, 2001). The most important aspects of the GWSS that account for the rapid epidemic of PD are twofold: (1) the GWSS has a propensity to fly long distances into a vineyard, thus spreading PD far and affecting a large percentage of grapevines, in contrast to the traditional spread of PD to the first several rows of a vineyard, and (2) GWSS can feed on woody grapevine branches, accounting for what appears to be vine-to-vine spread of PD in California, a phenomenon previously unknown.

Management of PD that is spread by the GWSS is lacking a fundamental strategy and solid tactics. Although grape growing in areas with "traditional" California vectors (e.g. blue-green, red-headed, and green sharpshooters) is possible and practical, growing in the presence of high populations of the GWSS is not. Moreover, the GWSS is currently spreading throughout California, and will without doubt spread PD in other areas, thus putting grape-growing areas in the entire state at risk (Calif. Dept Food Agric. 2000). Scientists are exploring avenues that attack the plant-pathogen interface (B. Kirkpatrick & E. Civerolo, UC Davis, D. Cooksey, UC Riverside), yet currently there are no therapies available to "cure" infected plants. This makes vector control not only helpful but also imperative. An important goal to minimize PD spread by the GWSS will include reducing GWSS population densities in vineyards with insecticides. Removing diseased vines will be an important way of minimizing acquisition of the PD bacterium by the GWSS, but chemicals that reduce vector feeding may also keep infectivity of vectors at a low level.

OBJECTIVES

In experiments that examined the impact of neonicotinoids on GWSS mortality through time after treatment, we noticed little or no GWSS excreta on sleeve cages containing the sharpshooters on experimental plants treated versus those not treated, even if mortality was not induced. This provided impetus to ask the question: Can sublethal doses of neonicotinoids keep the GWSS from feeding and thus inhibit the PD epidemic? The objective of this ongoing project is to ascertain the degree to which neonicotinoids inhibit the spread of Pierce's disease by influencing GWSS feeding, and thus the acquisition and transmission of *X. fastidiosa*.

RESULTS AND CONCLUSIONS

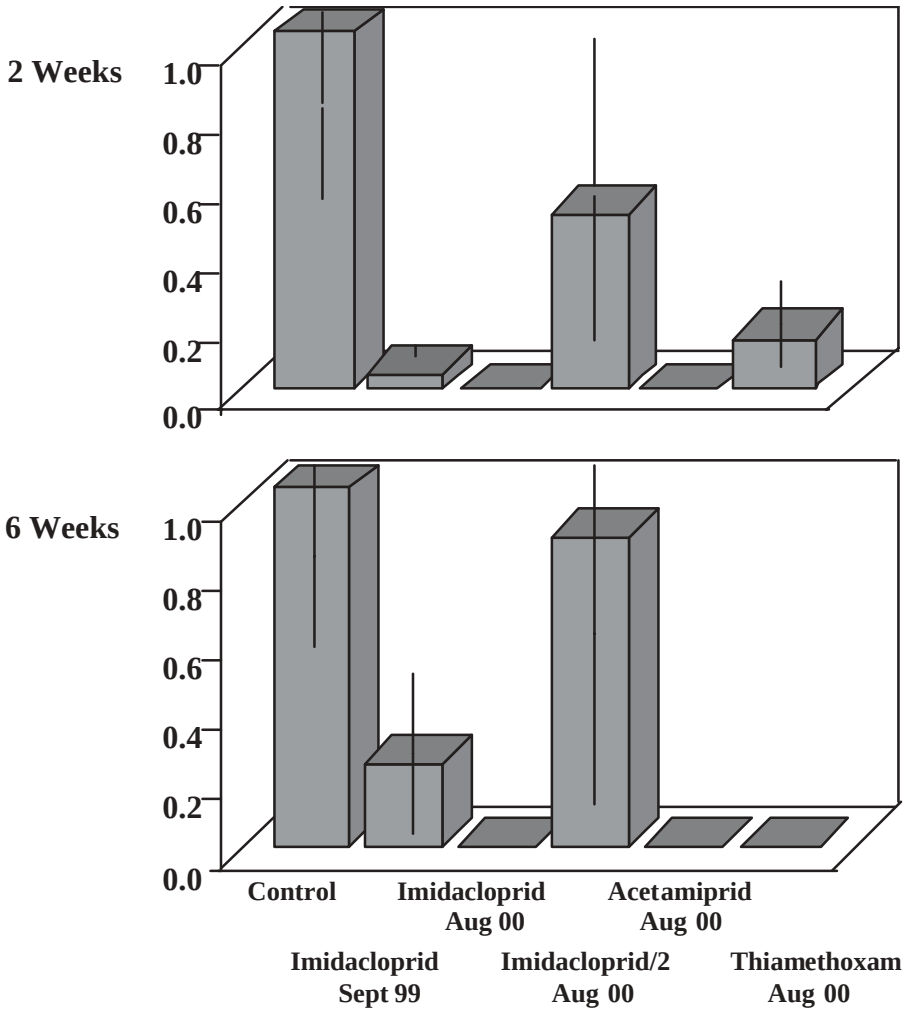
In an experiment conducted at the University of California, Riverside, from August through September 2000, sharpshooters were caged for 2 hours on field-grown grapevines treated with neonicotinoids or left untreated. These neonicotinoids included soil-applied formulations of imidacloprid (Admire 2F, Bayer, Inc.) at full label rate (561 g AI/Ha) applied to plants in September 1999 or August 2000, and one-half label rate (280 g AI/Ha) applied in August 2000; acetamiprid (Assail 70 WP, Aventis, Inc.) applied as a foliar spray at full label rate (56 g AI/Ha) in August 2000; and thiamethoxam (Platinum 2SC, Syngentia, Inc.) applied to the soil at full label rate (289 g AI/Ha) in August 2000. Sharpshooters on neonicotinoid-treated grapevines generated substantially less excreta than sharpshooters on plants that were not treated at both 2 and 6 weeks after the August 2000 insecticide applications (Figure 1).

Most striking is our observation that imidacloprid applied to grapevines in September 1999 had a substantial impact on GWSS feeding almost a year later (Figure 1). This may, in fact, be more important to protecting plants from *X. fastidiosa*-carrying sharpshooters than inducing mortality.

We have not documented the impact of neonicotinoids on acquisition or inoculation of the PD bacterium. Yet our greenhouse experiments with the strain of *X. fastidiosa* that induces oleander leaf scorch show that transmission of *X. fastidiosa* by the GWSS was blocked by applications of foliar-applied acetamiprid, soil-applied imidacloprid, and thiamethoxam (Bethke et al. *In press*).

Insecticides to control GWSS population densities and disease spread in vineyards will undoubtedly be part of a management strategy to control outbreaks of PD. Two aspects of insecticides are necessary: (1) they must affect GWSS immediately after they arrive on a vine; and (2) they must remain efficacious for a long time. Since 1998, we have experimented with insecticides to reduce GWSS population densities (Blua et al. 2000). Future experiments will examine in more detail the impact of neonicotinoids on GWSS feeding behavior and the acquisition and transmission of *X. fastidiosa*.

Figure 1. Volume of excreta produced by GWSS on grapevines treated with various neonicotinoids relative to untreated control vines 2 and 6 weeks after August 2000 treatments.



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EPIDEMIOLOGY OF *XYLELLA FASTIDIOSA* DISEASES IN CALIFORNIA

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INTRODUCTION

Several diseases of agronomic, horticultural, and landscape ornamental plants in California are caused by *Xylella fastidiosa* (*Xf*) (Purcell and Hopkins, 1996, Purcell et al. 1999). Effective disease management strategies are dependent upon knowledge of inoculum sources, biology and ecology of *Xf* insect vectors (e.g., glassy-winged sharpshooter [GWSS]) and natural enemies, and characteristics of disease progress. In the short term, commercially available materials may be useful in vineyards to reduce *Xf* transmission by the GWSS (e.g., Surround, containing 95% Kaolin), and to prevent *Xf* infection of grapevines and Pierce's disease (PD) development (e.g., systemic acquired resistance inducers, such as Messenger, containing 3% harpin).

OBJECTIVES

1. Determine the incidence and severity of PD in the Area-wide GWSS Management Pilot Project area to determine the temporal and spatial rate of disease increase.
2. Monitor the incidence and severity of PD in untreated and particle film barrier (Surround) treated vines.
3. Initiate evaluation of induced systemic acquired resistance with a commercially available harpin-containing product (Messenger).
4. Determine the oviposition host preferences of GWSS in citrus in Southern California, and determine the geographic distribution of GWSS oviposition in citrus throughout Southern California.
5. Survey the extent of GWSS egg parasitism and predation in Southern California.

RESULTS AND CONCLUSIONS

The incidence of PD in the different vineyards in the Area-wide GWSS Management Pilot Project Area ranged from 2% to 80% and the disease severity ratings ranged from 1 (less than 25% of the canopy exhibiting PD symptoms) to 4 (dead).

The spatial and temporal development of PD in Red Globe (3 plots) and Flame Seedless (1 plot) was monitored from May 15 to September 15, 2001, in two commercial vineyards naturally infested with *Xf*, and the data were analyzed as described by Campbell and Madden, 1990; Tubajika et al. 1999. Incidence and severity were regressed on time without transformation and after Gompertz [Y= -1n(-1n(-1n(y))], Logistic [Y=1n(y/1-y), monomolecular [Y=1n(1/(1-y))], and Logarithmic [Y= 1n(y)] transformations as previously described (Campbell and Madden, 1990; Tubajika et al. 1999). Similarly, PD incidence and severity were regressed on distance without transformation and after logit(y)-log(x), log(y)-log(x), log(y)- linear (x), and logit(y) - linear(x) models. Coefficient of determination, the standard error of Y-estimate, and significance of estimate slope parameter, the mean square errors, and the patterns of residuals versus predicted values were used to evaluate the goodness-of-fit of data and to choose the regression model. No disease gradients were discernible in any of the plots. A theoretical model of disease progress in time based on the Gompertz model for time adequately described several epidemics of PD during all experiments (Table 1).

Table 1. Average parameters of five models for regression of transformed Pierce's disease incidence in three plots in a commercial vineyard over time.

Model	Intercept	Slope	R^2	SEE
Gompertz	-12.287	0.061	0.945	0.478
Logarithmic	-12.574	0.052	0.558	1.505
Logistic	-20.694	0.095	0.833	1.392
Monomolecular	-8.120	0.044	0.911	0.443
Linear	-1.995	0.012	0.925	0.109

Because of low PD incidence and severity of PD in plot C37, data on incidence and severity of PD were not used. There was a clustering of diseased vines in the center of plot 2 and a random distribution of diseased vines away from the center—that is, there did not seem to be any directionality to the increase in PD incidence from the presumed focus in 2001. Disease spread tended to be greater within rows than across rows and disease spread quickly and unilaterally down rows. A clustering of diseased vines observed in the center could have been due to the presence of young vines in the area.

Three grapevine varieties (Flame Seedless, Thompson Seedless, and Chenin Blanc) in four blocks were treated three times beginning March 30, 2001, with Surround. Overall PD incidence in Surround-treated plots was 30% lower than in untreated plots. PD incidence was less in Thompson Seedless (1%) than in Flame Seedless (8%) and Chenin Blanc (9%). There was no difference in PD incidence between Surround treated and untreated Thompson Seedless plots.

A field trial was established in a vineyard in Tulare County to determine the effect(s) of Messenger on PD incidence using three rates (2.25, 4.50 and 6.50 oz/50 gal) of the material. Treatments were arranged in a randomized complete block design with 5 replications. The incidence of PD in grapevines was 16%, 2% and 9% in grapevines treated with 2.25, 4.50 and 6.50 oz Messenger/50 gal, respectively. PD incidence in untreated control vines was 27%. The highest rate (6.50 oz Messenger/50 gal) did not affect the development of PD. This may be explained by the presence of *Xf* –infected vines before the trial was established.

Field surveys were conducted in citrus groves (oranges, grapefruits, lemons, tangerines) in Southern California (San Marcos, Fallbrook, Pala, Temecula, Riverside, Redlands, Indio) of GWSS oviposition preferences and the extent of egg parasitism and predation. GWSS egg masses left leaf scars that remained on citrus trees for up to several years, because citrus trees retain their leaves for years and the scars do not heal. GWSS oviposition activity in the citrus groves surveyed was highest in Pala, followed by Riverside, Redlands, Temecula and San Marcos, with Fallbrook and Indio having the lowest level of GWSS oviposition activity. In the surveyed trees, GWSS preferred to oviposit in grapefruit, followed by orange, lemon, and tangerine (Table 3).

Table 2. Geographic distribution of GWSS in citrus groves in Southern California.

Location	Avg. No. GWSS Egg Mass Scars per Tree (N=240)
Pala	73.0
Riverside	55.5
Redlands	29.3
Temecula	10.0
San Marcos	6.0
Fallbrook	4.0
Indio	4.0

Table 3. Host preference for GWSS oviposition in citrus groves in Southern California.

Citrus Type	Avg. No. GWSS Egg Mass Scars per Tree (N=240)
Grapefruit	46.8
Orange	27.1
Lemon	9.0
Tangerine	2.3

Based on examination of leaf scars, when eggs successfully hatched there were exit holes only at the margins of the egg masses. Parasitoid wasps left exit holes in the middle of the egg masses. The size of wasp exit holes also could be used to determine the species of parasitic wasps. When eggs were eaten by predators, the entire leaf epidermal surface above the egg masses was removed.

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DEVELOPMENT OF AN ARTIFICIAL DIET FOR THE GLASSY-WINGED SHARPSHOOTER

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INTRODUCTION

The glassy-winged sharpshooter (GWSS) (*Homalodisca coagulata*) is a serious pest of grapes and a number of other crops. Its most destructive characteristic is that it is an efficient vector of the bacterium that causes Pierce's disease (*Xylella fastidiosa*). The management of this pest will, in all likelihood, require a multilateral set of approaches. Several of these approaches would be enhanced if the GWSS could be reared on a large-scale basis, especially with an artificial diet. Development of such a diet presents challenging barriers, including formulation of a liquid diet that closely simulates the xylem sap that is the target of GWSS. Understanding the feeding biology of this pest is complicated by the fact that it has cosmopolitan selection of hosts: i.e., it is a promiscuous feeder. However, formulation of a suitable diet is complicated by the fact that this species, which is a strict xylem sap-feeder, must be accommodated with a diet presentation system that simulates the vascular system of plants. Because the development of the diet and the feeding system must be done simultaneously, the probability of success of the entire project is reduced.

The approaches to be taken to increase the chances of complete success include the analysis of xylem sap from several host plants, modeling the diets to be bioassayed after those analyses, attempting to use non-flowing and flowing systems of diet delivery, and microscopic studies of the feeding choices made by GWSS when using their natural hosts. Should all of these approaches work suitably, the need for an oviposition system will be born, and it will become a further aim of this project to develop an in vitro-based system that will allow harvesting of GWSS eggs with maximum efficiency.

OBJECTIVES

1. Development of an artificial diet that will permit complete development of GWSS and reproduction of continuous generations of healthy individuals.

2. Development of a feeding system that will allow efficient delivery of diet to the GWSS.

3. Development of an oviposition system for in vitro egg production and efficient harvesting of the eggs.

RESULTS AND CONCLUSIONS

At the time that this report is being written, the funds have just been put in place, so there has been no research progress. We have set up our greenhouse, gotten the permit to have the GWSS shipped, and we have set up our analytical equipment to do the required amino acid and carbohydrate analyses.

FUNCTIONAL GENOMICS OF THE GRAPE-*XYLELLA* INTERACTION: TOWARDS THE IDENTIFICATION OF HOST RESISTANCE DETERMINANTS

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INTRODUCTION

Pierce's disease (PD), caused by *Xylella fastidiosa (Xf)*, is one of the most important diseases of grapevines (Purcell and Hopkins, 1996). Currently, the development of resistant varieties through classical breeding is limited by the absence of resistant phenotypes in *Vitis vinifera*. On the other hand, several wild grape species, not suitable for wine production, are known to either resist or tolerate infection by *Xf*. Therefore, an alternative approach for the development of resistance in cultivated grapes is to identify transcriptional pathways correlated with susceptible or resistant interactions in *Vitis* species. In principle, comparison of these two distinct interactions will reveal functional elements of the host resistance response, or conversely host functions that confer susceptibility (Cummings and Relman, 2000).

The experimental strategies outlined below will use genomics technology to identify genes in *Vitis* species that may be causal to host susceptibility (in the case of *V. vinifera*) or resistance/tolerance (in the case of native *Vitis* species). Such information will considerably increase our knowledge of the *Xylella*-grape interaction, and potentially provide the basis for developing resistance to the PD pathogen in *V. vinifera*.

OBJECTIVES

1. Construction of cDNA libraries from infected and non-infected grape plants of both susceptible *V. vinifera* and tolerant/resistant *Vitis* species (e.g., *V. shuttleworthii* or *V. aestivalis* complex).

2. A total of 30,000 DNA sequencing reactions will be completed in the first year of the project from cDNA products of the above libraries. The resulting sequence information (i.e., Expressed Sequence Tags (ESTs) [Marra et al., 1998]) will be submitted to the National Center for Biotechnology Information (NCBI) in a simple annotated format.

3. An online relational database will be developed in Oracle 8 to distill relationships within the data, and in particular to estimate a minimum gene set expressed during *Xylella*-grape interactions. A Web-based interface to the project database will make the results of this project available to all Pierce's disease researchers, with the intent of stimulating interaction among scientists and accelerating progress towards control of the *Xylella* pathogen in cultivated grapes.

4. Subsequent to EST sequencing and electronic data mining, we will employ functional genomics strategies to first verify and then dissect host gene expression in both susceptible and tolerant/resistant grape genotypes.

RESULTS AND CONCLUSIONS

In the first phase of the project, a total of 120 individuals from both Chardonnay and Cabernet plants (60 plants for each variety) in the Napa Valley of California were randomly selected. For each grape variety, 30 plants were selected located close to riparian areas with a previous history of PD infection and 30 plants were selected located distally from the riparian areas without previous PD infection. At two-week intervals, plants were analyzed for PD using a PCR-based approach with *Xylella*-specific primers (Kim et al., *In Preparation*). By early July the first symptoms of PD infection were observed, and PCR analysis confirmed that two Chardonnay and three Cabernet plants were infected by *Xylella*. Leaves and petioles from these plants were collected and stored at –80 °C until further use for cDNA library construction. These same plants

gave positive PCR results in subsequent weeks, thus confirming the original diagnosis. By late September the frequency and severity of PD symptoms had increased, and tissue was again sampled for cDNA library construction immediately prior to the grape harvest. cDNA libraries are being constructed from these infected and non-infected plants, at time points corresponding to early and late disease development (i.e., early July and late September). DNA sequencing reactions are being carried out at the UC Davis College of Agricultural and Environmental Sciences Core Genome Facility (http://cgf.ucdavis.edu). By March 2002 a total of 30,000 cDNAs will be sequenced and analyzed. These data, corresponding to differences in the transcriptional profiles between infected and non-infected plants, are expected to include host resistance and susceptibility factors. Thus, they will provide the basis for new lines of experimental inquiry focused on testing the efficacy of specific host genes for PD resistance.

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BIOLOGICAL CONTROL OF PIERCE'S DISEASE WITH NON-PATHOGENIC STRAINS OF *XYLELLA FASTIDIOSA*

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INTRODUCTION

Competitive exclusion of plant pathogens with nonpathogenic or less virulent strains has been demonstrated for a number of bacterial, fungal, and viral pathogens. Many nonpathogenic mutants retain the ability to colonize either external or internal plant tissues, and if established first, can effectively compete for colonization and establishment of pathogenic strains. One advantage of this approach for biological control is that the biocontrol agent and target pathogen occupy the same niche and have similar requirements for growth and survival. In addition, the specificity of the biocontrol interaction reduces the possibility of undesirable non-target effects. We propose to construct several nonpathogenic derivatives of *Xylella fastidiosa (Xf)* and test them for preemptive competitive exclusion of pathogenic strains in grape. In practice, such strains could be established in plants at the nursery level or potentially inoculated to mature vines. To construct nonpathogenic mutants, we are taking advantage of the completed genome sequence of the citrus variegated chlorosis (CVC) strain of *Xf* and the rough genome sequence of the almond leaf scorch strain, which is closely related to the PD strains. We predict that genes that are likely to be required for pathogenicity can be identified through comparison of these sequences with known pathogenicity gene sequences from its nearest relative, *Xanthomonas campestris*, or other plant or animal pathogens. PCR methods are being used to amplify these genes from the Pierce's disease strain of *Xf*. Deletions are being created in the genes, and homologous recombination will be used to introduce each deletion independently into the Pierce's disease strain. Each mutant will be tested for virulence and systemic colonization of grapevines, as well as the ability to competitively reduce populations of a pathogenic strain and reduce expression of symptoms.

OBJECTIVES

1. Construct deletion mutations in putative virulence genes of *Xylella fastidiosa*.
2. Test mutant strains for virulence in grapevines.
3. Test mutant strains for biological control of pathogenic strains in grapevines.

RESULTS AND CONCLUSIONS

In our first year, we have cloned a number of putative virulence genes that were identified from the full genome sequence of the CVC strain of *Xf* and from additional sequence information for an oleander and an almond leaf scorch strain available from the DOE-JGI. The genes we have amplified or cloned (in bold) and others we intend to target are as follows:

Genes encoding potential adhesins:

- ***pil*** genes encoding type IV pili
- Three large genes encoding hemagglutinin-like proteins, most closely related to *pspA* in *Neisseria meningitidis*
- Other afimbrial ahesin genes similar to: *hsf* and *hia* from *Hemophilus influenzae; uspA1* from *Moraxella catarrhalis*.

Xanthan gum biosynthesis genes:

- The entire xanthan gum biosynthetic operon (**9 *gum*** genes)

Regulatory genes known to control virulence in *Xanthomonas* or other pathogens:

- *gacA*
- ***rpfB, rpfF***
- ***rsmA***
- ***sphIM*** (Dam methylase)

Mutations have been constructed in several of the cloned genes, and we are attempting to insert them into a wild-type strain by electroporation and homologous recombination. Our initial attempts at transformation with standard plasmid vector systems were not successful. However, we have recently achieved stable transformation of *Xylella* with the vector pCL1920. We also had success with the transformation and mutagenesis system that Dr. Bruce Kirkpatrick at UC Davis recently published. We are therefore confident that our strategy will yield the desired mutants during this next year. These will then be tested for virulence and colonization of grapevines.

EPIDEMIOLOGY OF PIERCE'S DISEASE IN SOUTHERN CALIFORNIA: IDENTIFYING INOCULUM SOURCES AND TRANSMISSION PATHWAYS

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INTRODUCTION

Previous studies on the epidemiology of Pierce's disease (PD) of grape in Northern California have described systems dealing with different primary vector species and different alternate host plants than those that are found in the Southern California systems. Understanding the role that other plant species in the Temecula area may play in spread of Pierce's disease of grapes could be critical to management decisions. In addition to dealing with different host plants, the feeding habits and host range of the primary vector of the pathogen in Southern California differ from other primary vector species in Northern California. Studies with the insect vector species present in Northern California suggest that the pathogen was primarily spread by vectors moving into vineyards from outside habitats, rather than spreading from vine to vine. There is little information available on the relative ability of the glassy-winged sharpshooter to acquire or transmit the Pierce's disease pathogen from vine to vine, or from alternate hosts to grape. Because in many cases the vineyards of the Temecula area are in close proximity to citrus groves, it is critical to know the relative inoculum pressure that citrus and other plant hosts may provide in that area. Knowledge of the source of disease inoculum from vectors, whether from inside or outside the vineyard, will be critical to development of management strategies for disease control, such as the choice and management of plant species surrounding vineyards. Results of these studies, combined with data on seasonal fluctuations of sharpshooter populations, will also allow us to estimate the time of year and the regions where pathogen pressure is the greatest, and management strategies can be adjusted appropriately.

OBJECTIVES

1. Determine which plant species near vineyards harbor *Xylella fastidiosa (Xf)* and serve as potential reservoirs of inoculum for the spread of Pierce's disease to grapes.

2. Measure the ability of the glassy-winged sharpshooter to acquire and transmit *Xf* to and from grape, citrus, almond, and other plant species identified as potential hosts and sources of inoculum for the spread of Pierce's disease.

3. Comparison of the sensitivity and specificity of various methods to screen large numbers of plant and insect samples for the presence of Pierce's disease.

RESULTS AND CONCLUSIONS

Detection of *Xylella fastidiosa* in various plant species. Our regular sampling of over 60 plant species at 10 sites in the Temecula area is continuing. Samples are macerated in buffer and plated on selective media for *Xf*. All samples are also processed for serological (ELISA) and/or DNA-based (PCR) detection. Thus far we have detected *Xf* in several plant species in Temecula, including grapevine, almond, oleander, and Spanish broom. For these hosts, positive results were found using ELISA, PCR, and culture methods. Detection in mirror plant by ELISA was supported by PCR results, but we were not able to culture *Xylella* from these samples due to culture contamination. Spanish broom gave consistently strong ELISA reactions comparable to that of symptomatic grapevines, suggesting that the bacterium achieves a high titer in this new host. Weak positive results using ELISA were sporadically found with wild mustard, coyote brush, and elderberry; however, we were not able to confirm these results with other methods, suggesting that they could have been false positives.

Transmission studies. We have initiated greenhouse transmission studies testing the relative ability of *Xylella* to infect grape, citrus, almond, oleander, blackberry, bougainvillea, *Vinca* sp., toyon, coyote brush, *Brassica nigra*, brittlebush, mule fat, sage, California buckwheat, sugar bush, and laurel sumac. Our preliminary results suggest that grape-to-grape transmission can occur, as was suspected based on the observed widespread occurrence of PD in Temecula vineyards. We can now rapidly and reliably assay the glassy-winged sharpshooter for the presence of the pathogen, so we can follow it

during each step of the transmission process. We have also initiated experiments testing the ability of insects to transmit *Xylella* from Spanish broom to grape.

Evaluation of detection methods. We are continuing to evaluate the effectiveness of various methods for detecting *Xylella* in plants and in the insect vector. Immunocapture PCR is now incorporated into our regular screening procedures. We also have had success in developing strain-specific detection that allows us to differentiate between infections with either the Pierce's disease or oleander leaf scorch strains of *Xylella*. This was done by designing a new set of PCR primers to amplify a gene involved in xanthan gum biosynthesis that is present in all *Xylella* strains characterized (based on comparative genomic analysis). However, the sequence of the gum gene differs slightly between strains, and this can be easily detected by digesting the amplified gene with specific restriction endonucleases. Amplification of the gum gene from a sample indicates that *Xylella* was present, and subsequent digestion of the amplified product and gel electrophoresis yields two bands for the grape strain and one band for the oleander strain, or vice versa, depending on which one of two restriction endonucleases are chosen. This ability to detect which plants and insects are truly infected with the Pierce's disease strain will be essential for interpretation of our survey results in the Temecula area.

CONTROL OF PIERCE'S DISEASE THROUGH DEGRADATION OF XANTHAN GUM

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INTRODUCTION

Pierce's disease of grapevine and other leaf scorch diseases caused by *Xylella fastidiosa* are associated with aggregation of the bacteria in xylem vessels, formation of a gummy matrix, and subsequent blockage of water uptake. In the closely related pathogen, *Xanthomonas campestris*, xanthan gum is known to be an important virulence factor, probably contributing to bacterial adhesion, aggregation, and plugging of xylem. The recently published genome sequence of the citrus variegated chlorosis (CVC) strain of *X. fastidiosa* revealed that this pathogen also has genes for xanthan gum production. This project is to identify bacteria that produce xanthan-degrading enzymes to target this specific virulence factor of *X. fastidiosa*. This approach has the potential to significantly reduce damage caused by Pierce's disease in grapes and potentially in other hosts of *X. fastidiosa*, such as almonds and oleander. If xanthan gum is important in the aggregation of the pathogen in the insect vector, then our approach may also reduce the efficiency of transmission of Pierce's disease. Our first approach will be to develop endophytic bacteria that produce these enzymes in the xylem of grapevines, but another approach is to engineer grape plants to produce these enzymes. Through the cloning and characterization of genes encoding xanthases and xanthan lyases, we will facilitate possible efforts to transform grapevines to produce these enzymes.

OBJECTIVES

1. Characterize xanthan-degrading enzymes from endophytic bacteria isolated from grape.
2. Explore applications of naturally occurring endophytic bacteria that produce xanthan-degrading enzymes for reduction of Pierce's disease and insect transmission.
3. Clone and characterize genes encoding xanthan-degrading enzymes for enzyme overproduction and construction of transgenic endophytes and plants.

RESULTS AND CONCLUSIONS

Comparative genomics for investigation of xanthan production in different strains. To determine whether the xanthan gum from the Pierce's disease strain of *X. fastidiosa* is likely to be structured as we have predicted from the CVC strain sequence, we compared the available genomic sequences of the closely related almond leaf scorch strain and the more distantly related oleander leaf scorch strain. All of these *Xylella* strains have the same nine *gum* genes conserved, and they all lack *gumG, gumI,* and *gumL* that are found in *Xanthomonas*. In *Xanthomonas, gumI* is thought to add the terminal mannose residue on the sugar side chains of the xanthan polymer. *gumG* and *gumL* add acetylate and pyruvate to the terminal mannose. The lack of these three genes in *Xylella* suggests that its xanthan gum lacks the terminal mannose on the side chains, which is predicted to reduce its viscosity slightly.

Initial isolation of xanthan-degrading bacteria. We predicted that a potential source of bacteria that degrade xanthan gum would be in natural settings where xanthan gum is present, such as plants infected with *Xanthomonas campestris* or *X. fastidiosa*. Following the techniques used by industrial microbiologists studying xanthan degradation, we found that both grapevines infected with Pierce's disease and oleanders infected with oleander leaf scorch contain bacteria that are capable of degrading xanthan gum. These were obtained through enrichment in minimal broth media containing commercial xanthan gum as the only carbon source. After two serial transfers into fresh media, we plated these cultures on an agar medium containing xanthan and have obtained pure cultures of bacteria that grow on xanthan gum as the sole carbon source and clear the turbidity that the xanthan produces in the medium. We have several different types of bacterial xanthan degraders from these initial attempts. However, these bacteria were selected for degradation of commercial xanthan gum, which is produced from *Xanthomonas*. Therefore, we needed to repeat these isolations using xanthan gum from *Xylella,* which is predicted to differ chemically.

Enzymatic activity against xanthan from *Xylella*. Purifying significant quantities of xanthan from the slow-growing *X. fastidiosa* is not practical. Therefore, we have constructed a mutant of *Xanthomonas campestris* lacking the *gumI* gene that is responsible for adding the terminal mannose. A large section of the *gum* operon was cloned from *Xanthomonas*, and an antibiotic resistance cassette was inserted into a deletion in the *gumI* gene. This construction was used to introduce the mutation into wild-type *X. campestris* by homologous recombination. We are confirming that this strain produces xanthan that is chemically similar to that from *Xylella*. Viscosimetric assays are also being conducted to compare the viscosity of *Xylella* and *Xanthomonas* xanthan, as well as to provide a quantitative measurement for the activity of xanthan-degrading enzymes. Using this new source of modified xanthan gum, we are in the process of sampling symptomatic grapevines from diverse sources to develop a broad collection of xanthan-degrading bacteria. Other tests will be performed to characterize the nature of xanthan-degrading enzymes from these bacteria. We anticipate that we will recover both xanthanases that break the cellulosic backbone of the xanthan polymer, and xanthan lyases that cleave the sugar side chains.

IMPACT OF MULTIPLE-STRAIN INFECTIONS OF *XYLELLA FASTIDIOSA* ON ACQUISITION AND TRANSMISSION BY THE GLASSY-WINGED SHARPSHOOTER

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INTRODUCTION

Xylella fastidiosa (Xf) is a xylem-limited bacterium that causes a number of plant diseases such as Pierce's disease (PD) of grapevines, almond leaf scorch, alfalfa dwarf, citrus variegated chlorosis, leaf scorch of live oak, pear leaf scorch, and oleander leaf scorch (OLS). Recent studies by Purcell et al. have shown that oleander leaf scorch is caused by a different strain of *Xf* than the strain that causes PD. Thus far, two strains of *Xf* have been identified in Southern California; one that causes Pierce's disease of grapevines and almond leaf scorch, and another one that causes oleander leaf scorch (OLS). The strain that infects oleander does not appear to infect grape, and the strain that infects grape does not appear to infect oleander.

We are interested in documenting the ability of glassy-winged sharpshooter (GWSS) to acquire and transmit more than one strain of *Xylella* at a time, and to determine the effects of a previous infection with one given strain of *Xf* on the subsequent acquisition and/or transmission of a second strain. We will document important aspects of the transmission process to determine 1) if the insects can only acquire and transmit one strain of the pathogen at a time, or if they are capable of transmitting more than one strain at a time, and 2) if the order of exposure is important to acquisition or transmission. This type of information is critical to the development of management strategies, particularly those that attempt to utilize mechanisms involving competitive less virulent strains of bacteria, or other methods of control that interfere with vector competence.

OBJECTIVE

Assess the ability of glassy-winged sharpshooter inoculated with multiple strains of *Xylella fastidiosa* to transmit any strain of the pathogen.

RESULTS AND CONCLUSIONS

Establishment of plants. A supply of healthy grape and oleander plants was obtained and established for use as test plants in transmission studies. All plants were confirmed negative for *Xylella* using ELISA. We also established a supply of grape plants (*Vitis* spp.) infected with the PD strain of *Xf*, and oleander plants *(Nerium oleander*) infected with the OLS strain as sources of inoculum. Repeated sampling of plant tissue has been done in source plants to confirm that they are positive by ELISA.

Large numbers of GWSS adults from the first generation were collected in the field and caged on plants in the greenhouses to allow oviposition. Eggs from these insects were collected just prior to hatching and moved to new cages with a variety of clean plants. The offspring were allowed to develop until adulthood. These insects were considered to be "clean," as they were never exposed to sources of *Xylella*. High mortality rates of insects occurred during development from the egg-to-adult stage in these colonies, resulting in low numbers of available clean adults. Thus, field collected insects were also used in some experiments to get preliminary data.

Transmission studies. Studies are being conducted in a series of experiments where groups of 25-100 GWSS adults are caged for 2 days on OLS infected oleander plants. These same insects are collected and subsequently moved to PD infected grape plants to feed for 2 days. Conversely, other groups of insects are first caged on PD infected grape plants, and subsequently caged on OLS infected oleander plants.

Sub-samples of these insects were removed after exposure to both species of plants and frozen for future analysis for the presence of either or both strains of the pathogen using culturing and PCR methods. We have demonstrated that the immunocapture and PCR methods we use can detect *Xf* in individual insects that fed on infected plants. In addition, digestion of positive PCR products with restriction endonucleases, followed by resolution by agarose gel electrophoresis, allows clear differentiation between the PD and OLS strains.

Additional sub-samples of insects from infected plants are transferred individually to healthy recipient oleander and grape test plants in a biological assay to test the ability of insects to transmit the pathogens to new hosts. Insects are allowed to feed on test plants for 4-5 days, then are removed and frozen for analysis by PCR.

Recipient test plants are maintained in the greenhouse, observed for symptom development, and tested periodically for the presence of *Xylella* using ELISA. Tissue from two sets of test plants has been sampled at two months after inoculation. Thus far, two grape plants and one oleander plant have already tested positive. It is expected that more plants will test positive on future sampling dates, since it is common for plants to take longer than two months to become systemically infected and test positive. All plants will be repeatedly tested for infection for up to one year after inoculation. Plants that test positive with ELISA will be subsequently tested with PCR to determine the strain(s) of *Xylella* present.

Summary of First Year Progress:

- Established a supply of healthy and infected plants for transmission studies, and confirmed status with ELISA.
- Initiated acquisition and transmission studies.
- PCR methods were developed to differentiate strains of *Xylella* in individual insects and plants.
- Thus far, two grape plants and one oleander test plant have already tested positive with ELISA. It is expected that more plants will test positive on future sampling dates, since it is common for plants to take longer than two months to become systemically infected and test positive.

ROOTSTOCK VARIETY INFLUENCE ON PIERCE'S DISEASE SYMPTOMS IN GRAFTED CHARDONNAY (*VITIS VINIFERA* L.) GRAPEVINES

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INTRODUCTION

Rootstocks are already widely in use in viticulture to manage damage from soil-borne pests and provide adaptation to particular soils. Grape rootstocks can impact the symptom expression of diseased scions in at least one disease (fanleaf degeneration). In other crops, rootstock variety has been reported to impact expression of *Xylella fastidiosa* diseases in scions (Gould et al. 1991). Pierce (1905) reported that rootstock variety affected expression of "California vine disease" (now known as Pierce's disease) in grape. Grape rootstock trials in Mississippi showed a large effect of rootstock trial on vine longevity in a region recognized for high Pierce's disease pressure (Magoon and Magness 1937, Loomis 1952, 1965). If grape rootstocks could contribute Pierce's disease resistance or tolerance to their scions, this would be a major benefit to viticulture in Pierce's disease prone areas. Elite wine, juice, and table grape varieties could be grown in areas where viticulture is currently restricted to Pierce's disease resistant and tolerant varieties whose consumer appeal is low.

OBJECTIVES

1. To evaluate the impact of rootstock variety on expression of Pierce's disease symptoms in the scion.
2. To assess any relationship between Pierce's disease symptoms on ungrafted rootstocks and the expression of Pierce's disease on susceptible scions grafted to those rootstocks.

RESULTS AND CONCLUSIONS

Chardonnay (*Vitis vinifera*) vines grafted on nine rootstocks and own-rooted Chardonnay vines were planted in Tallahassee, Florida in the vineyard of the Center for Viticulture, Florida A&M University in the spring 2001 planting season (Table 1). Ungrafted vines of the same nine rootstocks plus St. George were planted at the same location. The vineyard site has a high incidence of Pierce's disease and glassy-winged sharpshooters inhabit the site.

Pierce's disease (PD) symptoms were evaluated on September 5, October 5, and October 26, 2001 for Chardonnay vines and September 5 and October 26, 2001 for ungrafted rootstocks. Symptoms on leaves were assessed and vines given a numerical score from 0 to 5, with 0 representing no symptoms, 1 = minor symptoms up to 15% of leaves with marginal necrosis (MN), 2 = 15-30% of leaves with MN, 3 = 30-50% of leaves with MN, 4 = 50-75% of leaves with MN, 5 = over 75% of leaves with MN or vine dead. There were four replicates. Each replicate consisted of two vines of the same treatment, either grafted to the same rootstock or the same rootstock variety ungrafted. The mean score of the two vines is recorded as the score for that replicate.

Chardonnay vines showed symptoms on all rootstocks (Table 1). Every Chardonnay vine showed symptoms at some level. It is premature to make conclusions about the longer-term impact of rootstock on scion health, however. At the experimental vineyard site, PD pressure is sufficiently high that even some muscadine grapevines (*Vitis rotundifolia*) show PD symptoms. However, these muscadine vines do not succumb to PD, but recover. Additional scoring of symptoms in the grafted vines will be needed to determine if there is an effect of rootstock variety. Symptom evaluation will occur in the spring and fall of subsequent growing seasons.

Ungrafted vines of rootstock varieties exhibited a range of symptom levels (Table 1). Some O39-16 vines did not display symptoms of Pierce's disease, and this rootstock characteristically had less severe PD symptom expression. In contrast, Ramsey and St. George showed more severe PD symptoms. Interestingly, St. George has been considered to be PD tolerant under some conditions. The population of bacteria, level of inoculation, or cultural or climatic conditions could be impacting the PD symptom expression in St. George in this situation. The low level of symptom expression in O39-16 is consistent with the parentage of this rootstock. O39-16 is a *V. vinifera* x *V. rotundifolia* cross and may have derived its resistance to PD from *V. rotundifolia*, which is often regarded as being highly resistant to PD. Loomis (1952, 1965) reported that a different rootstock with *V. vinifera* and *V. rotundifolia* parentage extended the life of susceptible scions in

Mississippi. Correlations of symptom expression in ungrafted rootstocks and scions on those same rootstocks would be premature at this time.

Additional experiments are planned to further investigate the possible influence of rootstock on PD expression in susceptible scion varieties. More rootstocks will be grafted to Chardonnay, including Dog Ridge, 161-49C, Lenoir, Tampa, Florilush, Blue Lake, Lake Emerald, MidSouth, Daytona, and Miss Blanc. Dog Ridge and 161-49C have been reported as increasing vine longevity in areas of high PD pressure (Loomis 1952, 1965). Lenoir was suggested by Pierce (1905) as a rootstock to manage this disease. Tampa and Florilush are rootstocks developed in Florida, which are known to survive ungrafted in the face of high PD pressure. Blue Lake, Lake Emerald, MidSouth, Daytona, and Miss Blanc are scion varieties developed in the southern U.S. that are tolerant or resistant to PD. In addition to Chardonnay on these rootstocks, Cabernet Sauvignon will be grafted on all twenty rootstock varieties. The additional rootstock varieties will also be planted ungrafted to evaluate correlation of symptom expression in grafted scions and ungrafted rootstocks.

Table 1. Symptom expression in grafted Chardonnay scions, own-rooted Chardonnay, and ungrafted rootstocks. 0 = absence of symptoms, 5 = 75-100% of leaf area symptomatic.

Grafted Chardonnay, by rootstock variety	**Mean Symptom Expression**			**Ungrafted rootstocks, by variety**	**Mean Symptom Expression**	
Screening date (2001)	Sept 5	Oct 5	Oct 26		Sept 5	Oct 26
44-53M	2.0	3.7	4.5	**O39-16**	1.13	1.6
5C	2.0	2.5	3.9	**5C**	1.3	1.4
3309C	2.3	2.6	3.6	**Freedom**	1.4	2.8
O39-16	2.3	2.4	3.7	**3309C**	1.5	2.3
110R	2.3	3.3	4.3	**5BB**	1.8	2.3
Ramsey	2.4	3.1	4.0	**101-14**	1.8	2.2
Freedom	2.5	2.8	4.2	**44-53M**	1.9	2.7
Own-rooted	2.6	3.7	4.4	**110R**	2.3	3.1
5BB	2.9	2.4	4.0	**St. George**	2.4	1.9
101-14	2.9	3.4	4.5	**Ramsey**	2.7	2.9

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GENOME SEQUENCE OF A STRAIN OF *XYLELLA FASTIDIOSA* ASSOCIATED WITH PIERCE'S DISEASE IN CALIFORNIA

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INTRODUCTION

Several economically important diseases of agronomic and horticultural crops, as well as landscape and forest trees, are caused by different strains or pathogenic variants of *Xylella fastidiosa* (*Xf*). In California, these include (but are not necessarily limited to) alfalfa dwarf, almond leaf scorch, oleander leaf scorch, and Pierce's disease (PD). Other *Xf*-caused diseases that are potential threats to California agriculture are citrus variegated chlorosis (CVC), phony peach, and plum leaf scald. Recently, the complete genome sequence of *Xf* strain 9a5c, which causes CVC in Brazil, was determined (3). Such information is useful for increased understanding of *Xf*-host interactions in order to develop new disease management strategies. However, the nature of, and mechanism(s) involved in, differing host ranges of *Xf* strains are not completely understood. Therefore, comparative information about the genome structure, specifically the complete genome sequence, of another *Xf* strain (besides *Xf*-CVC strain 9a5c) could contribute to elucidation of *Xf*-host and *Xf*-insect vector interactions. Accordingly, the complete genome sequence of a strain of *Xf* associated with PD in California was determined through a cooperative project between the USDA-ARS (including AVF and CDFA) and FAPESP.

OBJECTIVE

Determine the complete genome sequence of a *Xylella fastidiosa* strain associated with Pierce's disease in California.

RESULTS AND CONCLUSIONS

Genome assembly of a strain of *Xf* associated with PD in California (*Xf*-PD [Temecula1]) was achieved through shotgun and cosmid sequencing by the AEG network from the ONSA-FAPESP program. The draft generated so far has 2,507,178 bp, which corresponds to 93.6 % of the previously sequenced *Xf* strain associated with citrus variegated chlorosis (*Xf*-CVC). In addition, only a miniplasmid consisting of 1.345 bp was found in the *Xf*-PD (Temecula1) genome. This plasmid is similar to the previously described miniplasmid in the genomes of other *Xf* strains.

Xf-PD v1.0 draft genome assembly was obtained after using "Scaffold" program (2) on our database consisting of 191 contigs generated from shotgun reads and cosmid ends. From the scaffolded genome, 14 cosmids and 2 plasmids were selected for further sequencing in order to close the genome. The overall base quality achieved for this genome is phred quality above 50 for 99.7 % of the nucleotide bases. Low quality regions are being screened to determine regions to have base quality improved.

The *Xf*-PD genome harbors large rearrangements compared to the *Xf*-CVC genome. These rearrangements include some translocation events as well as insertion/deletion (INDEL) events. INDEL events can either be represented by single missing or inserted gene or sets of genes, most being related to phage sequences. These structural differences were first identified by cross match analysis and the genes involved in these rearrangements are now being identified by the annotation of the genome.

Open reading frames (ORFs) were identified in the genome using the "GeneMark" program (1) and subsequently analyzed by a group of 40 annotators. Annotation is being carried in a first set of 1,935 identified ORFs. From these, 6.0% are exclusively ORFs from the *Xf*-PD genome while the *Xf*-CVC genome has 25% exclusive ORFs. Both the *Xf*-PD and *Xf*-CVC genomes have similar distributions of mobile genetic elements, which include phage, transposon and plasmid-related sequences.

There are ORFs in the *Xf*-PD genome that are present in more copies than in the *Xf*-CVC genome. Also, there are missing genes in the *Xf*-CVC genome that are missing in the *Xf*-PD genome. These are being carefully analyzed and the final description is dependent upon completion of the annotation process. Interestingly, there is a region in the *Xf*-CVC genome, which is missing in the *Xf*-PD genome. This region encompasses 76 genes corresponding to at least a deletion of 70,000 bp. Also, no group II intron was detected in the *Xf*-PD genome. On the other hand, there is at least one new phage insertion in the *Xf*-PD genome not described in *Xf*-CVC genome. These comparative analyses will help target specific genes in either strain to be studied as well as common genes that enable these bacteria to live in the plant xylem and in the insect foregut.

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APPLICATION OF *AGROBACTERIUM RHIZOGENES*-MEDIATED TRANSFORMATION STRATEGIES FOR (A) RAPID HIGH THROUGH PUT SCREEN FOR GENETIC RESISTANCE TO PIERCE'S DISEASE IN GRAPE THAT MAINTAINS THE CLONAL INTEGRITY OF THE RECIPIENT HOST, AND (B) RAPID SCREENING FOR VIRULENCE DETERMINANTS IN *XYLELLA FASTIDIOSA*

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Funds for this project were received 1 August 2001.

This brief report will summarize the results that have been accomplished for the two-month period August 1 to October 1, 2001 dating from the receipt of funds.

INTRODUCTION

The goal of this project is to identify novel genes from either grape or heterologous plants that, when expressed in grape, will lead to disruption of infection or spread of the xylem-limited bacteria, *Xylella fastidiosa.* There is no useful genetic resistance in commercially used grape clones, and introgression of resistance from grape relatives by sexual crossing introduces substantial genetic variation. Introgression of resistance would be most useful if it were introduced directly into vegetative tissue without requiring recurrent selection to attempt to return to the original host genotype. We have developed a functional screen for cDNAs that block either bacterial multiplication, movement or symptom expression using an *A. rhizogenes* mediated transformation strategy that also enables the direct introgression of cloned resistance genes into a susceptible host plant while maintaining the clonal integrity of the recipient plant following transformation.

OBJECTIVES

The research plan includes (a) a rapid functional screen for genes that confer resistance to Pierce's disease (PD) in transformed grape tissue and (b) an analysis of potential pathogenicity factors of the PD causative agent, the bacterium *X. fastidiosa.* Approximately 85% of the research effort is devoted to sub-objective (a) and 15% to sub-objective (b).

Sub-objective (a): Screening for resistance genes in *Agrobacterium rhizogenes*-induced *X. fastidiosa*-infected hairy root cultures. (85% effort)

The goal is to rapidly identify resistance genes in grape genotypes that block any one of several required steps in the infection and spread of *X. fastidiosa* in the xylem.

RESULTS AND CONCLUSIONS

Transformation with *A. rhizogenes*

The method of delivery of the cDNA libraries into grape is now established in our laboratory. In the pasts two months we

have confirmed that grape is readily transformed by *A. rhizogenes* and that foreign genes (green fluorescent protein, GFP), including our new cDNA libraries, can be expressed readily in grape by this method. In the case of the roots expressing GFP driven by the 35S promoter, all roots were highly fluorescent when viewed under a fluorescence microscope.

Transformation of infected grape with *A. rhizogenes*

We have established *Xylella* infections in the xylem of *V. vinifera* (Chardonnay) and transformed test genes into roots derived from infected stem sections by *A. rhizogenes*. Transformed root induction occurred equally well on both infected and healthy stem sections. Interestingly, and perhaps fortuitously, the roots from the healthy stem sections remain alive and growing after 2 months, however the roots that emerged from the infected stem sections appeared normal for about two weeks but then they stopped growing and eventually died. We have now repeated this result and believe it constitutes a direct assay for genes from the resistant background that block movement into or accumulation of bacteria in the very young roots that leads to root stress and eventually death. If this works, as now visualized, we will be able to screen the cDNA libraries without the GFP-transformed *X. fastidiosa*.

Construction of cDNA libraries

Isolation of grape mRNA proved to be a considerable challenge, not only for us but also for Dr. Cook's research group who have similar needs for clean RNA preparations from grape. In the past two months we have developed a procedure that now enables high yields of high quality RNA from grape. Dr. Cook's group now uses the procedure successfully; details of the procedure can be obtained by email. We have now begun the library construction from both healthy and infected grape tissue. The mRNA is being converted into cDNA and cloned into a binary plant transformation vector, a derivative of pBIN19, with the CaMV 35S promoter for high level, constitutive expression. Libraries are being made from *Vitis vinifera* (Chardonnay), *Muscadinia rotundifolia* (Cowart), and *Vitis shuttleworthii* (Hanes City) as indicated in the original proposal. These materials are being used in Dr. Walker's research and the libraries will be available to his group. The first screens will be done with the Cowart cDNA library with susceptible Chardonnay as a recipient host.

Transformation of *X. fastidiosa* with the green fluorescent protein (GFP)

Using the technique developed by Zhang et al. (2000), Guilhabert and Kirkpatrick introduced a kanamycin resistance gene into *X. fastidiosa*; by electroporating a Kan cartridge with inverted repeats and a bound transposase that catalyzes the insertion of the Kan resistance gene. However, our current results indicate that this method, although effective at generating very low level Kan resistance, is not effective in introducing GFP with expression at a level necessary to visualize the bacteria in the xylem of grape. We have now constructed a modified transposon that expresses Kan resistance and GFP from T7 RNA polymerase promoters. Also present at the very end of the transposon is a promoter-less T7 RNA polymerase gene. Following electroporation, we expect random insertions into the genome some of which will have integrated in a manner whereby a low level of T7 RNA polymerase (higher levels are toxic) is expressed, resulting in a large amount of Kan resistance and GFP expression. This method is currently being tested in *E. coli*.

Sub-objective (b) Identification of virulence genes in *X. fastidiosa* (15% effort)

Objective (b) is designed to identify those genes of the *Xylella fastidiosa* bacterium whose products are essential for virulence. The research described under objective b is directed by Dr. Bruening.

RESULTS AND CONCLUSIONS

A program aimed at recovering *X. fastidiosa* mutants requires a rapid simple assay for such mutants. Beginning in July of 2000, we developed an assay for biological activity of *X. fastidiosa* that requires only two days. Cell suspensions (0.1 to 0.4 A600 turbidity) are pressure infiltrated into inter-vein panels of 3-5 cm long leaves of *Chenopodium quinoa*. A chlorosis with small yellow to white spots, limited to the infiltrated area, develops in about 40 hr. Some tested *Xanthomas* species, which are closely related to *Xylella*, evoked no reaction on *C. quinoa*, whereas a few caused chlorosis or late necrosis (observations of Dr. Edwin Civerolo). Pseudomonads evoked necrosis. All six of the virulent *X. fastidiosa* strains tested to date induce chlorosis with spots on *C. quinoa*. More than 500 *X. fastidiosa* transposon mutants from the laboratory of Dr. Bruce Kirkpatrick were tested. None of these mutants failed to give chlorosis, suggesting that no transposon mutant tested has an insert in any region of the bacterial genome needed for chlorosis induction or that the factor(s) needed for chlorosis induction are essential to *X. fastidiosa*.

We observed that the chlorosis-inducing activity survived being heated to 100°C for 6 min or being incubated in detergent solution at 65°C for 30 min. These results suggested that the activity does not require a protein but might, for example, be due to a polysaccharide. However, incubation with any of three proteases eliminated or nearly eliminated the activity, suggesting that a protein component of unusual stability to heating and detergent contributes to the chlorosis-inducing activity. Experiments designed to identify the putative protein component of the *X. fastidiosa*-derived activity are in progress and will take advantage of genomic information available. Other research in progress explores the possible biological relationships between chlorosis on *C. quinoa* and the leaf inter-veinal chlorosis that is one of the symptoms of Pierce's disease in grape.

EFFICACY OF INSECTICIDES USED FOR GLASSY-WINGED SHARPSHOOTER CONTROL IN CITRUS

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INTRODUCTION

Glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*, is currently infesting more than 130 square miles of grapes, citrus, and urban landscapes in Kern County. Citrus is a preferred oviposition host for GWSS, thereby acting as a source of adults that move into grapes and potentially vector Pierce's disease (PD). The incidence of PD in Kern County was thought to be very low, however, after 4+ years of heavy GWSS densities in the General Beal Road area, the incidence is beginning to escalate. Kern County grape growers are aggressively treating their vineyards with pesticides and requesting that neighboring citrus growers treat their orchards as well. Research is needed to understand how efficacious citrus insecticides are against GWSS and how these additional treatments will disrupt citrus IPM. While vector control is not the long-term solution to the PD problem, it will be important in the short-term to slow the spread of the disease in Kern County and help delay the advance of GWSS into other agricultural areas of the San Joaquin Valley.

OBJECTIVE

1. Conduct pesticide trials in commercial citrus in Kern County to determine the efficacy of various registered insecticides against glassy-winged sharpshooter.

RESULTS AND CONCLUSIONS

A group of 10 citrus orchards in Kern County were periodically surveyed by UC Cooperative Extension personnel for pests and beneficials during 2000 and 2001 as a part of an IPM Demonstration program. The sampling method consisted of using a sweep net in the winter months and conducting a 2-minute visual search in the spring-fall months of 20 trees per orchard. Yellow sticky cards were monitored monthly to measure densities of *Gonatocerus ashmeadi*. Glassy-winged sharpshooter was present in all orchards during early 2000 and so we were able to observe how the standard insecticide practices, as well as sprays targeting GWSS, affected their populations.

Table 1 shows the GWSS egg mass and adults+nymphs counts for 2000. Citrus thrips insecticide treatments are generally applied late April and early May. Blocks 1-8 applied selective insecticides for thrips (Veratran®, Success®, and Agri-Mek®) and these insecticides had little or no effect on GWSS. Blocks 9 and 10 applied broad spectrum carbamate (Carzol®) or pyrethroid (Baythroid®) insecticides for citrus thrips and these treatments were effective against GWSS for one-two months. Treatments of Admire® for GWSS were applied to blocks 1, 4, 7, and 8. The Admire® helped to reduce GWSS throughout the summer months, but began to break down by November when harvest was beginning. Growers that wanted to ship to packinghouses in uninfested areas were forced to treat with Lannate® (blocks 8 and 9) to disinfest their crop. These treatments helped to reduce the winter population of GWSS in those two blocks, however, by the following April the populations of GWSS were increasing again (Table 2). California red scale was treated with either Esteem® (blocks 1, 9, and 10) or Lorsban® (block 8). Neither insecticide was very effective in reducing GWSS.

Table 2 shows the GWSS densities during 2001. Block 1 conducted an aggressive and successful program to eliminate GWSS by treating with Lannate® in April followed by Admire® in May. Blocks 2, 3, 4, 7 and 8 continued to use a very soft pesticide for citrus thrips (Success®, Agri-Mek® or Veratran®) and these insecticides did not reduce GWSS. Blocks 5, 6, 9, and 10 applied broad spectrum pyrethroid insecticides (Baythroid® and Danitol®) for thrips control in May and

these were effective in reducing GWSS initially, but these residues began to break down and GWSS began to increase in July. Block 3 applied Provado® (foliar imidacloprid) for citricola scale control in July and it reduced but did not eliminate GWSS. Sites 6 & 8 used Esteem® for red scale and these insecticides had no effect on GWSS. All sites that applied Admire® (Blocks 1, 4, and 7) were successful in reducing GWSS to very low levels. Block 8 applied Evergreen® (pyrethrin + piperonyl butoxide) for GWSS and only partially reduced the population.

To summarize, the best suppression of GWSS in citrus has been through the use of a foliar broad spectrum pesticide (pyrethroid or carbamate) in the spring to reduce overwintering adults, followed by a systemic Admire® treatment to reduce their numbers in the summer. No treatment program has prevented GWSS from returning to the citrus in the fall and overwintering in that crop. Insecticide treatments applied specifically for in-season or preharvest reduction of GWSS have increased grower applications from an average of 1.4 to 2.0 treatments per orchard in 2000 and an average of 1.7 to 2.4 in 2001. This increase of 0.6-0.7 treatments per orchard of course has a direct economic cost to the citrus grower, but it also has a long-term cost. The insecticides that work well to control GWSS are not compatible with vedalia beetles. Thus, we are likely to see outbreaks of cottony cushion scale develop in Kern County that will then require treatment with additional broad spectrum insecticides such as Malathion and Supracide. This, in turn, will disrupt attempts to control citrus thrips, red scale, and mites with natural enemies. Eventually, GWSS and other pests will develop resistance to these insecticides because of repeated use and we will see outbreaks of other pests. Because of the seriousness of the GWSS as a vector of disease, a number of growers in Kern County have abandoned an excellent IPM program and stepped back onto a pesticide treadmill.

Table 1. Densities of GWSS in Kern County IPM demonstration orchards – 2000.

Site	Apr 23	May 7	May 21	Jun 11	Jun 25	Jul 9	Jul 23	Aug 13	Sep 3	Nov 5	Pre-harvest	Dec 17
1	*1/0	4/1	1/4*	0/4	1/0	4/0	0/0	0/0	0/5*	0/9		0/16
2	0/0	0/0	0/0*	0/29	43/28	23/0	8/10	0/38	0/136	0/21		0/24
3	6/0*	1/3	2/0	0/32	49/21	40/5	6/12	6/24	3/12	0/7		0/14
4	0/0**	0/1	0/0	0/9	1/2	1/0	1/0	0/0	0/0	0/1		0/1
5	0/0*	0/0	0/0	0/0	0/3	3/2	21/12	14/1	4/5	0/16		0/30
6	0/0*	0/0	0/0*	0/0	0/0	11/0	4/0	1/0	5/1	0/28		0/12
7	*1/3*	0/3	1/1	0/2	1/0	0/0	1/0	0/0	0/0	0/8		0/5
8	2/3*	0/1*	2/0	0/0	0/0	11/2	9/0	1/0	0/2*	0/10	*	0/0
9	1/0*	3/0	0/0	0/0	2/0*	4/0	7/0	0/0	1/0	0/1	*	0/1
10	0/0*	0/0*	0/0	0/0	0/0	17/9	17/1	16/3	10/32	0/10		0/21

2000 Pesticide Treatments (* Indicates when treatment occurred)

Site 1: (April 21) Admire® for GWSS, (May 31) Veratran® for citrus thrips, (Sept 29) Esteem®+oil for red scale

Site 2: (May 24) Success® + oil for citrus thrips

Site 3: (May 2) 1/2 Success® and 1/2 Agri-Mek® + oil for citrus thrips

Site 4: (April 25) Admire® for GWSS, (May 4) Success® for citrus thrips and katydid

Site 5: (May 4) Success® + oil for citrus thrips

Site 6: (April 27) Agri-Mek® + oil and (June 7) Veratran® + molasses for citrus thrips

Site 7: (April 19) Admire® for GWSS, (Apr 29) Success® + oil for citrus thrips

Site 8: (Apr 26) Admire® for GWSS, (May 6) Agri-mek® + oil for citrus thrips, (Sept 24) Lorsban® for red scale, Lannate® (Nov) for GWSS

Site 9: (May 1) Baythroid® for citrus thrips, (July 6) Esteem® + oil for red scale, Lannate® (Nov) for GWSS

Site 10: (Apr 27) Carzol® for citrus thrips + Lorsban® for katydid and GWSS and (May 20) Esteem® for California red scale

Table 2. Densities of GWSS in Kern County IPM demonstration orchards – 2001.

Adults			Egg masses/Nymphs+Adults									
Site	Jan 15	Feb 19	Apr 2	Apr 23	May 7	May 21	Jun 4	Jun 28	Jul 9	Jul 30	Aug 13	Sep 3
1	5	1	9/0*	10/12	0/0*	0/1	0/0	0/0	0/0	0/0	0/0	0/0
2	17	19	12/4	8/14	2/1*	2/8	3/17	38/33	32/26	*0/0	1/0	2/0
3	5	19	3/2	5/0	5/0*	3/5	2/29	38/44	18/17	7/11	2/17	4/15
4	2	0	0/0	1/0*	3/2**	2/0	1/0	0/0*	1/2	0/0	0/0*	0/0
5	1	0	0/0	1/0*	0/0	0/0	0/0	0/0	0/0	1/0	1/0	5/0
6	0	2	9/0	1/0*	3/2	0/0**	0/0*	0/0	0/1	6/2	3/0	6/0
7	7	5*	0/2	0/0	0/0*	0/0	0/0	0/0	0/0	0/0	0/0	0/0
8	1	0	8/4	18/1	12/2*	5/0*	2/0	1/0	14/7*	20/1	9/1	6/0
9	0	0	2/1	4/0	2/0*	1/0	0/0*	0/0	0/0	2/0	0/0*	5/2
10	0	1	9/0	6/0	1/0*	1/0	0/0	2/0	7/7	6/0	9/2	22/5

2001 Pesticide Treatments (*Indicates when treatment occurred)
Site 1: Lannate® for GWSS (Apr 8), Admire® for GWSS (May 7)
Site 2: Success® + 0.8% 440 oil (May 16) for thrips and katydid, Provado® (Jul 16) for citricola scale
Site 3: Success® +0.6% oil (May 20) for thrips
Site 4: Veratran® +Molasses for thrips (May 3) + Kryocide® for katydids, 1/2 rate of Admire® for GWSS (May 8), Success® for thrips (May 12), 1/2 rate of Admire® for GWSS (July 6), Esteem® (Aug 24) for red scale
Site 5: Success® + Baythroid® + 1% oil for thrips & katydids (May 4)
Site 6: Success® + Baythroid® + 1% oil for thrips & katydids (May 4), Success® +1% oil (May 23) for thrips, Veratran® + Molasses (Jun 1) for thrips, Esteem® +Latron® B1956 (Jun 11) for California red scale
Site 7: Admire® for GWSS (Mar 29), Success® + .8% oil for thrips (May 4)
Site 8: Agri-Mek® + 1.6% oil for thrips (May 7), Esteem® + Cygon® (May 25) for red scale and thrips, Evergreen® (Jul 23) for GWSS
Site 9: Baythroid® + .5% oil for thrips (May 7), 1/2 block Baythroid® + 1/2 block Success® (June 8), 1/2 block Esteem® (Aug 29) for California red scale
Site 10: Danitol® for thrips, worms, katydid and GWSS (May 15)

EVALUATION OF EFFICACY OF SEVIN® TREATMENTS IN PORTERVILLE GWSS INFESTATION

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INTRODUCTION

In 1999, the agricultural community in Temecula, California became aware that glassy-winged sharpshooter (GWSS) was a serious problem with regard to transmitting the bacterium *Xylella fastidiosa* and was causing epidemics of Pierce's Disease in grapes. This discovery spawned a search for GWSS in other areas of California during 2000. On May 16, 2000 yellow panel traps revealed an infestation in the Porterville area that was surveyed by Tulare County Agricultural Commissioner/Sealer's Office (TCAC) staff, with the assistance of the California Conservation Corp (CCC). It was determined that a 9-square- mile urban area of Porterville was infested with GWSS. After much discussion and examination of insecticide options, the TCAC decided to treat GWSS-infested residential yards with carbaryl (Sevin®) because it was registered for both food and ornamental plants. The purpose of the treatment program was to prevent the pest from spreading into additional nearby communities, and commercial citrus and grapes. The effectiveness of this treatment had not been previously studied.

OBJECTIVE

1. Study the effectiveness of multiple treatments of Sevin® for suppression of GWSS in two neighborhoods in the Porterville area.

RESULTS AND CONCLUSIONS

The Tulare County Agricultural Commissioner's office staff and the California Conservation Corp conducted visual surveys of 600 yards during early June of 2000 (ultimately 16,500 properties were surveyed). Treatments of Sevin® were applied only to yards that had visual signs of GWSS (egg masses, nymphs or adults). During the period of June 20-26, 2000, a commercial Pest Control Operator applied Sevin® to approximately 300 properties. A team of entomologists from the Kearney Agricultural Center sampled 30 residential properties on July 17 (approximately one month after treatments) in two different neighborhoods to measure densities of GWSS and determine efficacy of the insecticide treatments. We found evidence of GWSS (cast skins, old or new egg masses, and live individuals) in all 30 residences, yet only 50% of these residences were sprayed in June. This suggests that the California Conservation Corp crews were about 50% effective in finding evidence of GWSS when they first began surveying. As a result of this finding Tulare County Agricultural Commissioner's office and California Conservation Corp conducted another full survey to identify additional infested properties. Egg masses were found on *Canna*, calla lily, *Camellia*, ivy, tree-of-heaven, citrus, four o'clocks, *Viola*, grape, *Agapanthus*, ash, pear, redbud, apricot, privet, *Euonymus*, tiger lily, *Hibiscus*, crape myrtle and apples. Nymphs were found on fruitless mulberry, four o'clocks and apples. Adults were found on fruitless mulberry and grapes. On July 17th, approximately one month after the first treatment of Sevin®, there were fewer properties and fewer live GWSS found in the 14 yards that had been treated compared to the 16 yards that had not been treated. Since many yards were not treated, and coverage was not complete (tall trees), it is not surprising that the GWSS were not eliminated from these neighborhoods with one treatment of Sevin® (Table 1).

Sevin® was applied to over 1,000 Porterville yards during mid to late July. A survey of the two study neighborhoods in August revealed very few live stages of GWSS in untreated yards and yards that had received only one treatment, and no GWSS were found in yards that received two treatments of Sevin®. We found egg masses on apricot, *Citrus*, *Camellia* and four o'clocks. We found nymphs on grapes, and adults on fruitless mulberry trees and oleander. At this time of year, it was difficult to find any live stages of GWSS. This may have been because the pesticides effectively reduced their numbers, and/or because the majority of the GWSS were in the adult stage and adults are difficult to see. Where we did find GWSS in yards that were treated only once, the finds tended to be next to yards that were untreated. These data suggest that where coverage was good and applications of Sevin® were repeated, the program successfully suppressed GWSS.

In five locations, a total of 111 GWSS egg masses were enclosed in mesh bags immediately after treatment with Sevin®. We examined the bags weekly for four weeks and found no survival of nymphs. This suggests that if the egg masses are treated directly, the nymphs are very susceptible to carbaryl. Evidence of parasitism by *Gonatocerus ashmeadi* was found at each location. No live parasites were found suggesting that Sevin® was also toxic to this parasite.

In April of 2001, Tulare County Agricultural Commissioner's office staff re-surveyed all of the Porterville neighborhoods and treated only 474 properties. They treated both infested (213) and adjacent (261) properties. They found that the GWSS population had not expanded beyond the original 9 square miles of neighborhoods and far fewer properties had infestations than in 2000. These data suggest that multiple treatments with Sevin® in the urban area helped to contain GWSS.

Table 1. Surveys for GWSS in two Porterville neighborhoods after first (late June) and second (late July) sprays of Sevin®.

Treatment	Total properties	No. of GWSS Infested properties	Live Stages of GWSS
July 17 survey			
Unsprayed	16	14	131
Sprayed once	14	9	49
Aug 24 survey			
Unsprayed	10	2	3
Sprayed once	6	2	8
Sprayed twice	14	0	0

A MONOCLONAL ANTIBODY SPECIFIC TO GLASSY-WINGED SHARPSHOOTER EGG PROTEIN: A TOOL FOR PREDATOR GUT ANALYSIS AND EARLY DETECTION OF PEST INFESTATION

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INTRODUCTION

Effective control of glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*, will require an integrated pest management approach. A major component of true integrated pest management is the exploitation of the pest's natural enemies, which, when utilized to their greatest potential, can increase the effectiveness of other control tactics. Unfortunately, very little information exists on GWSS natural enemies (Triapitsyn et al., 1998). This is especially true for their predaceous natural enemies. Evidence of predation of GWSS eggs has been observed in the field; however, the composition of the predator complex, and the relative impact of each predator on GWSS mortality are unknown.

In the past, we have developed a library of monoclonal antibodies (MAbs) specific to the egg stage of *Lygus hesperus*, *Pectinophora gossypiella*, and *Bemisia argentifolii* (Hagler et al., 1991, 1993, 1994) for use in studying predation in the field (Hagler et al., 1992; Hagler & Naranjo, 1994a,b). MAbs provide an avenue to qualitatively assess the impact of predator species on populations of key insect pests; provide a quick, efficient, and cost effective technique for screening numerous predators in a conservation biological control program (Hagler & Naranjo, 1994a,b); and provide a method to compare the efficacy of *in vitro*-reared predators with that of their wild counterparts in an augmentative biological control program (Hagler & Naranjo, 1996).

Attempts to monitor GWSS populations and their natural enemies in Southern California are complicated by the presence of a native species of sharpshooter, the smoke tree sharpshooter, *Homalodisca lacerta*. The eggs of this species are virtually indistinguishable from those of *H. coagulata* with the naked eye. Thus it is difficult to separate the relative rates of predation and parasitism of GWSS and smoke tree sharpshooter in areas where these two species overlap. The similarity also prohibits positive identification of GWSS eggs intercepted during quarantine inspections of plant shipments. A pest-specific MAb can be used to accurately identify pests that are difficult to differentiate by the naked eye (Greenstone 1995). A MAb specific to GWSS egg would be an invaluable tool for early monitoring of pest infestation and decision-making in pesticide application.

OBJECTIVES

1. Develop a monoclonal antibody specific to GWSS egg protein to use in an enzyme-linked immunoassay (ELISA) to:
   a) identify key predators of GWSS by analyzing their gut contents for GWSS remains.
   b) differentiate GWSS eggs from taxonomically and visually similar species.

RESULTS AND CONCLUSIONS

Antibody Production

Five BALB/cByJ female mice were immunized 4 times between June 8 to August 31, 2001 by intraperitoneal injection of 100-µl of a 1:1 emulsion of Freund's Complete adjuvant and phosphate buffered saline containing 100-µg of crude GWSS egg protein. Mouse serum (i.e., polyclonal antibody) was collected 3 days after the final immunization and the titer of each

mouse for the presence of anti-GWSS egg antibodies was determined by ELISA. All 5 mice had a strong positive response to the GWSS (Fig. 1). The titer of each mouse for GWSS egg antigen will be tested again in mid October. The mouse with the strongest positive response at a 1:1250 dilution will be selected for fusion. The fusion of anti-GWSS egg antibody producing cells with myeloma cells will be identical to that described by Hagler et al. (1991, 1993, 1994). Hybridoma cell lines will then be monitored daily to observe for the emergence of hybridoma clones. Selected hybridoma cell lines will then be mass screened by ELISA for recognition of molecules within other insect species (e.g., predators, several sharpshooter species, etc.). After screening hybridoma cell lines, cells secreting only GWSS-specific antibody will be harvested for mass production.

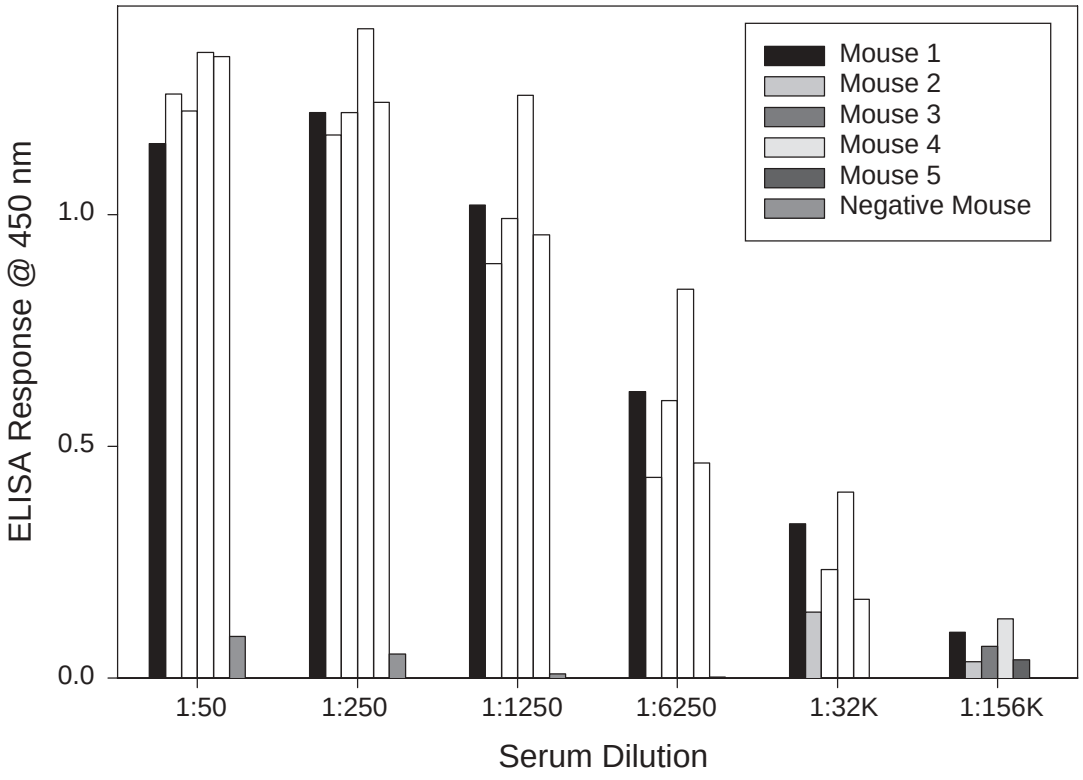

Figure 1. ELISA reactivity of mouse antisera to GWSS.

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ISOLATION AND CHARACTERIZATION OF GWSS PATHOGENIC VIRUSES

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INTRODUCTION

Insect pathogens such as insect-selective viruses often cause natural epizootics in the field and can be used as a biological control agent or vector for carrying plant or crop protection agent. In order to identify an epizootic, scientists have classically waited for an outbreak of disease and then tried to isolate the causative agent from diseased insects. Alternatively, one can take advantage of the fact that in any population there are multiple infective agents and look for these agents using biochemical and molecular methods. At present there are no known viral epizootics of GWSS. In this project, we are attempting to isolate and characterize viral pathogens of the GWSS using a new approach we call 'virus mining". In this approach we assume that GWSS pathogenic viruses will be found and can be isolated from any given population as long as the population examined is sufficiently large. Additionally, we are attempting to establish continuous GWSS cell lines that can efficiently support the replication of the GWSS viruses that we isolate. A susceptible cell line is a key to the further characterization and manipulation of a virus and will be beneficial in other areas of GWSS study.

OBJECTIVES

1. Isolate and characterize viruses infective against GWSS.
2. Establish continuous cell lines from embryonic GWSS tissues.

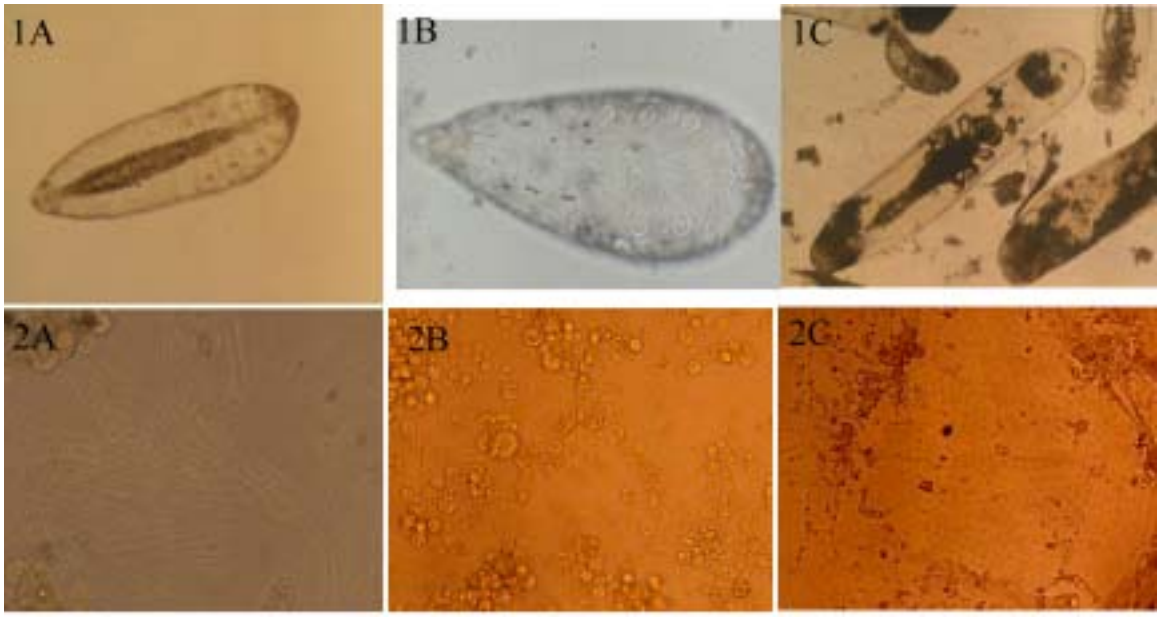
RESULTS AND CONCLUSIONS (April-September of 2001)

GWSS adults (Objective 1) and egg masses (Objective 2) were field-collected during two outings, one on April 25-26 (Filmore, CA) and the other on August 15-17 (Riverside, CA) of 2001. Adults from the April collection consisted of insects that had overwintered whereas those collected during August were the progeny of the overwintering insects. At present the effect of overwintering on virus ecology is unknown. GWSS showing unusual behavior or morphology indicative of virus infection were not observed. Egg masses from the April collection generally contained from 10-16 eggs whereas the egg masses from August contained significantly less, generally only 4-8. Interestingly, although an attempt was made in the field to select egg masses that were not parasitized by wasps (i.e., masses with any dark-colored eggs, eggs with holes, etc. were not collected), roughly 40% of the primary cultures showed wasp larvae (Figure 1A), pupae (Figure 1B) or adults (Figure 1C). This suggested that parasitization of one or more egg per mass occurs at significantly higher levels in the field. Additionally, during the August outing, female GWSS bearing white spots on their forewings were collected for oviposition in rearing cages. White wing spots are an indicator of oviposition within 3 minutes to 36 hours (Hix, 2001). Egg masses were stored at 5ºC under humidity for less than 48 h prior to processing for the generation of primary cultures.

In preliminary experiments to isolate a GWSS pathogenic virus (Objective 1), approximately 1000 GWSS adults were homogenized and resuspended in PBS buffer. The average adult weighed 31.3±6.8 mg. Following ultracentrifugation through a 20-50% sucrose gradient at least 7 distinct major and minor bands were observed. Three very minor bands were collected, resuspended in PBS, and pelleted. Following phenol and chloroform extractions, the bands were found to contain nucleic acid. Endonuclease and ribonuclease treatments and agarose gel electrophoresis indicated that the bands contained RNA of approximately 800 nucleotides. Extractions were performed with three additional batches of approximately 1000 adults each and similar results were obtained. Since all of the experiments produced similar results, we don't believe that the isolated RNAs are of virus origin. It appeared that the bands were composed of ribosomes and polyribosomes on the basis

of abundance, density, and size of the RNAs. We are currently processing larger numbers of adults (approx. 3,000-5,000) per batch and will use the currently identified bands as markers. We will also subject potentially virus-containing bands to scanning electron microscopy.

Egg masses from both collection trips were surface sterilized and dissociated in culture medium in order to generate primary cultures (Objective 2). Three out of 17 primary cultures established from the April egg masses and 1 of 11 primary cultures from the August egg masses have survived and are proliferating. The lower survival rate of the primary cultures from the egg masses collected in August may be partly due to the reduced amount of embryonic tissue (resulting from fewer eggs per egg mass). The following parameters appear to be the best for the establishment of GWSS primary cultures: the egg mass should contain at least 10 eggs (per 1.5 ml of medium) at an early stage of development (see Fig. 1A of Hirumi and Maramorosch, 1971); 30 second sterilization with 70% ethanol; aseptic dissociation with forceps in ExCell 401 medium (JRH Biosciences) supplemented with 20% fetal bovine serum (FBS) followed by gentle pipeting, and culture at 28ºC in darkness. Sterilization with formaldehyde (2%) and mild trypsinization (0.01%) appeared to be detrimental. LH, TC-100, IPL-41 and TNM-FH media each supplemented with 20% FBS were less optimal. At present, the GWSS primary cultures are mainly composed of fibroblast-like (Figure 2A), round (Figure 2B), and epithelial (Figure 2C) cells with a doubling time of 2-3 week.



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POTENTIAL OF CONVENTIONAL AND BIORATIONAL INSECTICIDES FOR GLASSY-WINGED SHARPSHOOTER CONTROL

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INTRODUCTION

The glassy-winged sharpshooter (GWSS) is a primary vector of *Xylella fastidiosa* Wells et al., the causative agent of Pierce's disease (PD) in grapevines. The incidence of PD has increased with serious fruit and vine losses as GWSS numbers have increased in southern California (Blua et al. 1999). With continuing spread of GWSS, Purcell et al. (1999) suggested that diseases caused by *X. fastidiosa* are likely to become more prevalent. There is an urgent need to develop short- and long-term GWSS management that is economically, ecologically and socially acceptable. The essential cultural and biological components of developing integrated pest management (IPM) strategies for the short-term, at least, will need an efficacious GWSS chemical control component with attendant insecticide resistance management (IRM), and integrated crop management (ICM) inputs.

OBJECTIVES

The current studies were conducted to identify selective, conventional and biorational insecticides against immatures as potential components of GWSS management programs and evaluate their effectiveness for control. Previously, we studied insecticides for control of adult GWSS in grapes (Akey et al. 2001).

RESULTS AND CONCLUSIONS

We conducted a series of field trials using natural GWSS populations (eggs to nymphs to adults during a 6-mo. period in the spring/summer of 2001). Trials were conducted on small citrus, 2-m ht. orange trees. Experiments were conducted in a 3-replicate randomized complete block design at University of California, Agricultural Operations, Riverside, California. Plots were 0.114 ac in size; 25 by 22 ft, 3 trees per plot with guard rows on each side. Counts were made weekly following insecticide applications.. Mean separation tests were made following significant F values by analyses of variance.

Treatments were made with a windmill blast-type sprayer (John Bean Div., FMC) (compliant with Good Lab Practices, GLP). Spray delivery was at 180-200 psi at 300 gal/ac with 5 swivel-nozzle bodies (Tee Jet) on one side; 10 nozzles, each had a core 23, disc 6, and slotted strainer. Insecticide trade and generic names, chemistry classes, formulations, product amounts, AI/ac, and source companies are given in Table 1. An adjuvant, Silwet L 77, (Loveland Ind.) was used in all formulations. Spray penetration was estimated using water sensitive paper (Spray Systems, Inc).

Esteem showed 100% control of egg masses and may be a true ovicide for GWSS (unpublished data). This needs more investigation. Pyrethroids, neonicotinoids, and the IGR Applaud (buprofezin) were highly effective against nymphs of GWSS. Future studies should be conducted on rates of Applaud application for GWSS control. Neem products were slowly (accumulatively) effective against large nymph production (neem products had no efficacy or repellency on GWSS adults on grapes; unpublished data, summer/fall 2000).

Our previous experience, and that of many others, in pest insect crop protection using chemical control has shown that different insecticides, within the same chemical class, often have different qualities that contribute to their usefulness when applied alone or in tank mixes with another insecticide in a different or even in the same class. Analyses of the impact on the total pest and beneficial arthropod populations present in vineyards and associated ecosystems and selection of the

best-fitting insecticides is a formidable challenge for implementation of ecologically oriented control programs. Such insecticide selections have been very useful in other emergency insect outbreak control efforts (Akey et al.1997, Ellsworth et al. 1997). Grower options that can accommodate management needs in different areas that may vary in climate, pest complex, and environmental and economic variables are essential, but require extensive research to develop.

Table 1. Trade names, chemistry classes, formulations and rates per acre of foliar insecticides evaluated for immature and adult glassy-winged sharpshooter control in Citrus, Riverside, CA, 2001.

Name		Chemistry		Per acre		
Trade	Generic	Class	Formulation†	Product	lb AI	Company
			Conventional Insecticides			
Capture®	bifenthrin	Pyrethroid	2 EC	6.4 fl oz	0.100	FMC
Baythroid®	cyfluthrin		2 E	3.2 fl oz 1.6 fl oz.	0.040 0.020	Bayer
Fujimite	fenpyroxi-mate	Oxime	5 EC	4.0 pt	0.200	Nichino America
Assail®	acetamiprid	Neonicotinoid	70 WP	1.2 oz	0.050	Bayer
Provado®	imidacloprid		75 WP	10.0 oz	0.460	Bayer
			Biorational Insecticides			
Trilogy, clarified extract of Neem oil (no Azadirachtin)		Neem products	70%	5.0 gal	27.3	Certis USA
Agroneem	neem extract azadirachtin		15.0% 0.15%	4.0 qt 4.0 qt	1.100 0.110	AgroLogistics
Neemix	azadirachtin		4.5%	16.0 fl oz.	0.046	Certis USA
Applaud	buprofezin	chitin inhibitor	70 WP	2.86 lb	0.200	Nichino America
Esteem	pyriproxyfen	JH analog	0.86 EC	16.0 fl oz	0.054	Valent USA

† EC-Emulsifiable Concentrate, SP-Soluble Powder, W or WP-Wettable Powder, SC-Soluble Concentrate.

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DEVELOPMENT OF TRAPPING SYSTEMS TO TRAP THE GLASSY-WINGED SHARPSHOOTER *HOMALODISCA COAGULATA* ADULTS AND NYMPHS IN GRAPE (AVF V107)

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INTRODUCTION

The glassy-winged sharpshooter (GWSS) *Homalodisca coagulata* is native to the southeastern United States where it is a known vector of various strains of the bacterium *Xylella fastidiosa*. Since its introduction into California, it has become established in large numbers in certain areas. Pierce's disease has been a problem in California for more than 100 years, but the GWSS is a more efficient vector of *X. fastidiosa* because it is a stronger flier than native California sharpshooters, and it can feed on the xylem of seemingly dormant woody stems.

The wine industry in Temecula, CA has been seriously impacted by Pierce's disease (PD) losing about 30% of its vineyards to date. The combination of PD and GWSS in California poses a serious threat to the grape industries. About 98,000 acres of table grapes are currently cultivated in California with 14,000 acres of table grapes in the Coachella Valley (Riverside County). The Coachella Valley has a history of PD and there is currently a high population of glassy-winged sharpshooters. The combination of the bacterium and new vector creates a serious disease threat to grape in the area. A similar situation has occurred in Kern County, California as well.

One of the crucial components and cornerstones of integrated pest management is the monitoring for the presence and density of a pest. Proper detection methods allow for optimum integration of biological, cultural, physical, chemical and regulatory measures to manage a pest. Yellow sticky traps have been used extensively in the southeastern U.S. for monitoring leafhoppers including GWSS in peach (Ball, 1979) and citrus (Timmer et al., 1982). However, the reliability of these methods to detect the GWSS in California is questionable, and traps specifically designed for GWSS do not currently exist. To compound the situation, current methods are not standardized. For example, different sizes and shades of yellow sticky traps are being used in monitoring programs. The AM designation on certain traps actually refers to the apple maggot for which the trap was designed. Furthermore, the relationship of trap catches to actual populations of GWSS in grape or citrus are currently unknown.

Trap designs based on the behavior and biology of the insect in question have a much higher chance of success than relying on trial and error of traps designed to monitor other insects. Female GWSS secrete and deposit brochosomes on the forewings just prior to egg laying (Hix, 2001). These spots are then scraped off during egg laying. Furthermore, white spots are secreted before each egg mass is laid, and female GWSS can only produce rod shaped brochosomes after mating. It is therefore feasible to relate preovipositional females with white spots and residues to egg masses in associated vegetation analysis. The white spots are very visible on females caught in traps (Hix, 2001). Many leafhopper species produce brochosomes, but only females are known to produce the rod shaped brochosomes.

OBJECTIVES

This research addresses: 1) which hue of yellow is the most attractive to GWSS; 2) what is the field longevity of a trap before weather and photo degradation impact trap reliability; 3) how does trap catch relate to populations of GWSS in citrus and grape; 4) GWSS spectral sensitivity; 5) how does temperature affect trap catch; 6) the feasibility of using certain wavelengths of light to enhance trap catch of GWSS in vineyards and associated orchards; 7) develop and evaluate sticky barriers to trap and detect GWSS nymphs within a vine or tree canopy.

RESULTS AND CONCLUSIONS

Three trap types were field tested in 2001. Traps were deployed in citrus groves and grape vineyards with known high populations in addition to groves and vineyards with low populations. Trap types tested included flight intercept traps (5 colors), plates (11 colors), and nymph traps (3 colors). Traps were checked weekly and visual count of egg masses, nymphs, and adults were made. Trapped GWSS were sexed, and females with forewing spots of brochosomes or residue were noted.

The data from the intercept traps and colored plates clearly indicated that GWSS are attracted to yellow as well as orange. Attraction to these colors was statistically significant and demonstrated that even though the AM type trap may have reliability issues, it is clearly not a "blunder trap."

The yellow and orange colored plates were very successful in catching adult GWSS. Yellow plates caught statistically more GWSS than AM traps while orange traps usually caught more than the AM traps (Figure 1). The interesting thing is that the yellow plates were more reliable at catching GWSS at low population levels than the AM traps. The nymph traps reliably caught 1st through 5th instar nymphs in moderate to low populations. These traps are easy to deploy in grape canes in situations where it could take hours of searching to locate nymphs. Low populations of GWSS nymphs in a vineyard may pose threats of moving *X. fastidiosa* from vine to vine within trellises. The fluorescent yellow and canary yellow intercept traps attracted large numbers but the collection mechanism only caught about 15% of the bugs that encountered the panels, which made the traps unreliable. However, intercept traps were capable of catching live insects.

Additional progress was made in determining that adult and nymphs are attracted to upper UV, and certain wavelengths in the yellow and orange ranges. The preliminary data indicated relationships between the number of ovipositional females trapped and egg laying in associated vegetation.

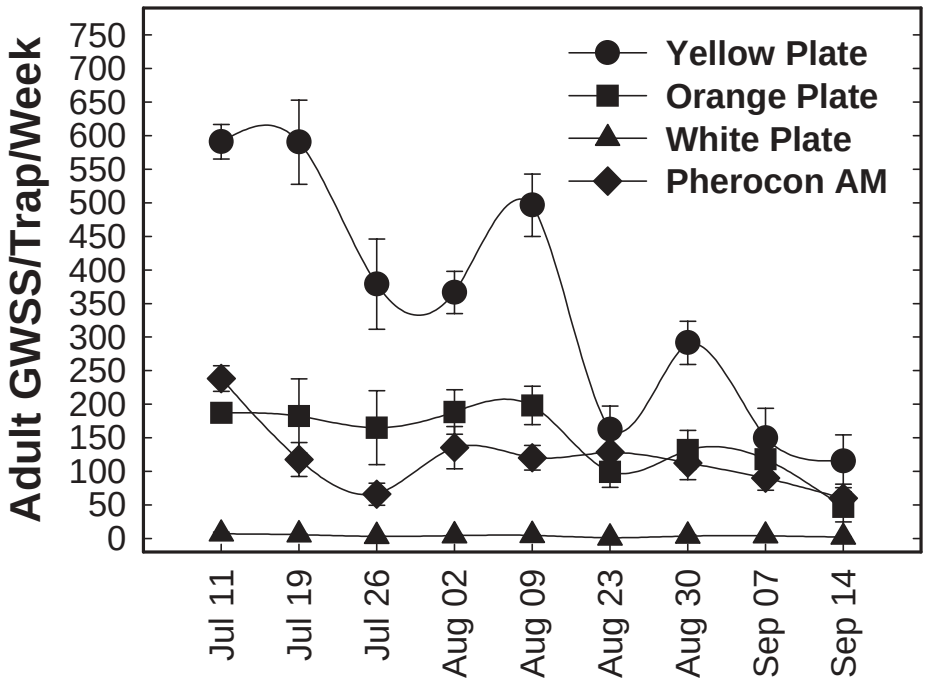

Fig. 1. Comparisons of Yellow, Orange, and White Plates and AM traps. Bars= ±SE n=5.

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MATING BEHAVIOR OF THE GLASSY-WINGED SHARPSHOOTER, *HOMALODISCA COAGULATA*

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INTRODUCTION

Mating behavior in leafhoppers is mediated by vibrational signals transmitted through plants (Claridge and de Vrijer 1994). Leafhopper calls are species-specific and have proven useful in resolving taxonomic problems. Furthermore, analysis of intra- and interspecific variation in male calls has provided clues about speciation processes. However, little is known about mate-finding tactics at the habitat level or the specific cues used by males to locate females after mate recognition. Theoretical and some experimental research on leafhoppers and planthoppers clearly indicate that seasonal patterns of abundance and dispersal are intimately linked to a species mating system (Ott 1993). Thus, determining rules that govern mating behavior may ultimately contribute an understanding of population and community level processes. Also, the application of basic knowledge of leafhopper mating behavior to an applied problem such as developing a novel monitoring device for the glassy-winged sharpshooter is unexplored.

OBJECTIVES

1. Determine the role of vibrational signals in mate recognition, attraction, courtship, and copulation. This objective will be accomplished by describing variation in vibrational signals associated with mate recognition, attraction, courtship, and copulation and by quantifying behavioral transitions that lead to mating. Playback experiments will be done to confirm the involvement of observed signals in mediating the above behaviors.

2. Assess the feasibility of developing improved monitoring traps by using vibrational signals to attract adults. This objective will be accomplished by determining the effect of sticky traps augmented with vibrational signals on the capture of glassy-winged sharpshooters.

RESULTS AND CONCLUSION

Research was initiated July 2001. Calls emitted by males have been recorded and are being characterized (Figure 1). Further progress will be reported in the symposium.

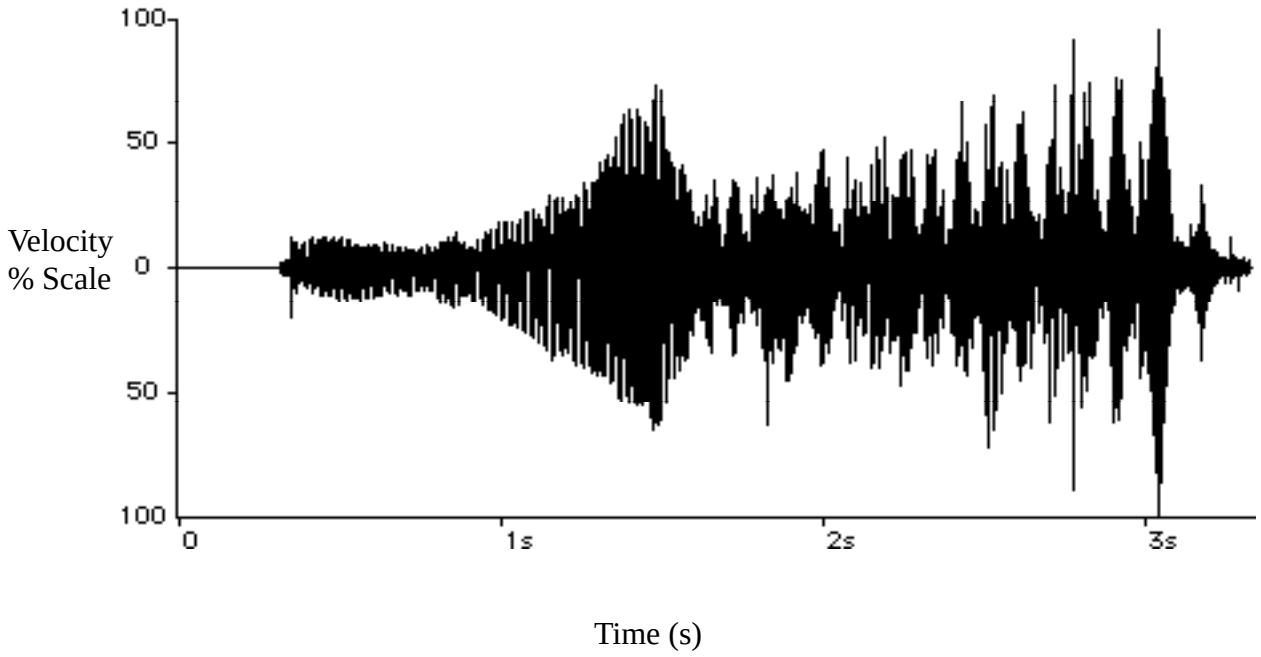

Figure 1. Oscillogram of a signal emitted by a male *H. coagulata*

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CLASSICAL BIOLOGICAL CONTROL OF THE GLASSY-WINGED SHARPSHOOTER

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INTRODUCTION

The leafhopper tribe Proconiini composes the "sharpshooters". There are 54 described genera and hundreds of species within this specific taxonomic group (Young, 1968). All are confined to the New World. The genus *Homalodisca* is found from the United States to Brazil and Argentina. Outside California, the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*, is known to occur from the southeastern and south central United States into northeastern Mexico. The exact limits of its range are known primarily through label data in insect collections.

A collaborative program to collect natural enemies from its area of origin in the United States and Mexico has been underway since at least 1999, initiated by the California Department of Food and Agriculture, and in cooperation with the University of California, Riverside, USDA-APHIS, and USDA-ARS and Mexico. These efforts have led to the successful collection and release in California of a number of native parasitoids of the GWSS. In south Texas, GWSS is rare. A survey of local egg parasitoids during the past two years has demonstrated that the eggs are heavily attacked by *Gonatocerus triguttatus* throughout the sharpshooter's breeding season and appears most responsible for the low population levels of GWSS in the area. This parasitoid was only recently identified from GWSS (Triapitsyn and Phillips, 2000). No nymphal parasitoids were detected.

It is not known if parasitoids from other sharpshooter genera and species will attack GWSS eggs. However, many genera within the Proconiini are very closely related to *Homalodisca* and possess similar habits. *Homalodisca* is among at least 10 leafhopper species that transmit citrus variegated chlorosis in South America. It is felt that parasitoids of eggs, and possibly other stages of sharpshooters from similar climate and habitat types from South America, may possess the ability to also attack GWSS because (1) they can be collected from areas that allow them to be pre-adapted to California climate conditions, and (2) they may be able to search for and successfully attack GWSS eggs in micro-habitats such as citrus. Thus, contacts were made and a plan was formulated to explore for and collect sharpshooter parasitoids from South America and import them into quarantine for identification and evaluation for their potential against GWSS. Additionally, a project was initiated to conduct a survey of insect collections to determine the historical range of the GWSS.

OBJECTIVES

The program will encompass the total array of activities available for classical biological control from:
1. Climate matching.
2. Taxonomic review.
3. Foreign exploration.
4. Quarantine evaluation.
5. Release and post-release evaluation.
6. It is also proposed to support collections in South America, provide technical support for insect and host plant colonization, quarantine evaluation of parasitoids, and in release and evaluation in California.

RESULTS AND CONCLUSIONS

During 2000-2001, the initial survey year, parasitoids would be collected for identification only, with shipments of live material beginning during the 2001-2002 season. Parallel with initial sampling, climate matching will be conducted to identify areas in South America that match subclimate types where grapes are grown in California.

In cooperation with USDA-APHIS, Mission Plant Protection Center, Mission, TX, a collaborative project was developed with the USDA-ARS South American Biological Control Laboratory (SABCL), Hurlingham, Argentina, to collect natural enemies for identification and importation for evaluation in US quarantine. More recently, a cooperative agreement was developed with Dr. Mark Hoddle, University of California, Riverside, to share responsibility for receipt, identification, evaluation and possible non-target effects of imported material. The USDA-ARS Systematic Entomology Laboratory would collect label data from GWSS in museum collections to provide information for an accurate distribution map of GWSS.

The survey of data label information from insect collection is still underway. A survey of egg parasitoids of the sharpshooter *Tapajosa rubromarginata* (Signoret) in northern Argentina was conducted. This genus is most closely related to *Oncometopia*. *T. rubromarginata* possesses a wide host range including citrus. Adults were caged over small potted citrus plants and transported to several sites, including citrus. From about 220 specimens shipped in alcohol to Dr. Serguei Triapitsyn, about 10 were species of *Gonatocerus*, all belonging to the subgroup that attack GWSS in the United States. The most common species closely resembles *G. triguttatus*. Among the trichogrammatids were species in the genera *Oligosita* and *Zagella*.

The examination of Klammadiagrams and a review of the CLIMEX program (Sutherst et al. 1999) resulted in the identification of areas in the country of Chile that possess identical subclimate types as those where grapes are grown in California. Also, areas in northern Argentina matched much of the climate of the southeastern United States where GWSS is considered to be indigenous.

Permits to import live parasitoids into quarantine facilities in California (Riverside) and Texas (Mission) have been submitted to USDA-APHIS-PPQ. Exploration for egg and nymphal parasitoids in Argentina and Chile by USDA-ARS personnel located in Argentina was initiated again in September, 2001 with the goal of shipping live parasitoids for evaluation pending receipt of permits.

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BIOLOGICAL, CULTURAL, AND CHEMICAL MANAGEMENT OF PIERCE'S DISEASE

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INTRODUCTION

The systemic movement of *Xylella fastidiosa* (*Xf*) within the plant xylem system is essential to this bacterium's ability to cause disease and probably for its indefinite survival in natural environments. Numerous microscopic studies of plants affected by Pierce's disease revealed high concentrations of bacteria in some xylem cells, but it is notable that in all of these studies, adjacent xylem elements are often devoid of bacteria. The important basic question of how the bacteria move from cell to cell is still unanswered.

Our initial hypothesis is that bacterial multiplication is an important requisite for cell-to-cell movement. Do *Xf* populations in nonsystemic hosts reach high or low densities within infected cells? Do *Xf* populations in systemic hosts with low populations of *Xf* (as determined by dilution plating) attain high populations in few cells or lower populations in many cells? We will examine the behavior of *Xf* in nonsystemic hosts such as willow and mugwort and in plants with low, but systemic populations, such as blackberry. The occlusion of xylem cells with *Xf* in willow, for example, would illustrate that bacterial aggregates that completely fill xylem cells is not sufficient for systemic movement.

The *Xf* oleander strain is not systemic in grape and vice versa (A.H. Purcell et al., unpublished data). The typical fate of *Xf* in most woody plant species is to multiply without systemic movement (A.H. Purcell, unpublished). We will investigate if this is true for the oleander strain in grape and the grape strain in oleander. Both of these strains are systemic in their pathological hosts but not in the opposite host. We will seek to identify whether oleander strains multiply in grape using dilution plating on solid culture medium (PW) and confocal fluorescent microscopy.

A recent development that aids the study of bacterial movements in plants is the emergence of scanning confocal laser microscopy (SCLM). The new techniques to study bacterial biofilms could provide valuable information on the distribution of *Xf* within xylem tissue. The application of the SCLM coupled with image analysis techniques permits the study of living, fully hydrated microbial biofilms.

Success in introducing novel genes into *Xf* (objective 4) to create new but grape-virulent strains with reporter gene constructs allowing easy *Xf* detection in grape tissues would greatly facilitate studies of *Xf* movement in plants. Such a system could enable *Xf* detection with SCLM in plants without fixing, dehydrating, staining, or otherwise preparing plant specimens. Thus the same tissues could be examined repeatedly to follow *Xf* movements, especially those events associated with the cell to cell movements that are critical to disease. If *Xf* can be genetically engineered to express the green fluorescent protein (GFP) gene, the movement of bacteria could be followed through the plant similar to the methods used in studies on the movement of *Erwinia amylovora* in apple. Our use of GFP-mutants for histological studies of movements or of biofilm formation will depend upon success of objective 4.

An understanding of biofilms may also help explain *Xf* movement and pathogenicity. A biofilm is an aggregate of attached cells produced when bacteria adhere to a surface, initiate glycocalyx (exopolysaccharide) production and form microcolonies. *Xf* appears to produce biofilms that are unique compared to other documented biofilms in that they are inhabited only by a

single bacterial species and occur within plant xylem and insect guts. Others have speculated the matrix material surrounding aggregations of *Xf* within plants improves the bacterium's extraction of nutrients and provide physical protection. We suspect that biofilm formation is also critical to *Xf*'s movement from cell to cell within plants and necessary for its survival within the vector foregut, from which it is transmitted to plants by insects. We examine *Xf*'s occurrence within a spectrum of host plants - from those in which it multiplies rapidly but does not exhibit systemic spread (willow), to those in which it multiplies and moves but does not reach high enough levels to cause disease, to pathological hosts such as grape. We will also develop methods to produce and examine biofilms under in vitro conditions so as to be able to experimentally manipulate environmental conditions and determine their effects on biofilm formation. If we are successful in developing *Xf* transformation protocols (objective 4), we should be able to provide conclusive genetic evidence for the potential role of biofilms in plant pathogenesis or insect transmission by knocking out biofilm biosynthesis gene(s) using transposon mutagenesis.

OBJECTIVES

1. Understand how *Xf* moves, and the patterns of its movement, in systemic (grape, blackberry) and non-systemic (willow) plant hosts using microscopy. (AHP, PCA, BCK)

2. Understand how temperature influences the movement and survival of *Xf* and the incidence and/or severity of PD. (AHP)

3. Determine whether vegetation barriers between riparian areas and vineyards and/or insecticide-treated "trap crops" at the vineyard edge can reduce the incidence of PD. (AHP, EAW, BCK, MAW)

4. Develop transformation / transposon mutagenesis systems for *Xf* using existing or novel bacterial transformation vectors. Use *Xf* mutants to identify bacterial genes that mediate plant pathogenicity, movement, or insect attachment. (BCK)

5. Isolate and identify endophytic bacteria that systemically colonize grapevine. Develop methods to genetically transform grape endophytes to express anti-*Xf* peptides. (BCK)

6. Develop a genetic map to *Xf* resistance using *V. vinifera* x (*V. rupestris* x *M. rotundifolia*) seedling populations and AFLP (amplified fragment length polymorphism) markers, identifying resistance markers, and possible identification of resistance genes. Utilize DNA markers for resistance to rapidly introgress *Xf* resistance into several *V. vinifera* winegrapes and/or utilize genetic engineering procedures (when available) to move above identified *Xf* resistance genes into winegrapes. (AW)

7. The purpose of this objective is to:
   a. Determine the resistance of 10 grape genotypes to PD after mechanical inoculation and natural infection with *Xf*. Elucidate the xylem chemistry of these grape genotypes and statistically correlate both chemical profiles and specific molecular markers to PD resistance. (PCA, MAW)

   b. Determine the resistance of common host plants (willow, resistant; blackberry, susceptible) to *Xf* and discern the relationship of specific chemical profiles to resistance. Utilize these techniques to examine resistance mechanisms of resistant seedlings identified in 6a. (PCA, AHP)

   c. Validate the influence of chemical profiles and specific chemical markers on the growth and survival of *Xf* by tests in in-vitro culture. (PCA)

RESULTS AND CONCLUSIONS

Objective 1: Understand how *Xylella fastidiosa* moves, and the patterns of its movement, in systemic (grape, blackberry) and non-systemic (willow) plant hosts using microscopy (Purcell)

Confocal microscopy of red willow noculated with *Xf* showed that *Xf* can multiply to very high levels within individual xylem elements but does not move to adjacent cells in non-systemic hosts. Thus, the low populations of *Xf* recovered from

willow by culturing represent many *Xf* cells within a very few colonized cells rather than fewer numbers of *Xf* in a larger number of cells. High magnification scanning electron microscope and modified staining methods of *Xf* in grape revealed "tentacle-like" structures (fimbriae) at the narrow ends and division plane of dividing *Xf* cells collected from expressed xylem sap of infected grape but not when collected from cultured *Xf* cells from PW medium. *Xf* aggregated on the surface of xylem vessels in PD-infected grape petioles as well as dormant infected grape tissue (green and woody stem pieces) early or late in colonization of grape xylem. The bacteria were embedded in a matrix of fibers that covered them in a net-like fashion. This matrix of *Xf* cells and extracellular materials (a biofilm) adhered to the xylem cell walls. The fimbriae of attached *Xf* connected only in contact with plant tissues. Biofilm formation appears to be a consistent feature of colonization of grape by *Xf*. Cells of *Xf* grown in a new culture provided by Brazilian collaborators produced abundant extracellular fibrils but not fimbriae, and these cells attached strongly to glass, unlike cells grown in traditional *Xf* media (PW). Our findings suggest that *Xf* produces attachment structures only under specific environmental cues.

Objective 2. Understand how temperature influences the movement and survival of *Xylella fastidiosa* and the incidence and/or severity of PD (Purcell)

Field inoculations of grape at different times of the year at Oakville (1997), Davis (1997-98), Fresno (1998-99) and Hopland (1999) consistently confirmed that infections during April through May and to a much lesser in June resulted in persistent infections of *Xf* the following year; whereas most June, July or August inoculations resulted in non-persistent infections unless the base of canes were inoculated. At the Hopland site, recovery of early-season infected vines was the first evidence of possible climate-mediated recovery of PD-infected vines that was not explainable by pruning eliminating the *Xf* infections. Inoculations of vines during April 2000 in San Luis Obispo County and Santa Barbara County suggest that cool temperatures (not exceeding 16 ^{0}C) following inoculation may not allow systemic infection by *Xf*. The highest populations of *Xf* occurred at the bases of symptomatic canes and decreased towards the tip of sampled canes in experimentally inoculated or naturally infected vines.

Experimental freezing of dormant, PD-infected potted vines cured the plants of PD at rates of 0 to 70% after exposures to temperatures ranging from -2 to -10 ^{0}C for 3 successive freezing exposures. Cane segments treated identically at the same time never recovered, suggesting that some aspect of plant physiology, rather than the direct action of freezing alone, kills *Xf* by freezing in grape xylem. The survival of *Xf* after freezing in various liquid media further supports this view.

Objective 3: Determine whether vegetation barriers or trap crops can reduce the incidence of PD in riparian areas. (Weber)

Two trap crop trials have been established on either side of a large vineyard in Napa. One trial borders the Napa River, the other Milliken Creek. Vines are spaced 9 feet (rows) by 5 feet (in-row). In each trial, St. George rootstock is planted at the ends of adjacent rows to create the trap crop treatments. Trap crop treatments include the first 6 vines in 12 adjacent rows. In each trial, there are three replicate trap crop planting and three control plantings where Chardonnay or Pinot Noir are planted to the end of the row. The vineyard was planted in May 1999. By October 2000, trap crop vines along the Napa River had reached the upper trellis wire and had filled in well. They were pruned in December along with the rest of the vineyard leaving wood on the "fruiting" wire. On the Milliken Creek side, deer did considerable grazing damage throughout the year and the vines did not develop as well.

Admire (soil-applied imidacloprid) was applied to trap crop vines in November 1999. Due to concerns about its efficacy, this will be replaced with a spring 2001 treatment of Provado (foliar-applied imidacloprid). BGSS were monitored in 2000 in both trials using yellow sticky cards placed at the ends of rows. BGSS were detected in all treatments, although counts were relatively low. Per-trap catch totals ranged from 2 to 23 BGSS for the trapping period March-October. A PD disease survey was conducted in both trials in Sept. 2000 extending approximately 40 vines into the vineyard. Only 5 vines were found showing PD symptoms. Monitoring and mapping will continue in 2001.

Objective 4: Develop transformation and transposon mutagenesis for *Xylella fastidiosa* (Kirkpatrick)

Procedures were developed to successfully introduce the transposon, Tn5, into two different strains of *Xf*. Electroporation conditions that were similar to those that have been used to electroporate DNA into several *Xanthomonas* species were found to be efficacious for electroporating DNA into *Xf*. Several attempts to transpose *Xf* with two different Tn5 and two Tn10 suicide constructs failed to produce transposed *Xf* cells. However, using identical electroporation conditions, we obtained several hundred Tn5 mutants of both the Fetzer and Temecula *Xf* strains using a "transposome" complex composed

of a transposase protein/DNA complex containing Tn5. Southern blot analysis showed these were random, single, Tn5 inserts throughout the *Xf* genome. Sequence analysis of *Xf* genomic DNA that flanked the Tn5 insertion identified several genes that were found in the CVC strain of *Xf*. We now have approximately 500 Tn5 mutants stored at –80; inoculation of grapevines and other analyses of the mutants will begin in a few months after several thousand mutants have been obtained.

Three, 1.8 kb *Xf* plasmids were cloned and sequenced from the UCLA *Xf* strain. The largest open reading frame (ORF) on these small *Xf* plasmids had significant homology with another phage replicase gene, suggesting this *Xf* ORF encodes a plasmid replicase gene. Initial cloning of these plasmids probably interrupted the promoter for this ORF and we have since recloned these plasmids at another location on the plasmid. We are now introducing the Kan^R gene from the Tn5 construct described above, which we know is expressed in *Xf*, into the cloned *Xf* plasmids. In this manner we hope to construct an *Xf/ E. coli* shuttle vector that will greatly facilitate molecular genetic analyses of *Xf*.

Objective 5: Isolate and identify endophytic bacteria that systemically colonize grapevine xylem. Identify natural, or genetically engineer, endophytes to be antagonist to *Xylella fastidiosa* (Kirkpatrick)

Several hundred bacterial isolates were obtained from both healthy and *Xf*-infected grapevines located in Napa and Yolo counties. Specific grapevines of two cultivars were sampled bimonthly during 1999 and 2000. In addition, healthy appearing grapevines that were growing in the middle of vineyards that were decimated by PD were also randomly sampled. Four different media were used to cultivate the bacteria, however no significant differences in the types or numbers of bacteria were observed using the different media. Gram stains were performed on all of the isolates so that the appropriate Biolog plates can be used to initially identify the isolates, at least to the genus level. Beginning in the spring these isolates will be pin-prick inoculated into grapevines growing in the greenhouse. After several weeks, attempts will be made to recover the bacteria from sections of xylem that are 2 or 3cm from the point of inoculation. Any bacteria that are found to systemically colonize grapevine xylem in reasonable concentrations will be tested for natural antagonism towards *Xf*. We are now screening a random peptide library for synthetic peptides that are inhibitory to *Xf*. If these peptides are identified, attempts will be made to genetically engineer grape endophytes to express these peptides within grapevine xylem.

Objective 6: Genetics of Resistance to *Xylella fastidiosa* (Walker)

A Design II mating design with a set of 6 females by a set of 6 male parents, from which we will select sets of seedlings to study the inheritance of *Xf* resistance, is completed. The mean expression of resistance within a given female across all males and similar comparisons of males across females will allow us to draw conclusions about the inheritance of *Xf* resistance.

We define *Xf* resistance as the ability of a genotype to limit the movement of *Xf*, particularly in a downward direction, and have tested a set of resistant and susceptible individuals to determine whether this definition is valid. The known susceptible genotypes were *V. rupestris* 'A. de Serres' (the female parent of the 89 population), Chardonnay and the *V. rupestris* x *M rotundifolia* genotype 8909-19, potentially resistant genotypes were 8909-04 and 8909-11, and the resistant genotypes were 890915 and 890917. After 4 weeks, *Xf* was easily detected in the three susceptible genotypes. By 16 weeks, the differences among resistant and susceptible genotypes are very clear in terms of both symptom expression in leaves and unevenly lignified stems and ELISA readings. ELISA is cheaper, faster, quantifiable and more simple than PCR detection of *Xf*, and can reliably detect 10,000 cfu/ml of *Xf* in ground plant sap. 150 samples with duplicate readings can be processed in a day at a material cost of $0.28 per plant sample. IC-PCR detection is 10 more sensitive, but is not quantifiable and costs about $1.44 per sample. Nested PCR gave very good results and is 100 times more sensitive than IC-PCR, but few samples can be run per day, and the cost of materials and labor is high ($2.88 per sample). We also examined spot-PCR which would allow several hundred samples to be run per day, but the sensitivity is equivalent to ELISA and cost $2.70 per sample. (All costs exclude labor).

We are mapping *Xf* resistance in the *V. rupestris* X *M. rotundifolia* 9621 population (previously used for *Xiphinema index* resistance). We have AFLP marker data on about 70 of the 150 individuals and are testing 4 replicates from each of these 150. *Xf* resistance data from 70 individuals are complete and the others are due for analysis in about 4 months. We plan to place *Xf* resistance on our existing AFLP based map. If the resistance trait does not place on the existing map, we have seeds for a second mapping population based on a cross of the 8909-15 X 8909-19, a resistant by susceptible genotype which will allow greater power in mapping the resistance genes. However, this seedling population will have to be grown out from seed, propagated and screened for resistance and marker information. We also have (8909-08 and 8909-23) to Chardonnay, which should also segregate widely.

We bench-grafted Chardonnay on each of the following rootstocks: AXR#1, St. George, 3309C, 101-14Mgt, Schwarzmann, 44-53 Malegue, Riparia Gloire, 1616C, Lenoir, 5BB, 5C, Börner, 110R, 1103P, Harmony, Freedom, and Ramsey, and Chardonnay (control), Riesling, Sylvaner, Chenin blanc, and Colombard. Own-rooted Chardonnay, Riesling, Sylvaner, Chenin blanc and Colombard were also inoculated. We have evaluated all three reps for PD expression and ELISA sampling to determine the extent of downward movement is continuing. The vines were cut back to basal buds, are now re-growing and will once again be evaluated for symptoms and by ELISA.

Objective 7: Determine whether xylem chemical composition is involved with PD resistance or susceptiblity in grape varieties and common *Xylella fastidiosa* plant hosts (Andersen)

In an effort to determine whether xylem fluid chemistry is related to resistance to *Xylella* we investigated the chemistry of *Vitis* genotypes covering a wide range of resistance/tolerance. Xylem fluid chemistry of 10 grape genotypes belonging to 5 species has been characterized, although only the amino acid data was presented. Total amino acids varied over 4-fold, and many amino acids only occurred in less than 10uM or trace quantities. A complete correlation with resistance will await compilation of organic acid and sugar data. Total amino acids in xylem fluid collected from Chardonnay grafted on 4 different rootstocks showed an effect of rootstock and of infection with *Xf*. Xylem fluid of Chardonnay on 3309 rootstock tended to be most dilute of the rootstocks and *Xf* infection increased total amino acids in xylem fluid of Chardonnay on all rootstocks except SO4. Arginine was the amino acid that showed the biggest increase in amino acids with *Xf* infection. A more complete analysis of the effect of xylem chemistry of grape genotypes on resistance to *Xf* and the change in chemistry with *Xf* infection awaits further research.

An investigation is underway to determine whether resistance to *Xf* is influenced by xylem fluid chemistry across plant species common to both Florida and California. Xylem fluid chemistry of 32 host plants species/cultivars was analyzed and natural *Xf* (resistant/susceptible) was noted by PCR analysis. About 50% of the plant species/cultivars were *Xylella* negative. Those that were *Xylella*-negative were mechanically inoculated with *Xf* during the fall of 1999. After 4 weeks only one species was positive, *Vitis rotundifolia* (wild grape). Each of the *Xylella* negative species/cultivars have also been sampled fall 2000 and are currently being analyzed via PCR. Those species that still do not harbor *Xylella* may be inoculated with different strains for confirmation of resistance to multiple *Xylella* strains.

In vitro nutrient requirements of *Xylella* have been studied for 3 months without much success. A UCLA strain of *Xf* was subjected to 3 months of experimentation using Chang and Donaldson's chemically defined media. This UCLA strain grew well on PD or PW+ but did not grow on the chemically defined media. We switched strains to ATCC 35881 and tested this strain on all the media. The result was that it grew well on all the media and it even grew slowly on a media consisting of glutamine as the only amino acid source. Thus, nutritional fastidiousness is extremely strain dependent. Additional experiments will be performed with a different strain (probably the Temecula strain) after consultation with B. Kirkpatrick, S. Purcell and A. Walker.

Lytic peptides were extremely effective against *Xylella*. They act by disrupting cell membrane integrity of bacteria but not eucaryotic cells of higher animals. Cecropins were one to three orders more effective than tetracycline. *Xf* incubated with Cecropin A and B at 1 uM resulted in 100% inhibition of growth; incubation with *Xylella* at 0.1 uM resulted in 95% inhibition. Indolicidin was a fairly strong inhibitor of *Xf* with 100% inhibition at 9.5 uM. Magainin II was followed by Magainin I in potency. For tetracycline 100% inhibition was achieved at 112 uM. These compounds may serve as a potent naturally occurring pesticide against *Xylella*.

CDFA Objective 1: Determine the effects of lethal and sub-lethal doses of insecticides on insect transmission of *Xylella fastidiosa* to plants (Purcell)

Preliminary transmission tests to characterize GWSS' transmission of *Xf* to grape suggested that some adult GWSS were very poor transmitters. We are investigating several unidentified bacteria isolated from plants and sharpshooters as possible antagonists to *Xf* transmission by GWSS. Experiments confirmed the prediction that molting stops GWSS' transmission of *Xf*. GWSS experimentally transmitted *Xf* to woody stems (scaly bark) of grapevines at rates estimated at 6% per insect per day, while GWSS exposed to green shoots of the same aged vines transmitted at rates of about 7%.

Doses of the systemic insecticide imidacloprid applied to soil (Admire^{R}) that killed from 40% to 60% of GWSS on potted grape within 24 hours or smaller, "sub-lethal dosages" drastically reduced sharpshooter feeding and movements. The efficacy of all dosages mimicking field rates of Admire peaked 3 weeks after application, then decreased. Lab experiments

with GWSS having a choice or no-choice of treated or untreated grape produced no evidence that imidacloprid repelled GWSS, except at perhaps extremely high doses not attained with full label rates of application. Sub-lethal or slightly toxic doses prolonged GWSS survival compared to untreated controls but greatly reduced the ability of GWSS to jump or fly. Experiments to test the effects of Admire treatments on GWSS of *Xf* were inconclusive because of low rates of transmission of *Xf* to untreated controls.

CDFA Objective 2: Evaluate potential *Xylella fastidiosa* bactericides and developments to introduce these materials into grapevines (Kirkpatrick)

Plant microelements such as zinc, copper, manganese and iron, as well as three antibiotics were tested for inhibition against *Xf* 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 phytoxicity 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 *Xf* or if the ions are too tightly bound.

THE DEVELOPMENT OF PIERCE'S DISEASE IN XYLEM: THE ROLES OF VESSEL CAVITATION, CELL WALL METABOLISM AND VESSEL OCCLUSION

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INTRODUCTION

This proposal is directed toward discovering the plant responses to infection that are fundamental to the progression of Pierce's Disease (PD) in grapevine. The disease is caused by the growth of the bacterium *Xylella fastidiosa* (*Xf*) in the xylem vessels of stems, petioles and leaf blades. The disease progresses rapidly, causing severe water deficits in infected shoots and vine death–often within two years. However the progression of the disease and the mechanism(s) by which the disease produces water deficits and death in infected tissues have not been well established.

The prevailing notion is that vessels become occluded with bacteria or products of metabolism. However, it is unclear how the bacterium moves among vessels, whether vessels cavitate upon introduction of the bacterium by the insect vector or during artificial inoculation, and whether the bacterium must enter vessels in order to cause disease symptoms. The bacterium is reported to be larger than the openings in pit pore "membranes". Thus, it is likely that cell wall digestion is necessary for movement of the bacteria through the vine. This digestion may be a key component of disease progression.

Based on work that we have done (VanderMolen et al., 1983) with the *Fusarium oxysporum* vascular wilt pathogen that also causes blockage of its plant host's vascular system, we anticipate a linkage between cell wall metabolism, xylem occlusion, vine water stress and leaf abscission. If the disease develops in a manner similar to fusarium wilt, the plant may respond to oligosaccharide "signals" (Melotto et al., 1994; Ryan and Farmer, 1991) produced upon digestion of its own cell walls and, consequently, vessels that have not been infected with bacteria may nevertheless become occluded. Therefore the "occluding" material could be of grapevine origin and component of the plant defense responses to infection. There also is reason to suspect that each vessel cavitates upon bacterial entry, rendering the vessel unable to transport water (Schultz and Matthews, 1986 and 1993; Zimmerman, 1983). Thus cavitation may be the fundamental cause of impaired water transport and leaf death. The proposed work will establish whether the vessels become occluded with material of bacterial or *Vitis* origin. It will also determine the role of vessel occlusion and cavitation on the progression of PD.

OBJECTIVES

1. Determine the impact of infection by *Xylella* on the water status of grapevines using both destructive and non-destructive measurements of stem and leaf water potential and water conductivity.
2. Determine the chemical nature of the xylem-occluding material.
3. Determine the nature of the grape cell wall degradation that is caused by *Xylella*: What cell wall components are digested and what wall-digesting enzymes do the bacteria make or cause the grapevine to make?
4. Determine if oligosaccharide signals influence the progression of the disease.
5. Determine whether the plant hormone ethylene is produced when the bacterium infects grapevines and, if so, whether grape responses to the ethylene influence the development of xylem occlusions and other PD symptoms.

RESULTS AND CONCLUSIONS

We began our efforts on July 1, 2001. The approach we have taken in the first part of the study is to determine which technical approaches will be most useful to test individual components of our hypothesis about how PD progresses following infection with *Xf*. Our "model" for PD development is:

***Xf* introduction to vessels—>vessel cavitation—> initial water deficit—> *Xf* population increase—> production of enzymes by *Xf* (signals ?) —> cell wall digestion —> oligosaccharide signals —> ethylene synthesis rise—> a "wave" of vessel occlusion beyond the infection site —> global collapse of vine water transport—> leaf abscission—>vine death**

Thus far we have performed one large scale inoculation test in which a population of Merlot vines was infected with *Xf* using the pinprick technique (Purcell and Saunders, 1999). Infection rate was low and PD symptom development was slow (little disease after 10 weeks) but we were able to make several important observations and test techniques. Initially we have used immunocapture PCR (Smart et al., 1998) to determine *Xf* presence in inoculated vines. Vines were sampled every two weeks and tissues (internodes, nodes and leaves at, above, and below the point of inoculation) were tested for *Xf* presence. In addition, water conductance through stem segments was measured. PD symptoms were clear in a few vines sampled 8 weeks after inoculation. At this time, PCR revealed a substantial bacterial population in the internodes that had been inoculated, but bacterial DNA was not found in extracts of other vine tissues. We have done tests of the PCR system's sensitivity and know that a few hundred bacteria can be readily detected. We also have shown that the inoculation technique efficiently introduces a large *Xf* population to the **interior** of the vine (i.e., bacteria are not removed from the inoculation site by scrubbing with detergent). Therefore we conclude that bacteria were introduced during inoculation, that the number of bacteria that moved from the site was quite low, and that at least one PD symptom (reduced water movement) can be detected (Figure 1) in the absence of a large *Xf* population. We have begun a second inoculation trial with a population of young Merlot vines and hope to have greater success in promoting PD development.

Among the approaches we will use to enhance PD development is controlled water stressing of the vines and alternative approaches to vine inoculation. We have found that mock pinprick inoculation using water-soluble dyes instead of bacteria leads to rapid movement of dye both up and down the cane. Thus the introduced dye (and presumably also a suspension of *Xf*) has access to the vascular system and at least some vessels remain functional. However, we have used PCR to show that *Xf* that has been introduced by pinprick does not spread out of the inoculated internode within 20 hours of inoculation. We presume that because the bacteria are particulate and in suspension (rather than in solution like the dye) they are not readily taken into the xylem and that vessels near the pinprick will have cavitated and be non-functional, at least for a time. On the other hand, bacteria introduced directly into the xylem by the sharpshooter insect vector may be more immediately mobile if damage to water-conducting elements is not extensive.

We have carried out a trial where bacteria were introduced to young canes by cutting the stems under water and placing them in an *Xf* suspension. In this situation, we were able to detect *Xf* with PCR at least four internodes above the cut internode within three hours. We will use a more quantitative PCR approach to determine the relative concentrations of *Xf* in each of the internodes, but it is clear that bacteria can move relatively freely through functional vessels. In a parallel test, dye moved to the top of the canes (>12 internodes) within minutes. This experiment also indicated that some continuous xylem vessels extend through several grapevine internodes or that the pit membranes that allow one vessel to pass water to its neighbors are not barriers to *Xf* movement. We will examine this point further because one tenet of our hypothesis is that cell wall degradation, of pit membranes within the xylem, is an important factor in bacterial movement. One difficulty that all researchers of PD must face is the slow progress of the disease. Our tests with introduction of *Xf* via cut stem ends makes clear that a substantial inoculum can be introduced into the xylem. Therefore we will begin testing an infection technique that utilizes direct "xylem feeding" of bacteria into vines. We have experience with xylem feeding of compounds into woody stems of almond. We should be able to seal grape stem flaps through which bacteria are introduced and the wounds may heal. This approach could allow more rapid testing of aspects of our PD model (above) that are downstream of the proposed "***Xf* population increase**" stage.

An important aspect of our model is the idea that the bacteria produce enzymes which digest the primary cell wall present in pit membranes and that this facilitates systemic *Xf* spread and generates oligosaccharide signals that cause grapevine responses leading to vessel blockage and reduced water transport. We have evidence that cell wall digestion occurs in canes that contain substantial *Xf* populations and show PD symptoms. Graduate student Caroline Roper and Dr. Carl Greve have found that low molecular weight sugars of the sort found in the secondary cell walls of vessels (particularly

xylose) are found in the small amount of xylem sap that is expressed from infected stems and that the amount of xylose in the cell walls of these stems is also reduced. Presumably xylan polysaccharides are being digested in these stems. One problem with this analysis is that severely infected tissue may support growth of microbes other than *Xf* and so the digestion we see may not be due to the PD causal agent. The work with inoculation by stem feeding may make clearer both that wall digestion occurs and *Xf* is responsible. We have also looked for cell wall-digesting enzymes in the medium of bacterial cultures. Thus far we have not identified any activities. However, bacterial wall-digesting enzymes are generally under close control and we do not have a good minimal medium for *Xf* growth to which we can add possible inducers of wall-modifying enzymes (generally wall digestion products). We hope to obtain guidance from other PD researchers so that we can mimic in culture the environment the bacterium would experience *in vivo* and test the potential for *Xf* to digest grapevine walls.

Many aspects of our work require that we correlate *in vivo* the presence of *Xf* and obstructions in the xylem. Work in other labs is attempting to introduce genes encoding green fluorescent protein into the *Xf* genome. This will greatly facilitate histological screening for *Xf*. However, undergraduate Phillip Bates, working with Rost and Greve has developed a number of techniques to enhance our examination of vine vascular system anatomy. The primary antibody used in our immunocapture PCR (above) recognizes and binds to a surface component of *Xf*. We have obtained a second antibody that is tagged to the histochemical dye Texas red. When this dye-antibody conjugate is used in conjunction with the primary antibody we can stain *Xf* bacteria in tissue sections (Figure 2). Additional histochemical approaches have allowed us to see tyloses (cellular "balloons" protruding through pit membranes from adjacent parenchyma cells into vessels, Figure 3) and gel-like vessel occlusions in PD-infected stems (Figure 4). Because these gels stain with both the non-specific stain methylene blue as well as the pectin-specific stain ruthenium red, we presume that they are of grape plant, rather than *Xf* origin and are like the vessel-occluding gels we identified in *Fusarium*-infected tissues several years ago. (VanderMolen et al. 1986).

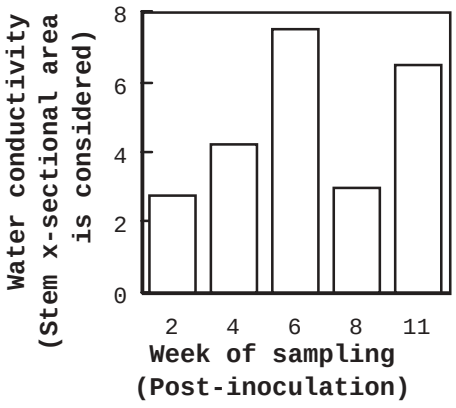

Figure 1. Water transport is reduced in infected grape stems sampled 8 weeks after inoculation. Water transport increased as stems grew (weeks 2-6). The symptomatic week 8 sample showed reduced water transport. Week 11 samples did not show symptoms.

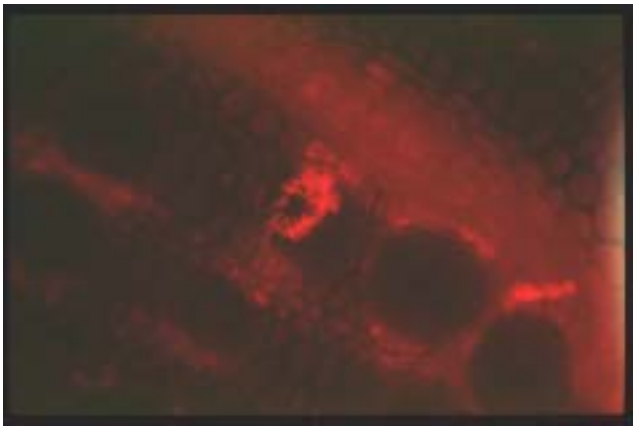

Figure 2. This section of an inoculated young grape stem cuts across three water-conducting vessels. One of the three contains *Xylella fastidiosa* cells which are seen as the red fluorescent ring revealed by immunostaining with the Texas red-anti-rabbit antibody conjugate.

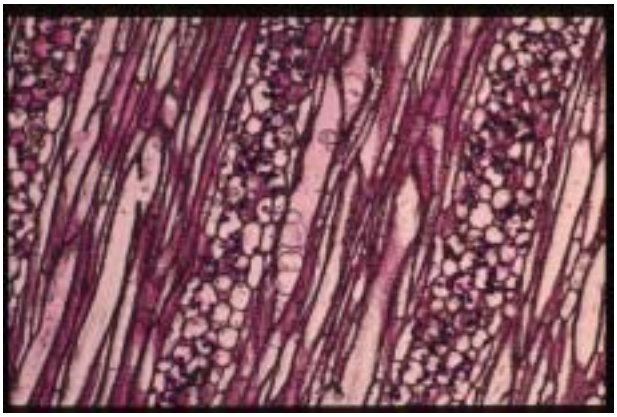

Figure 3. The longitudinal section across the xylem of a heavily infected grape stem reveals several tyloses. The section is stained with periodic acid-Schiff's reagent and the tyloses are seen as membrane surrounded "bubbles".

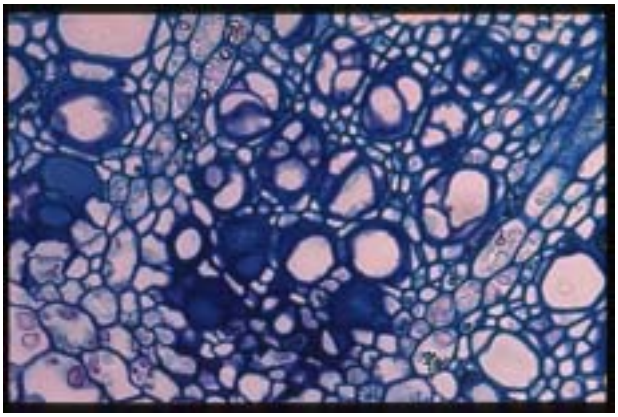

Figure 4. This section across the xylem of a heavily infected grape stem is stained with methylene blue. Tyloses are seen in section and grazing cut in some vessels and occluding "gels" are seen as amorphous stained bodies in other vessels.

Our work began in July and so we have only limited findings to describe in this report. However, we anticipate results from our second large inoculation trial and several of the specific approaches described above by the time of the December meeting. We also hope to report on results from experiments testing links between *Xf* presence, ethylene production in vines, and altered vessel anatomy and water transport. We anticipate also results from preliminary work aimed at understanding further the chemical nature of vessel occlusions.

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A SURVEY OF INSECT VECTORS OF PIERCE'S DISEASE (PD) AND PD-INFECTED PLANTS FOR THE PRESENCE OF BACTERIOPHAGE THAT INFECT *XYLELLA FASTIDIOSA*

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INTRODUCTION

Pierce's disease (PD) is an incurable disease of grapevine caused by strains of *Xylella fastidiosa*. The bacterium gains entrance into the grapevine through the feeding activities of the blue-green sharpshooter, *Graphocephala atropunctata* (Purcell, 1975) and the glassy-winged sharpshooter, *Homalodisca coagulata* (Purcell, 1979). PD is endemic to California, however, with the recent detection of the glassy-winged sharpshooter (GWSS) in California, patterns of PD distribution are likely to change and host plant infection and/or associated plant death rates are likely to soar (Purcell, Personal Communication).

Bacteriophage (phage) therapy is considered an unconventional pathogen countermeasure where viruses are used to kill specific bacteria, primarily pathogens. Recent successful endeavors using phage to control *Lactococcus garvieaea* infection in yellowtail (Natai et al., 1999) and the discovery that the natural antibiotic in dog saliva is a bacteriophage (Matzinger, and Arnheiter, 2000) lend momentum toward the exploration and use of novel ways to control many different bacterial infections. Our proposal addresses the possibility that phage exist in nature that infect *X. fastidiosa* and that these phage may be useful for PD control or management.

OBJECTIVES

1. To screen wild *Graphocephala atropunctata*, *Homalodisca coagulata*, and plants with PD for the presence of bacteriophage.
2. To test any/all acquired bacteriophage for its/their ability to infect and destroy *Xylella fastidiosa.*

RESULTS AND CONCLUSIONS

X. fastidiosa isolated from grapevine with PD were found to be infected with what we believe to be two different bacteriophage. One phage type possesses a shape and size resembling phage in the family Microviridae, while the size and shape of the second phage resemble those in the family Podoviridae. Bacteriophage in the Podoviridae are known to infect *Xanthomonas* spp. which are bacterial relatives of *X. fastidiosa*. Currently, we are attempting to isolate these phage. Once isolated, we will attempt to infect a pure culture of *X. fastidiosa* to monitor and describe the destructive nature of the viruses.

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DEVELOPING A NOVEL DETECTION AND MONITORING SYSTEM FOR THE GLASSY-WINGED SHARPSHOOTER

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INTRODUCTION

Pheromones and other semiochemicals are invaluable tools for quarantine and monitoring insect populations. Attractants are critically needed to detect early invasions of the glassy-winged sharpshooter (GWSS). Once detected using current technologies, their population densities may be too high to achieve eradication or effective control. Although it is unlikely that chemical communication is the major means of sex recruitment in the GWSS, plant-derived chemical signals are utilized for host location. The primary objective of this project is to develop attractants for detection and monitoring of the glassy-winged sharpshooter, *Homalodisca coagulata.* The goal is going to be pursued by the extraction, isolation, identification and synthesis of leaf compounds from plants that attract sharpshooters and other leafhoppers. Synthetic compounds, originally identified in plants, as well as other candidate compounds, will be tested in the field in order to evaluate their potential for monitoring population levels of the GWSS.

OBJECTIVES

1. The primary objective of this project is to develop attractants for detection and monitoring of the glassy-winged sharpshooter, *Homalodisca coagulata*.
2. One approach (strategy I) is based on the extraction, isolation, identification and synthesis of leaf compounds from plants that attract sharpshooters and other leafhoppers.
3. The other potential attractants will be screened by a binding assay with an olfactory protein, odourant-binding protein (OBP), involved in the filtering of chemical signals in the insect antennae. Initially, OBP(s) from the GWSS will be isolated, cloned and expressed in bacteria.

RESULTS AND CONCLUSIONS

Plants that attract sharpshooters and other leafhoppers were extracted and fractionated (chromatographic separations). Identification of the active constituents was carried out by gas chromatography-mass spectrometry, vapor-phase infrared spectroscopy and by chemical derivatizations. Based on these data, chemical structures of the compounds were proposed and confirmed by synthesis of the authentic compounds.

Candidate compounds were incorporated in release-controlling plastic pellets (Leal et al., 1996) and evaluated as baits with yellow sticky traps in randomized blocks (Leal, 1999). Field bioassays are being conducted in Ventura County. This is an excellent location to test attractants because the area has well established populations (among the first identified in California) which are not heavily treated as they must be in locations such as Riverside and Kern counties where transmission of *Xylella fastidiosa* to grapes is of primary concern.

Field observations indicated that sharpshooters lay eggs in non-host plants and that location of these oviposition sites is chemically mediated. This prompted us to characterize potential attractants from plants preferred for oviposition. Plants were extracted either in Ventura County or other locations where field ecology of sharpshooters have been studied. Chemical analysis of the plant extracts led us to the characterization of semiochemicals of potential application in monitoring the GWSS (Figure 1).

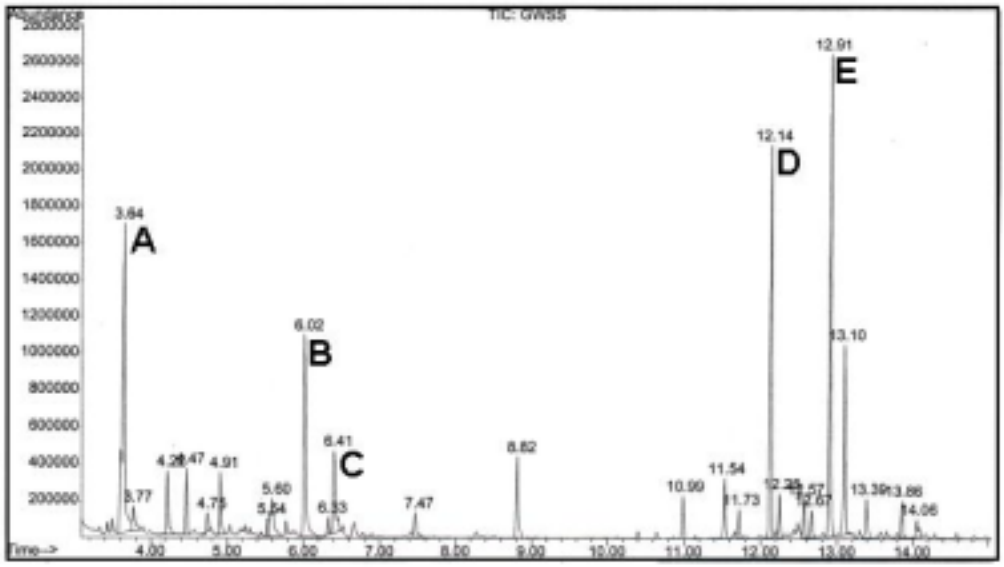

Figure 1. Gas chromatography-mass spectrometry (GC-MS) analysis of an extract from a plant preferred by GWSS for oviposition. The major constituents of the extract were identified and synthetic compounds are being tested as potential attractants for GWSS. To date, combinations of compounds A, B, C, D, and E have been tested.

Field evaluations of these semiochemicals and other potential attractants are underway. Tests of known pheromones were unrewarding. Among the plant formulations tested so far, traps baited with combinations of compounds A, B, C, D, and E (Figure 1) showed initially more catches than control traps, but the effect of trap position (false positive) could not be completely ruled out. Further experiments with randomized blocks and perhaps greater replication will be carried out when the adult populations once again reach higher densities. Screening of additional compounds from host plant extracts will also be carried out.

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COLD STORAGE OF PARASITIZED AND UNPARASITIZED EGGS OF THE GLASSY-WINGED SHARPSHOOTER, *HOMALODISCA COAGULATA*

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INTRODUCTION

The egg parasitoid, *G. ashmeadi*, is a mymarid wasp that accounts for 95% of the observed parasitism of the glassy-winged sharpshooter (GWSS) in California. This has stimulated researchers to try to develop methods for rearing large quantities of this parasite for release in areas where augmentation is needed or where other control measures cannot be used. In the absence of techniques for propagating *G. ashmeadi* via artificial means, rearing this insect in large quantities also requires that the GWSS or another acceptable host be cultured to provide the eggs for this parasite. Protocols designed for efficient mass-rearing generally include techniques which enable the production managers to hold their insects for varying periods of time to synchronize various aspects of the rearing procedure and for distribution to the release site when needed. Having the capability to hold a particular life stage or stages in abeyance during mass-rearing is especially important when synchronizing the life cycles of two insects such as in a parasite-host relationship. Low temperature storage is an integral part of the process of mass-rearing insects for use in agricultural pest control programs. It is the practical application of information provided by researchers studying arthropod cryobiology, dormancy, host-prey interactions, and mass-rearing methods. Storage of parasitoids of the GWSS will allow insect production managers to gain flexibility and enables them to supply a purely biological product on demand. The effectiveness of any biological agent used for pest control purposes depends on being released at the proper time. Unforeseeable environmental influences such as those impacting on pest migration, population fluctuations, and plant growth amplify the need for precise timing, especially when releases of insects are to be integrated into multi-disciplinary control programs. Thus, this project is designed to yield information to aid in the mass-rearing and timely release of egg parasitoids of the GWSS.

OBJECTIVES

1. Determine the cold tolerance of *G. ashmeadi* within host eggs of GWSS under specific environmental and developmental parameters. Assess whether chilling has latent effects on the quality of the adult parasitoid.

2. Determine the most effective method for cold storage of unparasitized GWSS eggs by examining post-storage acceptability by the parasitoid, parasite survival, reproduction, and host-seeking behavior.

3. Determine the efficacy of extending the shelf-life of unparasitized eggs by pre-conditioning the female GWSS through altering the environmental and/or nutritional standards prior to cold storage.

RESULTS AND CONCLUSIONS

This research project was only recently begun because funding was received in September of this year.

THE ROLE OF CELL-CELL SIGNALING IN HOST COLONIZATION BY *XYLELLA FASTIDIOSA*

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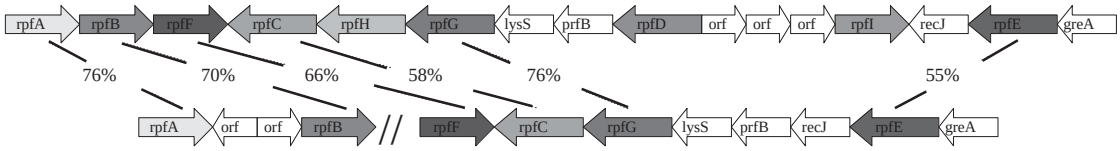
INTRODUCTION

Endophytic bacteria such as *Xylella fastidiosa (Xf)* colonize the internal tissues of the host, forming a structure very similar to a fixed biofilm inside the plant. Cells secrete extracellular polysaccharides that may serve to enhance surface attachment, to protect the cells from host defenses or to serve as a matrix to concentrate nutrient ions (Denny, 1995). But what happens when the local environment can no longer support further population growth? A key determinant of success for an endophyte is the ability to move within the plant, sending out "scouts" to colonize new areas within the host. For a xylem-restricted species such as *Xf*, movement must be from vessel to vessel, which would require degrading and traversing the pit membranes connecting them. We expect activities such as degradation of a pit membrane to be most successful when carried out by a community of cells since individual cells may be incapable of completing the feat on their own and may be detected and easily eliminated by the host. Therefore, cells must have a means by which to assess the size of their local population, and to coordinately regulate the expression of genes required for such processes only at the appropriate time. Our study aims to investigate cell-cell communication in *Xf* to determine its role in colonization and pathogenicity in grapevine.

Xf shares sequence similarity with the plant pathogen s. *Xanthomonas campestris* pathovar *campestris (Xcc).* In *Xcc,* the expression of pathogenicity genes is controlled by the Rpf system of cell-cell communication, enabling a population of cells to launch a pathogenic attack in a coordinated manner (Barber et al., 1997). The *rpf* (regulation of pathogenicity factors) genes of *Xcc* encode the components of a cell-cell communication system. Two of the Rpf proteins work to produce a diffusible signal factor (DSF; Barber et al., 1997). As the population grows, the local concentration of DSF increases. Other Rpf proteins are thought to sense the increase in DSF concentration and transduce a signal, resulting in expression of pathogenicity factors (Slater et al., 2000).

The *Xf* genome not only contains homologs of the *rpf* genes most essential for cell-cell signaling in *Xcc*, but also exhibits striking colinearity in the arrangement of these genes on the chromosome. Furthermore, the *Xf* genome



Arrangement of rpf *genes on the chromosome of* Xcc *(top) and* Xf *(bottom). Numbers in parentheses indicate amino acid identity. Slashes mark a break in continuity.*

lacks homologs of the *luxI/luxR* genes and other genes shown to be involved in production and perception of AHLs (Dow and Daniels, 2000). Thus it is likely that *Xf* employs a cell-cell signaling apparatus similar to that of *Xcc.* Based on our knowledge of density-dependent gene regulation in other species, we predict the targets of Rpf regulation would be genes necessary for colonizing the xylem and spreading from vessel to vessel. For example, expression of extracellular polysaccharides, cellulases, proteases and pectinases might be induced by the signal. Similarly, we would expect the density-dependent genes to be expressed during the time when a population of *Xf* is ready to move into uncolonized areas of the plant.

It is conceivable that cell-cell signal interference may be used by other organisms to inhibit density-dependent behaviors, such as pathogenicity or spreading through the habitat. Several recent studies indicate that other organisms can disrupt or manipulate the cell-cell signaling system of bacteria (Leadbetter and Greenberg, 2000; Manefield et al., 1999). Examination of *Xf* population size in plants where *Xf* lives as an endophyte versus those in which *Xf* causes the xylem blockage symptoms of Pierce's disease demonstrates a positive relationship between population size and symptom development (Fry and Milholland, 1990). We hypothesize that an interaction between *Xf* and other organisms, such as another endophyte or the host plant itself, may modulate density-dependent behaviors in *Xf* by interfering with cell-cell signaling.

OBJECTIVES

1. To determine the chemical identity of the signal used in cell-cell signaling in *Xf*.

2. To investigate which behaviors are controlled by signaling factors.

3. To isolate other strains of bacteria that are capable of interfering with cell-cell signaling in *Xf*.

RESULTS AND CONCLUSIONS

Objective 1. We have obtained and are in the process of testing "signal sensing" strains of *Xcc* to determine whether *Xf* uses the same butyrolactone signal as *Xcc*. These strains carry a reporter gene under the control of a promoter that is up-regulated in response to the butyrolactone signal factor produced by *Xanthomonas*. We are also in the process of constructing plasmids for generating signal sensing strains of *Xf* using a reporter gene fused to promoters of genes we believe should be up-regulated in response to the signal in Xf.

Objective 2. We are in the process of constructing strains of *Xf* in which the *rpfB* gene, which is required for production of the signal in *Xcc* is knocked out. These will be subjected to phenotypic analysis and tested for their ability to infect and move within host plants. We are also determining the patterns of transcriptional regulation of genes in the rpf operon as well as other genes that are mostly likely involved in pathogenicity such as cellulases and polygalacturonases. The regulation of the genes is being assessed by producing fusions of these genes upstream from a promoterless *gfp* reporter gene. The gene fusions are being introduced into the chromosome via the use of integrative plasmids of partial stability that allow gene replacement in *Xf*. The plasmids that we are developing for this purpose possess features that should allow integrative recombination into target genes in the chromosome of *Xf* but also permit sensitive estimation of target genes by use of reporter gene fusions protected by transcription terminators.

Objective 3. We have collected grapevines from Pierce's Disease affected vineyards. We are in the process of recovering bacteria that grow inside these vines by 4 different methods to generate a comprehensive collection of grapevine endophytes. These endophytes are being cultured on 5% TSA. The each strain will be tested for the ability to interfere with cell-cell signaling in *Xcc* and *Xf* in an agar assay using diffusible factor-regulated genes using the signal sensing strains from objective 1.

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ROLE OF *XYLELLA FASTIDIOSA* ATTACHMENT ON PATHOGENICITY

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INTRODUCTION

Xylella fastidiosa is a gram negative bacterium which causes serious diseases of plants such as Pierce's disease (PD), citrus variegated chlorosis (CVC), or almond leaf scorch and inhabits many other insect and plant host (Purcell 1997). The control of plant diseases caused by this bacterium will ultimately require treating plants with chemical or biological methods, or manipulating plants genetically. In this proposal, we propose to identify inhibitors to the attachment and colonization processes of *X. fastidiosa*. A striking feature of *X. fastidiosa* is its polar attachment via the production of fimbriae (Kitajima et al. 1975, Purcell et al. 1979, Davis et al. 1981, Backus 1985, Purcell and Suslow 1988, H. Feil *unpublished data*). This is an adhesion mechanism that appears to be unique to *X. fastidiosa* and clearly requires traits special to this organism. This suggests the existence of either compounds or conditions that would prevent *X. fastidiosa* to form this polar fimbriae bundle and to attach to its host might confer disease resistance. A method of controlling *X. fastidiosa* would be to target these special traits that allow *X. fastidiosa* to adhere to its host. By interfering with the binding of *X. fastidiosa* to its host, these inhibitors would reduce *X. fastidiosa* virulence and therefore prevent the bacterium from establishing and causing disease.

OBJECTIVES

1. Determine the effects of targeted mutations of selected attachment genes (i.e. *fimA, pilH, pilS* and related genes) on *X. fastidiosa* attachment.

2. Determine differences in pathogenicity between *X. fastidiosa* attachment-deficient mutants and wild type PD strains.

3. Identify specific plant chemicals, pH, and various compounds that either promote or inhibit *X. fastidiosa* attachment in vitro and in plants.

RESULTS AND CONCLUSIONS

To achieve Objective 1, we produced site-specific mutants of *X. fastidiosa (Xf)* attachment gene. We improved on the method described in the transformation of the CVC strain of *Xf* to kanamycin resistance with plasmid *p*16Kori (Monteiro et al. 2001). In a similar fashion we disrupted the *fim*A gene of the *Xf* grape strain Temecula with the kanamycin resistant gene via homologous recombination (Figure 1). We are currently adding the *gfp* (green-fluorescent protein) gene to the vector plasmid to use it as a reporter gene for the *fimA* promoter. We were successful in producing *fimA*$^{-}$disrupted transformants. It is the first time that a site directed transformation of a grape strain of *X. fastidiosa* has been demonstrated. The transformation efficiency was approximately 40 transformants per mg of plasmid DNA (*p*Vector). Figures 2 and 3 indicate that the transformants appear to be the result of a double crossing over event. The size of the *fim*A in the transformant corresponds to the size of the *fim*A gene to which was added the size of the Kanamycin gene. We are confirming the results using southern blot hybridization.

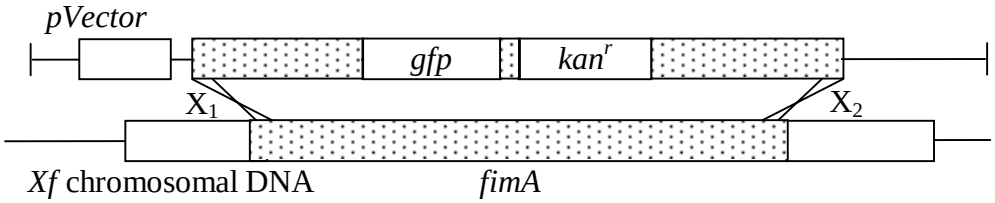

Figure 1: Schematic representation of the double cross over between the disrupted *fimA* gene of *p*Vector and the *fimA* chromosomal gene via homologous recombination.

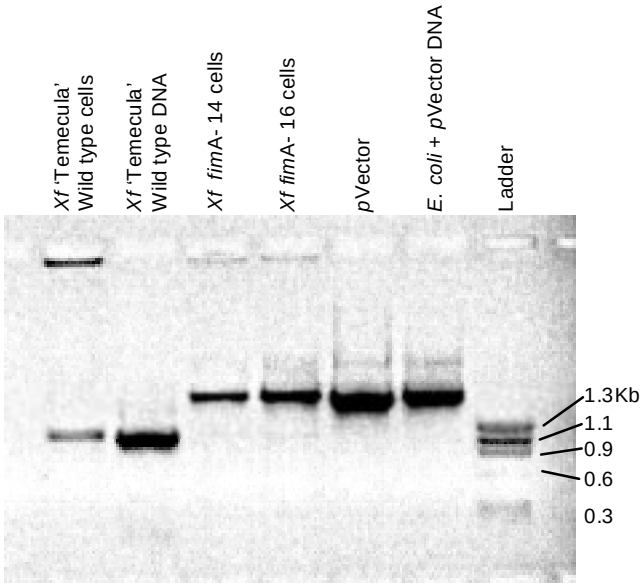

Figure 2: Agarose gel of PCR products using *FimA* primers

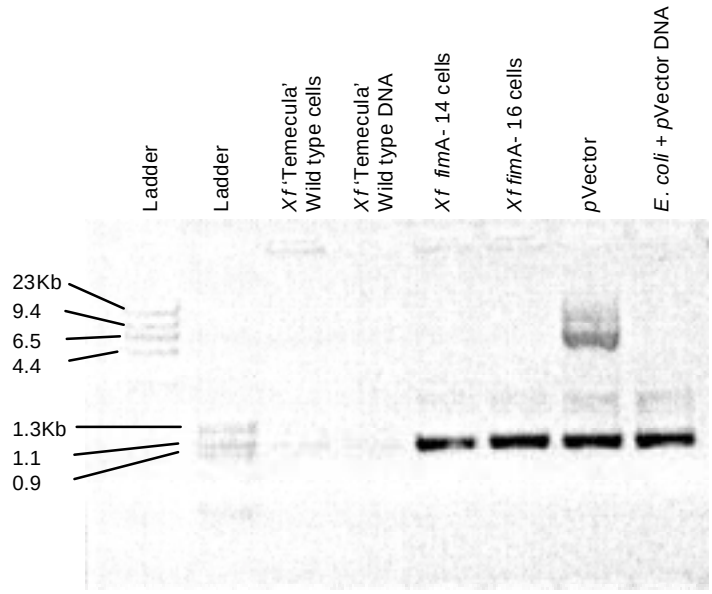

Figure 3: Agarose gel of PCR products using *Kan* primers

We are in the process of testing the *fimA*$^-$ transformants for pathogenicity by inoculation of grape plants. The nature of the *fimA* product is also investigated using SDS-PAGE of the supernatant of an agitated cell suspension of either wild type or *fimA*$^-$ mutants.

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SPATIAL AND TEMPORAL RELATIONS BETWEEN GLASSY-WINGED SHARPSHOOTER SURVIVAL AND MOVEMENT, XYLEM FLUX PATTERNS AND XYLEM CHEMISTRY IN DIFFERENT HOST PLANTS

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INTRODUCTION

The glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say) (Homoptera: Cicadellidae), has been identified as the principal vector for the xylem restricted bacterium, *Xylella fastidiosa*, the causal agent of Pierce's and other related diseases. Pierce's disease is threatening California's wine and grape industry, and has already inflicted heavy damage to certain areas of the state. Our project seeks to identify those aspects in the GWSS-host plant interaction that explain variations in GWSS density and performance (i.e., immature survival and adult reproduction), that is, its population dynamics. Following a species' population dynamics requires a reliable and accurate method of estimating its field densities. Currently, such estimates rely mainly on yellow sticky traps or net beatings with subsequent insect counts. We lack a means of calibrating these density estimates. Thus, as a first step in our study, we have developed a method of estimating GWSS populations in a tree using an "absolute" sampling method. Using a parachute to cover a citrus tree, we fog it to recover from it all GWSS stadia except eggs. We correlate GWSS densities from this method with those using the standard yellow sticky traps and net beatings for adult GWSS. We will present data on density estimates for this insect, in regions of the state heavily infested with GWSS.

A second aspect of our research seeks to determine host plant choice and the density of GWSS on the host plant. We will use a specially designed Schölander bomb to measure xylem fluid pressure and to extract xylem fluid for chemical analyses. We will use this technique to explore possible correlations between changes in GWSS density and reproduction on a host plant and its relationship to physical and chemical properties of that plant. These techniques will give us insight into host plant choice by GWSS.

OBJECTIVES

1. Quantify xylem flux patterns and characterize xylem fluid chemistry to determine potential correlations with GWSS movement from surrounding alternate host plants into vineyards.

2. Quantify egg production, nymphal survival, and adult production and movements in different host plants with xylem flux and chemistry.

RESULTS AND CONCLUSIONS

As a first step in this project, we have developed a system to obtain "absolute" samples of all but the egg stage of GWSS. We will sample the egg stage separately. The "absolute method" was first developed in collaboration with Dr. David Akey and Dr. Mathew Blua, using small citrus trees. After refining this method, we scaled it up to obtain samples from commercial-sized orange trees. We use a surplus military parachute to cover an entire tree and then fog the tree to recover all the insects. We then count all of the GWSS in the sample. Our sampling indicates we recover 80 to 90% of the GWSS present in these

trees. At this point, the method is being used to sample the GWSS present in orange trees at two locations: one in an orange grove in the Bena Road area, near Bakersfield, Kern County, and second in a mixed orange/lemon grove in an Agricultural Operations field, Citrus Experiment Station, UC Riverside. We intend to use this method over two complete years, to follow GWSS population dynamics, and to correlate the changing GWSS densities over time with our characterization of xylem flux and xylem chemistry.

We have begun our xylem pressure measurements and extractions of xylem fluid for chemical analyses at both the Bena Road area in Kern County and the Citrus Experiment Station, at UCR. In the near future, we intend to expand these xylem studies to other host plants, initially focusing on grapevines.

SEASONAL CHANGES IN THE GLASSY-WINGED SHARPSHOOTER'S AGE, STRUCTURE, ABUNDANCE, HOST PLANT USE AND DISPERSAL

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INTRODUCTION

The glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say) (Homoptera: Cicadellidae) is an exotic insect in California, likely introduced from southeastern United States in the late 1980's. In California, it reportedly feeds on 73 plant species in 35 families and has the ability to spread *Xylella fastidiosa*, plant-pathogenic bacteria with differing pathovars each of which infects one or more economically important plant species. GWSS vectors Pierce's disease (in grapevines), phony peach disease, almond leaf scorch and oleander leaf scorch. Moreover, the vector is very mobile and occurs throughout the year; thus, to understand the epidemiology of these diseases, it is crucial that we are able to track GWSS' movements among its various hosts. To accomplish this end, we have developed a mark-recapture technique to track field movements of adult GWSS using fluorescent colored dust as a marker. Greenhouse trials show no significant difference in mortality and longevity of marked versus unmarked (=control) adults. We also monitored spatial movements of adult GWSS in 1) a citrus grove east of Bakersfield, California, 2) between a citrus grove and adjacent vineyards east of Bakersfield, California, 3) in a barren field in southern California and 4) in a lemon/Valencia orange grove interface on the Citrus Experiment Station at UC Riverside.

OBJECTIVES:

1. Develop a technique for marking adult glassy-winged sharpshooters rapidly and for releasing and recapturing them.
2. Develop a sampling system for glassy-winged sharpshooters eggs and monitor sharpshooter egg density initially on citrus.
3. Monitor field movements of adult glassy-winged sharpshooters.

RESULTS AND CONCLUSIONS

The use of several colors of dust to mark GWSS has proven to be a reliable and cost effective technique. In greenhouse studies, mortality was unaffected by the marking technique. No differences in mortality between marked and control insects were observed over a 60-day period. In the field, we recovered insects with recognizable marking 28 days after release. We concluded that this method could be used to mark and track GWSS field movements.

We conducted an experiment to track adult GWSS movements within a non-chemically treated citrus grove in the Bena Road area, near Bakersfield, Kern County. Weekly, from June through August 2001, we marked and released adult GWSS into a grove from which they were initially captured. A different colored marker was used each week to identify the date on which a GWSS had been released. A total of 44 yellow sticky traps were placed at increasing distances around the release point in the grove and the total number of GWSS and the number of recaptured (marked) GWSS on the traps were counted weekly. Over the course of the experiment, a total of 3,050 insects were marked. We recaptured 40 marked individuals out of the 10,311 GWSS captured on the sticky traps (1.3% of total GWSS marked). None of the marked insects was recaptured more than 40 meters from the release point.

In a choice experiment to determine GWSS' preference for Valencia versus lemon, we released marked insects in the interface between a lemon and a Valencia orange grove. Between July and August 2001, we marked a total of 8,330 adult GWSS with different colors of florescent dust each week. A total of 120 yellow sticky traps were placed on all the trees surrounding the release point up to 40 meters distance from the point. Preliminary results from this experiment suggest that, in the absence of disturbance, most adult GWSS do not move over long distances.

A barren field experiment was designed to test the dispersal behavior of marked versus unmarked adult GWSS in the absence of suitable host plants. We placed a total of 107 yellow sticky traps in concentric circles with each trap placed at 17 meters equidistant from the two adjacent traps on that circle. The concentric circles were 10, 20, 40, 60, 80 and 100 meters distance from the release point. Between July and September 2001, we released a total of 20,350 adult GWSS, half of which were marked with florescent dust. Traps were checked 24 hours after each release and every adult GWSS was counted. We recover a total of 327 insects (1.61% of the total released). Our results show that marked individuals are equally likely to be captured as unmarked individuals and that adult GWSS are able to fly at least the 100 meters over which the traps were placed in the absence host plants within the first hour of release.

GENETIC TRANSFORMATION TO IMPROVE THE PIERCE'S DISEASE RESISTANCE OF EXISTING GRAPE VARIETIES

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INTRODUCTION

Genetic engineering offers the possibility of introducing a gene that will confer increased Pierce's disease (PD) tolerance to existing grape varieties. If other research strategies being considered for PD management are not sufficiently successful, genetic engineering may be the only viable alternative.

In many crops, disease resistance is achieved by identifying sources of resistance within the crop species or its wild relatives and then moving the resistance to the crop by several cycles of conventional breeding. This approach produces entirely new varieties. In California, new varieties of table grapes and raisins are readily accepted but new winegrape varieties are not. As in Europe, the California wine industry is based on the classic wine cultivars and wines are identified by the grape cultivar name. Thus new wine cultivars are incompatible with the way wine is marketed and there is little interest in them in the California wine industry.

We are investigating the use of genetic engineering to alter the disease response of existing grape varieties without otherwise changing their viticultural or enological characteristics. Legal authorities in Europe and the U.S. have expressed the opinion that a genetically engineered winegrape produced by the addition of a gene to a traditional wine variety may be permitted to retain the original variety name if it is indistinguishable from the original version in appearance and flavor.

Genetic engineering methods are now well established for many major crops-thousands of field trials of transgenic crops have already been conducted and many such plants have now been commercialized. Many of these transgenic crop plants carry introduced genes for disease resistance. Perhaps the most dramatic example is that of the papaya industry in Hawaii, the state's second largest fruit crop. Papaya producers were faced with the complete devastation of their industry by papaya ringspot virus disease. A genetically engineered resistant papaya was developed (Luis et al., 1997) and is now the predominant papaya variety in Hawaii, well accepted by both growers and consumers alike. The Hawaiian papaya industry has been rescued by genetic engineering.

The xylem vessels of PD-infected grapevines are blocked by a polysaccharide substance that may be a product of cell wall breakdown. The origin and composition of this substance are the subjects of other research projects. Plant cell wall degradation is an early step in the development of many plant diseases and, of several cell-wall-degrading enzymes produced by pathogens, the best known are the polygalacturonases (PGs). Many plants have polygalacturonase inhibiting proteins (PGIPs) that inhibit pathogen PG enzymes and enhance plant defense response. The expression of a pear PGIP gene in tomato has been shown to reduce the development of fungal disease (Powell et al., 2000). The discovery that the *Xylella fastidiosa* genome appears to encode a polygalacturonase and several other cell wall degrading enzymes (Simpson et al., 2000), suggests that PGIP might reduce vascular plugging in PD-affected grapevines or otherwise restrict the development of the disease. We are studying the Pierce's disease response of transgenic grapevines expressing pear PGIP.

OBJECTIVES

1. Evaluate the effect of an introduced polygalacturonase inhibitory protein (PGIP) gene on the development of Pierce's disease in transgenic grapevines.
2. Evaluate the effect of promoters and signal sequences on the targeting of transgene products to xylem tissue.

RESULTS AND CONCLUSIONS

Proembryogenic cultures of *Vitis vinifera* cvs. Chardonnay and Thompson Seedless were transformed with *Agrobacterium tumefaciens* containing a pear PGIP gene construct under the control of the CaMV 35S promoter. Fifty-nine independent putatively transformed lines were obtained and plants were regenerated. Plants from 37 of these lines have been transferred to the greenhouse. Of 25 lines that have been tested to date, 18 are positive for PGIP activity. PGIP activity was not detected in untransformed controls. Western blot analysis demonstrated the presence of the protein in roots, leaves and young stems of the transgenic plants but not in untransformed controls. The post-translational glycosylation of the enzyme is apparently similar to that of the endogenous PGIP in pear fruit. Plants from some lines have been inoculated with *Xylella fastidiosa* and will be evaluated for Pierce's disease symptoms.

New gene constructs are being made that combine the pear PGIP gene with regulatory sequences from a cucumber gene that encodes a xylem-specific protein. These will be used in a new round of transformation experiments to determine whether the cucumber sequences enhance the presence of PGIP in the xylem of transgenic grapevines.

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INSECT-SYMBIOTIC BACTERIA INHIBITORY TO *XYLELLA FASTIDIOSA* IN SHARPSHOOTERS

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INTRODUCTION

The appearance of Pierce's disease vectored by a relative newcomer to California, the glassy-winged sharpshooter, *Homalodisca coagulata*, has lead to the project outlined here. We hope to develop a new strategy to combat the spread of Pierce's disease by delivering anti-disease strategies aimed at neutralizing the disease organism, the bacterium *Xylella fastidiosa*, its production of a form of xanthan gum that clogs the xylem spaces of the plants or disrupts connection of the pathogen to the mouthparts of the vector insect. The use of bacteria associated with insects to disrupt disease transmission is itself a new approach to disease control. The main principle, paratransgenesis, which is the genetic alteration of bacteria carried by insects, was originally designed by Frank Richards to disrupt the transmission of Chagas disease by Triatomine bugs. The possible use of paratransgenesis for preventing Pierce's disease was seen to be feasible when Carol Lauzon and John Peloquin in a preliminary study showed that the vector insect was cycling typical plant bacteria through the midgut. The project then is to exploit this initial finding by seeking ways to deliver anti-Pierce's strategies via one of the innocuous bacteria found common to the host plant. This approach calls for expertise in environmental microbiology, plant pathology, molecular biology, entomology and microbiology. The research leaders listed above have the proper credentials to make this strategy function.

OBJECTIVES

1. Culture, identify, then genetically transform insect-associated bacteria, especially gut bacteria, from glassy-winged sharpshooter, *Homalodisca coagulata* (GWSS).
2. Find gene products that inhibit or kill *Xylella fastidiosa*, or disrupt its attachment in GWSS.
3. Work out transformation systems for the bacteria identified in No. 1 above.
4. Study the movement of plant bacteria between the vector and host plants.
5. Set up disease cycles in the greenhouse to test anti-Pierce's strategies.
6. Test the application of anti-Pierce's agents.

Realistic application of the strategies described above will require permits to release transgenic bacteria. It is obvious that studies on the effects of such organisms on the environment are called for. We are working on such permits now for use in other projects.

RESULTS AND CONCLUSIONS

The first six months of this project (initiated in March of 2001) was spent recruiting participants. Blake Bextine will join the project in mid-November. For his Ph.D., Blake worked out an artificial system to study transmission of the Yellow Vine disease of cucurbits by the squash bug, *Anasa tristis*. That disease is also caused by a bacterial pathogen identified as *Serratia marcescens*. We feel Dr. Bextine is the ideal person to work out a disease transmission protocols to test anti-

Pierce's strategies. He will work directly with Dr. Lauzon and Dr. Peloquin. Early in the project Donald Cooksey hired Ludmila Kuzina as a postdoctoral to work on rearing the pathogenic bacterium, *Xylella fastidiosa*. We were already well aware of how difficult this rearing is and are fortunate to find a young scientist willing to accept the challenge. Dr. Kuzina is testing the various anti-Pierce's gene products and agents that are identified. Dr. Lampe secured the services of a trusted technician early in the project and has been working on transformation protocols in cooperation with John Peloquin. Dr. Cooksey's other related projects are a good match to his desire to find methods of alleviating the Pierce's disease symptoms in the affected plants.

Specific research progress:

Methods of collection and isolation. Field collections of GWSS were done by trapping the insects by hand directly from vegetation in clean, fresh unused Ziploc polyethylene baggies. This was compared to the more efficient sweep net collection to determine any possible source of contamination. We went to some trouble to ensure that gut samples of bacteria were not contaminated with surface bacteria. Initial studies established that samples were indeed from the gut alone. The gut and contents were plated on nutrient agar. The inoculated agar was incubated until bacterial colonies arose. Whereupon these colonies were isolated, purified by restreaking and subsequently analyzed through biochemical and nucleic acid analysis to establish the identity of the bacteria. Subsequent to discovery of bacteria in the gut, collections of GWSS were made throughout the year to establish whether or not these bacteria were always found in GWSS as a part of the normal gut flora. In the case of at least *Alcaligenes xylosoxidans denitrificans*, this appears to be so. Besides *A. x. denitrificans*, a number of other gut bacteria were identified.

The most recent analysis of GWSS gut bacteria have included three yet to be fully characterized bacteria. *Ralstonia* was formerly known as *Alcaligenes* and it has similar biochemical characteristics to this genus. Further biochemical characterization should allow us to identify it to species. The *Arthrobacter* sp. and *Bacillus* sp. are Gram-positive bacteria. A great deal known about the biology of *Bacillus*, and a number of dependable expression vectors are known. *Arthrobacter* species are considerably less well understood. The distribution and frequency of occurrence of these other bacteria must be further studied to understand how they may be used in a program to control Pierce's disease and/or the GWSS. Unlike *Alcaligenes xylosoxidans denitrificans*, *Chryseomonas luteola* has been very slow growing on typical laboratory media and is quite difficult to culture.

The *Alcaligenes* have thus far been found in all the GWSS collections. This suggests a possible symbiotic relationship with GWSS that also needs to be further investigated. We have been concentrating on culture and transformation of *Alcaligenes xylosoxidans denitrificans* because of ease of its laboratory culture and the observation that it has been found in all the GWSS so far collected. Additionally, the biochemical characteristics (such as especially good growth in low nutrient conditions) of these GWSS bacterial isolates suggest a plant affiliation or origin for these bacteria. We speculate that the GWSS may be picking up these bacteria from their host plants. As GWSS feeds solely on xylem, the source of these bacteria may very well be plant xylem. Investigation continues.

Alcaligenes xylosoxidans denitrificans strains have been characterized biochemically. The 16S rRNA sequence of JP134, *Alcaligenes xylosoxidans denitrificans* from GWSS was determined. The closest sequence to this 16S rRNA sequence matched in GenBank by BlastN was an uncharacterized *Pseudomonas* sp. In keeping with the need to study this possible aspect of the biology of these bacteria, Bruce Kirkpatrick has made arrangements with us to follow the movement (if any) of GWSS gut bacteria in plants. We hope to have a genetically marked (EGFP or similar fluorescent protein) available for him to use in his studies soon.

Transformation vectors and attempts. [pEGFP/Zeo]. This plasmid was originally used to successfully transform Gram negative endosymbionts of Tephritid gut (Peloquin, et al., 2000). An electroporation protocol developed for *Psuedomonas aerogenosa* and *Alcaligenes eutrophus* was used in attempts to transform *Alcaligenes xylosoxidans denitrificans* with this plasmid using Zeocin, a derivative of bleomycin/phleomycin as the selective antibiotic. We encountered some problems with Zeocin and are testing alternative selective antibiotics to which our isolates of *Alcaligenes xylosoxidans denitrificans* were susceptible. Kanamycin was found to be a good selective agent in disc diffusion assays. Fortunately, the GWSS bacteria grew well on the Mueller Hinton agar used for this assay.

Derivatives of pBBRMCS2. We found that our *Alcaligenes xylosoxidans denitrificans* were also susceptible to kanamycin and derivatives by growth studies on plates supplemented with this antibiotic at standard concentrations as above with Zeocin. Additionally, an analysis of *Alcaligenes xylosoxidans denitrificans* 16S rRNA sequence suggested that the closest

relatives to this bacterial ribosomal RNA in the genetic data bank were that of a *Psuedomonas* species. We then investigated alternative vectors with origins of replication and promoters different from those usually used in Enterobacteriaceae in a hope that an alternate origin of replication and antibiotic selection other than Zeocin might have better selection properties and stronger expression of transgenes. The plasmid we are developing is a derivative of the previously published pBBRMCS plasmids. We successfully transformed *Alcaligenes xylosoxidans denitrificans* isolates with plasmids derived from these elements. We hope to next engineer these plasmids so that they express useful genes for pest control. At first we will use fluorescent proteins like DsRed and EGFP. Immediately, these fluorescent protein markers can be used to trace the movements and behavior of the transformed bacteria and later to produce substances that reduce or eliminate the vector potential of GWSS infected with the transformed bacteria similarly to work done in other systems.

Endogenous mariners of the GWSS. We screened the genome of the GWSS for the presence of *mariner* transposable elements by PCR using fully degenerate primers designed against highly conserved motifs of *mariner* family transposases (Robertson 1993). PCR products were obtained with one set of the primers and the products cloned and sequenced. The GWSS contains a *mariner* that falls into the *irritans* subfamily of *mariner* transposons. All of the copies we sequenced contained multiple frame shifts indicating they were inactive. These copies are derived from a very divergent *irritans* subfamily element.

We know that the possibilities for interactions between the endogenous mariners of the GWSS and those we intend to use in the gut symbionts are likely to be non-existent for several reasons. First, there is no known way for the elements in the cells of the GWSS to interact with those inside the bacteria. Second, even if such an interaction were possible, the elements in the GWSS are inactive. Finally, the elements in the GWSS are extremely divergent in comparison to the elements proposed for use in GWSS symbionts and as such could not interact with them (Lampe et al. 2001).

A transgenic system for GWSS bacterial symbionts. We are constructing two genetic transformation systems based on the *mariner* family elements, *Famar1* and *Himar1*. The final constructs that will be used to make stable insertions into *Alcaligenes* are described below. The plasmids have several features that help it fulfill the requirements that meet our purposes. They have an RP4 origin of transfer so they can be mated from *E. coli* to *Alcaligenes*. They also have a R6K origin of replication so that they can only replicate in special strains of *E. coli*. The drug marker is carried between two FRT sites, the sequences that are used by the FLP recombinase of yeast. Thus, once insertions of the *Famar1* transposon are obtained, the drug marker can be removed by recombination. Since the transposase gene lies outside the inverted terminal repeats (ITRs), the insertions will be stable after the plasmids are lost. Using this system we should be able to obtain insertions at random positions in the chromosome of *Alcaligenes* that are completely stable. These systems are currently being tested in *Alcaligenes*.

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KEYS TO MANAGEMENT OF GLASSY-WINGED SHARPSHOOTER: INTERACTIONS BETWEEN HOST PLANTS, MALNUTRITION AND NATURAL ENEMIES

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INTRODUCTION

Leafhopper vectors of *Xylella fastidiosa* (including GWSS) behave very differently from most herbivorous insects. GWSS and other xylophagous leafhoppers have evolved many unusual adaptations such as host switching to maximize nutrient uptake, unprecedented assimilation efficiency of nutrients, and excretion of ammonia that enable GWSS to subsist on xylem fluid. The physiology and behavior of GWSS that make it an efficient vector also make it less amenable to conventional management tactics. Adult GWSS may feed on hundreds of different host species, are long lived and exceptionally mobile and fecund. Natural seasonal fluctuations in plant xylem chemistry determine the seasonal use of host plants by GWSS adults. Xylem chemistry can be affected and/or manipulated by environmental factors, culture and management practices (fertilizer, water, pruning, rootstock) and weather extremes. Xylem fluid has the most dilute concentrations of dietary nitrogen and carbon of any plant tissue. Malnutrition is a primary source of mortality of immature GWSS. The nutrient requirements of immature GWSS are different and much more restricted than those of adults such that successful development might often require host switching. Whereas adult GWSS utilize many different host species, early instar nymphs survive and develop on only a few. GWSS females select both feeding hosts and ovipositional hosts, yet the interactions between these two choices may be complex and conflicting. We have established that adults prefer to feed on xylem fluid with specific chemical characteristics (high amide concentrations). However, nymphs develop poorly on these high amide diets. Nymphs develop more successfully on xylem fluid with low amide concentrations and proportionally higher concentrations of many of the more dilute amino acids that are deemed essential for the development of most insects. We have also established the physiological basis for this phenomenon: adults can efficiently use nitrogen and carbon from high amide concentrations, whereas young developing nymphs cannot. Females can consume more nutrients (thus, produce more eggs) on high amide diets, yet oviposition on these same hosts will result in up to 100% nymphal mortality (from malnutrition). Oviposition (as it affects parasitism rate) and survivorship of nymphs (malnutrition) are key mortality factors for GWSS with potential for manipulation to suppress leafhopper populations and *X. fastidiosa* diseases. It is these important key interactions and identification of the host plant species important in mediating such interactions that must be elucidated to manage GWSS in any geographical location.

OBJECTIVES

1. To determine the effects of host plant assemblages and host plant chemistry on distribution, performance and behavior of *Homalodisca coagulata*, glassy-winged sharpshooter (GWSS) and its natural enemies.
2. To determine the relationship of host plant xylem chemistry, and leaf morphology on host selection, feeding and ovipositional behavior of GWSS and its parasites.
   a. Assess host plant acceptance and subsequent feeding rate, host plant selection and acceptance for oviposition and the survival and performance of early and late instar nymphs as a function of host plant species.
   b. Quantify the impact of these plant variables on the behavior and parasitism rate of eggs by *Gonatocerus ashmeadi*.

RESULTS AND CONCLUSIONS

GWSS oviposits in many plant species, yet the majority of GWSS egg masses tend to be concentrated on a lower number of select host species that apparently offer the quality of xylem fluid (food) required for survival of nymphs. Food quality for nymphs appears to be an important factor affecting the population increase of GWSS. In this first year we concentrated on developing field methods and data towards determining the host selection behavior and use for oviposition of known host plants preferred for feeding by the adult GWSS.

Two experimental sites were established near Monticello, Florida. In north Florida, crape myrtle, *Lagerstroemia indica*, is an abundant and high quality host for GWSS adult feeding, but is clearly not a host species frequently used for oviposition. Therefore, one of the sites chosen was a uniform stand - ca. 10-15 acres - of crape myrtle as the adult feeding host. Four different species of host plants: holly (*Ilex* sp.), *Euonymus* sp., *Pyracantha* sp. and Bradford pear (*Pyrus* sp.*)* were chosen as potential oviposition hosts for the GWSS. Twenty trees of each species (a total of 80) were mixed and sorted into10 islands that were randomly distributed within the large block of crape myrtle to establish oviposition 'islands' for GWSS. Plants were fertilized and maintained under overhead irrigation that was supplemented by hand watering. Each plant on the islands and the surrounding crape myrtle were examined for the presence of GWSS adults and nymphs weekly. Every leaf on the plants was carefully examined every two weeks and GWSS egg masses found were counted, marked and dated, and left undisturbed. The experiment was established on 21 June and sampling continued until late September 2000. Yellow sticky traps were placed at each island to monitor flight and presence of GWSS adults near the islands.

The second site was also located at NFREC- Monticello. The experiment was established in a ca. 3-acre open turf-covered field surrounded on 3 sides by pecan and a forest on the other. Sampling began 14 June 2000 and was continued as described above until 28 September 2000. Ten different species of known GWSS host plants in pots and maintained as described above were grouped in 9 plant "islands". Each plant island contained several species of host plants in an effort to simulate the natural aggregated distribution of host plants as found in nature. A total of 149 trees were distributed among the 9 islands. All plants had drip irrigation. Because the islands were located in open grassland without other feeding hosts, crape myrtles were included in each island as food for GWSS adults. The plant islands were separated ca. 75m from each other. Yellow traps were placed as above to monitor GWSS adult presence and flight periodicity.

Results from both sites indicated a statistically significant preference of GWSS for laying eggs on holly plants over all other plant species offered as oviposition hosts. The second host most frequently used by GWSS for oviposition was Bradford pear. A few egg masses were also found on other hosts. *Pyracantha* was chosen as oviposition host only very early in the summer and in the fall. Parasites were able to utilize the GWSS eggs on all hosts.

To complement the small assemblages of mixed hosts blocks in Monticello-FL, a single large plot "epicenter" was constructed in Quincy, FL to test assemblages of hosts for oviposition and adult GWSS on a larger scale. A circular plot (500 m circumference) was constructed in an isolated grass-covered field in late June 2000. Eighteen ovipositional hosts (12 *Euonymus* sp. and 6 *Pyracantha* sp.) in containers were centered in the middle of the plot, and crape myrtle cv. Natchez in containers were arranged in concentric rings at 10, 20, 40, and 80 m away from the ovipositional hosts. The crape myrtles were arranged at 10 m intervals along the concentric rings. GWSS adults and nymphs were counted twice weekly from 1 July until leaves began to senesce (15 October). Remains of egg clutches were counted just prior to leaf senescence in mid-October on both the adult and ovipositional hosts. All plants were maintained by drip irrigation.

Common host species of GWSS were also established by in-ground planting in solid blocks in Quincy to provide data on seasonal fluctuations on a variety of host species. Host species established included peach, plum, apple, grape, holly, *Pyracantha* sp., citrus, *Euonymus* sp. and *Eucalyptus* sp. and also were maintained on drip irrigation. The grape planting included 21 cultivars of grapes (minimum of 6 reps per cultivar) to examine varietal selection within *Vitis* spp. Visual counts of all stages of GWSS were made one to two times weekly during the peak season (July through October) and once every one to two weeks after that to identify potential overwintering hosts.

Preliminary analysis of GWSS populations within the epicenter (circular grid) showed leafhopper aggregation, as is often noted for GWSS populations. However, aggregation patterns varied within the grid throughout the season and were not consistently related to distance from the center (ovipositional hosts). The most noticeable effect in the epicenter as compared to the contiguous blocks was that GWSS populations persisted on the potted crape myrtle well into the autumn. Populations in the solid blocks of crape myrtle declined by mid to late August, as we have previously documented on crape myrtle. In the epicenter, however, populations remained high (>50 % of peak populations) into October when crape myrtle leaves began to senesce. Counts of egg masses indicated that more egg masses were present on the 18 ovipositional hosts (*Pyracantha* and *Euonymus*) than on all 96 crape myrtle. Presumably, nymphs developing on ovipositional hosts may have supplied new adults in late summer that dispersed to the surrounding crape myrtle. Alternatively, the phenology and/or physiology of the potted plants was changed relative to the in-ground plants which maintained them attractive to GWSS for the extended time period. Exposure of these plants to GWSS was ca. 2 months longer than typically noted on crape myrtle. This suggests that proximity of adult and ovipositional hosts may greatly increase exposure of adult hosts to GWSS.

Counts of GWSS within solid blocks established that crape myrtle, apple and *Eucalyptus* were the predominant hosts throughout the summer. *Eucalyptus* was of particular interest as both nymphs and adults were present throughout the season. We note that in this initial year of planting, plant size of young plants may also have been a factor. Citrus seedlings, for instance, were not utilized as much as expected based on our previous examination of more mature citrus in our region. Similarly, grape was not used as frequently as expected by adult GWSS, but this may have (in part) reflected the size of first year plantings.

GWSS Rearing and Host Plant Parameters:
Our first-year efforts focused on standardizing and quantifying methodology for rearing GWSS. This methodology is essential for all research groups attempting to study GWSS development and nutrition, as well as for research on GWSS parasitoids. We have previously had good success in rearing GWSS, yet procedures need to be further standardized to allow year-round mass rearing of GWSS.

We have consistently achieved the highest success rates in rearing GWSS on glabrous *Glycine max*. This is not a naturally occurring host, as typically *G. max* has trichomes that inhibit feeding by GWSS (particularly by nymphs). Glabrous lines are, however, excellent and consistent rearing hosts. We have manipulated xylem nutrients by a variety of inoculation and fertilization techniques. Glabrous *G. max* grown under a variety of cultural conditions will support GWSS development. We have obtained best results if seeds are *Rhizobium* inoculated prior to planting, and only urea fertilizers are applied to soil. Recently we established that a variety of glabrous lines will support GWSS development, although there is some variation in developmental success with genotype. While some developmental success is noted for a variety of *G. max* genotypes and cultural conditions, combinations that result in comparatively low developmental rates often have a highly skewed sex ratio with a paucity of successfully developing females.

The biggest problem in rearing GWSS year round is strict attention to lighting conditions. In north Florida, GWSS that are field collected later than mid-September will diapause, whereas individuals collected in summer or reared under long day length conditions will continue to oviposit throughout the year as long as proper light conditions are maintained. When diapause occurs, we have recently established that we can break diapause if three weeks of short daylength are followed by long daylength (we employ 16:8 light:dark regime) *so long as* the proper ovipositional hosts are present.

Glabrous *G. max* can be grown in the greenhouse year-round, and is relatively pest free. Growth of *G. max* is slow under greenhouse/cage conditions and plants can be maintained for the length of a GWSS generation (ca. 35-50) days without having to replace within cages. While glabrous *G. max* is not a typical host of GWSS, they have provided excellent (and consistent) baseline data on developmental rates, instar durations, growth rates, consumption rates and other measurements on developing GWSS (we have also used these hosts to establish specific nutritional requirements of developing GWSS).

We initiated the second phase of this experiment, which is to assess these same insect measurements on California relevant host species. We have collected xylem chemistry data on the host plants used in the field plots and these are ready for analyses. We are currently investigating Chardonnay grapes, Navel oranges, Spanish Pink Lemon and Crape Myrtle. Data from these hosts will be compared to rates of development on *G. max* in order to assess the value of each of these species as developmental hosts for GWSS. Upon completion, other California relevant hosts will be examined with a similar protocol. These quantitative results are essential, as the California host list for GWSS continues to expand. Quantitative analysis is needed to prioritize the role of each potential host for implementation of GWSS control measures.

Natural Enemies:
By exposing GWSS eggs to parasite adults, we determined the duration of the susceptibility of GWSS eggs to the parasites *Gonatocerus ashmeadi and Gonatocerus morrilli*. It was determined that parasitoids can successfully parasitize 100% of GWSS eggs for at least 7 days after oviposition. We also made observations on the relative phenology of parasite species and investigated their mating and oviposition behavior. Parasitism on GWSS egg masses by these two parasitoids has been studied on egg masses of different age during the development period range (9-10 days at summer temperatures). Parasitoids were able to effectively parasitize 100% of the egg masses laid on holly plant leaves from the first day after being laid until the day before sharpshooter emergence. Emergence of parasitoids from parasitized egg masses 8-10 days old was somehow limited due to leaves hardening because of age.

Beginning in July at Monticello Florida, 99% of field collected GWSS egg masses on 5-gallon potted experimental plants of Hollies, *Pyracantha*, *Euonymus*, Citrus, Bradford pear, and Crape Myrtle were found with 100% of eggs parasitized. Parasitism rate remained at this high rate for the rest of the season until the end of the oviposition period, which in Monticello was the second week of November.

Parasitoid distribution and survival may be affected by available alternative food sources such as nectar and pollen, especially at times during low host density of hosts. We initiated experiments looking at overwintering behavior and requirements of GWSS and its parasitoids. In a greenhouse kept with open screens allowing parasitoids in an out but without heat of any kind, the last GWSS egg masses where oviposited the second week of November when parasitoids were still active. Those egg masses were a total of seven, 4 and 3 oviposited respectively on two 1-gallon holly plants (*Ilex opaca* var. *cornuta*). Overwintering *Gonatocerus* sp. parasitoids emerged from 2 of the 7 egg masses on February 11th, 2001 and GWSS nymphs emerged from the other 5 egg masses. This indicates for the first time in the U.S. that GWSS as eggs and *Gonatocerus* sp. within parasitized egg masses can overwinter at north Florida winter temperatures.

Laboratory experiments showed that adult parasitoids preferred whitefly honeydew to 50% honey solution and that parasitoids fed honeydew provided from excised leaves with live whiteflies lived twice as long as those fed simple honey solution. Parasitoids may need food sources to sustain them and the low numbers of parasitoids and parasitism of GWSS eggs observed in California in early season may be related to this need. Perhaps parasitoid abundance could be enhanced by providing alternative food sources. It was also found at Monticello, Florida, that an earwig, *Doru taeniatum,* and tree cricket species appear to be important predators of GWSS egg masses particularly early in the season. Because California has native earwig species and early season impact from parasitoids is low, earwigs deserve more research attention for their potential impact on GWSS.

HOST SELECTION BEHAVIOR AND IMPROVED DETECTION FOR GLASSY-WINGED SHARPSHOOTER, *HOMALODISCA COAGULATA* (SAY)

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INTRODUCTION

The glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*, a major leafhopper vector of Pierce's disease, *Xylella fastidiosa*, in the Southeast, has recently colonized many locations in California with devastating results. Better and more effective monitoring tools are needed to detect GWSS presence at very low population levels for regulatory and quarantine purposes and to accurately estimate local GWSS population density. Other than direct observation or sweep net collection, the only available survey and detection tool is a yellow sticky trap. CDFA is presently using the Pherocon AM yellow trap configured in two dimensions to capture GWSS adults. Yellow traps capture significantly more GWSS than other colors. Other data suggests that GWSS behavior is driven primarily by vision, and while GWSS apparently can visually discriminate between host plants and other objects, GWSS does not appear to use plant volatiles in host finding. No research has been directed towards improving the yellow trap or to evaluate trap precision at detecting GWSS relative to GWSS population density. We are investigating trap size, shape, and spectral reflectance pattern along with placement parameters (height, background, proximity to vegetation) to optimize a field detection method for GWSS. We are also investigating GWSS behavior during host plant searching and selection.

OBJECTIVES

1. To improve and optimize a trapping method(s) (size, configuration, spectral reflectance pattern, field placement, etc.) to detect and monitor GWSS.
2. To determine the mechanisms used by GWSS in host plant finding and selection.

RESULTS AND CONCLUSIONS

Local populations of GWSS in Florida are usually much lower than populations observed in California under most host plant-habitat situations. We attribute this to the higher plant diversity present in Florida that allows GWSS to feed on many host plant species without intense aggregation on irrigated crops, such as citrus and grapes present in the dry areas of California. As a result, local GWSS populations in Florida more closely approximate founding GWSS populations that may occur in California during early colonization of new locations.

Adult GWSS may feed on hundreds of different host species and are long-lived and exceptionally mobile. Mark-recapture experiments indicate that GWSS is capable of moving away from a release point at least 200 m in less than 2 hours. Natural seasonal fluctuations in plant xylem chemistry determine the seasonal use of host plants by GWSS adults. GWSS move in short flights from plant to plant but also appear to move over longer distances using extended flights. As a result, caution must be followed in any interpretation of trap catch relative to estimation of the potential numbers of GWSS in the proximal and distal surrounding vegetation.

***Current Trapping*:**
CDFA is using the yellow Pherocon AM trap which provides a two dimensional surface that is 18 x 22 cm or 396 cm^2. These traps are inexpensive and easy to use because the stickem is already applied to the trap. Trap size facilitates shipment, handing and storage. Any new trap configuration must balance the need for ease of use and cost versus improved detection and monitoring. Therefore, our goal was to evaluate trap configurations that would improve detection and monitoring but be practical to use in large numbers.

***Trap Color*:**
In previous and present research we have found that GWSS are only captured in significant numbers in traps yellow in color. We have found that bright yellow hues that have little or no reflectance below wavelengths of 500 nm (blue-violet-UV) are preferred by GWSS. As little as 20% reflectance in the wavelengths below 500 nm decrease trap captures. Experiments using traps with a range of colors and intensities of gray support these conclusions.

***Trap Shape and Size*:**
We have found that traps of 3-dimensional cylinders perform better then rectangles and other two dimensional configurations and provide at least 3x increase in trap catch over the regular Pherocon AM. We have found that trap size affects trap capture. Trap capture increases with trap surface area without regard to shape. By testing combinations of shape and size we have found that larger traps (plastic pots as large as 30 x 30 cm) perform better than smaller traps and that cylinders perform better than two dimensional traps. Trap capture increases proportionally as cylinder diameter increases from 5 - 7.6 -10.7 cm and cylinder length increases from 15.2 - 30.5 - 61 cm.

***Trap Placement (background and contrast)*:**
We have found that trap placement relative to host plants and other landscape structures is very important. Placement of two dimensional traps in the open increased trap capture rate by 2x over captures by traps placed against tree foliage or in the interior of host plants. Traps placed at heights near the top or just above the vegetation captured more GWSS than traps placed surrounded by vegetation or near the ground.

***Practicality and Recommendations*:**
Given our results and recognizing the needs of regulatory for large numbers of cost-effective and biologically effective traps, we suggest that detection and monitoring can be significantly improved. Until further experimentation is complete, the Pherocon AM trap as presently configured can be used more effectively if a cylinder-shaped trap (7-8 cm diameter x 30-40 cm length) is made from two Pherocon AM traps. This can be accomplished easily using a stapler to attach the traps together, but would require the use of rubber gloves to avoid the stickem. Several alternatives could be easily developed. Mailing tubes 15 x 30 cm painted Glidden Safety yellow would perform better than the current Pherocon AM trap but would require more space to ship and handle. A cylinder trap could be configured in the field using the appropriately sized, prefabricated yellow rectangles with stickem that could be shipped and handled by placing the sticky sides of two traps together. With either of the alternative traps, cost and effort would remain relatively the same as required for the Pherocon AM trap, however, trap capture would be improved by a factor of 2-4.

SHARPSHOOTER ASSOCIATED BACTERIA THAT MAY INHIBIT PIERCE'S DISEASE

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INTRODUCTION

Xylella fastidiosa is the etiological agent of Pierce's disease, an important disease of grapes in the United States. This disease limits viticulture in Florida and the rest of the southeastern US. It was observed in the Temecula Valley of California in 1997. Though this disease had been known in southern California (California Vine Disease, Anaheim Disease) since the 1880's, it had not been reported before in the Temecula Valley wine grape area. The appearance of Pierce's disease in Temecula coincided with increased populations of the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*. Because of the mobility and vector capacity of this insect, Pierce's disease has become a cause for great concern to wine industry in California.

We plan to culture, identify, then select or genetically transform insect-associated bacteria, especially gut bacteria, from glassy-winged sharpshooter to produce substances that inhibit or kill *X. fastidiosa.* We also intend to use bacterial-plasmids derived from gram-negative bacteria and the novel *himar* transposon/transposases-mediated system derived from the *mariner* insect transposon.

OBJECTIVES

1. Identify insect associated bacteria in GWSS and related insects in Southern California or other areas where the vector insects are endemic.
2. Evaluate identified bacteria for the production of antibiotics inhibitory to *Xylella* for potential hazards to plants animals and humans and for their potential for genetic manipulation through techniques for genetic transformation. Those that can be genetically transformed will be investigated for their ability to express transgenes that produce substances that inhibit, attack, destroy, or prevent the transmission of *X. fastidiosa.*
3. Evaluate various peptide antibiotics demonstrating effective inhibition of *X. fastidiosa* that could be candidates for introduction and production in the GWSS-associated bacteria.

RESULTS AND CONCLUSIONS

Methods of collection and isolation of GWSS was simplified. At the beginning of our work on GWSS gut bacteria, the prevailing thoughts were that the propensity for GWSS to promiscuously acquire environmental bacteria was represented as very high— these insects would pick up practically anything. On further study, it was found that this propensity referred to surface contamination, not gut bacteria. That is, a wide range of bacteria of environmental nature could be cultured from the surface of unsterilized GWSS but not necessarily from solely their internal structures. However, the previous studies of GWSS bacterial flora had been performed to detect any bacteria associated with GWSS and were not necessarily performed with an eye to the exclusion of surface contamination. Our investigations of gut bacteria had been done after having taken especial pains not to destroy bacteria in the gut or other internal organs of this insect.

The thought that GWSS indiscriminately picks up environmental bacteria guided our original collections of this insect. Because of concern over casual contamination of collected GWSS, field collections of GWSS were done by trapping the insects by hand directly from vegetation and placing them in clean, unused Ziploc polyethylene baggies. These baggies are quite clean if not sterile. We were working with the concern that GWSS could pick up environmental bacteria from surfaces, particularly that of a sweep net, which could contaminate the gut flora. The time needed for this hypothetical contamination of GWSS gut to happen was completely unknown so we erred on the side of caution in our initial collection procedure. We wanted to collect uncontaminated insects so as to be certain of the origin of the bacteria we isolated from their guts. This method of collection was used for several collection cycles in the Southern California area. However it was extremely laborious and it was very difficult indeed to capture these very active and alert insects in a plastic baggie, sometimes an entire afternoon was used in pursuit of a single GWSS where populations of GWSS were low (vineyards).

Hence, in an effort to determine the necessity of such elaborate and inefficient collection measures, an experimental collection was made in which 10 insects were captured with the baggies and 10 were collected in a sweep net at the same site on University of California, Riverside's campus agricultural research lands. If the contamination of the gut was rapid, we would expect to see differences between the gut flora of the insects collected with the baggies and those with the net. Subsequent microbiological study of both groups of insects revealed no differences in the gut flora. Therefore, subsequent GWSS collections for purposes of obtaining gut bacteria were made with the much more efficient sweep net.

In retrospect, this discovery makes sense. The initial assumption that GWSS picked up environmental bacteria promiscuously was based on previous surveys in which the unsterilized insects were rolled over the surface of a petri dish and the resulting bacterial colonies were then isolated, purified, and identified. In contrast, our studies were performed on insects which were collected in the field, first by grabbing the insects with clean baggies, and then storing them for a short time in clean fresh baggies with a sprig of host plant material as food for the GWSS until further processing. Within two hours of collection, GWSS were removed from the baggies with sterile instruments, then they were cooled to 4 ^{0}C to reduce their mobility. They were then placed with sterile instruments in sterile mineral oil at about 20 ^{0}C and frozen at -80 ^{0}C. The mineral oil sealed in the contents of the gut at both the anus and mouth and prevented any bacteria from subsequently entering the GWSS during storage or transit. For gut bacteria isolation, the frozen insects were removed from the oil, and then exhaustively surface-sterilized in 70% ethanol/3% sodium hypochlorite solution for five minutes. The surface sterilized (and quite dead) GWSS were then transferred to sterile insect Ringer's solution and aseptically dissected to remove the gut. The gut and contents were plated on nutrient agar and incubated for the growth of bacterial colonies. These colonies were purified by restreaking and analyzed through biochemical and nucleic acid analysis to establish the identity of the bacteria. Subsequent to discovery of bacteria in the gut, collections of GWSS were made throughout the year to establish whether these bacteria were always found in GWSS as a part of the normal gut flora. In the case of at least *Alcaligenes xylosoxidans denitrificans*, this appears to be so.

Various bacteria like *Alcaligenes xylosoxidans denitrificans, Chryseomonas luteola, Arthrobacter* sp., *Bacillus* sp. and *Ralstonia* sp.are reported. The most recent analysis of GWSS gut bacteria have included three yet to be fully characterized bacteria. *Ralstonia* was formerly known as *Alcaligene* and it has similar biochemical characteristics to this genus. Further biochemical characterization should allow us to identify it to species. The *Arthrobacter* sp. and *Bacillus* sp. are gram-positive bacteria. Since there is a great deal known about the biology of *Bacillus,* and a number of dependable expression vectors for transformation of this bacteria, this particular bacterium may have promise as a vehicle for production of substances inhibitory to *X. fastidiosa* within either the plant or GWSS, or to actively destroy the GWSS through engineering of this *Bacillus* to produce a toxin detrimental to GWSS. Perhaps some sort of *Bacillus thuringiensis* toxin might be employed in this fashion. *Arthrobacter* species are considerably less understood. The distribution and frequency of occurrence of these bacteria must be further studied to understand how they may be used in a program to control Pierce's disease and/or the GWSS.

The *Alcaligenes* have thus far been found in all the GWSS collections. This suggests a potential symbiotic relationship with GWSS that also needs to be further investigated. Unlike *Alcaligenes xylosoxidans denitrificans, Chryseomonas luteola* has been very slow growing on typical laboratory media and is quite difficult to culture. We have been concentrating on culture and transformation of *Alcaligenes xylosoxidans denitrificans* because of ease of its laboratory culture and the observation that it has been found in all the GWSS so far collected. Additionally, the biochemical characteristics (such as especially good growth in low nutrient conditions) of these GWSS bacterial isolates suggest a plant affiliation or origin for these bacteria. We speculate that the GWSS may be picking up these bacteria from their host plants. As GWSS feeds solely on xylem, the source of these bacteria may very well be plant xylem. Further study is needed to investigate this.

REPRODUCTIVE BIOLOGY AND PHYSIOLOGY OF GLASSY-WINGED SHARPSHOOTER

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INTRODUCTION

Glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Homoptera: Cicadellidae), is a vector of Pierce's disease of grape. Glassy-winged sharpshooter (GWSS) also vectors other diseases, including phony peach, alfalfa dwarf, almond leaf scorch and oleander leaf scorch. All these diseases are caused by various strains of the bacterium, *Xylella fastidiosa* (Wells) (Purcell and Hopkins, 1996).

Although GWSS is an important vector of plant diseases, little is known about its reproductive biology. It has been reported that GWSS has two generations per year in Southern California (Blua et al., 1999). Oviposition occurs in late winter to early spring, and again in mid-to-late summer. Adult females live several months and lay small eggs side by side in groups of about 10, ranging from 1 to 27 (Turner and Pollard, 1959). The greenish, sausage-shaped eggs are deposited in the leaf epidermis of the host plants.

The morphology and anatomy of GWSS reproductive system has not been described. The first part of our research project is to study the structure and function of the female reproductive system.

OBJECTIVES

1. Collect and prepare GWSS specimens for studying the morphology and anatomy of female GWSS.
2. Study and describe the female genitalia focusing on the structure of the ovipositor.
3. Study the gross anatomy of the female reproductive organs.

RESULTS AND CONCLUSIONS

Female and male GWSS were collected in June, July, and August 2001. The total numbers of male and female GWSS collected were recorded (Figure 1). A subsample of female specimens were taken, dissected and examined under a stereomicroscope to study their reproductive system. Some dissected specimens were further treated with KOH and stained to observe the detailed structures.

The abdomen of the GWSS contains 11 segments. The first two and last two segments are reduced. Dorsally, the 1st abdominal tergum is folded beneath the 2nd and both of the 1st and 2nd tergites are hidden under the presternum. The 3rd tergum is clearly visible and is followed sequentially by the 4th through the 9th tergites. The 9th tergum is modified to form a pygofer. The pygofer extends ventrally to enclose the genitalia. Following the 9th tergum are the greatly reduced 10th and 11th tergites (Figure 2 a). Ventrally, the 1st and 2nd sternites are reduced in width. The 1st is hidden beneath the 2nd and both sternites are tucked into a fold at the base of the abdomen. The 3rd through 6th sternites follow sequentially after the 2nd sternum (Figures 2 b and c).

Modification of the genitalia begins with the 7th sternum, which is extended over the base of the ovipositor concealing it from view externally (Figures 2 b and c). The 7th sternum extends with v-shaped, tooth-like projections over the genital capsule and a part of the pygofer (Figures 2 b). The 8th sternum is reduced to a lip-like fold (Figure 2c). The genital

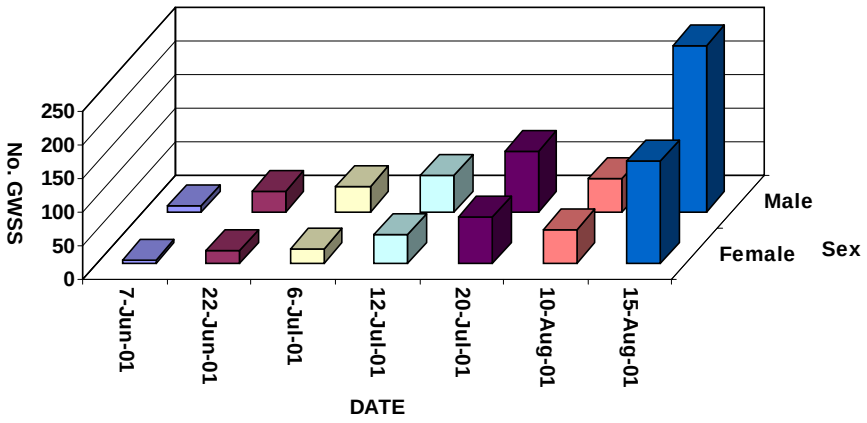
capsule appears to arise from the intersegmental membrane between the 7th and 8th sternites (Snodgrass, 1933; Tsai and Perrier, 1996). The valvulae arise from the intersegmental membranes between the 7th and 8th, and 8th and 9th sternites.

The 1st valvula articulates with the 1st valvifers. The 1st valvula is long and strap-like in appearance with a distinct tapering point at the posterior end (Figure 3). The pair of the 1st valvulae is connected anteriorly and become separated approximately one third down their length. The 2nd pair of valvulae is found internal to the 1st pair of valvulae. They articulate with the 2nd valvifers at one ramus. The articulation of the 2nd valvula with its valvifer is very close to that of the 1st valvula, and as a consequence, they lie very close to each other. The two pairs of valvulae are held together by a ridge. About a third down its length dorsally, the 2nd valvula takes the shape of a saw blade with approximately 35 teeth. The distal end of the 2nd valvula is somewhat rounded. The 3rd pair of valvulae is fused together by a membrane on the dorsal edge to enclose the 1st and 2nd valvulae. The 3rd valvula articulates with the 2nd valvifer anteriorly at a ramus opposite to that of the 2nd valvula. The 3rd valvula is darkly pigmented posteriorly and ventrally. Anteriorly and dorsally, it is membranous and connects to the pygofer. The pygofer is the outermost apparatus of the female genitalia. It is almost a ring-like structure and encloses the 3rd, 1st and 2nd valvulae in that order. Dorsally, it appears as one continuous tergum, but ventrally, it splits at a middle line exposing the ovipositor (1st and 2nd valvulae) and the 3rd valvulae. Ventrally, the pygofer articulates with the 2nd valvifers at one ramus.

The internal reproductive organs consist of a pair of ovaries (each consists of ca. 10 ovarioles/ovary), two lateral oviducts, a common oviduct, a spermatheca, accessory gland(s), and the genital chamber (Figure 4).

Currently, we are studying the morphology and anatomy of the male reproductive system. More research will be carried out to study oogenesis and spermatogenesis by histological and cytological methods.

Figure 1. Total Number of specimens of Glassy-winged sharpshooter collected on citrus at UCR in the summer of 2001.



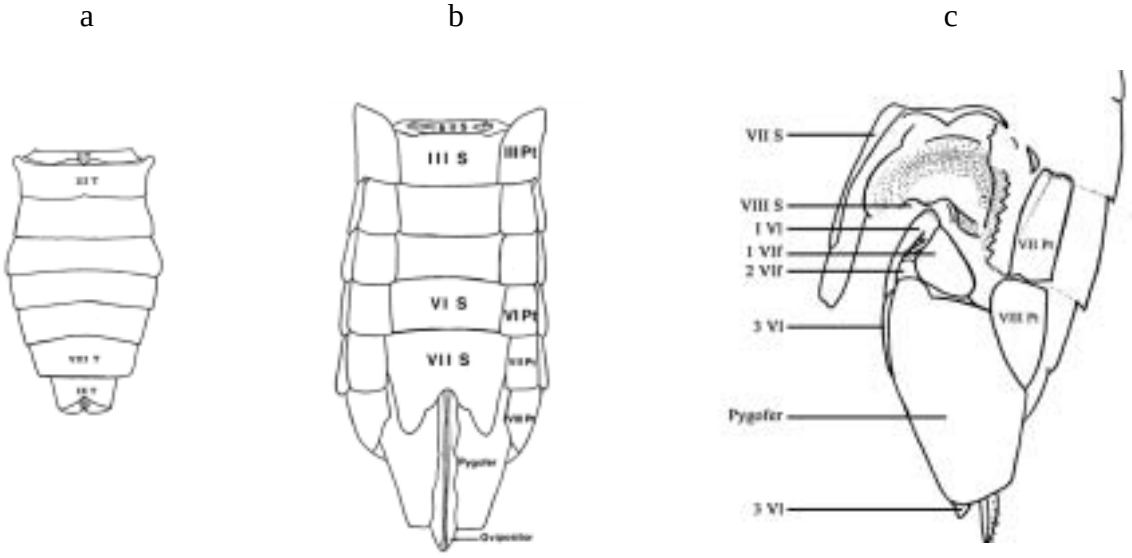


Figure 2. a. Dorsal view of the abdomen of a female GWSS. b. Ventral view. c. Lateral view to show the ovipositor.

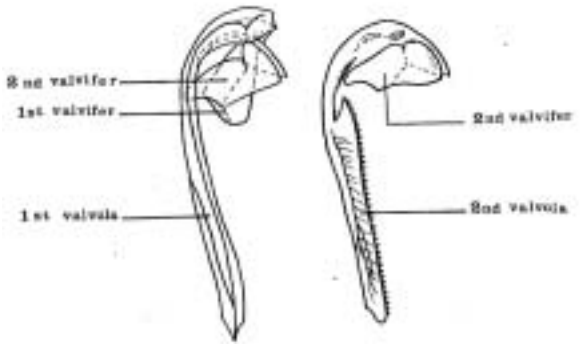


Figure 3. The ovipositor of a female GWSS.

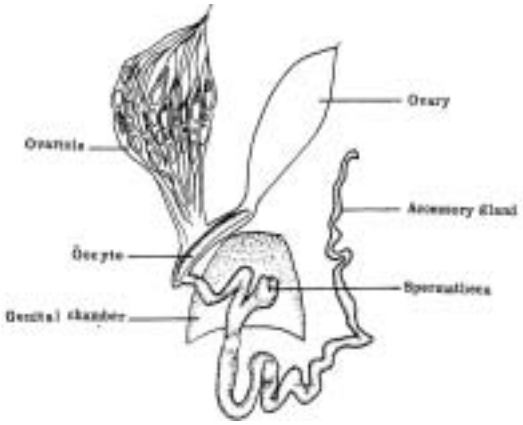


Figure 4. The reproductive organ of a female GWSS.

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EPIDEMIOLOGY OF PIERCE'S DISEASE IN THE COACHELLA VALLEY

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INTRODUCTION

The table grape industry in the Coachella Valley is represented by 14,400 acres of producing vines (California Department of Food and Agriculture 1999), which generated grapes valued at $131 million in 1998 (Jose Aguiar, Riverside County Farm Advisor, personal communication). In the past, Pierce's disease (PD), a disease caused by the xylem-limited bacteria, *Xylella fastidiosa* Wells et al., has occurred in the Valley, but incidence has been limited to fields bordering weedy areas. *X. fastidiosa* is transmitted from infected to healthy plants by sharpshooters, a group of insects in the family Cicadellidae.

In 1997, PD was documented in the wine grape-growing region of the Temecula Valley in southern California. Unlike the Coachella Valley and other areas in the state where PD is known to exist, the Temecula growers suffered devastating losses. A survey of 8 Temecula vineyards, conducted in September 2000 found plant decline or death due to PD ranging from 51%-87% (Perring et al. 2001). The most plausible explanation for the swiftness and severity of the PD epidemic in Temecula is the unique epidemiology created when the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say) is introduced into an area with endemic PD sources (Purcell and Saunders 1999). When this occurred in Temecula, the epidemic mimicked the southeastern US, where GWSS-transmitted PD is the major factor limiting grape production.

The glassy-winged sharpshooter was identified in the Coachella Valley in the early 1990's (Blua et al. 1999). There are no apparent biological or climatological factors that will limit the spread of PD in grapes in the Coachella Valley. Our work reported here was designed to document the current levels of PD in the Coachella Valley and to describe the seasonal cycle of the GWSS. This will allow us to identify characteristics of vineyards with high and low disease incidence for the purpose of designing strategies to minimize PD spread.

OBJECTIVES

During the first year of our project, we focused on two objectives.

1. Determine the incidence and distribution of Pierce's disease (PD) in the Coachella Valley.
2. Describe the spatial and temporal abundance of GWSS in the Coachella Valley and determine site characteristics that may be contributing to GWSS abundance.

RESULTS AND CONCLUSIONS

PD Incidence*:* Sampling for PD was done in two ways. Growers and their field workers were invited to attend one of a series of workshops where they were exposed to PD symptomatology. Each attendee was given a PD booklet (Varela et al. 2001) to take to their vineyards to help further train their workers. As suspect plants were found, growers contacted us and we traveled to the vineyard, confirmed their visual detection, and collected tissue for ELISA analyses. No PD was detected in any of the samples.

The second sampling method involved the selection of 25 vineyards distributed throughout the Coachella Valley. At each vineyard, we intensively sampled 3 blocks of approximately 100 vines per block. Trained researchers conducted the visual surveys of these approximately 7,500 plants, and tissue was collected from plants suspected of having the disease. Approximately 180 samples were collected and subjected to ELISA and bacterial plating. None of the samples were positive with both techniques.

GWSS Abundance: For this study, we placed traps every square mile throughout the Coachella Valley. These 163 traps were changed each week and the numbers of GWSS and the related smoke-tree sharpshooter (STSS), *Homalodisca lacerta* (Fowler) were counted. Sampling started on May 14, 2001 (Julian date 134). Counts showed that average numbers of GWSS were low throughout the Valley and over the time period that we have sampled. The average number/trap/day ranged between 0.01 and 0.16, and as expected, count data were quite variable (Figure 1). This reflects the fact that many of the traps had no sharpshooters on them. Also, there was no difference between sharpshooter numbers found on the north versus the south sides of traps, therefore a wind-driven component to sharpshooter flight is not suspected at this time (data not shown).

There were substantially more GWSS than STSS on most dates (Figure 1). An interesting decline occurred in GWSS numbers between dates 197 - 204 (July 16 - 23), and we are looking at weather patterns to determine if GWSS was responding to any particular condition. When we looked at vegetation and ecological types surrounding the traps, we found some interesting relationships. Three are described here. We determined that traps next to urban landscapes (residences, edge of communities, etc.) had similar numbers of sharpshooters as did traps not adjacent to these areas. Second, we found that traps adjacent to grapes had no more sharpshooters than did traps not adjacent to grapes, thus it does not appear that vineyards are contributing to sharpshooter numbers at this time. Third, we found that traps adjacent to citrus caught significantly higher numbers of sharpshooters than traps not adjacent to citrus (Figure 2). This suggests that citrus contributed to the sharpshooter abundance in the Coachella Valley. Recent work has determined a relationship between citrus and PD incidence in the Temecula Valley (Perring et al. 2001), and there are aggressive campaigns to treat large areas of citrus elsewhere in the state. Our data do not support such action in the Coachella Valley. In our trapping studies, we found that only 33% of the traps adjacent to citrus caught GWSS while the other 64% of the traps next to citrus did not catch any GWSS (Figure 3). Thus our recommendation at this time is to monitor citrus carefully, and implement management strategies only where sharpshooters reach high numbers.

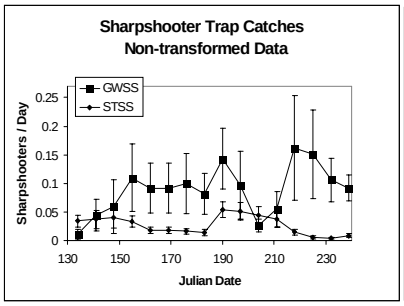

Figure 1

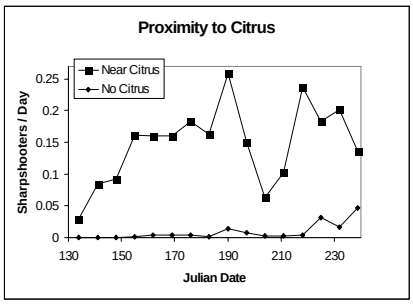

Figure 2

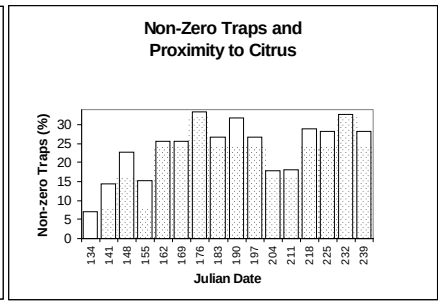

Figure 3

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SURVEY OF EGG PARASITOIDS OF GLASSY-WINGED SHARPSHOOTER IN CALIFORNIA

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INTRODUCTION

Collections over the past year of fresh glassy-winged sharpshooter (GWSS) egg masses have been made from Kern (on citrus), Los Angeles, Santa Barbara, and Ventura (on avocado, citrus, laurel sumac, and macadamia) Counties. No novel parasitoids have been encountered, all parasitoids belonged to the genus *Gonatocerus* (Mymaridae).

OBJECTIVE

1. Survey GWSS egg masses in California to determine indigenous parasitoid fauna.

RESULTS & CONCLUSIONS

Leaves with GWSS egg masses were collected from pre-selected survey sites and host plants and held in the laboratory for parasitoid emergence by Dr. Phillips. Parasitoids were preserved in 75% ethanol and identified to species by Dr. Triapitsyn.

Ventura County (Fillmore): *G. ashmeadi* (Girault) was the prevalent parasitoid in all samples collected from March through August (about 95-97% of specimens), sometimes mixed with a few *G. morrilli* (Howard) (3-5% of the specimens). In three samples *G. morrilli* was the only parasitoid. Generally, collections of *G. morrilli* have continued to increase in Ventura Co. during late May, June and early July relative to 2 years ago. One sample (5/3/2001 on laurel sumac) had one specimen of *G. novifasciatus* Girault.

Kern County (Bena Rd.): parasitoid numbers have increased significantly during 2001 in Kern County from what they were a year ago in 2000, with many collections during late spring and summer resulting in 100% parasitism rates. Only one parasitoid species, *G. ashmeadi*, was present in the samples taken from March through August. The number of specimens collected was very low in March-April samples compared with the samples collected during the summer.

Riverside County (collections were made by various individuals with no relation to this project and submitted for identification): *G. ashmeadi* has been by far the dominant parasitoid but the occurrence of other species such as *G. novifasciatus*, *G. incomptus* Huber as well as *Ufens* spp. (Trichogrammatidae) was greater than in Ventura County. The same species, along with *G. morrilli*, were reared from eggs of smoke tree sharpshooter.

XYLELLA FASTIDIOSA BACTERIAL POLYSACCHARIDES WITH A POTENTIAL ROLE IN PIERCE'S DISEASE OF GRAPES

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INTRODUCTION

Pierce's disease (PD) causes symptoms of leaf scorch and fruit cluster wilt on wine, table and raisin grapes, and is caused by the bacterial pathogen *Xylella fastidiosa*. These fastidious, gram-negative bacteria occur only in the xylem of infected plants and are transmitted by xylem-feeding insects; leafhoppers and sharpshooters. The close association of plants with bacteria, either pathogenic or symbiotic, is often mediated by cell surface polysaccharides that may become modified during the infection process (Price, 1999). *Xylella* has 9 genes homologous to the *gum* genes of *Xanthomonas campestris* that direct the synthesis of a highly viscous exopolysaccharide gum (EPS). The biosynthesis and secretion of EPS is often tightly regulated either by the availability of nutrients or of specific small-molecule inducers in plant exudates. In addition, *Xylella* undergo developmental changes from rippled to smooth cell walls during the infection process (Huang et al., 1986), implicating a possible involvement of cell surface lipopolysaccharides (LPS). LPS consists of membrane-anchored lipid A, a core oligosaccharide, and a carbohydrate O-antigen repeat that typically contains phosphorylated, acetylated or methylated sugars that can profoundly affect its physical properties. Since it forms the outermost bacterial surface, the *Xylella* LPS may be an important factor in mediating interactions such as recognition and adhesion between the bacteria and host plant, or the bacteria and the insect vector.

OBJECTIVES

The stated hypothesis of our research is that bacterial polysaccharides produced by *X. fastidiosa*, particularly cell wall lipopolysaccharides and an exopolysaccharide analogous to xanthan gum, may be causative agents of Pierce's disease on grapes. The prioritized research objectives were stated as:

1. Characterize structurally the bacterial exopolysaccharide(s) from the xylem sap of PD-infected and non-infected vines (using two grape varieties, Chardonnay and Carbernet), and to ascertain its potential involvement in Pierce's disease.
2. Characterize structurally the lipopolysaccharide O-antigen from *Xylella fastidiosa* grape pathovar. isolated from the xylem of PD-infected and non-infected vines (two varieties, Chardonnay and Cabernet).
3. Characterize structurally the exopolysaccharides and O-antigen from *Xylella fastidiosa* grown in culture, and to assess potential changes in response to changing growth conditions and/or cultures additives.

RESULTS AND CONCLUSIONS

Carbohydrate and Genomic Analysis

Sequence data from the *Xylella fastidiosa* clone 9a5c (Simpson, 2000), a causative agent of citrus variegated chlorosis, indicated that this strain has 9 genes with close homology to the xanthan gum biosynthetic cluster of *Xanthomonas campestris*, i.e. *gumBCDEFHJKM*, but lacked *gumGIL*. In *Xanthomonas* the *gum* genes direct the synthesis of a highly viscous exopolysaccharide gum (xanthan) that is widely used in the food industry as a thickening agent. The production of a comparable gum by *Xylella* in the xylem of infected vines would likely block the plants' water uptake system and produce Pierce-type pathogenic symptoms. For the citrus pathovar (CVC clone 9a5c) these *gum* genes are clustered on a single operon. Examination of the preliminary genomic data from *X. fastidiosa* grape, oleander and almond pathovars. (at the time of writing these data are not yet published) identified similar components of *gum* genes, suggesting the exopolysaccharide (EPS) production is common to all four pathovar. strains. Hence the EPS may be symptomatic of *Xylella*-induced plant diseases, but is unlikely to be a determinant of host-pathovar. specificity.

Biochemical studies have shown that in *Xanthomonas* the first nine *gum* genes encode enzymes involved in the biosynthesis of the xanthan gum tetrasaccharide repeat unit, D-GlcA-(beta-1,2)-6-O-acetyl-D-Man-(alpha-1,3)-D-Glc-(beta-1,4)-D-Glc, while *gumGIL* encode for a terminal mannosyl transferase (GumI) and for two enzymes involved in decorating the mannosyl residue, the acetyltransferase GumG and the pyruvate ligase GumL. Hence the overall structure of xanthan gum is a cellulosic backbone that is branched at the 3-position on every other Glc residue with a 4,6-pyruvyl-O-acetyl-Man-GlcA-6-O-acetyl-Man side chain. The lack of *gumGIL* in the *Xylella* genome suggested that its exopolysaccharide should be closely analogous to xanthan gum, but lacking the terminal 4,6-puyruvyl-O-acetylmannose residue. Our initial aim was to determine whether xanthan lacking this outermost sugar residue would still form a viscous polymerized gum likely to clog xylem vessels, or whether the intrinsic viscosity would be lost.

Xanthan gum forms a very stable gel in aqueous solution that retains its gel-state even after centrifugation. The gel assume a double stranded conformation that is stabilized by high concentration of metal ions, whereas denatured, single-stranded forms exist at low ion strength or elevated temperature. Hence, the presence of metal ions such as Mg2+ or Ca2+ tend to stabilize the ordered, double-stranded conformation of xanthan, and thereby stabilize the gel-like properties. Acid treatment of xanthan to selectively cleave the outermost mannose residue, leaves a polysaccharide directly analogous to the proposed *Xylella* gum. We report that the gel viscosity was apparently unaffected by this treatment. After dialysis, the diasylate contained a single monosaccharide identified by thin layer chromatography (TLC) after methanolic-H_2SO_4 charring as D-mannose. The lyophilized residual gel was rehydrated to 0.5% w/v and gave a stable gel indistinguishable to the hydrated xanthan. These data suggest that the outermost β-Man is not necessary in maintaining the ordered conformation of the predicted *Xylella* gum which is therefore likely to have physical properties very similar to xanthan. If produced in the xylem of vine plants, even at relatively low concentration (0.5% w/v), it would therefore likely occlude them, blocking the plants' water uptake system and leading to the water-stress damage symptomatic of Pierce's disease.

Hydrolysis/Methanolysis

Polysaccharide analysis generally requires hydrolysis (or methanolysis) to the component monosaccharides (methylglycosides) prior to compositional analysis. Controlled acid hydrolysis of the predicted *Xylella* gum would produce D-glucose and D-mannose plus a characteristic disaccharide, D-GlcA-(beta-1,2)-D-Man arising from the extra stability of the glucuronic acid (GlcA) glycosidic bond. Xanthan gel was still viscous after acid hydrolysis in TFA or 1% aq. sulfuric and gave a streak at the origin on TLC plates. In 10% aqueous sulfuric the gel-form was denatured, and a single spot was observed on the TLC plates probably corresponding to co-eluting component monosaccharides. Methanolysis at concentrations less than 1 M HCl were not viscous (in methanol) but the majority of the polysaccharide was undissolved. In 1 M methanolic HCl 80-90% of the xanthan dissolved to a non-viscous clear solution containing the predicted D-Man, D-Glc, and GlcA-β1,2-Man, plus free GlcA as a minor component. The GlcA-β1,2-Man disaccharide should therefore be characteristic of this type of polysaccharide gum and might be useful as a "fingerprint" diagnostic of the *Xylella* EPS in xylem exudates or vine cuttings.

Genomic Analyis of Xylella Lipopolysaccharide (LPS) Biosynthesis

An initiative of the American Vineyard Foundation and California Dept. Food and Agriculture to sequence the grape pathovar. genome is presently in progress, and *X. fastidiosa* pathovars. of citrus, almond and oleander are completed. These genomic data are invaluable to understanding comparative polysaccharide biosynthesis by *Xylella*, particularly for predictions of diverse or pathovar-specific carbohydrate structures, such as LPS O-antigen. Our sequence analysis of the published genome of *Xylella* clone 9a5c (CVC strain) (Simpson , 2000) indicates that the gram-negative *Xylella* has the full genetic compliment required for lipid A biosynthesis (*lpxABCDK*), but is unique in having multiple copies of several of these genes (Table 1). Four copies of the N-acyltransferase gene (*lpxD*) are present instead of the usual single copy, and there are two copies of the O-acyltransferase gene (*lpxA*). One *lpxA/lpxD* pair is linked (XF1043 and XF1045) and is also associated with the disaccharide synthase gene (*lpxB*, XF1042). However, the 4'-kinase (*lpxK*, XF1082) and deacetylase (*lpxC*, XF0803) genes are positioned elsewhere on the genome, as are the repeat copies of *lpxA* and *lpxD*. The multiple copies of the acyltransferases may indicate the *Xylella* has the ability to hyper-acylate its lipid A under certain conditions, thereby increasing its lipophilic character. Alternatively, there may be specific LpxA and LpxD proteins expressed to transfer specialized lipid motifs, such as the C28:27-OH fatty acid found on *Rhizobium* lipid A (Simpson, 2000).

Table 1: Genes implicated in Xylella LPS and Surface Antigen Biosynthesis.

XF1289	44 %	2-dehydro-3-deoxyphosphooctonate aldolase (KDSA) {*Escherichia coli*}
XF0105	44 %	3-deoxy-D-manno-octulosonic acid transferase (KDTA) {*Escherichia coli*}
XF2299	50 %	3-deoxy-manno-octulosonate cytidylyltransferase (KDSB) {*E. coli*}
XF1419	26 %	acetyltransferase (LPXD OR FIRA OR OMSA) {*Escherichia coli*}
XF0918	32 %	acyl-[ACP]-UDP-N-acetylglucosamine (LPXA OR AQ_604) {*Aquifex aeolicus*}
XF1994	29 %	beta 1,4 glucosyltransferase (HI0653) {*Haemophilus influenzae*}
XF0612	24 %	dolichol-phosphate mannosyltransferase (dmt) {*Aquifex aeolicus*}
XF1638	34 %	dolichyl-phosphate mannose synthase related protein {*Pyrococcus abyssi*}
XF0257	75 %	dTDP-4-dehydrorhamnose 3,5-epimerase (rfbD) {*Xanthomonas campestris*}
XF0258	54 %	dTDP-4-keto-L-rhamnose reductase (rfbC) {*Xanthomonas campestris*}
XF0255	76 %	dTDP-glucose 4,6-dehydratase (RFBB) {*Xanthomonas campestris*}
XF0611	63 %	dTDP-glucose 4-6-dehydratase (rfbB) {*Synechocystis sp.*}
XF1637	29 %	glycosyl transferase (spsQ) {*Sphingomonas sp. S88*}
XF1082	38 %	lipid A 4'-kinase (LPXK OR HI0059) {*Haemophilus influenzae*}
XF0104	40 %	lipid A lauroyl acyltransferase (HTRB OR WAAM) {*Escherichia coli*}
XF1348	26 %	lipid A lauroyl acyltransferase (HTRB OR WAAM) {*Escherichia coli*}
XF1042	45 %	lipid A disaccharide synthase (LPXB OR PGSB) {*Escherichia coli*}
XF0879	26 %	lipopolysaccharide biosynthesis protein (rfbU) {*Escherichia coli*}
XF2434	37 %	lipopolysaccharide core biosynthesis(rfb303) {*Pseudomonas aeruginosa*}
XF0980	56 %	lipopolysaccharide synthesis enzyme (kdtB) {*Serratia marcescens*}
XF0778	26 %	O-antigen acetylase (oafA) {*Salmonella typhimurium*}
XF1413	45 %	polysialic acid capsule expression protein (kpsF) {*Aquifex aeolicus*}
XF2154	73 %	saccharide regulatory protein (opsX) {*Xanthomonas campestris*}
XF0176	43 %	sugar transferase (SC4A2.10c) {*Streptomyces coelicolor A3(2)*}
XF1045	41 %	UDP-3-O-(R-3-hydroxymyristoyl)-GlcN-acyltransferase (LPXD) {*Salmonella}*
XF1646	28 %	UDP-3-O-(R-3-hydroxymyristoyl)-GlcN-acyltransferase (LPXD){*Rickettsia}*
XF0486	31 %	UDP-3-O-[3-hydroxymyristoyl] GlcN-acyltransferase (lpxD) {*Chlamydia}*
XF0803	59 %	UDP-3-O-[3-acyl] GlcNAc deacetylase (lpxC) {*Pseudomonas aeruginosa*}
XF1043	44 %	UDP-N-acetylglucosamine acyltransferase (LPXA) {*Escherichia coli*}

Rfa-type genes involved in core assembly are also present, as are *kds*/*kdt* genes involved in CMP-KDO biosynthesis. We have identified both ADP-heptose synthetase (*rfaD*) and two heptosyltransferase (*rfaC, rfaF*) genes suggesting that *Xylella* has a conserved inner core structure α Hep-1,3-α Hep-1,5-α KDO-2,6-lipidA, similar to *E. coli* or *Salmonella*. Rfb-type genes that are likely to encode for synthesis of the outermost carbohydrate portion (the O-antigen) are also present on the genome of the *Xylella*. Structurally O-antigen tends to be very diverse, perhaps reflecting adaptation to particular environments and in this respect it is noticeable that several of the *Xylella rfb* genes are also conserved in *Xanthomonas campestris*. For example, the *rfbBCD* cluster (XF0255, XF0258 and XF0257) are probably involved in the synthesis of dTDP-L-rhamnose, a sugar known to be part of *Xanthomonas* LPS. As stated earlier, *Xanthomonas* LPS has been implicated in adhesion to its plant host.

SDS-PAGE Analysis of *Xylella* Lipopolysaccharide (LPS)

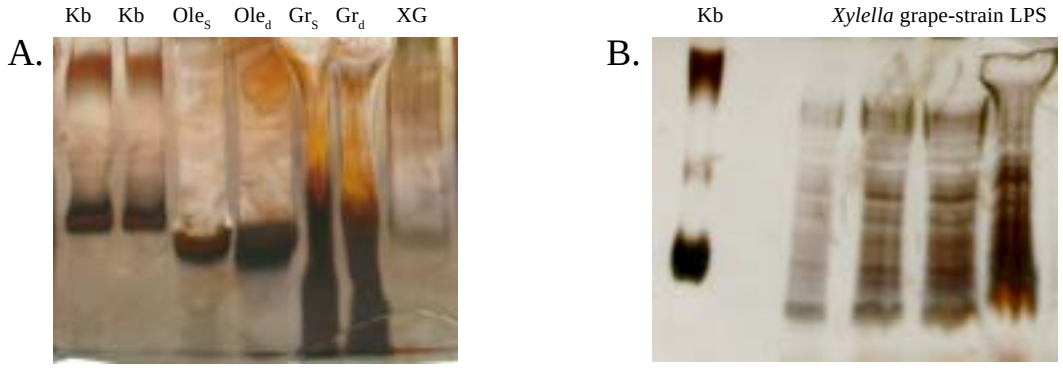

Figure 1. SDS-PAGE analysis of Silver-Alcian Blue stained LPS from *Xylella fastidiosa* grape (Gr) and oleander (Ole) pathovars. *Klebsiella* LPS (Kb) is included as a standard, and XG is xanthan gum. A. 18% continuous; B. 5%, 18% discontinuous.

X. fastidiosa undergoes developmental changes from rippled to intermediate to smooth walled during the course of Pierce's disease indicating a possible involvement of LPS, although *Xylella* LPS has never been identified previously. High molecular weight LPS typically consists of repeating units which are resolved into ladders by gel electrophoresis. SDS-treated LPS extracts of *Xylella* grape strain and oleander strain behaved very differently on 18% SDS-PAGE gels, indicating gross structural differences. On continuous gels LPS from the oleander strain (Ole_s and Ole_d) ran as a single low M.wt. band indicative of "rough" LPS lacking significant O-antigen (Figure 1A). In contrast, grape-strain *Xylella* LPS (Gr_s and Gr_d) comprised a ladder series of highly acidic (Alcian blue binding) O-antigen forms most readily resolved on discontinuous gels (Figure 1B). These data represent the first evidence of LPS production by *Xylella* species, and the first indication that diverse LPS structures may be host-pathovar. specific.

The *Xylella* LPS may be important in mediating interaction such as recognition and adhesion between the bacteria and host plant, or the bacteria and the insect vector, while EPS "xanthan" gum production is likely common to all strains. Our findings indicate that the EPS gum is not produced constituitively by *Xylella* but may be up-regulated during the development of the disease. In addition, SDS-PAGE analysis of cell wall extracts demonstrates the presence of *Xylella* LPS for the first time, and highlights considerable differences between LPS from oleander- and grape-specific Xylella strains. These structural differences may be potential determinants of the *Xylella* host-pathogen specificity and may provide a fuller understanding of Pierce's disease at the molecular level. Our present aims are therefore: 1. To investigate the *in planta* production of *Xylella* EPS gum during the later stages of the vine growing season; and 2. to chemically characterize *Xylella* LPS, and to compare cultured *Xylella* LPS with that from *Xylella* grown on grape xylem exudate or *in planta*.

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PRUNING FOR CONTROL OF PIERCE'S DISEASE

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INTRODUCTION

Because there are no practical therapeutic measures for grapevines with Pierce's disease (PD), we tested the effects of drastic pruning as a method of rapidly regenerating healthy vines from vines with PD symptoms. If such a practice were to be successful, it could speed the replacement of affected vines by preserving an established root system to support vigorous regrowth. Our studies are designed to provide useful information for growers even if our pruning experiments do not produce methods that eliminate the disease from infected vines. Some growers have experimented with pruning practices against PD and claim worthwhile successes but have not compared their results to negative controls. These efforts may be a waste of money for labor and lost time in vine replacement. Our goals were to determine either that pruning would reduce the time required to restore a diseased vine to productivity or that such practices are not worthwhile. Finally, the results of our proposed experiments would provide new data on the distribution and overwinter survival of *Xylella fastidiosa* relative to PD symptoms.

Preliminary pruning trials in 1977 discouraged further testing of pruning to eradicate PD from individual vines. The incidence of PD symptoms was mapped in a plot within a commercial vineyard of 'Ruby Cabernet' in Fresno County. Vines that had only a single cordon with PD symptoms were noted and the diseased cordon was sawed off of every other such vine during October. In the fall of 1978, about 35% of the marked vines had no PD symptoms, but pruning had no influence on the likelihood of recovery. The same percentages of vines recovered regardless of severe pruning.

In another pilot experiment begun in late October 1996, we mapped the occurrence of PD in about 1000 Cabernet Sauvignon vines in Napa Valley. We marked, photographed, and took samples of symptomatic leaves from vines that had very early symptoms of PD only on one or several canes in these quadrilateral-trained vines (4 cordons). The samples were stored frozen at -70 C to process with PCR at a later date to confirm our diagnosis of PD. We then immediately removed all canes with symptoms from half of the vines. The following fall, 12 of 19 of the early-pruned vines showed no symptoms of PD. However there were no disease symptoms in 16 of the 18 unpruned control vines. We concluded that removing canes with light symptoms was not a promising method to eliminate PD from infected vines.

Some growers in Napa and Sonoma county have claimed to have eliminated PD from about 60% of the vines by cutting the trunk of the vine near the ground and retraining a new shoot to form a new trunk. None of these growers left similarly diseased vines as unpruned controls, so the effectiveness of their drastic pruning to control PD could not be assessed. If this pruning practice were to be adopted on a widespread basis without further critical evaluation, it is possible that a lot of expense may be incurred with no real economic return.

OBJECTIVE

1. Determine if severe pruning can eliminate Pierce's disease from grapevines with symptoms of the disease.

RESULTS AND CONCLUSIONS

Severe pruning (just above the graft union) in the winter of 1998-99 successfully regenerated healthy grapevines from trellised vines in Napa Valley that had severe symptoms of PD during fall, 1998. Grape varieties used were Cabernet sauvignon, Merlot, Pinot noir, Chardonnay, and Cabernet franc. Vine ages were from 2 to over 8 years. Recovery rates ranged from 87 to 100% for vines with the least severe symptoms; from 71 to 95% for vines in the "moderate" severity category; and from 38 to 85% for the most severe category. For the least severe disease category, rates of recovery for pruned vines were not substantially or significantly greater than normal dormant pruning in some plots. Visual ratings of PD agreed with results from using a sensitive molecular diagnostic test (Polymerase Chain Reaction, PCR) for PD for 79% of the least severe category, 80% of the moderate category, and 97% of the severe category. These results appeared to demonstrate that it is feasible to regenerate healthy vines from vines with PD more quickly by severe pruning than by pulling and replanting the vines, but the results for the following year drastically changed this conclusion.

To test pruning as a PD control tool, we established as many plots in commercial Napa Valley vineyards as possible during fall, 1998. We defined three disease severity categories, based on the severity (extent) of PD symptoms from (1) light PD symptoms on one to a few leaves, (2) moderate PD symptoms on only one side of the vine, or (3) moderate to severe symptoms on both sides of the vine and with some fruit rasining. We photographed each vine in the experiment and pruned half of the vines in each category, leaving the remaining vines as controls for normal dormant pruning. In the fall of 1999 we evaluated the PD status of all vines in our plots. Two growers in three of our plots pruned most of our designated control vines, so we did not re-map PD in one of these and got limited information from two other plots.

The results for the first year were very promising (Weber et al. 2000), with recovery rates of 87% to 100% for light PD symptoms (category 1), 71-95% (category 2), and 38-85% (category 3) (Table 1). However, the second year (2000), most of these same vines in categories 2 and 3 had PD symptoms. It is possible that severe pruning in summer months may be a more effective approach, but this has yet to be tested. The success of severe pruning in eliminating PD probably is influenced heavily by grape variety. More susceptible varieties support faster movement of the causal bacterium (Purcell 1981). Pruning may also be more successful in climates in which the bacteria moves more slowly and has lower overwinter survival rates.

Based on our results, we conclude that severe pruning will not eliminate PD from the remaining vine at rates that are more profitable than removing and replanting. Severe pruning of category one vines (light symptoms) eliminates production for 2 years from all the severely pruned vines, over half of which will recover with normal dormant pruning. Severe pruning is not reliable or effective enough for programs that aim to reduce the amount of inoculum for vine-to vine spread of PD. Removing suspect vines as soon as possible is currently the only method to reduce PD inoculum levels in grape.

Table 1. First and Second Year Results for Regenerated Vines After Severe Pruning (Pr.) and for Controls (Check) of 1998 Symptoms in Five Vineyards. Pruning was in the winter of 1998-99.

Treat-ment	Cultivar	Number (No.) mapped in 1998 and percentage recovery (%) in three disease categories at the end of two growing seasons.								
		Category One			Category Two			Category Three		
		No. in 1998	% in 1999	% in 2000	No. in 1998	% in 1999	% in 2000	No. in 1998	% in 1999	% in 2000
Pruned	Cabernet Sauvignon	32	97	47	32	84	31	32	38	0
Check		32	81	78	32	31	25	28	0	0
Pruned	Cabernet Franc	30	87	70	10	80	30	9	55	0
Check		30	63	53	10	0	0	9	0	0
Pruned	Pinot Noir	30	97	47	14	71	42	62	85	0
Check		30	90	63	14	29	29	38	0	0
Pruned	Cabernet Sauvignon	14	100	64	20	95	60	17	70	13
Check		20	70	55	26	19	12	17	1	0
Pruned	Chardonnay	6	100	83	25	84	8	13	85	17
Check		6	50	50	19	16	16	13	0	0

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TRANSMISSION OF *XYLELLA FASTIDIOSA* TO ALMONDS BY THE GLASSY-WINGED SHARPSHOOTER

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INTRODUCTION

The glassy-winged sharpshooter or GWSS (*Homalodisca coagulata*) is a newly introduced vector of *Xylella fastidiosa*, the causal agent of almond leaf scorch (ALS) and Pierce's disease (PD) of grapes (Blua et al. 1999, Purcell and Saunders 1999). GWSS and ALS now occur in Kern, Tulare, and Fresno counties. The introduction of GWSS is expected to dramatically increase ALS from its very low levels currently in the southern Central Valley over the coming years. Our experiments are designed to determine the efficiency of transmission (acquisition and inoculation) of *X. fastidiosa* to almond by GWSS to new growth and to mature (> 1 year) woody tissues of almond and to estimate natural populations of this bacterium within almonds at different times during the growing season. Population densities of *X. fastidiosa* are important in how efficiently vectors of *X. fastidiosa* can acquire the bacterium from plants.

Pruning branches on trees with early symptoms was promising for control of ALS (Mircetich et al., unpublished progress reports to Almond Board in 1970s), but feeding of GWSS on larger branches may defeat the effectiveness of pruning and increase the importance of almond to almond transmission of *X. fastidiosa* (Purcell and Saunders, 1999).

OBJECTIVES

1. Determine the efficiencies of acquisition and inoculation of *Xylella fastidiosa* by the GWSS to almonds.
2. Quantify populations of *Xylella fastidiosa* in infected almonds in the field throughout a season.
3. Determine the ability of the GWSS to inoculate and acquire *Xylella fastidiosa* from mature (> 2 years) woody tissues of almond.

RESULTS AND CONCLUSIONS

In our transmission experiments, we exposed GWSS adults to almonds with ALS symptoms for 4 days and then transferred them in groups of 4 GWSS per plant for 1, 2, or 4 days to Peerless almonds in the lab. Infection was assessed after 3-6 months by culturing *X. fastidiosa* (Hill and Purcell 1995). Transmission rates of *X. fastidiosa* from almond to almond for these groups were 13% (3 of 24 test plants) for one day, 29% (6/21) for 2 days, and 67% (14/21) after 4 days access feeding on test plants. Test plants from experiments on acquisition rates from almond and inoculation rates from grape (a better source of the bacterium) to almond have not yet been diagnosed for *X. fastidiosa*. The rates of transmission from almond to almond are lower than those we have found for grape, using the same experimental methods, but transmission of *X. fastidiosa* to plants in experiments with GWSS tend to be more variable than with other vectors. Our results for both almond and grape suggest that some GWSS are not capable of efficient transmission, and we will investigate possibilities that other, competing microbes limit transmission by GWSS.

Experiments to determine if GWSS can inoculate *X. fastidiosa* into mature woody tissues of almond plants in the greenhouse resulted in transmission to both green shoots and older than one year trunks of almond seedlings in the greenhouse, but final results are still pending diagnosis by culture assays. We plan to test the ability of GWSS to transmit *X. fastidiosa* to dormant almond plants during the winter of 2002. The possibility of a year-round inoculation cycle would increase the spread of ALS by increasing the time during which infection of almond could occur more inoculation events would occur during the year. For grapes, this may be one of the main factors explaining why GWSS can create such devastating rates of increase of Pierce's disease in vineyards (Purcell and Saunders 1999).

We estimated the population sizes of *X. fastidiosa* per gram of plant tissue using dilution plating (Hill and Purcell 1995b) from plants with ALS at Davis, California in April, June, and September. Populations were low in April: log 10 (10,000) cells per gram. Populations in June and September averaged about log 6 (one million) colony-forming cells of *X. fastidiosa* per gram. The higher population levels in summer and early fall would promote higher rates of vector acquisition. Population

levels of log 4 are marginal for vector acquisition in grape (Hill and Purcell 1995a), suggesting that summer and fall months should be more efficient for almond to almond transmission. However, GWSS has been observed to occur most frequently in Kern County almonds in February.

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CHARACTERIZATION AND STUDIES ON THE FUNDAMENTAL MECHANISMS OF *XYLELLA FASTIDIOSA* TRANSMISSION TO GRAPEVINES BY THE GLASSY-WINGED SHARPSHOOTER

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INTRODUCTION

Much remains to be discovered about the vector transmission of *Xylella fastidiosa*, and an understanding of this process may be essential in using methods that attempt to control *X. fastidiosa* diseases by reducing vector exposure to crops (insecticides, repellents, barriers, biological control). Information is needed to relate the numbers and activity of GWSS to disease spread in order to establish guidelines for vector control. A better understanding of the transmission process might provide new ideas for limiting disease spread by reducing *X. fastidiosa* transmission from plant to plant.

Some of the important characteristics of transmission of *X. fastidiosa* by GWSS have not been described or estimated because so far there have been few studies of its transmission efficiency to grape or almond (Purcell and Saunders 1999). It has been suggested that the rapid impact of the GWSS in spreading Pierce's disease (PD) may have been due to GWSS' distinctive behavior of feeding on woody tissues and dormant plants rather than its abundance during the growing season. Transmission to woody tissues could increase the rate of chronic infection by *X. fastidiosa* if summer infections of the bases of grape canes or older wood establish chronic infections. Currently, it is thought that summer infections of the canes by traditional vectors in California do not often survive through the winter, explaining the lack of evidence to date for vine-to-vine spread of *X. fastidiosa* in California vineyards (Purcell 1981, unpublished data). If GWSS can infect dormant vines or acquire *X. fastidiosa* from or transmit *X. fastidiosa* to dormant vines, this would extend the period during which vineyards should be protected from GWSS. Reducing the number of vectors does not necessarily reduce the amount of transmission of *X. fastidiosa* proportionally (Purcell 1981). We intend to fill research gaps of information on vector transmission efficiency and better understand how physical and biological factors affect transmission and perhaps how to interrupt vector transmission.

The population levels of *X. fastidiosa* assessed by culturing bacteria from the head of the blue-green sharpshooter (BGSS) did not correlate with transmission (Hill and Purcell 1995). BGSS with levels of *X. fastidiosa* that were below a detection threshold of 100 cells per insect transmitted *X. fastidiosa* about as well as did BGSS with much higher populations of cultivable *X. fastidiosa*. The saturation of transmission efficiency by small numbers of *X. fastidiosa* implies that the active region in the vector from which the bacterium is transmitted is small. Understanding the efficiency and limitations of methods to detect *X. fastidiosa* is important, because estimates of what percentage of GWSS are capable of transmitting *X. fastidiosa* can otherwise only be made by transmission assays, which require special facilities and 6-10 weeks of incubation.

The observations that (i) sharpshooter vectors stop transmitting *X. fastidiosa* after molting until they are again fed on an infected plant and (ii) there is no latent period between acquisition of *X. fastidiosa* from infected plants and its inoculation into healthy plants (Purcell and Finlay 1979a) imply that the bacteria are transmitted from the vector's foregut. Although *X. fastidiosa* has been observed in the foregut of vectors (Purcell et al. 1979c; Brlansky et al. 1983), the location of *X. fastidiosa* within the vector's foregut from which the bacterium is transmitted has not been proven. GWSS and other sharpshooters in the tribe Proconiini seem to transmit *X. fastidiosa* less efficiently than members of the Cicadellini. The two tribes of sharpshooters differ greatly in morphology and some behaviors, so it is possible that GWSS may differ significantly in behavior or morphology in ways that affect its transmission of *X. fastidiosa*. We seek to determine the location(s) of *X. fastidiosa* within the foregut that are critical for transmission. We hypothesize that *X. fastidiosa* may be transmitted in a similar manner to non-persistent viruses (Gray and Banerjee 1999), but with *X. fastidiosa* persisting by multiplication. Our efforts to identify the site within the vector foregut will test this hypothesis. We are planning with others (Backus, Walker, Blua, as described in other reports) to use electronic penetration graphics (EPG) to monitor the insect's feeding activities, while identifying several phases of feeding such as ingestion and salivation and to identify critical phases of feeding during which inoculation of *X. fastidiosa* occurs.

An in vitro assay for studies of the transmission of *X. fastidiosa* is essential to many related areas of research, such as tests of the transmissibility of mutants of *X. fastidiosa* and efficiency of transmission of different strains of *X. fastidiosa*. Such methods would allow experimental control over the acquisition of *X. fastidiosa* by the vector. Unfortunately, such a system has not been developed yet. Davis et al. (1978) tested the possibility of in vitro acquisition of *X. fastidiosa*, but the sharpshooters tested did not transmit. Purcell and Finlay (1979b) used the same approach to study sharpshooter transmission of bacteria other than *Xylella*. The development of an efficient in vitro system will enable experiments to determine what characteristics make *X. fastidiosa* vector transmissible.

Understanding the transmission mechanisms of *X. fastidiosa* may also help develop biological control strategies for *X. fastidiosa* transmission. Microbes that occur on plant surfaces and can attach to the foregut surface of sharpshooters may compete with *X. fastidiosa* for a specific attachment site (or sites) in the GWSS foregut. This competition could exclude one of the microbes from this essential region in the vector's mouthparts, and the first microorganism to colonize it would in principle be the successful one. Our preliminary experimental data (unpublished) suggests that some GWSS are not able to transmit *X. fastidiosa*. Specifically; GWSS' acquisition of *X. fastidiosa* does not asymptotically approach 100% as does the BGSS, but rather 10-70%, with high variability among different groups of GWSS. We seek to confirm and understand this phenomenon, including the possibility that other microbes can compete with *X. fastidiosa* for an attachment site on the GWSS' foregut. We have isolated miscellaneous bacteria and yeast from the surface-sterilized heads of non-transmitting GWSS and from test plants fed upon by these insects in transmission tests. Our approach will be to look for reduced transmission after GWSS access to plants sprayed (or naturally infected) with different bacteria and fungi obtained from various sources (GWSS head, grapevine leaf surface and internal tissues).

OBJECTIVES

1. Characterize the transmission of *Xylella fastidiosa* to grapes by the glassy-winged sharpshooter (GWSS).
2. Develop in vitro assays to assess vector transmission of *Xylella fastidiosa*.
3. Test the possibility of biological control of *Xylella fastidiosa* transmission through competition for attachment site in vector's foregut. (No results in this report)

RESULTS AND CONCLUSIONS

GWSS transmission to and from grapes

Adult GWSS acquisition of *X. fastidiosa* was fairly efficient with short periods of time, but the rates did not increase after 24 hours of acquisition access period (AAP). Transmission during a 4 day period on healthy test plants for single GWSS were 14% for 1 hour AAP, 34% for 6 hours, 13% for 12 hours, 44% for 24 hours, 28% for 48 hours, and 32% for 96 hours. Inoculation efficiency of GWSS fed on diseased grape for 4 days was 2% after a 1 hour inoculation access period, 23% after 6 hours, 4% after 12 hours, 20% after 24 hours, 33% after 48 hours, and 35% after 96 hours (n=46 to 56 for each period). These data suggest that some GWSS (approximately 60%) are not able to transmit *X. fastidiosa* and that this level of vector competence varies among different groups of GWSS. We seek to confirm and understand this phenomenon.

GWSS transmission into woody tissues and acquisition of X. fastidiosa from dormant plants

Grapevines kept in a cold box (4°C) during the winter were tested for positive root pressure (sap exudation from plant after pin-prick). GWSS usually did not survive on plants that did not bleed. Inoculations with 4 GWSS per plant in a growth chamber with controlled temperature (18°C) for 4 days, after an AAP of 4 days on a source plant, resulted in transmission to some dormant plants, even after returning the plants to outdoor conditions during February, 2001. Twenty eight of 48 groups of GWSS caged on green plants at the same times became infected with *X. fastidiosa*, identifying groups of insects that could transmit the pathogen under the experimental conditions. These 28 infective groups inoculated a total of 58 dormant plants, of which 10 developed PD, a total of 17% of the plants. Our analysis of test plants has not yet been completed.

For acquisiton tests from formant plants, we first had to test if the adults collected from the field (Bakersfield, CA) already had *X. fastidiosa* or not, this was done by letting the insects feed on green (non-dormant) plants for 4 days (pre-test). After this period, the insects were caged on PD plants that were dormant (same conditions as dormant plants in objective 4) for 4 days. Only one group of GWSS out of hundreds of tested GWSS inoculated *X. fastidiosa* into a pre-test plant. A total of 30 infected dormant plants were tested as sources of *X. fastidiosa*, with 30 groups of 4 GWSS. After the subsequent IAP on green plants, a total of 5 plants become infected with *X. fastidiosa*. We are repeating these experiments in 2002.

Other characteristics of GWSS transmission

Our experiments have established that GWSS loses its ability to transmit after molting (data not shown). Experiments are still in progress to determine if transmission capability persists for months after initial acquisition by adults and if there are day/night differences in transmission rates.

Our data demonstrated that BGSS and RHSS (red-headed sharpshooter) acquired *X. fastidiosa* from feeding on suspensions in sterile xylem sap of cultured *X. fastidiosa* cells but did not inoculate *X. fastidiosa* to grapes (Almeida and Purcell, unpublished). We believe that the planktonic *X. fastidiosa* cells did not attach to the foregut.

In vitro acquisition attempts

We conclude that GWSS does not differ in its transmission of *X. fastidiosa* to grape from other well-characterized vectors (like BGSS) in its retention of infectivity, lack of a definitive latent period, and loss of infectivity after molting. It differs by having lower and more variable transmission efficiency than BGSS. We plan to repeat these trials with GWSS. A better understanding of what conditions induce *X. fastidiosa* attachment is needed to progress in our objective of understanding how *X. fastidiosa* attaches to the vector foregut.

Effects of imidacloprid insecticide on GWSS' transmission of X. fastidiosa

In a separate project not covered in this conference, we found that the systemic insecticide imidacloprid (Admire®) reduced both visible feeding by GWSS and transmission of *X. fastidiosa* to grape. In 3 separate experiments, dosages of Admire applied to the soil of potted grapevines that killed about half of the GWSS confined on them after 24 hours (a typical field rate of mortality to this insecticide) reduced feeding by more than 95%. However, the reduction in transmission of *X. fastidiosa* was less than the reduction in either survival or feeding. Two- to four-fold smaller dosages of Admire still reduced feeding but did not reduce mortality or transmission in the same proportion. It is not clear if the reduction in transmission is caused entirely by mortality or by changes in feeding behavior. Further EPG studies (Walker, Blua project leaders) will be made of how imidacloprid changes feeding behavior and how these changes relate (or don't relate) to transmission.

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ALTERNATIVES TO CONVENTIONAL CHEMICAL INSECTICIDES FOR CONTROL OF GLASSY-WINGED SHARPSHOOTER

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INTRODUCTION

Glassy-winged sharpshooter (GWSS) is a major pest of grape because it vectors a serious grape disease called Pierce's disease. Successful control of GWSS requires disinfestation of vineyards, citrus orchards, and plant nurseries in both agricultural and urban areas. Although there are conventional chemical insecticides that could be used to disinfest grape vineyards before harvest or treat urban areas for GWSS, there are concerns over public and environmental safety. Use of organically approved materials or insecticide chemistries safe to human health and the environment would be desirable, especially in GWSS control situations near urban areas. Currently, there are only a few organically approved insecticides (Surround WP, plant oils, and insecticidal soap) and information on how effective these materials are against GWSS is lacking.

ARS and industry partners have developed two new insecticidal chemistries that offer significant insect control properties with improved safety to human health and the environment. Particle film technology (Surround WP) is based on the inert mineral, kaolin. Surround WP is exempt from tolerance, can be applied up to the day of harvest, has a 4 hour re-entry period, virtually no mammalian toxicity, and is listed as an approved organic production material. Surround WP is unique among insecticides in that it has the ability to repel insects from plants and prevents insect oviposition and feeding which could prevent transmission of Pierce's disease. It has proven to be as effective as imidacloprid in controlling GWSS in citrus in recent small block tests in California. Sucrose octanoate is nearing EPA registration, made of food grade materials, is exempt from tolerance, and has also shown levels of control of GWSS that is as good or better than many of the soft pesticides currently available (M-Pede, Valero).

OBJECTIVES

1. Effect of alternative insecticide chemistries on all life stages on GWSS;
2. Efficacy of alternative insecticide chemistries for quick knock-down kill of GWSS in the field;
3. Prevention of GWSS infestations with season-long and timed spray applications of Surround WP.

What was accomplished the first year of this grant was the completion of objective 3 and the partial completion of objective 1.

RESULTS AND CONCLUSIONS

Prevention of GWSS Infestation with Season-long Applications of Surround WP

In the 2000 season, research was initiated at 3 vineyard sites north of Temecula, California. Treatments of Surround WP and an untreated control were applied to 1–2 acre blocks of wine grapes with three replications at Luttgens, (Cabernet Sauvignon) Mt. Palomar (Reisling) and Calloway (Chardonnay) vineyards. Treatments were applied about every 2 weeks

from April to September using a rate of 50 lb. Surround/100 gal of water applied at 50 to 100 gpa. GWSS and leafhopper adults were monitored biweekly using yellow sticky traps. Direct counts of GWSS adults and nymphs were also conducted on 16 ft of vine row in both treatments. In addition, the percentage of grape leaves damaged by leafhoppers was assessed in July, August, and September. All vines in these studies were rated for Pierce's disease (PD) symptoms in May and September, 2000 and again in September 2001 by Ed Civerolo's group to determine treatment effects.

Early Season Applications of Surround as a Barrier to GWSS Movement from Citrus into Grape

In March of 2001, research was initiated at 3 vineyard sites bordering citrus near Bakersfield, California. In these studies, we examined the effect of a 600 ft and 800 ft Surround WP barrier treatments on GWSS adult movement from citrus into grape. Treatments of Surround WP were compared to a conventional chemical control program at 3 vineyard test sites using 4–12 acre plots/ treatment with 3 replications. Surround treatments of 50 lb Surround WP/100 gal was applied at 50 to 70 gpa on March 13, March 30 and April 14 at three test sites. In Test Site 1 (12 acre treatment blocks), yellow sticky traps were place in 2 transects per block and spaced every 100 feet that began where grape interfaced citrus and extended 1300 ft into the treatment blocks that went approximately 500 ft beyond the treated areas. In addition, the trap transects were extended into adjacent citrus groves for 100 ft. Test Sites 2 and 3 were similar to Test Site 1 except there were 4 replications and only 1 trap transect per treatment block. Data were only analyzed for Test Site 1 because GWSS control efforts in citrus adjacent to Test Sites 2 and 3 drove GWSS counts to levels too low to effectively sample.

Effect of Surround WP Applications on Nymphal Behavior

A series of studies were conducted on GWSS nymphs in free-choice and no-choice environments where they were offered Surround WP treated and untreated lemon foliage. The objectives of these studies were to determine if Surround treatments affected feeding preference and survival of GWSS nymphs. In a free-choice study, twenty GWSS nymphs were release at the base of a lemon seedling with one limb treated with Surround WP and one limb left untreated. Nymphs per limb were recorded 1 and 2 days after treatment. This experiment was replicated 6 times in field cages during July, 2001. In a no-choice study, twenty GWSS nymphs were released at the base of a lemon seedling that was treated with Surround or left untreated. Numbers of nymphs per seedling were recorded daily for 4 days after initiation of the study. This experiment was replicated 6 times in field cages during July, 2001.

Response of GWSS Adults to Different Colored Traps

A study was conducted to determine the response of GWSS adults to different colored sticky traps. Although it is known that GWSS adults are attracted to yellow, it is not known what other colors they are attracted to or if this attraction is temporal. Directly related to our Surround studies was the need to determine how GWSS adults respond to the color white because Surround turns plant foliage white. Round plastic colored targets 10 inches in diameter and coated with Tangle Foot sticky polymer were attached to bamboo poles 6 ft. above the ground. The colored traps were then placed within citrus groves at 3 sites beginning in April, 2001 and were sampled year-around. There were 9 colors with 4 replications per site.

Research on the different application strategies to control GWSS in grape and the studies on GWSS behavior have shown:

1) Bi-weekly Surround WP applications performed better than weekly applications of conventional insecticides in early spring.
2) Surround WP applied as an 800 ft barrier bi-weekly along heavily infested citrus performed better than weekly conventional insecticide treatments in preventing GWSS oviposition both within and beyond the barrier treat ment.
3) Bioassays determined that GWSS nymphs will not move to Surround treated foliage and if forced to stay on treated foliage they will eventually die.
4) GWSS adults orient to yellow and to a lesser degree orange. They did not orient to white, indicating that white Surround WP treated plants could be difficult for GWSS to locate.

This research, plus other independent studies conducted by other researchers in California have shown that Surround WP is an effective alternative for the control of GWSS in both citrus and grapes.

IMPACT OF LAYERING CONTROL TACTICS ON THE SPREAD OF PIERCE'S DISEASE BY THE GLASSY-WINGED SHARPSHOOTER

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INTRODUCTION

Solutions to managing and controlling Pierce's disease of grapes are often conceptualized as ways of breaking at least one two-way interaction among the insect, plant, and bacteria components that are required for successful disease spread and propagation. Hypothetical solutions may also involve altering the abiotic and biotic environment within which these interactions take place. On the basis of our understanding of Pierce's disease epidemics, as well as other insect transmitted plant pathogen systems, one single control tactic (especially focused upon the insect) will not be sufficient to substantially reduce vector populations such that the incidence of disease is below an economically acceptable level. One management and control strategy that potentially may be utilized to limit the damage brought about by Pierce's disease involves layering separate vector and disease management tactics together such that vector population densities are reduced, their interactions with grapevines are inhibited or disrupted, and the interface between grapevines and the disease organism, *X. fastidiosa*, is disrupted. Here we report on our efforts to simultaneously implement (i.e. "layer") various control strategies currently available to limit the spread of Pierce's disease transmitted by the glassy-winged sharpshooter, *Homalodisca coagulata*. Specifically we are investigating the efficacy in controlling Pierce's disease by simultaneously (1) applying systemic insecticides aimed at reducing the number of sharpshooters invading vineyards (functioning as deterrents and inducing mortality prior to feeding); (2) applying foliar barriers (application of kaolin) such that sharpshooter landing and feeding behavior on grapevines is disrupted; (4) rapidly killing sharpshooters that actually initiate feeding on grapevines; and (5) using chemotherapy (application of metalosate) on grapevines as a prophylactic to reduce establishment of *X. fastidiosa*. All of these control tactics are to be employed against a "backdrop" of region-wide biological control of glassy-winged sharpshooters.

OBJECTIVES

Our specific objectives are to determine the ability of a variety of treatment and treatment combinations on 1) their ability to reduce glassy-winged sharpshooter density and feeding and 2) their ability to reduce the rate of spread of Pierce's disease in newly planted vineyards. Treatment and treatment combinations to be evaluated are applied 1) imidacloprid at full rate, 2) imidacloprid at 1/2 rate, 3) a combination of imidacloprid (in the Spring) plus acetamiprid (in the Fall), 4) metalosate, 5) kaolin, 6) imidacloprid-acetamiprid combination PLUS kaolin, 7) imidacloprid-acetamiprid combination PLUS metalosate, 8) metalosate + kaolin, 9) imidacloprid-acetamiprid combination PLUS kaolin PLUS metalosate, and 10) control (water only).

RESULTS AND CONCLUSIONS (to date October 1, 2001)

Project Status

In April of 2001, a research site was selected at the Agricultural Operations facility located on the campus of the University of California, Riverside. The dimensions of this site are approximately 800 ft X 150 ft. During April and early May the site was cultivated, and prepared for planting. Drip irrigation was installed. One thousand grape vines were acquired from SunRidge Nursery in early May and planted on May 16, 2001. The variety utilized in this study is Chardonnay 04 on S04 rootstock. Vines were planted with 6 ft spacing between plants and 12 ft spacing between rows. A total of 10 rows of 100 vines per row was planted. Experimental treatments were initiated in August of 2001.

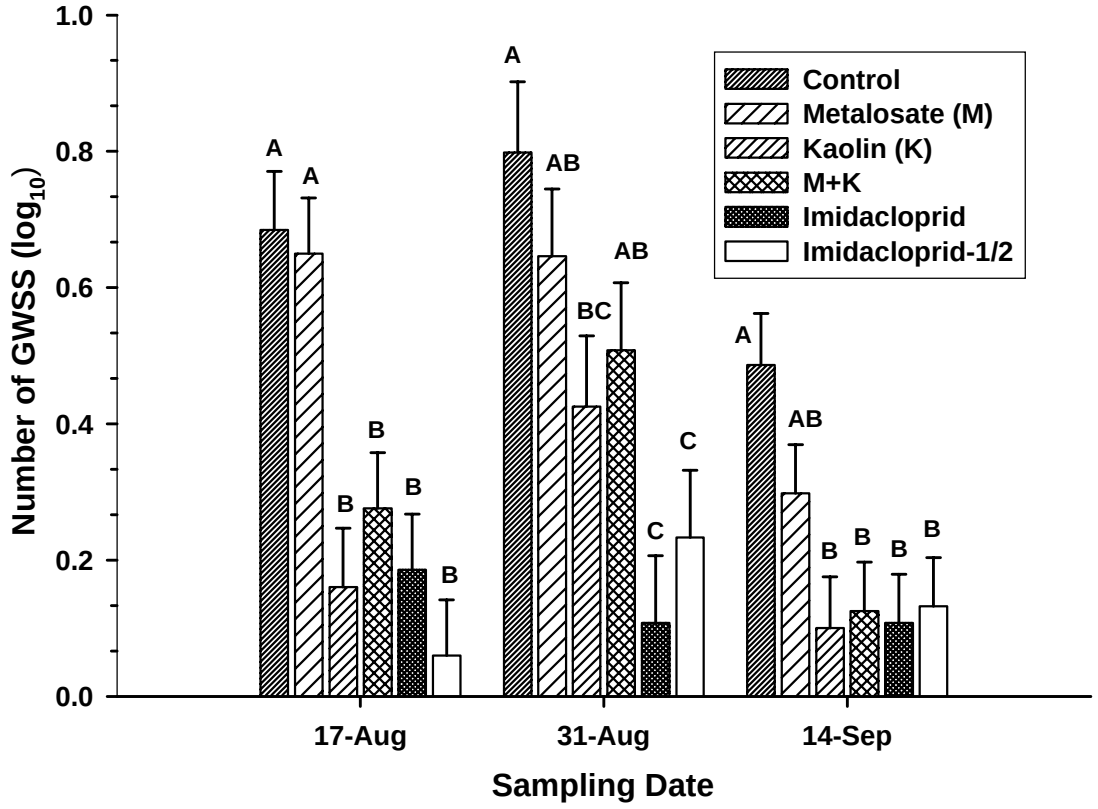
Treatments were applied using a randomized complete block design utilizing 10 experimental blocks. A block consists of 10 sections of vine-rows comprised of 10 plants per row. Treatments are applied individually to each plant within an entire row section. As rows will receive the treatment, rows will be considered as the replicate and plants nested within row. The following treatments will be applied 1) imidacloprid at full label rate, 2) imidacloprid at one-half label rate, 3) a combination of imidacloprid (applied in August) plus acetamiprid (in the Spring), 4) metalosate, 5) kaolin, 6) imidacloprid-acetamiprid combination PLUS kaolin, 7) imidacloprid-acetamiprid combination PLUS metalosate, 8) metalosate + kaolin, 9) imidacloprid-

acetamiprid combination PLUS kaolin PLUS metalosate, and 10) control (water only). One month following initial treatment application and every 2 weeks thereafter, the total number of sharpshooters found on each treated row will be determined. The impact of treatment on sharpshooter numbers is subsequently determined by repeated measures analysis of variance; the preliminary results showing the impact of treatment on sharpshooter numbers for the first six weeks following treatment application are presented below.

Additionally, for each treatment combination, sharpshooter feeding and behavior trials are conducted to evaluate the impact of treatments on feeding biology and host acceptance of the sharpshooter. The first set of these trials is currently underway and we hope to have preliminary results by December 2001. Similar trials will be conducted for each treatment every six months for the duration of the experiment (2.5 years).

Finally, in the fall of each year (beginning in 2002) of the experiment, an evaluation as to the efficacy of treatments to prevent the development of Pierce's disease will be made based on visual observations and sampling for *X. fastidiosa* within selected plants within each treated row.

Results from the repeated measures ANOVA indicated that there was a significant difference among treatments with respect to the number of sharpshooters found on experimental plants ($\mathbf{F}_{5,52}$=6.93, $\boldsymbol{P}$<0.0001). As replicates involving acetamiprid have not as of yet (Oct. 2001) received these treatments, these treatments have not been included in this analysis. As expected plants treated with metalosate (a potential prophylactic treatment for Pierce's disease) supported similar numbers of sharpshooters as untreated control plants (see figure below). The efficacy of metalosate for the prevention of Pierce's disease will be evaluated during the annual estimate of Pierce's disease incidence in test plants. Overall plants treated with kaolin demonstrated reduced numbers of sharpshooters relative to the untreated controls, and plants treated with imidacloprid exhibited the lowest numbers of sharpshooters. There were no significant differences in the numbers of sharpshooters found on plants treated with kaolin as compared to the numbers found on insecticide treated plants. No experimental treatment resulted in complete protection from sharpshooters; consequently, all treated plants are at risk of exposure to *X. fastidiosa*.



ECONOMIC IMPACT OF PIERCE'S DISEASE ON THE CALIFORNIA GRAPE INDUSTRY

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This report provides estimates of the economic impact of Pierce's disease on the California grape industry. It is the result of a study funded by the California Department of Food and Agriculture (CDFA). The report reviews both the current situation and provides estimates of future economic impacts if a new vector, the glassy-winged sharpshooter (GWSS), becomes established.

BACKGROUND

Pierce's disease (PD) is not new to California. It was first observed and recorded in the 1880s when it was responsible for destroying more than 40,000 acres of grapevines in the Los Angeles basin.[1] Localized infections of the disease have occurred in the Napa Valley since the 1880s. There have also been periodic epidemics over the last century where the disease has reached a higher incidence and become more widespread in the grape growing regions of the state. In the early 1990s, growers in Napa and Sonoma counties again began reporting symptoms of PD. The spread of PD into North Coast vineyards, while widespread, is mostly confined to riparian areas and near irrigated landscapes. The blue-green sharpshooter (BGSS) is the principal insect vector spreading the disease from the riparian habitats. Under BGSS, vine-to-vine spread is minimal, even though the disease is present in the vineyard. This is due to the nature of BGSS, which does not travel far and has a limited ability to transmit the disease due to the small size of its mouth. In addition, much of PD infection is eliminated through the pruning process. Small vineyards planted next to BGSS habitat traditionally have had the highest risk due to infestations from the habitat. The disease basically has an edge effect of about 300 feet; hence, if the vineyard is 600x600 feet, then it is all edge.[2] Since 1994, more than 1,000 acres of Napa and Sonoma county grapevines have been pulled and replanted (total 1999 bearing acreage equaled 66,700 acres) due to Pierce's disease with an estimated cost to growers of over $30 million in lost income, production, and replanting expense.

Up until the late 1990s, PD was known as a disease mostly prevalent in the North Coast grape growing areas. However, according to Bill Peacock, University of California of California Farm Advisor, Tulare County has battled PD since the 1930s. He claims the outbreak has been as severe as that in Napa, but the problem doesn't receive as much attention since it doesn't have the high profile that Napa does. The problem would be exacerbated with the introduction of a more efficient vector than the traditional blue-green, green, and red-headed sharpshooters which are not aggressive in their travel and eating habits. Enter the glassy-winged sharpshooter (GWSS), which recently became established in California and is a serious threat to vineyards since it moves faster and farther into vineyards than other species.

Since the early 1990s, GWSS has been seen in high numbers in citrus along the Southern California coast. During the past few years, it has become more abundant farther inland in Riverside and San Diego counties. In 1998 and 1999, high populations on citrus and adjacent vineyards were seen in southern Kern County. GWSS is expected to spread north into the citrus belt of the Central Valley and become a permanent resident of various habitats throughout northern California.[3]

Temecula Experience

In the summer of 1999, wine grape growers in Temecula, Riverside County, experienced the sudden die-back of grapevines confirmed to be caused by PD. It has been reported that more than 200 acres (out of a total of over 2,100) were lost to PD.

The economic impact of the loss in production from 1998 to 1999 can be seen in Table 1. As can be seen from this table, 11,113 tons of wine grapes (not counting raisin or table grape varieties) from District 16 (Riverside and San Diego counties) were crushed in 1998. This amount had decreased to 7,255 in 1999, or 35 percent. The value of this tonnage had decreased from over $9.8 million in 1998 to $6.3 million in 1999, a loss of $3.5 million in gross agricultural income, or a decrease of 36 percent! This lost production also has an impact on other economic variables such as employment and regional and state income. The lost production, if realized, would have been made into wine, which would have been sold into retail channels. It is estimated that, on average, the wine valued at the producer level has a multiplier of 4.3 to convert it to a value at the winery level.[4] This calculation results in a value of this lost production of $15.2 million at the winery

level. The impact of this lost production on California state income, using a multiplier of 2.5[5], is estimated to be $37.9 million (Total California income in 1997, the latest data available, was $846 billion).

Table 1. Wine Grape Statistics for District 16, 1998-99.

	1998	1999	2000
Bearing Acres White Wine	1,578	1,598	1,280
Bearing Acres Red Wine	572	547	534
Total Bearing Acres	2,150	2,145	1,814
Tons Crushed White Wine	7,109	4,173	5,230
Tons Crushed Red Wine	4,004	2,543	3,820
Total Tons Crushed	11,113	7,255	9,050
Grower Return White Wine ($/ton)	961	860	1,005
Grower Return Red Wine ($/ton)	746	882	846
Gross Grower Return White ($)	6,833,740	4,051,846	5,256,150
Gross Grower Return Red ($)	2,988,225	2,242,826	3,231,720
Total Gross Grower Return ($)	9,821,965	6,294,672	8,487,870
Total CA Wine Tons Crushed	2,527,056	2,616,831	3,318,507

Source: Grape Crush Report, California Department of Food and Agriculture, Sacramento.

Comparisons of 2000 crush data for District 16 to 1998 data temper the impact from 1998 to 1999. Wine grape gross income for District 16 still decreases, but by $1.334 million, or 13.6 percent, still a significant amount. Much of this improvement can be attributed to increases in tonnage in 2000 over 1999 and increased wine grape prices. A comparison of bearing acreage from 1998 to 2000 reflects more of the actual situation. Bearing acreage has declined by 336 acres, or 15.6 percent. Growers are reluctant to plant additional acres or replace vines until some strategy for dealing with PD is found.

While Temecula is a relatively small grape growing area compared to the rest of California (District 16 wine grape production accounted for 0.3 percent of the total crush in 2000), the lessons to be learned from this situation are valuable in estimating the economic losses from the introduction of GWSS. Growers in Temecula have no plans to replant grapevines until a remedy is found for PD. A major problem in Temecula is the presence of large numbers of citrus in close proximity to vineyards. Before GWSS, this situation was not a serious threat. However, citrus serves as a host for GWSS, which moves from it to grapevines. In Southern California, the large numbers of GWSS coupled with their aggressive habits laid the groundwork for the current disaster.

Of concern is the large numbers of citrus in the Southern San Joaquin Valley that are in close proximity to vineyards. However, there are a large number of additional hosts that pose danger to grapevines if GWSS is established on them as well.[6] Data on the establishment of GWSS is speculative at this time (data on the incidence of PD throughout California is also not well documented and anecdotal in many cases). Estimation of potential impacts will have to rely on scenarios synthesized from observable occurrences where data exists (Temecula) or surveys such as the Napa Valley Pierce's Disease Task Force Report.

Overview of the California Grape Industry

Statistics on the California grape industry are contained in Table 2. This table provides acres harvested and gross farm value for raisin, table, and wine grapes for 2000. Table 2. Harvested Acres and Gross Farm Value, California Grapes, 2000.

Table 2. Harvested Acres and Gross Farm Value, California Grapes, 2000.

	Acres	Gross Farm Value
Raisin Grapes	280,000	$489,384,000
Table Grapes	89,000	$438,280,000
Wine Grapes	458,000	$1,908,649,000
Total California Grapes	827,000	$2,836,313,000
Total California Agriculture	8,306,200	$26,243,717,000

Source: California Agricultural Statistics Service.

As can be seen from Table 2, the California grape industry accounts for $2.8 billion in gross farm value (nearly 11 percent of the California state total and growing rapidly with wine grapes the leader). Wine grapes is the largest sector at $1.9 billion (68 percent) followed by raisin grapes (17 percent) and table grapes (15 percent). While not shown here, wine grapes have been the fastest growing sector over the past 10 years with a growth rate at the producer income level of over 15 percent per year. In addition, exports of California wines have grown at an impressive rate of over 10 percent per year since 1995 and now rank second in state exports at $498.5 million (1999 value).[7] In terms of acreage, the California grape industry accounts for 827,000 harvested acres, or ten percent of the state total. Grape acreage has increased over 10 percent from 1998 to 2000.

Statistics of grape acreage, gross farm value, and citrus/avocado acreage by county can be found in Table 3. Citrus and Avocado acreage is displayed with grape acreage to show which areas are at greatest risk from the presence of large citrus and avocado acreage. Also, counties are roughly grouped into geographical areas. First, grapes are grown in a large geographical area. However the top five counties in terms of acreage (Fresno, Madera, San Joaquin, Tulare, and Kern) account for nearly 71 percent of the total grape acreage in California and 63 percent of the grape gross farm value. Three of these counties (Fresno, Kern, and Tulare) have large concentrations of citrus and avocados.

Table 3. California County Grape Acreage, Grape Gross Farm Value, and Citrus/Avocado Acreage, 1999.

County	Acres, Grapes	Grape Farm Value	Acres, Citrus/Avocados
Alameda	2,018	8,523,000	
Contra Costa	1,580	6,978,000	
Glenn	835	2,273,000	714
Napa	30,506	221,852,000	
Sacramento	22,630	90,409,000	
San Joaquin	83,000	291,197,000	
San Mateo	40	130,000	
Solano	3,390	14,130,000	
Sonoma	42,227	269,271,000	
Yolo	8,704	35,431,000	
Fresno	228,430	605,214,000	28,737
Kern	88,283	491,269,000	43,531
Kings	5,178	20,523,000	
Madera	92,230	228,567,000	600
Merced	16,200	46,090,000	
Stanislaus	13,900	35,420,000	
Tulare	81,334	442,652,000	115,697
Monterey	34,187	157,926,000	1,020
San Benito	2,494	13,455,000	
Santa Clara	1,600	12,531,000	
Santa Cruz	200	1,513,000	
San Luis Obispo	16,272	83,601,000	2,536
Santa Barbara	14,064	60,117,792	10,186
Los Angeles	79	348,000	
Riverside	16,349	146,739,000	38,225
San Bernardino	1,210	2,726,000	6,139
San Diego	189	161,154	42,293

Source: California At A Glance, Published by California Farmer, August 1999. Data taken from County Agricultural Commissioners' Reports.

Economic Impacts

The economic impacts from PD take on different dimensions and will vary by location, age of vine, and variety. Impacts are derived from replanting entire vineyards, vineyard management which includes monitoring, replacement of diseased vines, and training new growth, vector management which includes monitoring vectors and application of vector controls (in many cases pesticides), and sharpshooter host and riparian management (where appropriate). All of these tasks involve costs; all substantial that will increase the cost structure of producing grapes in California. Appendix Table 1 contains an overview of

wine grape production cost estimates by University of California of California Farm Advisors. Table 4 below contains University of California Farm Advisor estimates of establishing a vineyard (minus land costs), amortized cost contribution to annual costs, and replanting costs.

Table 4. Vineyard Establishment and Replanting Cost Estimates

Area, Variety	Establish Cost/Acre	Cost per Vine	Amortized Cost/Acre	Vines per Acre	Replant Cost/%
San Joaquin Valley Wine	$4,105	$7.27	$621	565	$18/2%
Lodi Cabernet	$5,949	$9.56	$381	622	$31/2%
Sierra Nevada Zinfandel	$10,173	$17.22	$1,013	622	$105/5%
Sonoma Chardonnay	$13,369	$14.72	$1,227	908	$103/4%
Lake Sauvignon Blanc	$8,640	$15.27	$834	566	$47/2%
Santa Maria Chardonnay	$11,985	$11.01	$736	1,089	$256/5%
San Luis Obispo Cabernet Sauvignon	$9,526	$10.94	$585	871	$64/2%
San Joaquin Valley Thompson Seedless	$3,839	$7.40	$378	519	$22/5%

Source: University of California Farm Advisor Sample Costs to Establish A Vineyard and Produce Wine Grapes.

As can be seen from Table 4, costs vary widely by location and variety. However, they give a good guide on the costs of replacing a vineyard and replanting vines within a vineyard. The last column deserves explanation. It presents the cost of replanting a percentage of the vines in a vineyard; hence, for the San Joaquin Valley Wine estimates, the cost is $18 to replant 2 percent of the vineyard (which in the case presented is 2 percent of 565 vines per acre).

The replacement of a vineyard due to PD involves more than just the replacement cost. It also involves lost yield and revenue in addition to the cost. A hypothetical example using the costs for Sonoma Chardonnay is contained in Table 5 below. It is assumed that the vineyard will suffer a 50 percent loss in yield the year prior to removing the vines. It also assumes a 7-ton per acre yield at the vineyard's maturity and a price of $1,060/Ton.

Table 5. Hypothetical Cost and Revenue Scenario of Vineyard Replacement.

	Year 0	Year 1	Year 2	Year 3	Year 4
Yield (tons/acre)	3.5	0	0	3	7
Revenue	$3,710	0	0	$3,180	$7,420
Revenue w/o PD	$7,420	$7,420	$7,420	$7,420	$7,420
Revenue Difference	-$3,710	-$7,420	-$7,420	-$4,420	$0
Replant Cost		-$1,227	-$1,227	-$1,227	-$1,227

In this example, the establishment of a new vineyard would cost $13,369 but be amortized over 22 years; the yearly cost would be $1,227. Not reflected in this example is a case where the vineyard being replaced was not completely amortized resulting in additional cost than reflected here. In this case, the total cost plus lost revenue over a five-year period is $27,878 per acre, a substantial and significant cost. In addition, replanting a vineyard is no assurance that the vineyard will not be re-infected, especially since the scientific literature states that younger vines are more susceptible (giving credence to the argument that Napa is especially vulnerable since it has replanted a significant amount of acreage due to Phylloxera.

Other costs involve the replacement of vines infected by PD and training new growth. Table 4 provides some estimates of this cost, which varies widely by location, variety, and percentage of vines to be replaced. An accurate assessment of this cost on California vineyards is dependent on assumptions relating to the percentage of vines infected each year and needing to be replaced. At this time, more information is needed.

Another cost is controlling the vector. This cost will involve the placement of traps or sweeping the edges of a vineyard with a net to determine infestations of sharpshooters. Control of vectors is detailed in the University of California Pest Management Guidelines. A combination of trapping and monitoring is recommended. Information needs to be developed on the density and number of traps to be used, and labor cost of monitoring.

Three materials are recommended for an IPM program to control sharpshooters based on their usefulness taking into account their efficacy and impact on natural enemies. The first is IMIDACLOPRID, which is a foliar product that gives a fast kill of sharpshooters but lasts only about two weeks. It is applied at the rate of 0.75 ounces per acre, but is limited to 2 ounces per acre per year. The commercial cost of this product is $32 per ounce; hence the cost per application is about $44 per acre assuming a $20 cost to apply it.

Another material is a drip system variation of IMIDACLOPRID and is applied through the irrigation drip system at a rate of 16 ounces per acre. Cost is $4.80 per ounce resulting in a cost of $76,80 per acre per application. Another material is DIMETHOATE, which is suggested for BGSS in coastal areas. Use of this material is under a special needs registration and cost data has not been obtained on it.

Scenarios for Estimating PD Economic Impacts

Since the introduction of GWSS as a new vector for the transmission of PD, a whole new set of dynamics have been introduced into the estimation of the economic impacts of PD. Previously, the impact has been estimated through surveys (in the case of the North Coast wine grape industry). These surveys are based on actual experience with the disease. In the case of GWSS, a number of assumptions will have to be made. The following scenarios suggest themselves:

1. All of California will be infected uniformly and to the same degree. This scenario assumes the PD does not vary by location and variety. There is evidence to suggest otherwise and this scenario is, in all probability, not realistic.
2. Separate the geographical areas and make assumptions based on the likelihood of PD being spread by vectors. Assumptions can be drawn from survey information and data presented in the Temecula case. In this scenario, the southern San Joaquin Valley would be treated as the spread of the disease in Temecula due to its exposure to substantial amounts of citrus and the rest of the state treated according to other hosts such as riparian areas.

Other scenarios may suggest themselves. However, economic analysis probably will be more accurate under the second scenario. Under this scenario, a greater impact will likely take place in the Southern San Joaquin Valley due to the proximity of other hosts such as citrus and approach the levels seen in Temecula. Under any scenario, control costs will be escalated for all areas. Producers will need to develop strategies for detection, control, and management of the disease, all of which will add significantly to costs of production. Currently, returns to grape producers, especially in the Southern San Joaquin Valley, are under great pressure due to overproduction. It is likely that many producers will not be able to survive an intensive outbreak of PD, which will significantly add to production costs and capital costs of replanting vineyards. While some of these producers may have been forced out of production in any event, an outbreak of PD will hasten their decline and exit from the industry.

Appendix Table 1. Summary of Farm Advisor Cost Studies for Wine Grapes.

Cost Item	SJV Wine	Lodi Cabernet	SJV Thompson	Sierra/Nv Zinfandel	Sonoma Chardonnay	Lake Sauvignon Bl.	S.Maria/S.B. Chardonnay	S.L.Obispo Cabernet
Prune & Tie	130	185	209	141	486	314	261	392
Suckering		49			38	60	90	85
Leaf Removal					202	150	150	
Frost Protection					59	68	54	
Weed Control	96	56	51	46	78	41	29	21
Mildew Control	106	44	82	106	296	71	202	95
Pest Control-vertebrates	14			36		5	31	16
Pest Control-insect	28	73	85	53	37	59		56
Birds								82
Irrigate	207	40	190	33	161	61	78	143
Fertilize	16	31	39		327	7	48	26
Green Tie/Shoot Thin		102		141	141			
Move Wires					338	48		
Pickup	34	13	31	393	187	45	17	17
Miscellaneous	48	9	34		46	143	13	12
Total Cultural	679	602	721	949	2396	1072	973	945
Operating Capital Int.	34	30	38	40	91	53	42	46
Harvest	450	280	281	441	418	812	825	1425
Total Operating	1163	912	1040	1430	2905	1937	1840	2416
Cash Overhead	221	469	240	879	1635	603	749	771
Total Cash	1384	1381	1280	2309	4540	2540	2589	3187
Amortized Overhead	819	507	498	2247	2070	1436	1066	1359
Total Costs w/o land	2203	1888	1778	4556	6610	3976	3655	4546
Amortized Land	387	305	186	378	2590	669	392	289
Total Costs with Land	2590	2193	1964	4934	9200	4645	4047	4835
Land Value	10,500	8,000	4,500	5,000	35,000	7,500	10,000	7,000
Study Year	1997	1994	1997	1996	1999	1998	1996	1996
Farm Acreage	120	200	120	5	30	40	95	18

Source: University of California, Cooperative Extension. Sample Costs to Establish a Vineyard and Produce Wine Grapes.

[1] The material in this section is largely taken from the University of California's Pierce's Disease Research and Emergency Response Task Force Report.

[2] As reported by Professor of Entomology Alexander Purcell, University of California, Berkeley.

[3] Source: University of California Statewide Integrated Pest Management Project, UC Pest Management Guidelines, Grape, Pierce's Disease, Updated 12/99.

[4] This estimate is taken from data contained "Economic Impact of California Wine", An MKF Research Report, Sponsored by the Wine Institute and California Association of Wine Grape Growers, January 2000.

[5] Figure supplied by University of California sources using IMPLAN.

[6] For a list of sharpshooter hosts, see "Pierce's Disease in the North Coast", University of California Cooperative Extension and Statewide IPM Project.

[7] Source: California Department of Food and Agriculture.

SURROGATE GENETICS FOR *XYLELLA FASTIDIOSA*: REGULATION OF EXOPOLYSACCHARIDE AND TYPE IV PILUS GENE EXPRESSION

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INTRODUCTION

Xylella fastidiosa presents a formidable challenge to the molecular geneticist. There are no published methods available for the basic operations of genetic exchange, mutant isolation, and complementation. The slow generation time, poor plating efficiency and requirement for complex culture media are further complications. Surrogate genetics (Maloy & Zahrt, 2000) provides a means to at least partially bypass these challenges. Here, one creates a hybrid organism, transplanting genes of interest from the poorly-studied species (e.g., *Xyella fastidiosa*) into a well-studied surrogate host (e.g., *Escherichia coli*). Given sufficiently related hosts, one expects the transplanted genes to function in the surrogate essentially as they do in the original. One may then exploit the advantageous properties of the surrogate to perform a large number of experiments, making and discarding hypotheses to define various aspects of gene function. Once gene function in the surrogate has been thoroughly explored, one can perform a focused set of experiments, informed by the results from the surrogate, to examine function in the native host. The use of *E. coli* as a surrogate host for studying gene regulation would open a range of experimental approaches that are currently unavailable in *X. fastidiosa*, and lead to more rapid advances in understanding the control of key pathogenicity determinants. We are analyzing the transcriptional regulation determinants for genes whose products may be involved in pathogenesis (e. g., *gum* genes, encoding exopolysaccharide; and *pil* genes, encoding type IV pili) as well as "housekeeping" genes involved in central metabolism (e.g., amino acid biosynthesis). During infection, *X. fastidiosa* produces an extracellular matrix that is hypothesized to contribute significantly to disease symptoms (see Bevan, 2000). The *X. fastidiosa* 9a5c genome encodes a *gumBCDEFHJK* operon homologous to the corresponding *Xanthomonas campestris* operon (Simpson et al., 2000). Note however that *Xan. campestris* pv. campestris *gum* null mutants exhibit at most a 50% reduction in virulence index when assayed on cabbage (Katzen et al., 1998). Type IV pili are responsible for twitching motility, natural transformation, and adherence in various species. The *X. fastidiosa* 9a5c genome encodes the various *pil* genes necessary for synthesis, secretion and assembly of type IV pili (Simpson et al., 2000).

OBJECTIVES

1. Apply bioinformatics to evaluate transcription control signals *in silico* for *X. fastidiosa* 9a5c
2. Construct and characterize a Φ (*gumB-lacZ*) operon fusion in *E. coli*
3. Construct and characterize a Φ (*pilA-lacZ*) operon fusion in *E. coli*

RESULTS AND CONCLUSIONS

A first approach to defining transcriptional regulatory mechanisms in *X. fastidiosa* is to visually examine the upstream nucleotide sequences of genes whose regulation has been well studied in other organisms. Common features in the sequences will reveal common regulatory strategies. Our initial analysis has focused on the *trp* and *his* operon transcription attenuation control regions which in enterobacteria and other species contain easily-recognized sequence features: regulatory leader peptide coding regions that are rich in codons for the regulatory aminoacyl-tRNA; stem-loop structures that serve as factor-independent transcription terminators; and alternative stem-loop antiterminator structures. However, the *X. fastidiosa hisGDCBHAFI* biosynthetic operon upstream regulatory sequence exhibits no leader peptide or terminator structures.

Therefore, *his* operon expression in *X. fastidiosa* is regulated by a mechanism other than transcription attenuation. The *X. fastidiosa trp* biosynthetic genes are not organized in a single *trpE(G)DC(F)BA* operon as in *E. coli*, but rather in three noncontiguous operons: *trpEGDC*, *trpF*, and *trpBA*, in an arrangement mimicking that of *Pseudomonas aeruginosa*. Again, however, the *X. fastidiosa trp* gene upstream regions do not contain apparent regulatory regions similar to those for controlling *trp* gene expression in either *E. coli* or *P. aeruginosa*. Thus, regulation of these amino acid biosynthetic pathways must occur through other mechanisms in *X. fastidiosa*.

Environmental and genetic controls of exopolysaccharide (EPS) biosynthesis remain largely undefined (reviewed by Becker et al., 1998). The laboratory of Michael Daniels (John Innes Centre) has identified a cluster of linked *Xan. campestris* pv. campestris regulatory genes, mutations in which affect production of several extracellular enzymes, including endoglucanases and proteases, along with EPS. The *rpfC* and *rpfG* genes (Slater et al., 1999) were thought to likely encode direct transcriptional regulators of pathogenicity gene expression. However, more recent analysis indicates that the RpfG protein is probably not a direct (DNA-binding) regulator of gum gene expression (Slater et al., 2000). We constructed a Φ (*gumB-lacZ*) operon fusion in *E. coli*, in order to use LacZ expression as a measure of *gumB* promoter activity. However, this construct expressed only low levels of LacZ enzyme. Given the uncertain nature of *gum* operon regulation, we elected to turn our immediate attention to study genes whose expression is more readily predicted from sequence inspection.

We chose therefore to study the regulation of *pil* gene expression. These genes control the formation of type IV pili in a variety of organisms, and the regulatory mechanisms have been studied in *P. aeruginosa* (Mattick et al., 1996) among others. It is hypothesized that these pili (fimbriae) are involved in adhesion to the gut and mouthparts of the insect vector (Bevan, 2000). Expression of *pilA* structural genes requires a specialized RNA polymerase specificity determinant (σ^{54}), which recognizes a strongly conserved -12/-24 nucleotide sequence. One of two *pilA* homologs (XF2542) in *X. fastidiosa* contains a σ^{54}-dependent promoter. We constructed a Φ (*pilA-lacZ*) operon fusion in *E. coli*, and observed that it expressed detectable levels of LacZ enzyme. We also cloned the regulatory *pilSR* genes (XF 2546-2545) from *X. fastidiosa*. The presence of these genes resulted in an approximately twofold increase in LacZ expression, suggesting that they may have activated the *pilA* promoter. Our current experiments are designed (1) to demonstrate that Φ (*pilA-lacZ*) expression requires σ^{54}; and (2) to optimize the expression of the *pilSR* genes in *E. coli*. Our goal is to observe a significant PilR-dependent stimulation of Φ (*pilA-lacZ*) expression. If this is successful, we can then employ surrogate genetics to better understand the control of type IV pilin synthesis.

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CHEMICAL CONTROL OF GLASSY-WINGED SHARPSHOOTER: ESTABLISHMENT OF BASELINE TOXICITY AND DEVELOPMENT OF MONITORING TECHNIQUES FOR DETECTION OF EARLY RESISTANCE TO INSECTICIDES

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INTRODUCTION

Among the control practices used against the glassy-winged sharpshooter (GWSS) that spreads Pierce's disease, chemical control offers an immediate remedial option against this pest. Insecticides were not used extensively until recently to control this pest and therefore efficacy of insecticides should be evaluated and monitored. Our present challenge is to study the effectiveness of selected insecticides representing various chemistries against the GWSS and to establish baseline toxicity of various insecticides. Knowledge of baseline toxicities would facilitate the selection of the most effective products for use in GWSS management. The results of tests conducted in the laboratory under controlled conditions can provide practical guidance to individual growers without conducting expensive large-scale trials to determine the suitability of an insecticide. Conventional bioassays provide rapid and field-based information to establish baseline toxicity data.

In order to establish baseline susceptibility data to various insecticides against the GWSS, evaluation and development of simple and suitable bioassay techniques to detect toxicological responses of the insects to various insecticides is critical. Ideally, bioassay techniques should require less handling of insects and also be sensitive enough to allow early detection of changes in an insect's response to insecticides over time. Changes in the toxicological response of a population to an insecticide is usually the first indication of resistance development. Hence, the priority of our project was to evaluate and standardize 3 bioassay techniques at the present time with the possibility of developing 2 more techniques in the future.

The purpose of the report is to describe three techniques, a petri-dish and a leaf-dip bioassay technique used for testing the relative susceptibilities of GWSS to a number of contact insecticides, and a systemic-uptake bioassay method for systemic insecticides. The baseline data obtained so far to selected insecticides against the GWSS can be useful for future studies on resistance monitoring and management of this pest. The availability of these three techniques will allow us to also examine various strategies of management with chemicals to avoid or delay insecticide resistance. Testing various chemical application strategies to manage GWSS populations will be critical to understand the evolution of resistance in GWSS.

OBJECTIVES

1. Develop reliable bioassay technique(s) to evaluate baseline toxicity of insecticides from major classes of insecticides against all life stages of GWSS.
2. Monitor all life stages of the GWSS populations collected from insecticide-treated citrus orchards and vineyards in Riverside, Redlands, San Joaquin Valley and Temecula to determine baseline susceptibility to various insecticides.
3. Investigate the rate of development of resistance to a selected OP, pyrethroid and a neonicotinoid by artificial selection in the greenhouse.
4. Develop electrophoretic techniques to identify esterase profiles in individual GWSS of all life stages including eggs.

RESULTS AND CONCLUSIONS

Petri-dish Bioassay: Petri dishes of 60 mm were used for this assay. Agar beds were prepared in the petri dishes for maintenance of citrus leaves for up to a week. Excised citrus leaf discs of the same size as the petri dish were dipped for 30 sec in five concentrations of each insecticide and allowed to dry for an hour. The dried leaf discs were placed on the agar bed in each petri dish. Tests were conducted using 10 contact insecticides and one systemic: esfenvalerate, cyfluthrin, bifenthrin, and fenpropathrin (pyrethroids), chlorpyrifos and dimethoate (organophosphates), endosulfan (cyclodiene), acetamiprid, imidacloprid, thiamethoxam (neonicotinoids), and pymetrozine. Five GWSS were exposed to the treated leaves in each petri dish covered with a plastic top. Each test was replicated 6 times and included water-only dipped controls. Mortality was assessed after 24, 48 and 96 h. In the case of acetamiprid and thiamethoxam, mortality was

assessed after only 4 and 16 h followed by 24 h because of their potency to the insects. No condensation was observed in the petri dishes while maintaining insects for exposure to the treated leaves even over a week.

Leaf-dip Bioassay: Leaf-dip bioassays of attached leaves on citrus plants were conducted in the greenhouse. Five serial dilutions of each of the 9 insecticides were used for dipping of the attached leaves and allowed to dry for an hour. Five adults or immatures were placed in clip cages and attached to the treated leaves. Mortality assessment was similar to that of the petri-dish bioassays.

For both the petri-dish and the leaf-dip technique, toxicity was determined based on the effects of exposure time and location effect (Table 1). Initial tests with GWSS were limited to populations collected from citrus orchards in Riverside. Tests on populations from citrus orchards in Redlands were initiated during late summer and compared with the Riverside populations. Considerable variation in susceptibility to insecticides was observed with both techniques. The petri-dish technique provided stable LC_{50}s with no or low control mortality in 24 h. Mortality increased in the controls over time. Monitoring data for chlorpyrifos indicated a difference of 13- and 15-fold between the two techniques in 24 and 48 h respectively. Toxicity of chlorpyrifos appears to be similar to both Riverside and Redlands populations. No significant differences in LC_{50}s were observed to fenpropathrin using the petri-dish technique between the two locations (LC_{50} = 0.019 to 0.042 ppm). However, a significant difference in LC_{50} was observed to esfenvalerate with the petri-dish test. Esfenvalerate was more potent to GWSS from Redlands (LC_{50} = 0.00003 ppm) compared to the insects from Riverside (LC_{50} = 0.002 ppm) showing a significant difference of 90-fold. Similarly, acetamiprid was also quite toxic to GWSS from Redlands with an LC_{50} of 0.003 ppm compared to 0.01 ppm for the Riverside insects using the petri-dish technique. Also the GWSS from Redlands were more susceptible to cyfluthrin (LC_{50}=0.004 ppm) indicating a 10-fold difference compared to Riverside insects (LC_{50}=0.038 ppm). In all tests, mortality increased with time, higher at 48 h compared to 24 h.

Systemic Bioassay: To assess the toxicity of imidacloprid action systemically, the leaf-dip method was modified to accommodate a system that allows excised leaves to take up imidacloprid through the petioles. The uptake and systemic translocation of imidacloprid through the leaf closely approximates the exposure pattern of GWSSs in an agricultural setting. The excised leaves were placed in serial dilutions of imidacloprid in aquapiks for 24 h. After 24 h uptake of imidacloprid, exposed leaves were placed in aquapiks containing water only. Exposure time of the insects to imidacloprid is similar to that of the other insecticides. Mortality was checked after 24, 48 and 96 h. Results as shown by LC_{50}s indicate that susceptibility to imidacloprid was not significantly different in 24 h between the Riverside and Redlands insects. However, with longer exposure time, a significant difference was observed between the responses of GWSS from the two locations (LC_{50} =0.0008 ppm for Redlands insects vs. 0.015 ppm for Riverside insects).

In general, GWSS are quite susceptible to all insecticides tested so far. However, insects from Redlands appear to be more susceptible to most compounds than the Riverside populations. Techniques were sensitive enough to detect even small differences in LC_{50}s between locations and exposure time. Monitoring results of GWSS also demonstrated seasonal variations in their responses to various insecticides.

Table 1. Susceptibility of glassy-winged sharpshooter from 2 locations to selected insecticides using 2 techniques.

			LC_{50} (ppm)	
Insecticide Class	**Insecticide**	**Field Location**	**Petri Dish**	**Leaf Dip**
Neonicotinoid	Acetamiprid	Riverside	0.01	0.09
		Redlands	0.003	0.008
	Imidacloprid	Riverside	1.646	—
		Redlands	0.612	—
	Thiamethoxam	Riverside	0.0037	—
		Redlands	0.0004	—
Pyrethroid	Cyfluthrin	Riverside	0.038	—
		Redlands	0.004	—
	Esfenvalerate	Riverside	0.0027	0.022
		Redlands	0.00003	0.00004
	Fenpropathrin	Riverside	0.042	0.168
		Redlands	0.019	0.012
Organophosphate	Chlorpyrifos	Riverside	0.001	0.013
		Redlands	0.001	0.015
Cyclodiene	Endosulfan	Riverside	0.006	—
		Redlands	0.003	—

LABORATORY AND FIELD EVALUATIONS OF IMIDACLOPRID AND THIAMETHOXAM AGAINST GWSS ON CITRUS AND GRAPES

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INTRODUCTION

The development of the neonicotinoid class of insecticides represents a powerful addition to our pest management capabilities. They are safer and more effective insecticides (Casida and Quistad, 1997) due to their lower mammalian toxicities, yet they exhibit higher toxicities against target insects. This permits their use at comparatively low rates and helps to limit environmental contamination. As systemic insecticides, they are more target-specific than conventional broadcast insecticides. This is because soil-applied neonicotinoid insecticides are taken up by plant roots and translocated throughout a plant. Thus, their toxic activity is by and large restricted to plant-feeding insects only. With their putative lower impact on beneficial insects relative to conventional insecticides, the neonicotinoids should be good candidates for incorporation into IPM programs for glassy-winged sharpshooter.

The potential of neonicotinoids to protect citrus and grapes and other perennial crops against glassy-winged sharpshooter is only beginning to be tested. Worldwide, imidacloprid has proven highly effective against a range of insects, but with especially strong performance against sap-feeding insects in both annual and perennial crops. The more recent products such as thiamethoxam and acetamiprid have also shown strong performance against homopterans and other taxa. For each of these insecticides, clearer understanding of their activities against targeted insect pests will lead to more proficient usage.

The overriding goal of research addressed by this project is to better understand the activity of neonicotinoid insecticides against glassy-winged sharpshooter in citrus and grapes. Direct effects on GWSS such as mortality and anti-feedant over the course of a treatment application need to be quantified. These are in turn dependent on the uptake and distribution dynamics within a tree or grapevine over the effective period of the treatment application. Knowledge of the duration and quality of protection afforded to large and complex plants such as mature citrus trees or grapevines is essential to optimizing the performance of neonicotinoid insecticides.

OBJECTIVES

1. Evaluate the titre and distribution of imidacloprid and thiamethoxam within citrus trees and grapevines over time.
2. Develop and conduct bioassays of GWSS on field-treated citrus trees and grapevines tissue and relate mortality to plant titres of imidacloprid and thiamethoxam.
3. Evaluate the behavior of GWSS adults and nymphs of citrus and grapevines treated with neonicotinoid insecticides.
4. Determine the impact of neonicotinoid insecticides on GWSS populations.

RESULTS

Two varieties of mature citrus, lemons and Valencia oranges, were treated with imidacloprid on 10 April 2001 at UC Riverside. Imidacloprid (Admire®) was applied through the irrigation system equipped with microjet emitters at a rate of 32 oz. per acre to 36 orange trees and 22 lemon trees. Beginning 25 April, GWSS samples were collected weekly with a bucket sampler from 12 each of treated and untreated orange trees, and from 7 each of treated and untreated lemon trees. Collections were sorted by nymphal stage and adult sex (for brevity, presented as nymphs and adults) and used to compare treatment densities (Fig. 1). Populations of GWSS consisted almost entirely of nymphs until mid-June in both citrus

varieties. There was little obvious treatment effect until 6 weeks post application, 25 May, when a significant drop in mean nymphal densities occurred relative to the untreated trees and to earlier samples from treated trees (Figure 1). Thereafter, nymphal densities declined progressively and at a greater rate compared to the untreated trees where conversion to adults also contributed to lower nymphal densities. Densities of GWSS adults in oranges begin to increase in mid-June as the earliest of the spring generation of GWSS completed their development (Figure 1). Initially, similar densities of GWSS adults occurred in both treated and untreated trees as much flight activity and movement between trees was observed orchard-wide. By mid-July, however, a significant drop in adult densities on treated oranges occurred and has been sustained ever since (Figure 1). The phenology of GWSS in lemon in terms of nymphal and adult occurrences was similar to that observed in orange (Figure 2). However, there was little obvious impact of the imidacloprid treatment on nymphal densities relative to the untreated lemon trees. In part, this could be due to slower uptake of the imidacloprid by lemon trees because of rootstock incompatibility (Cockeram, per. com.). The most obvious manifestation of this incompatibility is the general state of decline afflicting the lemon trees orchard-wide. But the impact of imidacloprid treatment became apparent by late July as adult densities increased on untreated lemon trees while remaining static on treated trees (Fig. 2).

In another study, colonization rates of GWSS on young Valencia orange trees treated with imidacloprid, thiamethoxam, dimethoate, or left untreated were monitored through summer, 2001. The young nursery trees were transported to a greenhouse, randomly assigned and divided into the 4 treatment groups, then treated using the appropriate rates for container plants for each insecticide. After 2 weeks in the greenhouse following treatment, the young trees were transplanted 18 May 2001 into 12 groups of 4 trees within a mature citrus orchard heavily infested with GWSS. Visual counts of GWSS on each tree began 1 June and continued each week through mid-August. Beginning 15 June, the number of dead GWSS beneath each tree was assessed. Although there was much variability among the 12 groups, densities of GWSS adults were consistently highest on thiamethoxam-treated trees and lowest on imidacloprid-treated trees (Figure 2a). This difference in relative densities between the 2 treatments was also apparent with the greater number of dead adults found beneath thiamethoxam-treated trees (Figure 2b).

Figure 1. Densities of GWSS nymphs and adults on imidacloprid treated and untreated Valencia orange (a) and lemon (b) trees. densities

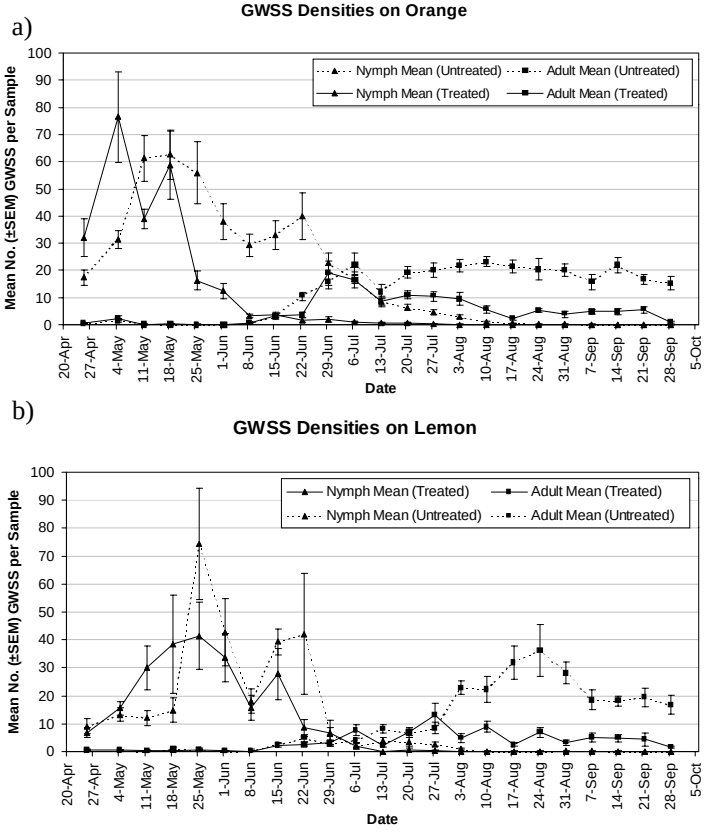
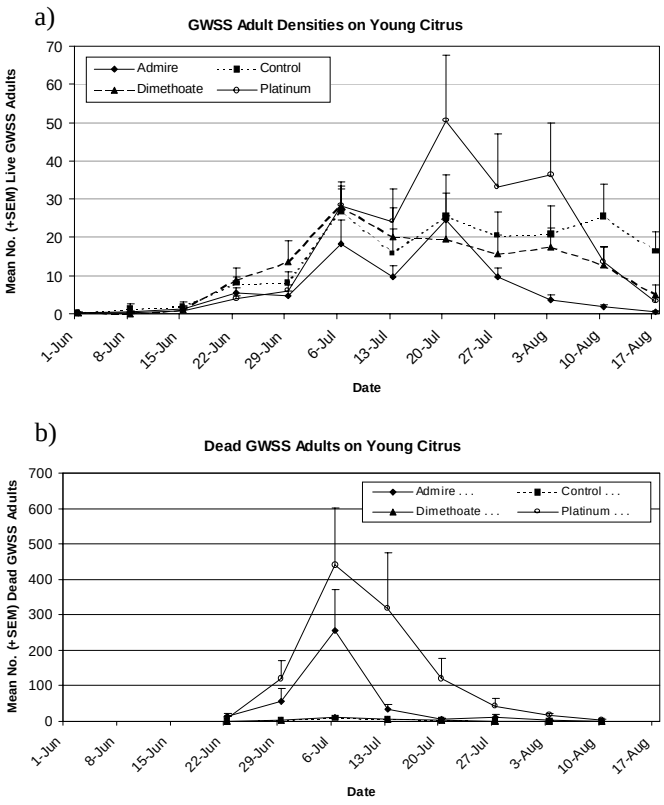


Figure 2. Live (a) and dead (b) GWSS adult on young citrus trees treated with 1 of 3 systemic insecticides.



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AREA-WIDE MANAGEMENT OF THE GLASSY-WINGED SHARPSHOOTER IN THE TEMECULA VALLEY

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INTRODUCTION

The Temecula viticultural area was the first in California to be seriously impacted by the glassy-winged sharpshooter (GWSS) *Homalodisca coagulata* and the spread of *Xylella fastidiosa*, the causative agent for Pierce's disease (PD). While PD problems were first identified in 1996, it was realized by 1999 that the situation was dire. As a result, this ongoing cooperative demonstration project was initiated in 2000 to examine the impact of area-wide management strategies on GWSS populations and PD incidences in the Temecula Valley. The Temecula advisory committee consists of representatives from wine grape growers, citrus growers, University of California-Riverside, USDA, CDFA and the Riverside County Agricultural Commissioner's Office.

Based on grower surveys, there were approximately 2800 acres of wine grapes in production in 1996 in the Temecula viticultural area. About 800 acres were removed due to PD by fall 2000 with 200 acres estimated for fall 2001 **(RLH).** The area encompasses about 2800 acres of wine grapes and 1600 acres of citrus.

The key strategy is to reduce and limit the vector (GWSS) and remove the reservoirs (infected vines). Another strategy in conjunction with the Riverside Agricultural Commissioner's Office was to facilitate the removal of abandoned citrus and vineyards in Temecula.

In the 2000 season, the opportunity to treat nearly the entire commercial citrus in the Temecula viticultural area was seized upon in an effort to destroy a substantial portion of the regional GWSS population. The emergency treatment of 1300 acres of citrus in Temecula, California with Admire (imidacloprid) during April and May 2000 represented a pivotal shift toward an area-wide management of GWSS. In March and April 2001, 269 acres of citrus were treated with Admire and an additional 319 acres were treated with foliar applications of Baythroid on an "as needed" basis. Many grape growers treated their grapes with Admire or made foliar applications of Baythroid, Provado, or Danitol. Recommendations were made to remove sick vines in order to remove bacterial reservoirs. Though response was slow initially, growers are aggressively removing sick vines.

Although wine grapes are the most vulnerable due to the risk of PD, other crops were scrutinized for contributions to GWSS population growth. Citrus was the most important year long reproductive host of GWSS in Temecula. Citrus also seemed to concentrate GWSS over the winter months when grapes and most ornamental hosts were dormant.

OBJECTIVES

1. Determine the impact of the 2000 area-wide management program on GWSS populations in citrus, grapes, and other plant hosts in the ecosystem in the 2001 season.

2. Determine the impact of the area-wide program on GWSS adult oviposition and nymphal development.

3. Determine the impact of the GWSS program on beneficial citrus insects, pest upsets and GWSS parasitism.

4. Evaluate the biological and economic effectiveness of an area-wide insecticide program of GWSS.

RESULTS AND CONCLUSIONS

GWSS populations were monitored weekly in citrus and grapes from March 2000 to December 2000 by trapping (500 traps), visual counts (adults, nymphs, egg masses), beats in citrus, and A-vacuuming in grapes. Although good in most cases, Admire was not 100% efficacious on citrus in 2000. Improper application of Admire or weak trees will affect uptake by citrus trees preventing it from reaching the target site. Lorsban was used in 2000 in areas where Admire failed

to provide adequate control of GWSS in citrus. GWSS populations tended to be clumped, with high numbers often found on weak trees. The results from the 2000 project indicated that every tree or acre does not need to be treated. GWSS numbers remained low in Admire treated citrus (Figure 1) during 2001.

Troublesome areas or hot spots were identified from the 2000 project and by early monitoring during January-April 2001. This led to the treatment of 269 acres of citrus with Admire in March and April 2001. An additional 319 acres of citrus were treated as needed with foliar applications of Baythroid (a pyrethroid) between May 21 and August 15, 2001. A helicopter was used to make Baythroid applications to all but 45 acres. An additional 106 acres of organic lemons received treatments of Gavacide C (440 oils are allowed by CCOF and OMRI). Two small abandoned vineyards with absentee owners and high incidences of PD and GWSS were treated with Danitol by helicopter. Several grape growers treated grapes with Admire or as needed with foliar applications of Danitol, Baythroid, or Provado.

Organic citrus groves were the problematic areas and populations remained high in these groves until August 2001 treatments were made with 440 oils. In April 2001, Surround (kaolin clay), 1.2% Gavacide C (a 440 oil) and 1.4% Gavacide C were applied to certified organic lemon (4 replicates of 3 acres each). The Gavacide C was promising with the lack of other alternatives in organic situations, and two organic lemon groves (30 and 52 acres) were treated with 1.25% Gavacide C on August 4, 2001 (750 gallons per acre). The oil resulted in a 62% and 71% reduction in adult populations in these groves. About 50% of the egg masses contacted by the oil were killed and subsequent oviposition was reduced. About 99% of wasps from oil-treated parasitized egg masses emerged compared to only 2% compared to Baythroid treated egg masses. Follow-up treatments will be made in these organic groves in Mar 2002 with 1.25% Gavacide C and Pyganic. This marked the first time that GWSS populations were impacted in organic situations.

The current situation in Temecula is serious, but cautious optimism prevails. First, GWSS populations are currently the lowest in Riverside County citrus. Second, 2001 grape vine removal due to PD was the lowest in the past three years. Temecula wine grape growers experienced record yields in 2000, and the 2001 crush may prove to be Temecula's finest to date. There will be some replanting in 2002 (2% or less) especially in high visibility areas for both aesthetic reasons and to explore the feasibility of reestablishing lost vineyards at this time.

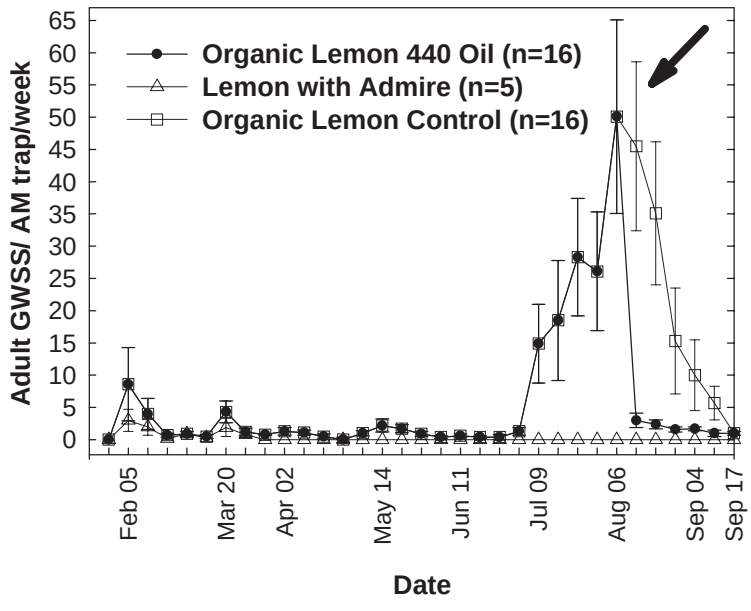

Figure 1. Seasonal Trends of Adult GWSS based on Pherocon AM trap data in organic lemons and Admire treated lemon. Treatment 440 oil was applied to organic lemons on August 7, 2001 as indicated by arrow. Bars represent ±SE.

THE GENETICS OF RESISTANCE TO PIERCE'S DISEASE

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INTRODUCTION

This project is part of the American Vineyard Foundation Long Term Project on Pierce's Disease. My component of this project focuses on understanding the genetics of resistance to *Xylella fastidiosa*, the causal agent of Pierce's disease (PD).

OBJECTIVES

1. Complete analysis of a series of crosses (Design II mating scheme) allowing the quantitative inheritance of *X. fastidiosa* resistance to be evaluated.
2. Complete a genetic map of a *Vitis rupestris* x *Muscadinia rotundifolia* seedling population using AFLP (amplified fragment length polymorphism) markers to allow the identification of DNA markers to *X. fastidiosa* resistance and eventual identification of *X. fastidiosa* resistance genes and their genetic engineering into vinifera cultivars.
3. Utilize genetic markers to *X. fastidiosa* resistance to accelerate the introgression of *X. fastidiosa* resistance into table, raisin and wine grapes.
4. Develop DNA markers from resistance sources other than *M. rotundifolia* for use in breeding table, raisin and wine grapes through the development of additional mapping populations and the bulk segregant analysis of DNA markers.

RESULTS AND CONCLUSIONS

Accomplishments to date have occurred in the following areas:

A. ***Inheritance studies of Xylella fastidiosa resistance in a Muscadinia rotundifolia background.*** Alan Krivanek, a PhD student, is studying the inheritance of *X. fastidiosa* resistance in *M. rotundifolia*. He has completed a broad series of crosses among 12 siblings from an F1 population of *Vitis rupestris* x *M. rotundifolia*, with 6 males crossed to each of 6 females. He will test a 4 x 4 mating scheme and evaluate a portion of the seedlings from each of the 16 possible seedling populations for resistance to *X. fastidiosa* by using needle inoculation followed by symptom evaluation and ELISA to determine the extent of *X. fastidiosa* movement. The results of this research will give estimates for the number of genes involved in resistance. Evaluation of the seedling populations has begun and will be completed over the coming year.

B. ***Defining Xylella fastidiosa resistance.*** We are defining *X. fastidiosa* resistance as the ability of a genotype to limit the movement of *X. fastidiosa*, particularly in a downward direction. A set of resistant and susceptible individuals to determine whether lack of movement is a valid resistance indicator has been tested. These tests used known susceptible, potentially resistant and known resistant genotypes. The known susceptible genotypes were *V. rupestris* 'A. de Serres' (the female parent of the 89 population), Chardonnay and the *V. rupestris* x *M rotundifolia* genotype 8909-19. The potentially resistant genotypes were 8909-04 and 8909-11 and the resistant genotypes were 8909-15 and 8909-17. A time course study with 4 replicates of these genotypes was sampled over 4, 8 and 16 weeks using greenhouse grown potted vines. The presence of *X. fastidiosa* was determined with optimized ELISA (see below) at the point of inoculation (poi), 10 cm above and below, and 20 cm above and below the poi. To allow quantification of the ELISA reading a three-fold dilution of *X. fastidiosa* in healthy plant sap – 1.8×10^6 cfu/ml; 1.8×10^5 cfu/ml and 1.8×10^4 cfu/ml was used. The ELISA reading threshold for a positive reaction was 0.100 at 450 OD, which corresponds to about 10,000 cfu/ml.

After 4 weeks, *X. fastidiosa* was easily detectable in the three susceptible genotypes. There was limited upward movement at this time, although movement seems greatest in 8909-19. At 8 weeks after inoculation downward movement in the susceptible genotypes was easily detected at 10 cm below the point of inoculation, and *X. fastidiosa* seemed to move most readily in *V. rupestris* 'A. de Serres'. By 16 weeks the differences among resistant and susceptible genotypes are very clear in terms of both symptom expression in leaves and unevenly lignified stems and ELISA readings. All three susceptible genotypes had mean ELISA values well above the OD 0.1 or 10,000 cfu/ml threshold at positions both above and below the point of inoculation. We are evaluating resistance after 16 weeks.

C. ***Optimizing detection of X. fastidiosa to better define resistance.*** We have optimized the sensitivity and reproducibility of ELISA for *X. fastidiosa* detection and can now reliably detect 10,000 cfu/ml of ground plant sap in 150 samples with duplicate readings, over the course of one day and at a cost of $0.28 per sample. Our definition of resistance depends upon a very accurate measure of the extent to which *X. fastidiosa* moves in the stem. We are using various PCR techniques (standard, IC-, and quantitative) to best evaluate movement. These efforts are being spearheaded by Nihal Buzkan, (post-doc). We are working towards a blot hybridization technique that will allow sensitive, rapid and inexpensive evaluation of movement in the hundreds of seedlings we must process. Nested IC-PCR techniques are able to detect 10 –20 cfu/ml of plant sap. However, the cost is 10X higher than ELISA and the throughput is 10X lower.

D. ***Developing a genetic map and markers for X. fastidiosa resistance.*** We are now finalizing a genetic map created from a cross of two *V. rupestris* x *M. rotundifolia* siblings (8909-15 x 8909-17). This map was initiated about 2 years ago and is now being completed with the addition of about 200 AFLP, SSR and ISSR markers, bring the total number of markers to about 500. This map was constructed to develop markers for resistance to the dagger nematode (*Xiphinema index*) and to enable the identification of the gene responsible for this resistance. Alan Krivanek tested about 50 individuals from the 8909 and 8913 populations and found strong resistance to *X. fastidiosa* in several including 8909-08, 89-15 and 8909-17. He was also able to document a wide range of responses from very susceptible to very resistant. Although the 8909-15 x 8909-17 seedling population is a cross of two resistant individuals, its progeny segregate widely for *X. fastidiosa* resistance. About 120 members of this sibling generated F2 have been evaluated for *X. fastidiosa* resistance and the character will be soon be mapped. Previous examinations of X. fastidiosa resistance in southeastern US grape species have found that at lest three genes are involved, and preliminary results from Alan also suggest that more than one gene is involved in *X. fastidiosa* resistance from *M. rotundifolia*. However, it may be possible to find markers very tightly associated with *X. fastidiosa* resistance, and preliminary analysis found that *X. fastidiosa* resistance does map in the 8909-15 x 809-17 population.

We are also examining several other populations that contain *X. fastidiosa* resistance from *V. rupestris* x *M. rotundifolia* selections. Alan Krivanek will be testing a population from 8909-15 x B90-116 (a larger berried seedless *V. vinifera* table grape from David Ramming) and then use Bulked Segregant Analysis (BSA) and AFLP markers to develop DNA markers for resistance. These will be compared to resistance markers developed with the mapping population. Resistance markers from the 8909-15 x B90-116 population may be easier to develop because of the much greater genetic difference between the two parents than in the mapping population and therefore greater segregation and genetic polymorphism. Shannon Reis, MS student, will also be using BSA to examine 8909-08 (another *V. rupestris* x *M. rotundifolia X. fastidiosa* resistant selection) x three Ramming *V. vinifera* seedless grapes. Her study will verify the existence and utility of the markers for *X. fastidiosa* resistance.

BREEDING PIERCE'S DISEASE RESISTANT TABLE AND RAISIN GRAPES

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INTRODUCTION

This project is jointly funded through the California Table Grape Commission and the California Raisin Marketing Board and the CDFA/APHIS Pierce's disease program. It is a collaborative effort between UC Davis and the USDA/ARS-Fresno, and is focused on breeding new PD resistant cultivars of table and raisin grapes. This project also builds off the Walker lab portion of the AVF Long Range Project on PD, which focuses on identification of DNA markers for *Xylella fastidiosa* resistance and the mapping of *X. fastidiosa* resistance leading to identification of resistance genes. Success in the AVF PD project will expedite progress in PD resistant table/raisin grape breeding by accelerating the selection process, fine-tuning parent selection, and allowing future resistance gene transformation efforts.

OBJECTIVE

Develop Pierce's disease resistant table and raisin grapes by crossing a variety of *Xylella fastidiosa* resistance sources with large berried and seedless *V. vinifera* table and raisin grapes.

RESULTS AND CONCLUSIONS

In 2000, we began a two-pronged approach for introducing PD resistance into our crosses. First resistance derived from *Muscadinia rotundifolia* via *V. rupestris* x *M. rotundifolia* selections that greatly suppress *X. fastidiosa* development. The second approach utilizes resistance derived from field resistant *Vitis* cultivars from various historic breeding programs in the Southeastern United States (SEUS). Fruit quality is better in these resistant sources, but still needs improving to meet commercially acceptable quality in California. We now have about 50 PD resistant cultivars at UCD and 13 at USDA-Fresno for use as breeding parents. There are also additional selections with high powdery mildew resistance. Fruit quality is coming from advanced seedless tablegrape genotypes at the USDA-Fresno.

Last year, about 180 embryo rescue seedlings resulted from crosses of a male *V. rupestris* x *M. rotundifolia* resistance source (8909-08) with advanced tablegrape genotypes; these were done at the USDA-Fresno. The seedlings were planted in Fresno and copies were given to UCD for *X. fastidiosa* resistance screening. To date, more than 100 of these have been screened for *X. fastidiosa* resistance. Genotypes that do not allow appreciable *X. fastidiosa* movement have been identified. Currently the Bulked Segregant Analysis (BSA) technique is being used to develop a genetic marker for this source of resistance. This marker will dramatically speed our work in selecting resistant seedlings.

Similarly, in mid-summer of this year, copies were made from about 130 seedlings in the UCD vineyard resulting from the 2000 cross of an advanced table grape cultivar onto a female *V. rupestris* x *M. rotundifolia* resistance source (8909-15). This population is also being screened and the BSA technique utilized to develop a genetic marker for resistance. Seedlings from both the male and female resistance source are expected to fruit in 2002 and crosses will be made onto them.

In the past year, over 35 of the SEUS field PD - resistant *Vitis* cultivars and accessions were subjected to the greenhouse PD screen. All were found to harbor ELISA – detectable levels of *X. fastidiosa* after two months of plant growth. A wide range of PD levels in this field resistant material were observed: from barely detectable, to levels equal to susceptible *vinifera* cultivars. Until a better definition of resistance is developed, we will use parents that allow the lowest levels of *X. fastidiosa* development. We have also established a field trial in a PD hotspot in northern California using 13 different SEUS genotypes that allow various *X. fastidiosa* titers in their xylem. Nine resistant and seven susceptible selections from the *V. rupestris* x *M. rotundifolia* population were also included.

In Spring 2000, 1,667 seedlings from PD crosses made using our two strategies were planted at UCD. Plants were derived from both embryo rescue and from seed. From the *V. rupestris* x *M. rotundifolia* resistance source strategy, 5 advanced seedless tablegrape selections were crossed onto a resistant female and a resistant male was crossed unto one seeded and one seedless tablegrape cultivar with 606 seedlings planted to the field. Within the *Vitis* source resistance strategy, 16 different female sources of resistance were used and 7 different male sources resulting in 43 different cross combinations and 1061 seedlings planted to the field. This wide diversity was chosen to allow for multiple trait selection as well as likely alternative PD defense mechanisms, and insure a broad base to the breeding program. Vineyard establishment of these plants has been excellent and most are large enough to fruit in 2002. Seedlings were also field established at the USDA-Fresno. These include the above mentioned embryo rescued seedlings (about 180), and 439 seedlings (pre-screened for powdery mildew resistance) from crosses of two *Vitis* resistance sources and one *V. rupestris* x *M. rotundifolia* selection.

2001 Crosses

At UCD, 6 different resistant females were crossed with 9 different advanced tablegrape selections creating 24 different cross combinations and yielding more than 6,100 seeds. Seven clusters of crosses of an advanced tablegrape female by resistant SEUS males and 3 shipments of resistant pollen from a number of the SEUS resistant vines were sent to USDA-Fresno for their crosses.

At USDA-Fresno, 16 resistant males were crossed to 8 seedless tablegrape selections to continue the embryo rescue *Xf* resistant program. These efforts produced 1,186 embryos. Ten PD resistant males onto 3 seeded tablegrape females to incorporate large berry size with PD resistance, and produced about 500 seeds.

VIRULENCE ANALYSIS OF THE PIERCE'S DISEASE AGENT *XYLELLA FASTIDIOSA*

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INTRODUCTION

The glassy-winged sharpshooter, *Homolodisca coagulata*, spreads the causative agent of Pierce's disease, the bacterium *Xylella fastidiosa* (*Xf*). Depending on whether the glassy-winged sharpshooter can establish itself in Northern California, Pierce's disease may represent a multi-billion dollar threat to the grape and wine industry and the associated tourist trade. The symptoms of Pierce's disease include a yellowing and gradual necrosis (scorching) of grapevine leaf edges, stunting of cane growth, and, particularly in the spring for vines infected for two or more seasons, inter-veinal chlorosis of leaves (Hewitt, 1970). (Lee et al., 1982) reported that detached grape leaves from grape cultivars that are particularly sensitive to Pierce's disease, showed typical marginal yellowing and scorching in less that 12 hr after petiole uptake of cell-free washing from *Xf* cells grown on agar plates. The activity did not survive autoclaving or multiple freezing and thawing and was not inactivated by incubation with proteinase K. No activity was observed for washings of uninoculated agar plates.

Applying the conditions of (Lee et al., 1982), (Goodwin et al., 1988) also observed phytotoxicity after petiole uptake of cell-free washing, but from both *Xf*-populated and uninoculated agar plates. Further, (Goodwin et al., 1988) found a correlation between increased midday stomatal resistance and symptom development on leaf margins and an approximately six-fold increase in leaf proline content (fresh-weight basis) associated with Pierce's disease. They discounted phytotoxins as significant contributors to Pierce's disease symptoms and state that "The biophysical and biochemical changes observed for diseased vines indicate that marginal leaf necrosis occurs when water stress develops. Diseased leaves are apparently water stressed because of vascular dysfunction which, when prolonged, may result in accelerated leaf senescence." (Goodwin et al., 1988) also found that higher stomatal resistance was associated with spring inter-veinal chlorosis but was not as pronounced as the stomatal resistance increase observed for leaves showing symptoms first at the margin.

OBJECTIVE

The objective of this project is to identify gene product(s) and gene(s) of *Xf* that contribute to its virulence.

RESULTS AND CONCLUSIONS

Experimental infection of grape to induce disease generally requires weeks or months to the appearance of symptoms. We wondered whether other plants might exhibit more rapid symptom development upon exposure to *Xf*, particularly to *Xf* infiltrated directly into the leaf lamella. A survey of plant species and lines commonly used as experimental hosts for plant pathogens revealed that *Chenopodium quinoa* (Cq) developed chlorosis corresponding to the infiltrated area within 48 hr of infiltration of 10^8 to 10^6 cfu/ml suspensions of *Xf* cells. Light microscope cytological studies carried out by Prof. Judy Jernstedt, UC Davis Agronomy and Range Sciences Department, revealed that the observed chlorosis of Cq leaves infiltrated with *Xf* is the result of chlorophyll loss from chloroplasts in all photosynthetic cell types without other observable effect. Xanthomonads are considered to be closely related to *Xylella* spp., and *Xanthomonas campestris* pv. *Vesicatoria* and some other, but not all, *Xanthomonas* spp. induce a similar chlorosis in Cq leaves. *Xanthomonas campestris* pv. *campestris* induced necrosis 3-4 days after infiltration. Currently we are testing the effects of petiole-feeding of grape leaves with *Xf* and fractions (see below) derived from *Xf*.

Chlorosis development varied significantly from leaf to leaf on a Cq plant and from plant to plant. However, comparisons of infiltrated opposite leaf halves appear to provide valid measures of the relative potency of *Xf*-derived preparations and forms the basis for a semi-quantitative assay used here. *Xf* cells from liquid culture had a slightly greater specific chlorosis-inducing (CI) activity than *Xf* cells from agar plates. However, the yield of *Xf* cells from plates was greater, and results

reported here are from plate-derived *Xf* cells. The CI activity was associated with cells, not washings of cells. Heating *Xf* cells at 100°C for 6 min slightly enhanced the CI activity. Treatment with sodium dodecyl sulfate (SDS) at 25°C or 65°C did not destroy the CI activity. Most proteins will not survive in active form after treatment at high temperatures or with SDS, but the CI activity was greatly reduced or destroyed by incubation with protease K, trypsin or chymotrypsin. Treatment with acetic acid or chloroform also was inactivating, but incubation with periodate, lysozyme, or ethanol did not reduce CI activity.

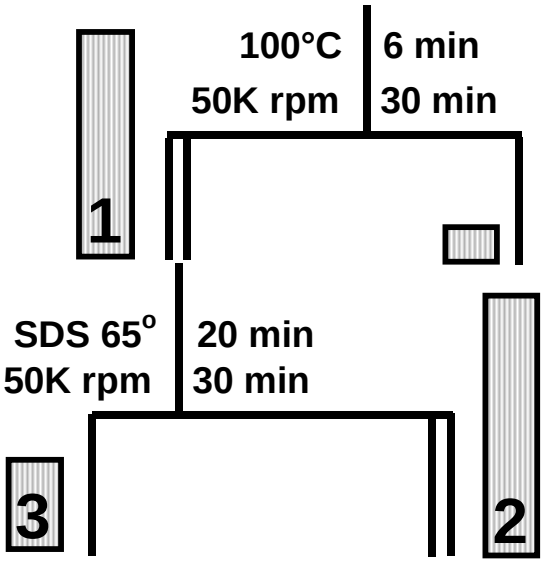
The results described above are consistent with an essential role for an unusually stable protein in whatever constitutes CI activity. Fig. 1 presents an analysis of proteins collected as a precipitate after treatment of *Xf* cells at 100°C or with SDS at 25°C and centrifugation at 50,000 rpm for 30 min. Fig. 2 is a flow chart showing treatment at 100°C and collection of insoluble material, which then was treated with SDS at 65°C. A single vertical line represents supernatant; a double vertical line represents precipitate. Bars show the relative CI activity of four fractions. Even after treatment with SDS at elevated temperature, most of the material remained insoluble. However, exposure to SDS and mercaptoethanol solubilized proteins for gel electrophoresis. As indicated by Fig. 1 and Fig. 3, elevated temperature and SDS removed the bulk of the protein without destruction of CI activity. Lane numbers in Fig. 3 correspond to sample numbers in Fig. 2. Current effort is aimed at correlating CI activity with a specific protein band(s), identification of the corresponding protein, with aid of *Xf* genome sequences (Simpson et al., 2000), and testing of *Xf*-derived, CI fractions for their effects on grape.

Fig. 1

1. *Xf* cells
2. 100°, 6 min
3. SDS RT, 30 min

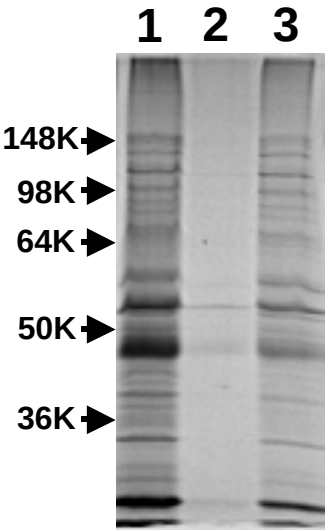
12.5% SDS-PAGE, Coomassie blue stain

Fig. 2



1. *Xf* cells, 100 deg 6 min, high speed pellet
2. #1 + SDS 65 deg 20 min, high speed pellet
3. #1 + SDS 65 deg 20 min, high speed supernatant

Fig. 3



12.5% SDS-PAGE Coomassie blue stain

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EFFECTS ON VERTEBRATES OF RIPARIAN WOODLAND MANAGEMENT FOR CONTROL OF PIERCE'S DISEASE

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INTRODUCTION

It is vital to the wine industry to be aware of the potential effects of control programs for disease, insects and other pests that might affect wildlife in riparian zones, as the health of these zones is important to properly functioning ecosystems as well as to the public and regulatory agencies. This study undertook to evaluate the effects on a variety of vertebrates of the removal of vegetation as well as planting of non-disease reservoir plants in riparian zones to control or reduce the incidence of Pierce's disease. Studies were initiated in 1997 with the establishment of eight plots, three on Conn Creek (open, managed, and not managed), and three on the Napa River (Ecological Reserve, managed, and not managed) in Napa County, and two (managed and not managed) on Maacama Creek in Sonoma County.

OBJECTIVES

1. Determine what influence habitat manipulation for Pierce's disease control will have on birds, mammals, reptiles and amphibians in riparian zones.

RESULTS AND CONCLUSIONS

Five sampling procedures were used per plot as follows:

i. Two Trailmaster bait stations once per month for 7 days (1997-1999)
ii. Fifteen Sherman live traps for small mammals one night per month (1997-1999)
iii. Three point census plots for birds once per week during the breeding season (spring-early summer 1998 and 1999)
iv. Eight nesting boxes for birds (Dahlsten and Copper 1979) checked weekly during the breeding season (1998 and 1999) and
v. Six 4-square foot reptile boards checked approximately twice per month (1998 season).

The bait stations showed the most common mammal to be the opossum, followed by squirrels, raccoons, rats, and foxes. Larger mammal activity was lowest in the open grassy plot, and highest in the Maacama Creek managed plot and the Ecological Reserve unmanaged plot. There were no significant differences in totals of all species between the managed and unmanaged pairs of plots. The most common mammal in the live traps was the deer mouse, *Peromyscus* spp. Activity was highest in the open unmanaged plot and lowest in the Conn Creek unmanaged plot, with no significant differences between the managed and unmanaged paired plots. The most common species in order were swallow species, European starling and American robin. Out of 66 and 72 species identified on the study plots during 1998 and 1999 respectively, with plots averaging about 38 species each. Numbers of birds did not vary significantly between treated and untreated plot pairs except for one pair in 1999 (plots 2 and 3). Numbers decreased significantly overall from 1998 and 1999, especially

in plots 4 and 5. Eight species of birds used the nest boxes and the highest occupancy rate was in the unmanaged area on the Napa River, followed by the managed area at Conn Creek. The Reserve area had the lowest occupancy rate. No patterns were evident in use between pairs of managed and unmanaged plots. The reptile boards were used by four species of lizards, two species of snakes, the Pacific tree frog, western toad, and a salamander. The most common organism was the western fence lizard. The highest use was in the managed plot on Conn Creek; with the other plots being similar to each other, with no significant differences in total numbers between managed and unmanaged plot pairs.

Analyses of our data support the hypothesis that the vegetation management used in this study does not affect numbers of mammals, birds, and reptiles significantly in these riparian areas. Significant overstory differences (plots without many large trees vs. all others with large trees) do show effects with some mammals and birds, and there are also some differences between the Napa Valley plots and the Sonoma plots.

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SEARCH FOR AND COLLECT EGG PARASITOIDS OF GLASSY-WINGED SHARPSHOOTER IN SOUTHEASTERN USA AND NORTHEASTERN MEXICO

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INTRODUCTION

Observations in northeastern Mexico and Texas in 1999 and 2000 revealed presence of *Homalodisca coagulata* (Say) (GWSS) there, but in extremely low densities during winter and spring months (Coronado-Blanco et al. 2000; Triapitsyn & Phillips 2000; Triapitsyn et al. in press). Almost all egg masses of GWSS and other related sharpshooters, such as *Oncometopia* spp., were heavily parasitized. The climate in central part of Tamaulipas, Mexico, is very similar to one in the valleys of southern California, particularly Temecula. Earlier surveys in Florida and Louisiana revealed several species of GWSS egg parasitoids there; some of those species do not occur in California (Triapitsyn et al. 1998).

As a result of the collections made in northeastern Mexico during 2000, quarantine and insectary colonies of three species, *Gonatocerus ashmeadi* Girault, *G. morrilli* (Howard), and *G. triguttatus* Girault (Mymaridae), were established in UC Riverside quarantine and insectary (Morgan et al. 2000; Triapitsyn et al. in press) and later propagated and released against GWSS in California by the California Department of Food and Agriculture (CDFA). Unfortunately, due to unavailability of the host material (i.e., GWSS eggs) during the winter of 2000-2001, the California colonies of *G. triguttatus* and other exotic parasitoids were discontinued.

OBJECTIVES

1. Recollect the target species of GWSS egg parasitoids, particularly *G. triguttatus* Girault, in northeastern Mexico and Texas, clear them through UCR quarantine, and reestablish their cultures (maintained by our cooperators from the CDFA) in southern California for a large-scale classical biological control program against GWSS in California.

2. Search for and collect additional biological control agents, among others *Gonatocerus atriclavus* Girault, *G. fasciatus* Girault, and *Ufens spiritus* Girault (Trichogrammatidae, also known as *Zagella* sp., see Triapitsyn et al. 1998), in the home range of GWSS (southeastern USA and northeastern Mexico) for introduction into California and establishing cultures in UCR quarantine.

RESULTS AND CONCLUSIONS

Two trips were made to date: 1) to Nuevo León, Tamaulipas, San Luis Potosí and Veracruz, Mexico, in February 2001 (D. Morgan, S. Myartseva, G. Simmons, S. Triapitsyn, and D. Yanega) and 2) to Florida, from Miami to Monticello and Quincy (M. Hoddle and S. Triapitsyn) and College Station, Texas (S. Triapitsyn and V. Berezovskiy) in August 2001. One more trip to Tamaulipas is planned for November 2001. In addition, several shipments of adult egg parasitoids, mainly *G. triguttatus* and *G. morrilli*, were sent to UCR quarantine by W. Jones (material reared from GWSS egg masses in and in the vicinity of Weslaco, TX) as well as extensive material from the exploratory trip to northeastern Mexico in March 2001 by D. Morgan and Ch. Pickett (CDFA) was processed in UCR quarantine. Getting the material from Tamaulipas and Texas early in the season helped in re-establishing a colony of *G. triguttatus* first in UCR quarantine and insectary and then at the CDFA facility in Riverside (D. Morgan).

The following species of egg parasitoids were collected during 2001 and propagated at UCR (if applicable):

Genus and species of egg parasitoid	Originally from: (State, location)	Original host	Propagated on GWSS at UCR quarantine (yes/no)
Gonatocerus ashmeadi	Tamaulipas	*H. coagulata*, *Oncometopia clarior*	Yes
	Texas (Weslaco)	*H. coagulata*	Yes
	Florida	*H. coagulata*	Yes
Gonatocerus atriclavus	San Luis Potosí, Tamaulipas, Veracruz	*Oncometopia* spp. including *O. clarior*	No (failed)
Gonatocerus morrilli	Tamaulipas	*Homalodisca* sp. and *Oncometopia* spp.	Yes
	Florida (Apopka)	*Oncometopia nigricans*	Yes
	Florida (Quincy)	*H. coagulata*	Yes
	Texas (Weslaco, College Station)	*H. coagulata*	Yes
Gonatocerus triguttatus	Tamaulipas	*H. coagulata*, *Oncometopia* sp.	Yes
	Texas (Weslaco)	*H. coagulata*	Yes
	Texas (College Station)	*H. coagulata*	Yes
	Florida (Apopka)	*Oncometopia nigricans*	Yes
Acmopolynema sema	Florida (Belle Glade)	*Homalodisca insolita*	Yes
Ufens spiritus (=Zagella)	Florida (Quincy)	*H. coagulata*	No (failed)
Ufens n. sp.	Tamaulipas	*Oncometopia* sp.	No (failed)

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EGG AGE PREFERENCE AND "WINDOW OF SUSCEPTIBILITY" OF *HOMALODISCA COAGULATA* EGGS TO ATTACK BY *GONATOCERUS ASHMEADI* AND *G. TRIGUTTATUS*

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INTRODUCTION

Understanding the reproductive biology of natural enemies is essential if observed outcomes in the field are to be explained accurately. *Gonatocerus ashmeadi* Girault and *G. triguttatus* Girault both attack glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* [Say] eggs, however it is not known whether they compete for host eggs of the same age, what egg ages are most preferred by females of each species, and which species would be most beneficial for GWSS control. *G. triguttatus* is associated with GWSS in Mexico and Texas and is now being released in limited numbers in Riverside County and elsewhere in California to combat GWSS. *G. ashmeadi* is native to California. Determining the egg age preference of this species will also be valuable in maximizing production for parasitoid releases. Therefore, the following experiments were conducted to determine GWSS egg age preference and the 'window of susceptibility' of eggs to attack by *G. ashmeadi* and *G. triguttatus*. Results presented here are for *G. ashmeadi* and are for egg age categories 1, 5 and 10 days of age. Replicates for 2, 3, 4, 6, 7, 8 and 9 days of age have been set up for both species, however, data is still being collected and analyzed. This will be completed and presented at the December 2001 Pierce's Disease Control Program Symposium.

OBJECTIVES

1. To determine the "window of susceptibility" or vulnerability in days of GWSS eggs to attack by *G. ashmeadi* and *G. triguttatus*.
2. To determine *G. ashmeadi* preference for young, medium and old GWSS eggs.

MATERIALS AND METHODS

For Objective 1, five leaves with 15 GWSS eggs of known age laid on Eureka lemon leaves were placed into a 3 inch ventilated vial cage and exposed to one mated female *G. ashmeadi* (~ 24 hrs of age) for two hours at 25°C. This experiment was replicated ten times for GWSS eggs 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days of age and repeated for *G. triguttatus*. The number of parasitoids that emerged from GWSS egg masses of each age and the sex ratio of emerged progeny was recorded.

Three egg ages (1, 3 and 5 days of age) were selected for Objective 2 to represent young, medium and old GWSS eggs. Ten eggs of each age category were presented simultaneously to each of one female *G. ashmeadi* for one hour. At this time the egg age that the parasitoid was found on was recorded, the parasitoid was removed, and the vials were kept at 25°C. The number of parasitoids that emerged from eggs in each age category was recorded after three weeks.

RESULTS AND CONCLUSIONS

Preliminary results showed that approximately 73% of one-day-old GWSS eggs and 26% of 5-day-old eggs were successfully parasitized by *G. ashmeadi*. Lower parasitism rates of 5 day old eggs may have resulted because GWSS embryos in eggs of this age had developed beyond a stage that most *G. ashmeadi* larvae were able to use after hatching, or higher rates of encapsulation were experienced post-oviposition, or venom injected at time of oviposition was not sufficient to halt GWSS embryo development and facilitate wasp development. Furthermore, Eidmann (1934) suggested that success in parasitizing host eggs that are close to terminal development depends on whether the parasitoid egg is oviposited directly into the

embryo, thereby killing it. If Eidmann (1934) is correct, then 26% of ovipositions by *G. ashmeadi* into GWSS eggs 5 days of age may have killed the embryo allowing the parasitoid to develop successfully.

G. ashmeadi successfully parasitised five day old GWSS eggs which indicates that this species has a wide host age preference. This is favorable for GWSS control because the period of vulnerability to attack by this parasitoid is long, therefore increasing the probability that an egg will be parasitised before it hatches.

Exposure of GWSS eggs 10 days of age to *G. ashmeadi* resulted in 0% parasitism as nymphs were emerging from egg masses of this age at 25ºC. Data for the entire 'window of susceptibility' for *G. ashmeadi* and *G. triguttatus* are still being collected. Once this has been completed the results will be analyzed to determine if different egg age preferences exist between these two species.

Results also showed that regardless of egg age and of how many of the 15 eggs were parasitized, female *G. ashmeadi* generally allocated two males to an egg mass and the remaining progeny were female. This is consistent with Stern and Bowen (1963) fixed sex allocation findings.

Results from Objective 2 showed that parasitism was slightly lower for eggs of 5 days of age compared with 3 days of age (Table 1), thereby supporting the results from Objective 1. However, the number of times the parasitoid was found on each treatment did not significantly differ between the three egg ages (Table 1). Also, parasitism did not significantly differ between eggs of 1 and 5 days of age (Table 1). This may suggest that this parasitoid species does not directly select between host age when all the age categories are able to be parasitised. However, other egg parasitoids, such as *Trichogramma* sp. have been found to distinguish between favorable and unfavorable eggs for parasitism and tend to prefer younger eggs (Pak, 1986; Hintz and Andow, 1990; Godin and Boivin, 1994). Therefore, this choice experiment will be repeated next season using the three egg age categories 1, 4 and 8 days of age to determine whether *G. ashmeadi* can differentiate between favorable and unfavorable eggs for parasitism.

Table 1. Percentage parasitism of 1, 3 and 5 days of age GWSS eggs by G. ashmeadi.

Egg age treatment	*Parasitism*	*Number of times the parasitoid was found on each treatment after one hour*
1	22.7%	5
3	27.9%	6
5	20.2%	5

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ELEVATION'S EFFECT ON SURVIVAL OF GLASSY-WINGED SHARPSHOOTER IN KERN COUNTY

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INTRODUCTION

The ability of glassy-winged sharpshooter (GWSS) to successfully over-winter within various crops in Kern County, California is of paramount interest to those seeking to control this pest. The eastern edge of the San Joaquin Valley is heavily planted with citrus, a preferred host plant for feeding and oviposition. This belt of host plants located within a suspected over-wintering survival zone could create a "highway" for rapid northern dispersal of GWSS. These extensive citrus plantings are located at various elevations along a thermal belt that experiences warmer winter temperatures than the valley floor. In addition, the valley floor is subjected to fog and continuous temperatures in the range of 3-5° C.

OBJECTIVE

1. Evaluate the effect of elevation and temperature on the winter survival of glassy-winged sharpshooter in Kern County.

RESULTS

GWSS were placed into replicated field cages at ten different elevations on December 8, 2000. Field cages were framed with PVC pipe (40"x40"x60") and covered with a fine mesh material, trade name Econet B. Four cages were erected at each site. Three different host plants were placed inside each cage at all locations. The type and size of host plants were: parent navel citrus, 5 gal. Established tree, oleander, 1 gal. and gardenia, 1 gal. Between 200 and 300 GWSS field collected adults were placed inside each cage. No attempt was made to determine age or sex of GWSS at the time of collection. The primary objective is to determine if there are areas where GWSS will not survive the winter in Kern County. Temperature and humidity measurements are collected at fifteen-minute intervals for each site. The test will run until the end of April or until new egg masses are found within the cages.

Table 1 summarizes the 10 test site locations, elevation above sea level and hours at or below 0°C. All cages and the plant material within them were retrieved from the ten test locations. All plant and cage material was inspected for GWSS egg masses, nymphs and adults and observations on GWSS survival were made. The results about the survival of GWSS in the cages are provided in Table 2.

Observations and counts suggest that GWSS may experience severe over-wintering mortality at the lower elevation test sites. It is not known what influences test techniques might have had on GWSS survival. Improvements to the study could include the use of larger potted plants. Beefwood trees (*Casuarina* spp.*)* could make an excellent addition to the host plants within any future GWSS study. This study will be repeated in 2001-02 with additional emphasis on elevations between 350-500 feet.

Table 1. Test site locations and elevations.

Location	*Elevation*	*Hours 0-°C**
UCCE Farm Advisors Office, S. Mount Vernon Ave.	371'.	140
Fairfax and Panama Rd.	404'.	191
Panama Rd.	449'.	180.5
AEWSD Ponds, Tejon Hwy.	497'.	37
USDA trailer, Vineland and Edison.	514'.	58.3
Rockpile Rd. and Panama Rd.	579'.	70.8
AEWSD plant, Redbank and Malaga.	606'.	19.0
AEWSD plant, Neumarkle Rd.	712'.	98.5
AEWSD plant, General Beale Rd.	834'.	2.8
Nuemarkle and Bena Rd.	902'.	9.8

Hours at 0° C from Dec. 4, 2000 - April 2, 2001

Table 2. Observations on GWSS survival in the cages.

Site #	*Observation*
1	No GWSS eggs, nymphs or adults found in any of the test cages. Last recorded sighting of a live GWSS in these cages, 1/02/01.
2	No GWSS eggs, nymphs or adults found within any test cage. Last recorded sighting of a live GWSS in these cages, 1/18/01.
3	No GWSS eggs, nymphs or adults found within any test cage. Last recorded sighting of a live GWSS in these cages, 1/18/01.
4	GWSS egg masses found on citrus in cages No. 3 & 4, 4/02/01. No GWSS found in cages No. 1 & 2.
5	GWSS egg masses found on citrus in cages No. 1 & 3, and one adult GWSS female recovered in cage No. 3, 4/12/01. No GWSS Found in cages No. 2 or 4.
6	No GWSS eggs, nymphs or adults found in any of the test cages. Last recorded sighting of a live GWSS in these cages, 2/28/01. Site over-sprayed by a dormant application to almond trees next to test cages.
7	GWSS egg masses found on citrus in all four cages. GWSS egg masses found on gardenias in cages No. 1,2 & 4. Two females and one male GWSS found in cage No. 1.
8	GWSS egg masses found on citrus in cages No. 1 & 3. No GWSS found in cages No. 2 &4.
9	No GWSS eggs, nymphs or adults found within any test cage. Last recorded sighting of a live GWSS in these cages, 3/14/01. This site was exposed to the East non-cropped desert area
10	GWSS egg masses found on citrus in cages No. 1, 2 & 3. GWSS egg mass found on gardenia in cage No. 2. Three females and one male found in cage No.1. Two females and one male found in cage No. 3. No GWSS found in cage # 4.

*****All cages were inoculated with 200 – 300 field collected GWSS adults on Dec. 8, 2000.

EVOLUTION AND HISTORICAL ECOLOGY OF THE PROCONIINI SHARPSHOOTERS

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INTRODUCTION

The tribes Proconiini and Cicadellini, commonly referred to as sharpshooters (Cicadellinae sensu Young 1968), together form the largest group of xylem-feeding leafhoppers and include most of the known vectors of xylem-born phytopathogenic organisms. Proconiini is a strictly New World group with most of its diversity confined to the tropical regions. Among ca. 350 described proconiine species, currently grouped into 56 genera, less than 10% (4 genera) occur north of Mexico, and only one genus (*Cuerna*) has substantially radiated in the temperate parts of the U.S. and Canada. In spite of its largely tropical distribution, the group is of significant economic importance in the U.S. because several *Homalodisca*, *Oncometopia*, and *Cuerna* species are able to transmit *Xylella fastidiosa (Xf)* (Nielson 1968), and two of them recently invaded new areas in the southern U.S. (Pollard et al. 1959; Sorensen & Gill 1996). Species of *Oncometopia* and *Acrogonia* are also principal vectors of *Xf* causing the citrus variegated chlorosis in South America (Gravena et al. 1998). Except for a few economically important species, the data on the ecology and bionomics of proconiines are virtually absent, although this picture is beginning to change. In particular, recent studies suggest that in at least 13 proconiine genera females display a unique type of maternal care by powdering their egg nests with brochosomes, specific Malpighian tubule products unique to leafhoppers (Rakitov 1999, 2000, and unpublished; Hix 2001). Although the exact adaptive significance of this behavior is not yet clear, its occurrence in several of the most speciose proconiine genera, including *Homalodisca*, *Oncometopia*, and *Acrogonia* , which contain principal vectors of *Xf*, suggests that its advantages may have facilitated speciation and contributed to the pest status of some species, perhaps by increasing their capability to colonize a wide range of vegetation. Building a robust estimate of the evolutionary relationships among Proconiini is important to provide a framework in which ecological and bionomical features, and the patterns of distribution of the group can be better understood. In the absence of fossil evidence this can be achieved by the phylogenetic analysis of the distribution of morphological, molecular, and other characters among modern species. Unfortunately, the only such analysis of Proconiini performed so far (Mejdalani 2000) focused on only one group of presumably related genera and therefore has not completely elucidated the relationships within the tribe.

OBJECTIVES

1. To assess phylogenetic relationships among proconiine leafhoppers, using morphological and DNA sequence data.
2. To study the evolution of “egg nest powdering”, and its role in the diversification of the tribe.

RESULTS AND CONCLUSIONS

Our preliminary morphology- and sequence-based analyses both indicated that Proconiini are derived from within Cicadellini. This result is consistent with biogeographical evidence, which suggests that the tribe is younger than the Cosmopolitan Cicadellini and apparently arose in South America after its separation from Africa (Nielson & Knight 2000). A much broader sampling is, however, needed to pinpoint a particular cicadelline group that might have given rise to Proconiini. Our morphological dataset strongly indicated that the Neotropical genera *Pamplona* and *Pamplonoidea*, previously placed in Cicadellini (Young 1977), and a small Neotropical group previously classified as subfamily Phereurhininae (Kramer 1976) should be placed in Proconiini, and that *Homalodisca*, as currently defined, includes certain species that should be placed in other genera. These results, which emphasize the need of a reclassification of the Proconiini, were largely congruent with an estimate based on the mitochondrial DNA sequences (Figure 1). Because of the small size of the molecular dataset, which included representatives of only 27% of the proconiine genera, the presented tree should be interpreted with caution. The analysis grouped the species into two major lineages, one of which included all of the known vectors of *Xf* The habit of coating egg nests with brochosomes appears to have arisen early in the evolution of this lineage; its origin and the vector status of some of the lineage’s members may both be related to certain changes in the ecology of the group. This behavior has apparently been independently lost in several taxa. Comparative studies of the egg-laying ecology in such taxa (e.g., *Cuerna*, *Phera*) and other proconiines may shed light onto its adaptive significance. Our future goals include building a more robust phylogenetic estimate by increasing both the taxon and character sampling, collecting more data on the ecology and bionomics of the group through field work and collaboration with sharpshooter researchers

in other countries, and performing more diverse analyses of the historical ecology, bionomics, and patterns of distribution of the Proconiini.

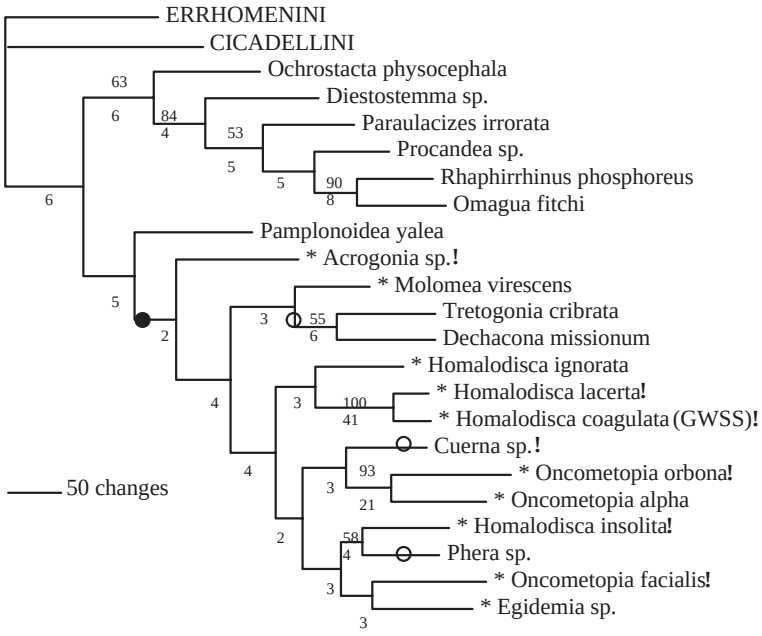

Figure 1. Preliminary estimate of phylogenetic relationships among the species of Proconiini, based on combined partial 12S, 16S, COI, and COII mitochondrial DNA sequences (ca 2000 base pairs) obtained for 21 proconiine and 2 outgroup species. The tree shown is a single maximum parsimonious tree; the length of branches is proportional to the number of nucleotide changes along each branch. The numbers above and under branches are bootstrap scores (shown only if greater than 50%) and decay indices, respectively; higher values indicate better support for the clade. Asterisks (*) indicate species using brochosomes in egg laying. The gain of this trait is shown by a closed circle, and three independent losses by open circles. Exclamation points (**!**) indicate known vectors of *Xylella fastidiosa*.

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PROGRESS REPORTS NOT INCLUDED

Progress Reports were not submitted for the following research projects:

Biology and Ecology of Glassy-winged Sharpshooter in the San Joaquin Valley
Daane, K.

Xylella fastidiosa Genome Analysis -- Almond and Oleander Comparison to Pierce's Disease Temecula1 and Citrus Strains
FAPESP/Van Sluys

Role of Type 1 Secretion in Pierce's Disease
Gabriel, D.

Screening Insecticides in Nursery Citrus for Efficacy Against Glassy-winged Sharpshooter
Grafton-Cardwell, E.

Glassy-winged Sharpshooter Impact on Yield, Fruit Size, and Quality
Hix, R.

Biocontrol of GWSS in California: One Cornerstone for the Foundation of an IPM Program
Hoddle, M.

Studies on Bacterial Canker and Almond Leaf Scorch
Kirkpatrick, B.

Production and Screening of Xylella fastidiosa Transposon Mutants and Microscopic Examination of Xf-Resistant and Susceptible Vitus Germplasm
Kirkpatrick, B.

Kern County Pilot Project
Luvisi, D., L. Wendell

Timing and Duration of Fresh Glassy-winged Sharpshooter Egg Masses in Lemon Fruit Rinds; Impact on Fruit Harvest and Shipments
Phillips, P.

Controlling the Spread of Xylella fastidiosa, the Causal Agent of Oleander Leaf Scorch, by Disrupting Vector Acquisition and Transmission
Redak, R. and M. Blua

Developing an Integrated Pest Management Solution for Pierce's Disease Spread by the GWSS in Temecula
Redak, R.