

Symposium Proceedings



**Pierce's Disease Research Symposium
December 15 – 18, 2002
Coronado Island Marriott Resort**

-- slightly revised --

*Proceedings of the
Pierce's Disease
Research Symposium*

-- slightly revised --

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Note 2: This edition of the Proceedings has been revised to increase formatting uniformity, correct punctuation and spelling errors, and provide more accurate information on the funding agencies for each project. It includes a revised version of the report entitled "Biological Control of *Homalodisca coagulata*" by W. Jones (page 88).

TABLE OF CONTENTS

Welcome Letter from Secretary William (Bill) J. Lyons, Jr.....	vii
--	-----

Breeding Cultivars of Grape Resistant to Pierce's Disease

Virulence Analysis of the Pierce's Disease Agent <i>Xylella fastidiosa</i> George Bruening	3
--	---

Functional Genomics of the Grape-<i>Xylella</i> Interaction: Towards the Identification of Host Resistance Determinants Douglas Cook, Francisco Goes-da-Silva, and Hyunju Lim.....	5
--	---

The Genetics of Resistance to Pierce's Disease Andrew Walker.....	7
---	---

An Expanded Genetic Map of <i>Vitis rupestris</i> x <i>Muscadinia rotundifolia</i> for Fine Scale Mapping and Characterization of Pierce's Disease Resistance Andrew Walker and Summaira Riaz	9
---	---

Application of <i>Agrobacterium rhizogenes</i>-Mediated Transformation Strategies for a Rapid High Throughput Screen for Genetic Resistance to Pierce's Disease in Grape that Maintains Clonal Integrity of the Recipient Host David Gilchrist	11
--	----

Biological Control of Pierce's Disease

Characterization of Fimbriae Production and Attachment of FimA⁻ and FimF⁻ Mutants of <i>Xylella fastidiosa</i> in vitro Steven Lindow.....	15
--	----

The <i>Xylella fastidiosa</i> Cell Surface Michele Igo.....	17
---	----

Understanding <i>Xylella fastidiosa</i> Colonization and Communication in Xylem Lumina Harvey Hoch and Thomas Burr	18
--	----

Management of Pierce's Disease of Grape by Interfering with Cell-Cell Communication in <i>Xylella fastidiosa</i> Steven Lindow.....	20
---	----

Biological Control of Pierce's Disease with Non-Pathogenic Strains of <i>Xylella fastidiosa</i> Donald Cooksey	23
--	----

Control of Pierce's Disease Through Degradation of Xanthan Gum Donald Cooksey	25
---	----

Paratransgenesis for Control of Pierce's Disease: Manipulation of Endophytic Bacteria for Paratransgenic Control of Pierce's Disease David Lampe and Thomas Miller	27
--	----

Insect-Symbiotic Bacteria Inhibitory to <i>Xylella fastidiosa</i> in Sharpshooters: Pressure Bomb Extraction of Xylem Fluid to Improve Bacterial Detection of <i>Xylella</i> in Plants Blake Bextine and Thomas Miller	29
--	----

Insect-Symbiotic Bacteria Inhibitory to <i>Xylella fastidiosa</i> (Paratransgenesis for Control of Pierce's Disease): Identification of Endophytic Bacteria Cycled by Glassy-winged Sharpshooters to Host Plants Carol Lauzon and Thomas Miller.....	31
--	----

Genetics of *Xylella fastidiosa* / Miscellaneous

Surrogate Genetics for <i>Xylella fastidiosa</i> Valley Stewart.....	35
Directing Potential Anti-<i>Xylella</i> Gene Products to the Xylem of Transgenic Grapevines Carole Meredith and Abhaya Dandekar.....	37
Biological, Cultural, Genetic, and Chemical Control of Pierce's Disease: Production and Screening of <i>Xylella fastidiosa</i> Transposon Pathogenicity and Attachment Mutants Bruce Kirkpatrick and Magalie Guilhabert.....	39
Sequence of the Genome of <i>Xylella fastidiosa</i> Causing Pierce's Disease of California Edwin Civerolo.....	41
Biological, Cultural, Genetic, and Chemical Control of Pierce's Disease: Xylem Fluid Chemistry Mediation of Resistance to Pierce's Disease Peter Andersen.....	43
Genome-Wide Identification of Rapidly Evolving Genes in <i>Xylella fastidiosa</i>: Key Elements in the Systematic Identification of Host Strains, and in the Search for Plant-Host Pathogenicity Candidate Genes Leonard Nunney, Richard Stouthamer, and Robert Luck.....	45
Fate of <i>Xylella fastidiosa</i> in Alternate Hosts Alexander Purcell	47
Trap Crops for Reducing Spread of Pierce's Disease Ed Weber	49
Biological, Cultural, Genetic, and Chemical Control of Pierce's Disease: Significance of Riparian Plants in the Epidemiology of Pierce's Disease Kendra Baumgartner.....	51
Breeding Pierce's Disease Resistant Table and Raisin Grapes Andrew Walker, Alan Tenschler, and David Ramming.....	53
Epidemiology of Pierce's Disease	
Characterization and Studies on the Fundamental Mechanisms of <i>Xylella fastidiosa</i> Transmission to Grapevines by the Glassy-winged Sharpshooter Alexander Purcell	57
Mechanisms of Pierce's Disease Transmission in Grapevines: An Analysis of the Movement of <i>Xylella fastidiosa</i> in Xylem Pathways Thomas Rost, Mark Matthews, and Joshua Stevenson	59
The Development of Pierce's Disease in Xylem: The Roles of Vessel Cavitation, Cell Wall Metabolism, and Vessel Occlusion John Labavitch, Mark Matthews, and L. Carl Greve.....	61
Epidemiology of Pierce's Disease in Southern California: Identifying Inoculum Sources and Transmission Pathways Donald Cooksey and Heather Costa	65
Sharpshooter Feeding Behavior in Relation to Transmission of Pierce's Disease Bacterium Elaine Backus	67
Epidemiology of Pierce's Disease in the Coachella Valley Thomas Perring, Carmen Gispert.....	70
Role of Type 1 Secretion in Pierce's Disease Dean Gabriel.....	72

The Epidemiology of Pierce's Disease Barry Hill and Jennifer Hashim	74
 Biological Control of Glassy-winged Sharpshooter	
Progress on the Development of a Monoclonal Antibody Specific to Glassy-winged Sharpshooter Egg Protein: A Tool for Predator Gut Analysis and Early Detection of Pest Infestation James Hagler, Kent Daane, and Heather Costa	79
Biological Control of the Glassy-winged Sharpshooter in Kern County, California Isabelle Lauziere, Matthew Ciomperlik, and Lloyd Wendel	81
Development of an Artificial Diet for the Glassy-winged Sharpshooter Allen Cohen.....	83
Interspecific Competition Between <i>Gonatocerus ashmeadi</i> and <i>G. triguttatus</i> for Glassy-winged Sharpshooter Egg Masses Mark Hoddle.....	86
Biological Control of <i>Homalodisca coagulata</i> Walker Jones	88
Host Selection and Low Temperature Storage of the Glassy-winged Sharpshooter, <i>Homalodisca coagulata</i> Roger Leopold and George Yocum.....	90
Is the Glassy-winged Sharpshooter Parasitoid <i>Gonatocerus ashmeadi</i> (Hymenoptera: Mymaridae) One Species or a Complex of Morphologically Indistinguishable Sibling Species? Mark Hoddle and Richard Stouthamer	93
Search for and Collect Egg Parasitoids of Glassy-winged Sharpshooter in Southeastern USA and Northeastern Mexico Serguei Triapitsyn and Mark Hoddle.....	94
 Biology, Ecology and Direct Impact of Glassy-winged Sharpshooter	
Biology and Ecology of Glassy-winged Sharpshooter in the San Joaquin Valley Kent Daane and Marshall Johnson	99
Glassy-winged Sharpshooter Impact on Orange Yield, Fruit Size, and Quality Raymond Hix.....	103
Seasonal Changes in the Glassy-winged Sharpshooter's Age Structure, Abundance, Host Plant Use, and Dispersal Robert Luck and Richard Redak.....	105
Spatial and Temporal Relations Between Glassy-winged Sharpshooter Survival and Movement, Xylem Flux Patterns, and Xylem Chemistry in Different Host Plants Robert Luck and Mark Hoddle	107
Keys to Management of Glassy-winged Sharpshooter: Interactions Between Host Plants, Malnutrition, and Natural Enemies Russell Mizell and Peter Andersen	109
Mating Behavior of the Glassy-winged Sharpshooter, <i>Homolodisca coagulata</i> Randy Hunt.....	111
Reproductive Biology and Physiology of Glassy-winged Sharpshooter Frank Zalom and Christine Peng	113
Ultrastructural Contributions to the Study of the Glassy-winged Sharpshooter and Pierce's Disease Thomas Freeman	116

Relationship Between Total Population Counts of Glassy-winged Sharpshooter and Numbers Obtained from Various Sampling Methods	
Matthew Blua, Rick Redak, Carlos Coveilla, and David Akey	118
Developing a Method to Detect <i>Xylella fastidiosa</i> in Glassy-winged Sharpshooter	
Matthew Blua, Blake Bextine, and Rick Redak.....	120
Developing a Stable Classification of the Glassy-winged Sharpshooter Genus <i>Homalodisca</i>	
Stuart McKamey	122
Host Plant Resistance to the Glassy-winged Sharpshooter in Grapes	
Jiang Lu	124
Sampling, Seasonal Abundance, and Comparative Dispersal of Glassy-winged Sharpshooters in Citrus and Grapes: Dispersal Progress Report	
Jacquelyn Blackmer, James Hagler, and Gregory Simmons.....	126
Use of Pesticides and Alternative Treatments to Control Glassy-winged Sharpshooter and Pierce's Disease	
Impact of Sub-Lethal Doses of Neonicotinoids on Glassy-winged Sharpshooter Feeding and Transmission of Pierce's Disease	
Matthew Blua and Gregory Walker	131
Control of Immature and Adult Glassy-winged Sharpshooters: Evaluation of Biorational and Conventional Insecticides	
David Akey, Matthew Blua, and Thomas Henneberry	133
Alternatives to Conventional Chemical Insecticides for Control of Glassy-winged Sharpshooter	
Gary Puterka	136
Chemical Control of Glassy-winged Sharpshooter: Establishment of Baseline Toxicity and Development of Monitoring Techniques for Detection of Early Resistance to Insecticides	
Nick Toscano, Nilima Prabhaker, Frank Byrne, and Steven Castle.....	139
Laboratory and Field Evaluations of Imidacloprid and Thiamethoxam Against Glassy-winged Sharpshooter on Citrus and Grapes	
Nick Toscano and Steven Castle.....	141
Efficacy of Insecticides Used for Glassy-winged Sharpshooter Control in Citrus Nursery Stock	
Elizabeth Grafton-Cardwell	143
Rootstock Variety Influence on Pierce's Disease Symptoms in Grafted Chardonnay (<i>Vitis vinifera</i> L.) Grapevines	
Peter Cousins and Jiang Lu.....	145
Monitoring and Database Management	
Sampling, Seasonal Abundance, and Comparative Dispersal of Glassy-winged Sharpshooters in Citrus and Grapes: Sampling Progress Report	
Steve Castle, Steve Naranjo, and Nick Toscano	149
Development of Trapping Systems to Trap Glassy-winged Sharpshooter (<i>Homalodisca coagulata</i>) Adults and Nymphs in Grape	
Raymond Hix.....	151
Spatial Distribution of Glassy-winged Sharpshooters in a Diverse Agricultural System, and Correlation Between Direct Observations and Sticky Trap Data	
David Bartels, Lloyd Wendel, and Matthew Ciomperlik.....	153

Plant-GWSS Interactions: Physiological and Biochemical Mechanisms Involved in Host Plant Selection with Particular Reference to Lemon and Orange Trees Nick Toscano, Jian Bi, Frank Byrne, and Steven Castle	155
Area-Wide Management of the Glassy-winged Sharpshooter in the Temecula Valley Raymond Hix, Nick Toscano, Rick Redak, and Matthew Blua.....	157
Impact of Layering Control Tactics on the Spread of Pierce’s Disease by the Glassy-winged Sharpshooter Rick Redak and Matthew Blua	159
Mycopathogens and Their Exotoxins Infecting Glassy-winged Sharpshooter: Survey, Evaluation, and Storage Russell Mizell and Drion Boucias	161
Exploration for Facultative Endosymbionts of Sharpshooters Alexander Purcell	163
The Area-Wide Pest Management of Glassy-winged Sharpshooter in Kern County Lloyd Wendel et al.	165
Monitoring and Control Measures for Pierce’s Disease in Kern County Jennifer Hashim.....	166
Additional Reports	
Detection of DNA Polymorphisms In Glassy-winged Sharpshooters (<i>Homalodisca coagulata</i>) By PCR-Based DNA Fingerprinting Methods Jesús de León and Walker Jones	171
Poster Abstracts	
AC and DC EPG Waveforms of Glassy-winged Sharpshooters on Chrysanthemum and Grape Fengming Yan, Javad Habibi, and Elaine A. Backus	175
Use of a Triparental Mating System to Introduce Green Fluorescent Protein Marked Transposon Insertions in the Plant Pathogen <i>Xylella fastidiosa</i> Xiaoting Qin, Wenbin Li, and John Hartung.....	175
Citrus and Coffee Strains of <i>Xylella fastidiosa</i> Induce Pierce’s Disease in Grapevine W.-B Li, C.-H Zhou, W. D. Pria, Jr., D. C. Teixeira, V. S. Miranda, E. O. Pereira, A. J. Ayres, C.-X He, P. I. Costa, and John Hartung.....	176
Ultrastructure of the Bacteriome-Associated Endosymbionts of the Glassy-winged Sharpshooter, <i>Homalodisca coagulata</i> (Homoptera: Cicadellidae) Rosemarie Rosell and Heather Costa.....	176
Epidemiology of <i>Xylella fastidiosa</i> Diseases in California K.M. Tubajika, E.L. Civerolo, G.J. Puterka, D. Bartels, J.M. Hashim, D. Luvisi, and G. Wittenborn	177



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Dear Attendees:

Welcome to the 2002 Pierce's Disease Research Symposium!

Following on the success of last year's event, CDFA is presenting this Symposium to give all interested parties an opportunity to hear firsthand from researchers who are continuing the concerted effort to solve the problems posed by Pierce's disease and the glassy-winged sharpshooter. I am gratified that so much collective ability and talent has been focused on this problem, and anticipate significant progress will continue to be made towards our goal.

Plant diseases caused by the bacterium *Xylella fastidiosa* have been characterized as some of the most significant new disease threats in the Americas. What we learn now from our studies will have broad applications to protect not just grapes but also many other crop, landscape, and native plants which are vulnerable to *Xylella*-related diseases.

I thank you for your interest in this important issue, and hope you will continue your participation in the community of researchers, stakeholders, plant protection professionals, and others who are working together to protect our precious plant resources.

Sincerely,

William (Bill) J. Lyons, Jr.



*Breeding Cultivars
of Grape
Resistant to
Pierce's Disease*



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

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Reporting Period: The results reported below derive from work conducted from November 1, 2001 to October 31, 2002.

INTRODUCTION

The bacterium, *Xylella fastidiosa* (*Xf*), is accepted as the causative agent of Pierce's disease of grape. In our survey of plants for their reaction to *Xf* cell suspensions pressure infiltrated into leaves, we found that *Chenopodium quinoa* (*Cq*) developed a chlorosis in 24-48hr that conformed to the area infiltrated with suspensions of 10^6 to 10^8 *Xf* cells/mL. Comparisons of infiltrated opposite leaf halves for the intensity of the developed chlorosis provided a useful semi-quantitative assessment of the relative potency of *Xf*-derived preparations. The chlorosis-inducing activity was associated with *Xf* cells, not washings of cells, and heating *Xf* cells at 100°C for 6 min slightly enhanced the activity. We observed that the chlorosis-inducing activity survived treatment with sodium dodecyl sulfate (SDS). Although the indicated stabilities do not suggest a protein as the active agent, the chlorosis-inducing activity was sensitive to each of three proteases and was lost after treatment with chloroform or acetic acid. These results suggested that *Xf* possesses a protein elicitor that is recognized in the intercellular spaces of *Cq* plants even when the protein is in a denatured state. Presumably metabolic events of *Cq*, subsequent to recognition, result in chlorosis.

Chenopodium ambrosioides (*Ca*) is known to be a natural host of *Xf* and a source of *Xf* inoculum that can be transmitted to grape under experimental conditions (Freitag 1951). We were able to infect *Ca* with *Xf* after inoculation by petiole injection. However, infiltrated leaves of *Ca* failed to develop chlorosis or other reaction. In contrast, *Cq*, which developed chlorosis after infiltration, did not become detectably infected after inoculation with *Xf*. In several systems, a pathogen protein that acts as an elicitor in one species may act as a virulence factor in a closely or distantly related line or species (De Wit, Joosten et al. 1994; van't Slot and Knogge 2002). Therefore, the *Xf* elicitor of *Cq* chlorosis may be a virulence factor in other, susceptible plant species, e.g., *Ca* and *Vitis vinifera*.

OBJECTIVES

1. Identify gene product(s) and gene(s) of *Xf* that contribute to its virulence.
2. Exploit knowledge of *Xf* virulence factor(s) in strategies for control of Pierce's disease.

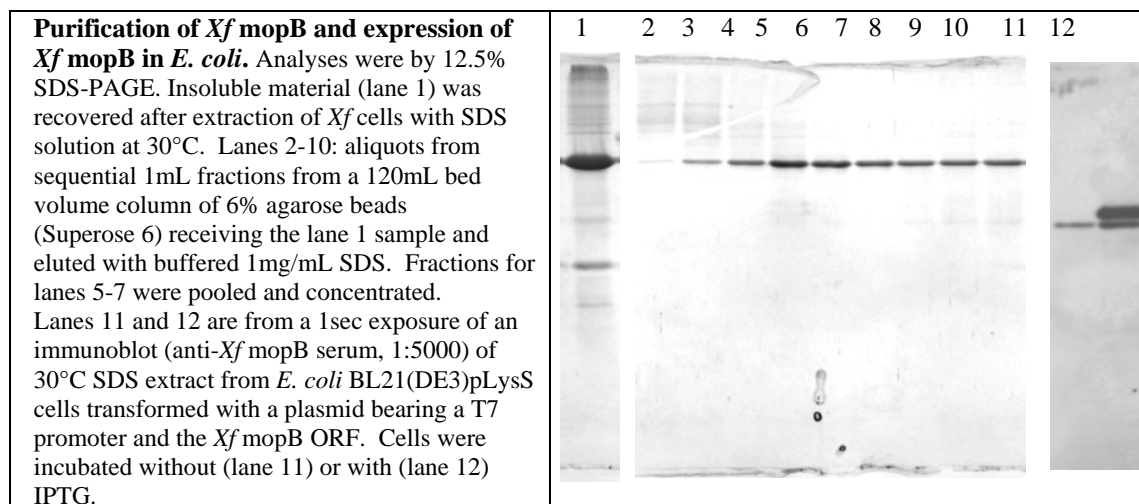
RESULTS AND CONCLUSIONS

A precipitate was collected by high speed centrifugation after incubating washed and suspended *Xf* cells for 30 min at 30°C in Tris-buffered sodium dodecyl sulfate (SDS) solution at pH approx. 8.6. Compared to intact *Xf* cells, the precipitate after SDS extraction presented a greatly simplified pattern of proteins after SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Extracts of gel regions were assayed by infiltration into *Cq* leaves. The bulk of the chlorosis-inducing activity was associated with material with mobility corresponding to molecular weight of about 40K. A prominent band (Coomassie blue stained) from the region was excised and subjected to digestion with trypsin and analysis by mass spectrometry (Young Moo Lee, UC Davis Molecular Structure Facility). Peptides were identified that correspond to about 40% of the *Xf* outer membrane protein mopB. The pyroglutaminyl-terminated peptide pyro-QEFDDR mapped to the mopB gene sequence (Simpson, Reinach et al. 2000) to predict a mopB protein of molecular weight 38.5K. Results from other experiments suggest that the pyroglutaminyl residue is the natural end of mature *Xf* mopB protein and is not created as an artifact of our analysis by cyclization of an amino-terminal glutamine residue. Edman degradation gave in very low yield the sequence MKKKILT..., consistent with a mopB protein of molecular weight 40.7K as a minor component. The 22 amino acid residue sequence at the amino end of the minor 40.7K protein, and not present in the abundant 38.5K protein, has the characteristics of a signal peptide. We conclude from the above results that the translation product of the *Xf* mopB gene is the 40.7K protein and that release of its 22 residue signal peptide results in insertion of the 38.5K mopB protein so tightly into the *Xf* outer membrane that it remains insoluble during a SDS treatment that releases most other *Xf* proteins.

translated mop B: MKKKILTAALLGGIAIIQVASAQEFDDRWYLAGSTG... 40.7K
mature mop B: pyro-QEFDDRWYLAGSTG... 38.5K

The *Xf mopB* amino acid sequence differs from the citrus strain *Xf mopB* at only seven sites. The next most similar proteins in databases form a group of more than 15 “ompA” proteins of Gram-negative bacteria. The ompA proteins show close to 30% similarity to *Xf mopB*, confined mostly to the carboxyl terminal region. The *Pseudomonas fluorescens* ompA protein OprF and *Xf mopB*, unlike most other ompA proteins, have a proline-rich region preceding the carboxyl end region of similarity. *P. fluorescens* competes against certain root-pathogenic fungi because of its ability to colonize root surfaces. De Mot and Vanderleyden (1991) purified OprF, a major outer membrane protein, and demonstrated that OprF binds tightly to roots and probably is responsible for some aspect of the root-adhesion capabilities of *P. fluorescens*, a supposition also consistent with mutational studies (Deflaun, Marshall et al. 1994). Therefore, we postulate that mopB may contribute to *Xf* virulence by adhering to xylem element interior surfaces.

The insoluble fraction obtained after 30°C SDS extraction of *Xf* cells was solubilized in hot SDS and chromatographed on 6% agarose beads. As indicated in the figure, fractions (lanes 2-10) were analyzed by SDS-PAGE to identify those showing greatest purity of *Xf mopB*. Pooled fractions were concentrated for production of rabbit anti-mopB. Attempts at cloning *Xf mopB* in *E. coli*, using constructions that encompassed the entire *Xf mopB* gene, including its putative promoter, were not successful. Therefore, the *Xf mopB* open reading frame (ORF) was placed under control of a bacteriophage T7 RNA polymerase promoter in *E. coli* strain BL21(DE3)pLysS, which bears a *Lac* promoter-driven T7 RNA polymerase gene, as well as a T7 lysozyme gene to prevent accumulation of active T7 RNA polymerase prior to induction of the *Lac* promoter. Induction of cultures with IPTG resulted in appearance of a new band with *Xf mopB* mobility as detected by immunoblotting (lanes 11,12; work of Paul Feldstein), but not to levels readily detected by staining with Coomassie blue. That is, *Xf mopB* accumulation may sicken *E. coli*. A band (lane 11), from uninduced culture and reacting with anti-*Xf mopB* antibody, may be due to cross reaction with the *E. coli* outer membrane protein ompA, which is slightly smaller than *Xf mopB*.



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

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Reporting Period: The results reported below derive from work conducted from December 2001 to November 2002.

INTRODUCTION

Pierce's disease (PD), caused by the bacterial pathogen *Xylella fastidiosa* (Xf), is one of the most destructive diseases of grapevines (Purcell and Hopkins, 1996). All genotypes of *Vitis vinifera* are susceptible to the PD pathogen and only certain non-vinifera species (e.g., *V. shuttleworthii* and *Muscadinia rotundifolia*), typically not suitable for wine production, are able to resist or tolerate this pathogen. Development of resistant varieties through classical breeding is complicated by the desire to retain varietal phenotypes in cultivated species, and by the generally poor agronomic properties (e.g., fruit quality) of these non-vinifera species. An alternative approach for developing disease resistant germplasm is to characterize the molecular basis of resistance and susceptibility in *Vitis* species, and to use this information to design rational strategies for crop protection. In this project we are pursuing a genomics approach to identify transcriptional pathways that are correlated with susceptible or resistant interactions in *Vitis* and *Muscadinia* species. The comparison of these two distinct interactions should reveal functional elements of the host resistance response, or conversely host functions that confer susceptibility.

The experimental strategies outlined below use genomics technology (e.g., cDNA sequencing to create Expressed Sequence Tags [ESTs] and transcriptional profiling using micro arrays) 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 *M. rotundifolia*). Such information will considerably increase our knowledge of the grape-*Xylella* interaction and potentially provide the basis for developing resistance to the PD pathogen in *V. vinifera*. A side benefit of these activities will be derivative information, such as a public database of grape ESTs, information for molecular marker development (e.g., SSR and SNP information), and anticipated public access to a grape oligonucleotide microarray.

OBJECTIVES

1. cDNA libraries will be produced from infected and non-infected grape genotypes. Library construction will focus on susceptible *V. vinifera* and related species (e.g., a *Vitis rupestris* x *Muscadinia rotundifolia* cross) that are tolerant/resistant to *Xylella* infection.
2. Sequencing reactions will be completed for a total of 60,000 cDNA clones obtained from the above libraries (30,000 from *V. vinifera* and 30,000 from the *V. rupestris* x *M. rotundifolia* cross). The resulting sequence information (i.e., Expressed Sequence Tags (ESTs)) will be submitted to the National Center for Biotechnology Information (NCBI).
3. An analysis pipeline and web-accessible database will be developed for the grape transcriptome. The initial focus of the database will be on the minimum gene set expressed during the grape-*Xylella* interactions.
4. Transcriptional profiling will be conducted to characterize host gene expression in susceptible and resistant/tolerant grape-*Xylella* interactions.

RESULTS AND CONCLUSIONS

We are taking an EST sequencing and transcriptional profiling approach to develop a detailed picture of the molecular events that underlie host susceptibility and host resistance to the pathogen *Xylella fastidiosa* in *Vitis* species. Currently we have constructed eight cDNA leaf libraries from infected and non-infected plants of *Vitis vinifera* at host developmental stages corresponding to key steps in disease development and in excess of 100,000 clones have been picked and archived for further analysis. DNA sequencing reactions are being completed and analyzed for a total of 30,000 cDNA products from these pathogen-related libraries. A similar strategy is being implemented to sequence and characterize an additional 30,000 cDNA sequences from related species of grapes that are resistant to *Xylella* infection. In collaboration with Dr. Andrew Walker, we will characterize PD-resistant progeny from a cross between *V. rupestris* and *M. rotundifolia*. In association with the National Center for Genome Resources (NCGR) we are implementing an online relational database (the X-Genome Initiative, XGI) as a means to organize and annotate the EST information resulting from the projects. As a temporary measure, we have established an in-house data analysis pipeline, consisting of EST contig assembly by means of the

PHRED/PHRAP algorithm, and BLASTN analysis against the entire *Arabidopsis* coding sequence and all publicly available sequences from *V. vinifera*. BLAST reports are stored on line and provide a simple homology-based analysis of the grape EST dataset. Subsequent to cDNA sequencing and electronic data mining we will employ a functional genomics strategy to monitor host gene expression during grape development using oligonucleotide microarrays. This work will be done in collaboration with the Department of Biochemistry, University of Nevada, Reno (as part of an NSF Plant Genome grant). The two projects are coordinating an international effort to develop a 70-mer oligonucleotide microarray. For purposes of this project, the array will provide a means to analyze the expression of thousands of grape genes during the grape-*Xylella* interaction. We anticipate that the strategies outlined above will define the transcriptional response of susceptible and resistant *Vitis* and *Muscadinia* species to infection by *Xylella fastidiosa*. This information will significantly advance our knowledge of grape-*Xylella* interactions, and it may reveal transcriptional pathways that are causal to host susceptibility or resistance/tolerance.

FUNDING AGENCIES

Funding for this project was provided by the USDA Animal and Plant Health Inspection Service, and the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.

THE GENETICS OF RESISTANCE TO PIERCE'S DISEASE

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Reporting Period: The results reported here are from work conducted from December 31, 2001 to November 1, 2002.

INTRODUCTION

This project was part of the American Vineyard Foundation Long Term Project on Pierce's Disease. Our component of this project focuses on understanding the genetics of resistance to *Xylella fastidiosa* (*Xf*), the causal agent of Pierce's disease (PD). The studies include understanding the inheritance of resistance to *Xf* in *Vitis rupestris* x *Muscadinia rotundifolia* and developing genetic markers for *Xf* resistance. It integrates into two other projects – a fine-scale mapping project for *Xf* resistance, and the breeding of PD resistant table and raisin grapes.

These *Xf* resistance studies are being carried out on potted and replicated plants under greenhouse conditions using needle inoculation with the 'Stag's Leap' strain of *Xf*. The plants are grown for 12 to 16 weeks and then evaluated for the presence of *Xf* and irregular shoot lignification symptoms. ELISA is used to test stem samples from 10 cm above and 10 below and at the point of inoculation. This system has proved to be highly reliable, efficient and quantifiable.

OBJECTIVES

1. Complete analysis of a series of crosses (Design II mating scheme) allowing the quantitative inheritance of *Xf* 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 *Xf* resistance and eventual identification of *Xf* resistance genes and their genetic engineering into *vinifera* cultivars.
3. Develop and utilize genetic markers to assist and accelerate the introgression of *Xf* resistance into table, raisin and wine grapes.

RESULTS AND CONCLUSIONS

Inheritance studies of Xf resistance in a M. rotundifolia background:

Last year, Alan Krivanek (PhD student) completed a broad series of crosses within a Design II mating scheme among siblings from the 8909 (*V. rupestris* x *M. rotundifolia*) population. From among those families he is currently screening a total of 2,100 plants for *Xf* resistance under a randomized complete block design using our greenhouse evaluation system. The crosses include 9 Resistant x Resistant families, 3 Susceptible x R families, 3 R x S families and 1 S x S family. From these crosses 3-4 cuttings from 20-38 seedlings from each of the 16 families have been propagated potted and inoculated twice with the 'Stags Leap' *Xf* isolate. Parents of the 4x4 Design II were chosen as follows: Females – 8909-02 R, 8909-07 S, 8909-15 R, 8909-16 R?; Males – 8909-01 R, 8909-08 R, 8909-19 S, 8909-26 R.

Genetic mapping of a V. rupestris x M. rotundifolia population (9621 – 8909-15 x 8909-17):

A genetic map of *V. rupestris* x *M. rotundifolia* has been completed and submitted for publication (M. Doucleff, Y. Jin, F. Gao and M.A. Walker. A Genetic Linkage Map of *Vitis rupestris* x *Muscadinia rotundifolia*. Submitted to Theoretical and Applied Genetics). This map is now being expanded and fine scale mapping undertaken to better localize *Xf* resistance genes and markers linked to them (Please see the report on this project by Walker and Riaz within these Proceedings).

Development of genetic markers for Xf resistance:

This study is being carried out in the 9621 mapping population (8909-15 x 8909-17, both *Xf* resistant). To date, about 70 primer combinations have been evaluated out of a goal of 150 to 200 primer combinations. It is expected that 2-4 markers flanking the *Xf* resistance gene within a 2-cM window will be identified. Several candidate markers linked to resistance in the male parent and the female parent have been identified. Candidate markers will be confirmed by separately evaluating marker patterns on each individual within the bulk. SCAR primers will be developed from the tightly flanking markers and run on the 145 genotypes previously screened for resistance. A total of 145 genotypes with marker data should yield a

mapping resolution of approximately 2cM. This resolution will be used to confirm the order and distance of the SCAR markers around the resistance locus on its linkage group.

Susceptible bulk: 9621-025, 9621-034, 9621-039, 9621-045, 9621-062, 9621-094, 9621-116, 9621-118, 9621-167, 9621-219, and 9621-277.

Bulked segregant analysis (BSA) was also carried out on a population composed of four different populations. The male in all four was 8909-08 (*V. rupestris* x *M. rotundifolia*). The females were advanced seedless *V. vinifera* table grape selections from D. Ramming: B90-116 (population 501), C63-83 (502), C33-30 (503), and P79-101 (504). A total of 120 plants were tested for *Xf* resistance under our greenhouse screen and evaluated with ELISA. From these, 14 were used in a resistant bulk and 16 were used in a susceptible bulk. These bulks were screened with 114 different AFLP primer combinations. Out of these 114, 11 were primer combinations that had already been mapped (see above) in a related cross (8909-15 x 8909-17). These primer combinations were from Group 15 of this map, as is *Xf* resistance. None of these 11 markers were close enough to show up in the BSA analysis.

Results from eight of the 114 markers tested suggest that they are candidate markers. These markers linked with *Xf* resistance in the bulk analysis, and need to be further tested on the individual genotypes. Most of these candidate markers were faint bands, indicating that they may not be present in all of the resistant genotypes, and therefore recombination events prevented tighter linkages.

Genetic markers were also sought in the 0023 population (8909-15 R x B90-116 S (advanced seedless selection from D. Ramming)). One hundred and eight seedlings were inoculated under our greenhouse system in a randomized block design. Plants were inoculated twice. After 16 weeks the plants were evaluated for *Xf* resistance based on cane lignification ELISA. Fifty-four of the 108 seedlings had mean bacteria numbers of less than 500,000 cfu/ml which in other populations is the cut off point for resistance. The genotypes: 0023-19, 0023-54, 0023-63, 0023-98 had stem bacteria numbers of less than 60,000 cfu/ml and were crossed to advanced table grape selections in order to establish a large breeding population. Approximately 1,800 seeds have been collected. Markers linked to resistance in the mapping and BSA portions of this project will be used on this population. Correlation of the markers with *Xf* resistance will be confirmed and calculated if different from the original mapping population.

FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board, and the USDA Animal and Plant Health Inspection Service.

AN EXPANDED GENETIC MAP OF *VITIS RUPESTRIS* X *MUSCADINIA ROTUNDIFOLIA* FOR FINE SCALE MAPPING AND CHARACTERIZATION OF PIERCE'S DISEASE RESISTANCE

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Reporting Period: The results reported here are from work conducted from June 1, 2002 to November 1, 2002.

INTRODUCTION

This project is funded by the UC Pierce's Disease Grant Program. It expands a genetic mapping effort originally funded by the California Grape Rootstock Improvement Commission, the Fruit tree, Nut tree and Grapevine Improvement Advisory Board, the California Table Grape Commission and the AVF. That study examined the genetics of resistance to the dagger nematode and found that this resistance segregated within several *V. rupestris* x *M. rotundifolia* F1 populations. A resistant and a susceptible sibling were selected from one of these populations, 8909, and they were crossed to produce the 9621 "F2" population (8909-15 x 8909-17). The 8909 and 9621 populations also segregate for resistance to *Xylella fastidiosa* (Xf). A genetic map of 116 individuals from the 9621 population was created primarily with AFLP markers. The work proposed here will increase the mapping population to 188 individuals and add at least 100 SSR markers. The addition of SSR markers will help to link the *V. rupestris* x *M. rotundifolia* map to other mapping efforts around the world through their universally comparable nature. This is a new project with funding finalized in September 2002.

OBJECTIVES

1. Expand an existing genetic map created within *V. rupestris* x *M. rotundifolia* focused on resistance to Xf by adding 100 more individuals and 100 SSR and 20 EST markers.

Completion of this objective will allow further identification of DNA markers that are tightly linked to Xf resistance so that marker-assisted selection strategies can be employed in the breeding program. It will also more fully support efforts to locate and identify the gene(s) responsible for Xf resistance.

RESULTS AND CONCLUSIONS

A genetic map of the 9621 population was completed and submitted for publication (M. Doucleff, Y. Jin, F. Gao and M.A. Walker. A Genetic Linkage Map of *Vitis rupestris* x *Muscadinia rotundifolia*. Submitted to Theoretical and Applied Genetics). This map was initiated several years ago, was based on the pseudo-testcross strategy, and used primarily AFLP markers. Over the past two years we have used 15 new AFLP primers, 7 new ISSR primers, and 9 new SSR primers to score over 200 additional molecular markers for 116 F2 individuals in the 9621 population. Ambiguous genotypes were rerun or left as unknown. After scoring and rechecking each marker, approximately 10% of the markers were discarded because they were not consistently scored. Chi-square tests found that about 20% of the markers had significantly distorted ($P < 0.05$) genotype ratios. The remaining markers with $P > 0.05$ (100 AFLP, 32 ISSR, and 18 SSR) combined with the existing 275 AFLP and 25 RAPD markers were used to create a framework map for each F1 parent using MapMaker UNIX 3.0 and PGRI.

A total of 474 polymorphic markers were scored with 298 segregating 1:1 and 176 segregating 3:1. Approximately 7.5% of the bands displayed skewed segregation ratios (Table 1). Of the 298 1:1 markers, 158 were heterozygous in the female (8909-15) and 140 were heterozygous in the male (8909-17). Overall linkages were robust with $p \leq 0.3$ and $X^2 \geq 0.001$ (equivalent to LOD score ≥ 3). At $X^2 = 0.00001$ and $p \leq 0.25$, 16 linkage groups were formed for 8909-15 and 20 linkage groups for 8909-17 (Table 2). A framework map for each parent was constructed based on a 90% confidence level for correct order using a PGRI bootstrapping algorithm. Markers not ordered with a confidence level $\geq 90\%$ were added to the framework maps as accessory markers. Together the two framework maps covered 1630 cM. This map was based on 116 individuals with 375 AFLP, 32 ISSR, 25 RAPD and 18 SSR markers. Two measures of Xf resistance (ELISA values indicating limited Xf movement beyond the point of inoculation; and the uneven lignification) both map to the same general area on the same Linkage Group.

Table 1. Data on mapping markers within the 8909-15 x 8909-17 mapping population.

Marker Information	1:1 8909-15 Female Markers	1:1 8909-17 Male Markers	3:1 Markers
Total markers scored	158	140	176
Percent distorted (χ^2 , df=1, p=0.05)	10.8	3.6	7.4
AFLP markers	125	111	160
ISSR, RAPD markers	25	21	14
Microsatellite markers	8	8	2
Framework markers	90	101	NA
Accessory markers	51	30	NA
Missing data %	3.8	5.4	4.0

Table 2. Data on linkage groups on the genetic maps

Framework Map Linkage Group Information	8909-15 Female Parent	8909-17 Male Parent
Total number of groups	16	20
Total size (cM)	730	900
Avg. group size (cM)	45.6	45.6
Avg. distance between markers (cM)	11.0	8.8
Avg. PCO	91.6 \pm 4.7	95.5 \pm 4.0

Before efficient efforts to locate *Xf* resistance genes can be undertaken, more individuals and markers are needed on the map. We are now in the process of adding this data. Thus far, DNA has been extracted from 188 individuals and we have produced the some marker data (Table 3).

Table 3. Data on number of markers tested and useful for the 8909-15 x 8909-17 mapping population.

Markers	Tested	Amplified	Useful for Map
SSR	111	92	65
EST (D. Adams)	20		14
Total	131		79

We are continuing to add SSR markers and are preparing to retest the entire population for AFLP markers.

FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board, and the University of California Pierce's Disease Grant Program.

APPLICATION OF *AGROBACTERIUM RHIZOGENES*-MEDIATED TRANSFORMATION STRATEGIES FOR A RAPID HIGH THROUGHPUT SCREEN FOR GENETIC RESISTANCE TO PIERCE'S DISEASE IN GRAPE THAT MAINTAINS THE CLONAL INTEGRITY OF THE RECIPIENT HOST

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Reporting Period: The results reported here are from work conducted from April 2002 through November 2002.

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, spread or symptom development of the xylem-limited bacteria, *Xylella fastidiosa*. There is no useful genetic resistance in commercially preferred 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 *Agrobacterium rhizogenes* mediated transformation strategy. This system enables the direct introgression of cloned resistance genes into a susceptible host plant while maintaining the clonal integrity of the recipient plant following transformation. In working with symptomatic grape leaf tissue for isolation of RNA for development of cDNA libraries, we examined the pattern and form of symptom development cytologically. The cytological pattern of symptom development suggested a similarity to tissue death in other plant systems that we have been studying for several years. As a consequence of our preliminary cytological studies we concluded that the death that was occurring in the pre-symptomatic and symptomatic areas of leaves on infected plants borne changes that we associate with the activation of a programmed cell death process that exhibits the morphological hallmarks of apoptosis, a widely studied gene mediated fundamental process of development and disease in animals and in plants. We have therefore included as a second objective an examination of the molecular basis of cell death in pre-symptomatic and symptomatic tissues along with the immediate assessment of the effect of expressing anti-apoptotic transgenes in Pierce's disease (PD) infected tissues on the development of death related symptoms in grape. The research plan includes a rapid functional screen for genes that confer resistance to PD in transformed grape tissue. 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.

OBJECTIVES

1. Transformation of grape with *Agrobacterium rhizogenes* for cDNA library screening.
2. Construction of a series of cDNA libraries from healthy and infected grape tissues exhibiting foliar symptoms.
3. Examine the morphological and cytological features of cell death in symptomatic leaves.
4. Investigate the potential of blocking PD symptom expression with anti-apoptotic transgenes.

RESULTS AND CONCLUSIONS

Transformation of healthy grape with A. rhizogenes:

The method of delivery of the cDNA libraries into grape is now established in our laboratory. We have confirmed that grape is readily transformed by *A. rhizogenes* and that foreign genes (e.g. GFP) and our new cDNA libraries, can be expressed readily in grape by this method. The proof of concept 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 the GFP gene 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 10 days but then they stopped growing and eventually died with the death beginning at the root tip. We have now repeated this result numerous times and conclude that 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 death, due either to signals from the bacteria or plant-expressed signals triggered by the presence of the bacteria in the vascular system of the root.

The pattern of death in the root tips is identical to the pattern we have observed and have published on in several host-pathogen systems that is characteristic of pathogen and toxin-induced death. This observation, which we believe is highly significant, suggests that the mechanism of cell death in PD is a form of programmed cell death with morphological features of apoptosis. We also have found in several other systems that we can simultaneously block this programmed cell death and disease using both anti-apoptotic transgenes and cell permeable chemicals (Richael et al. 2001). These results are described in two recent reports. The first report deals with anti-apoptotic chemicals (Richael et al. 2001), and the second report of this approach using an anti-apoptotic transgene to engineered broad spectrum disease resistance was published recently in the Proceeding of the National Academy of Sciences (Lincoln et al. 2002).

Construction of cDNA libraries:

The construction of a grape cDNA library proved much more difficult than originally assumed. We have to date constructed approximately 150,000 independent grape cDNAs cloned into a plant transformation binary vector, CB404, which is a derivative of pBIN19 and uses the CaMV 35S promoter for high level, constitutive expression. We will proceed to generate the 500,000 independent grape cDNAs needed for our complete screen. We have begun to move the cDNA library into *Agrobacterium rhizogenes* to transform infected grape explants for the purpose of finding cDNAs that will block the death of infected tissues. We intend to screen small batches of the library at first in order to ensure that the entire procedure is as efficient as possible. The first library constructed is from both healthy and infected *Vitis vinifera* (Chardonnay) grape leaves. Libraries are being made from *Muscadinia rotundifolia* (Cowart) and *V. shuttleworthii* (Hanes City) as indicated in the original proposal. These resistant source plant materials are being used in Dr. Walker's research and the libraries will be available to his group.

Transformation of the baculovirus anti-apoptotic gene p35 gene into infected grape:

In order to test the hypothesis that programmed cell death (PCD) mechanisms are responsible for the death that occurs in roots from *Xylella* infected grape stems, we directly transformed the baculovirus p35 gene into infected grape tissue explants in a manner similar to that reported by Lincoln et al (2002). Expression of p35 transgene in PD infected tissue explants blocked the *Xylella* induced root death, which indicates that signals directly from the bacterium or from the plant but induced by the presence of the bacterium triggered the root death which can be blocked by anti-apoptotic transgenes. Based on previous screens of cDNA libraries of tomato for endogenous anti-apoptotic genes we have 15 potential genes from tomato to test immediately in the *A. rhizogenes* transformed grape systems. Homologues of the tomato genes are currently being cloned from grape so that we will have the authentic grape genes to use also in the very near future.

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FUNDING AGENCIES

Funding for this project was provided by the USDA Animal and Plant Health Inspection Service.



*Biological
Control
of
Pierce's Disease*



CHARACTERIZATION OF FIMBRIAE PRODUCTION AND ATTACHMENT OF FIM^A⁻ AND FIM^F⁻ MUTANTS OF *XYLELLA FASTIDIOSA* IN VITRO

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Reporting Period: The results reported here are from work conducted from February 1, 2002 to October 30, 2002.

INTRODUCTION

Xylella fastidiosa is a Gram-negative bacterium which causes serious diseases of plants such as Pierce's disease (PD) of grape (*Vitis vinifera* L.), citrus variegated chlorosis (CVC), and almond leaf scorch and colonizes many other plant hosts as well as insect vectors (Purcell 1997). The complete genome sequences of several strains of this organism are now available and provide the base material to study the function of most genes in this organism. *X. fastidiosa* is known to produce fimbriae to attach unipolarly to cell surfaces (Purcell et al. 1979, Feil et al. 2002). The fimbrial genes are clustered in an operon containing 6 open reading frames (ORFs) (Bhattacharyya et al. 2002). Several of these ORFs have been identified to have homology with genes of other organisms that were shown to be involved in the production of fimbriae (Bhattacharyya et al. 2002). Fimbriae- and pili-mediated attachment of bacteria to host tissues is important for bacterial colonization and pathogenicity (Hultgren et al. 1996). We investigated the role of fimbriae and adhesins in the virulence of *X. fastidiosa* to plants such as grape. The aim of this study was to determine the importance of fimbriae on the attachment of *X. fastidiosa* to xylem vessels. Two fimbrial mutants, FimA⁻ and FimF⁻ (a homolog to the adhesin MrkD) were produced and further characterized (Feil et al. 2002). Pathogenicity test showed that the mutants were still virulent in grapes. Research is still underway to determine to what extent the process of colonization of plants is altered in FimA⁻ and FimF⁻ mutants. We expect that the speed with which the cells move through the plant and the time before symptom development is altered in the mutants; detailed measures of pathogen populations of the wild-type and mutant strains is underway in inoculated plants to determine these features. We described here the results of several attachment assays used to further characterize the attachment of these mutants compared to the attachment of the wild type to various substrates.

OBJECTIVES

1. Determine the role of fimbriae in the attachment of *Xylella fastidiosa* to grape xylem vessels.
2. Identify compounds that either enhance or inhibit the production of fimbriae.

RESULTS AND CONCLUSIONS

The large majority of site-directed mutants in *X. fastidiosa* obtained after introducing FimA or FimF genes modified to contain an insertion of a kanamycin resistance marker gene into the Temecula strain using a pUC18-based suicide plasmid have been the result of double recombination events. While this is a very fortuitous result given that we obtain a very high frequency of gene knockouts in our mutagenesis strategy, such results are unexpected given that in most other bacteria gene replacement occurs via a process that first generated single recombination events leading to cis-merodiploid strains. We are currently testing whether the high frequency of apparently simultaneous double recombination events is due to a linearization of the in-coming plasmid DNA.

To further characterize the attachment of the fimbrial mutants, FimA⁻ and FimF⁻ we chose glass and balsa wood as substrates for the assays. The attachment of the mutants to these substrates was compared with the attachment of the wild-type parental strain. Several media were also compared to determine if attachment to substrates was dependent on the nature of aqueous medium in which the cells were suspended. Fluorescence microscopy revealed that adhesion to glass and aggregate formation was greatly reduced for the mutants compared to the wild-type cells. Wild-type cells formed aggregates of large size at occasional sites on both glass slides and on wood surfaces. Most of the attached cells were found within such aggregates; very few cells were attached as solitary cells to the surfaces. In contrast, almost no cell aggregates were observed in the FimA⁻ and FimF⁻ mutant strains, and few solitary cells also had adhered to the surfaces. To determine the amounts of cells remaining attached to glass or wood, we quantified the amount of protein as an estimate of the number of cells present in a sample. The greatest reduction of attachment using this assay was found when FimA⁻ cells were grown in a low nutrient medium, whereas in the PW medium the attachment was similar for the mutants and wild-type cells. These results suggest that pili play an important role in attachment of *X. fastidiosa* cells to each other to form aggregates, and that pili may play

little role in attachment to other surfaces. Since cell masses are a main feature of *X. fastidiosa*-infected xylem vessels, the self-aggregation of the pathogen conferred by pili may be an important virulence factor. The self-association of cells of *X. fastidiosa* should also influence the extent to which cells move through the plant and contribute to blockage of water movement, thus influencing symptom development.

When scanning electron microscopy (SEM) was used to examine wild-type and FimA⁻ and FimF⁻ mutants, we observed that fimbriae production between the cells and the balsa wood for the wild type cells was enhanced when cells were grown in a low nutrient medium. Examination of the FimA⁻ mutant with SEM showed that fimbriae production was rare and that the fimbriae length was much reduced from that of wild-type cells grown under similar conditions.

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FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

THE *XYLELLA FASTIDIOSA* CELL SURFACE

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Reporting Period: This project was funded with a start date of September 1, 2002. Thus, the report will constitute our plans and goals.

INTRODUCTION

Pierce's disease is caused by the Gram-negative bacterium *Xylella fastidiosa*, which has been classified as a member of the gamma subgroup of the Proteobacteria and is phylogenically related to the *Xanthomonads*. *X. fastidiosa* is highly specialized and occupies two very different environmental niches. Specifically, the bacteria are capable of multiplying in both the foregut of xylem-feeding insects, such as the glassy-winged sharpshooter and in the xylem system of the host plant. The ability of *X. fastidiosa* to thrive in both the insect foregut and the xylem suggests that the bacterium has evolved regulatory mechanisms that help it to cope with the unique stresses experienced in these two very different ecological niches.

A common response of Gram-negative bacteria to such stresses is to change the composition of their cell surface, particularly the protein composition of their outer membrane. The outer membrane is the outermost continuous structure on the bacterial cell surface and serves as a selective barrier between the cell and the external environment. Changes in the protein composition of the outer membrane are known to have a profound effect on the sensitivity of Gram-negative bacteria to detergents, antibiotics, and bacteriophages. Therefore, in order to develop effective methods for controlling the spread of *X. fastidiosa*, it is important to obtain information concerning the protein composition of the *X. fastidiosa* outer membrane in general and how the composition of this membrane changes in response to environmental signals. The overall goal of this proposal is to identify the major outer membrane proteins of *X. fastidiosa*, to assign the individual proteins to specific genes on *X. fastidiosa* chromosome, and to determine how the relative abundance of these proteins changes in response to environmental signals.

OBJECTIVES

1. Identify the major outer membrane proteins of *Xylella fastidiosa* and assign them to a specific gene on the *Xylella fastidiosa* chromosome.
2. Determine how the protein composition of the *Xylella fastidiosa* outer membrane is influenced by environmental signals and signals from the infected grapevine.

RESULTS AND CONCLUSIONS

We have just received the funding for this project (October 2002).

FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

UNDERSTANDING *XYLELLA FASTIDIOSA* COLONIZATION AND COMMUNICATION IN XYLEM LUMINA

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Reporting Period: This project was funded with a start date of October 1, 2002. Thus, the report will constitute our plans and goals.

INTRODUCTION

Symptoms of Pierce's disease of grape caused by *Xylella fastidiosa* are generally recognized as being caused by restricted sap flow and resultant water stress due to plugging of xylem elements (Goodwin et al. 1988; Purcell and Hopkins 1996; Mollenhauer and Hopkins 1974). Such blockage is the result of massive bacterial aggregates and associated mucilage. It is not clear whether the extracellular polymeric mucilage is of bacterial and/or plant origin. Based on the analysis of the complete genome sequence of *X. fastidiosa*, gums produced by the *X. fastidiosa* are similar to the 'xanthan gums' produced by *Xanthomonas campestris* pv *campestris*, although they may be less viscous (Simpson et al. 2000). In addition, tylose development in xylem vessels in response to the presence of the bacterium further restricts sap flow (Mollenhauer and Hopkins 1976). These general concepts *X. fastidiosa* pathogenicity are readily recognized, although it is not understood how the bacterium becomes established in the turbulent habitat of a 'fluid conduit' i.e., xylem vessels and tracheae. Bacterial spread through xylem elements is also poorly understood, albeit enzymatic degradation of pit membranes is thought to be involved (Mollenhauer and Hopkins 1976). Colony formation is likely to be influenced by the physical constraints of the xylem element surface much like the formation of bacterial biofilms is influenced by surface characteristics (microtopography, chemistry, etc.) in other aqueous and fluid environments such as medical stents and prostheses, food handling equipment, and water supply systems (Ridgway and Olson 1981; LeChevallier et al. 1987; Caldwell and Lawrence 1988; Sternberg et al. 1999). Surface microtopography of these environments influence the temporal and spatial aspects of bacterial colonization (Bremer et al. 1992; Gorman et al. 1993; Korber et al. 1997; Arnold 1999). Surfaces become colonized as cells (in this case bacteria) attach initially via physio-chemical forces, and ultimately with extracellular polysaccharides or ligand-mediated interactions. The end result is the establishment of biofilms consisting of bacteria in a polysaccharide matrix that provide a protective habitat that is conducive for continued cell growth and colony formation.

The recently completed sequencing of the *X. fastidiosa* genome has revealed several open reading frames with putative functions that may be associated with bacterial colonization of xylem vessels and disease (Simpson et al. 2000). For example, at least one ORF with homology to the *luxR* family of transcriptional regulators has been identified (GenBank accession AAF83782). Such genes encode proteins (LuxR homologs) that when bound by acyl-homoserine lactone autoinducer molecules (AI), regulate transcription of diverse types of genes (Fuqua et al. 1996). Autoinducers are synthesized by enzymes that are encoded by *luxI* gene homologs. The *luxI* – *luxR* regulatory system was first discovered in the marine bacterium *Vibrio fischeri*, however now related systems have been discovered in diverse species of bacteria including plant and animal pathogens (Cha et al. 1998). Autoinducers diffuse bi-directionally across bacterial membranes and reach concentrations for efficient activation of LuxR regulators in environments of high bacterial density. Thus the ability of AI to activate the LuxR regulators is a cell density-dependent response referred to as quorum-sensing or autoinduction. The discovery of *luxR* homologs in *X. fastidiosa* strongly suggests that the bacterium produces AI and regulates genes in a density-dependent manner. This finding is intriguing because it suggests that a *luxI*-*luxR* type quorum-sensing regulatory system may be functioning in *X. fastidiosa* biofilm communities in xylem vessels.

The overall goal of the proposed research is to identify factors that affect colonization and plugging of grape xylem elements by *X. fastidiosa* and to use this information for development of effective control strategies for Pierce's disease. Our approach is to determine physical and chemical factors that influence *X. fastidiosa* attachment and colony development using an in vitro system, and to establish whether genes associated with these activities are regulated by quorum-sensing. The in vitro system that we propose has several advantages. It will allow the direct observation of bacterial community development in 'artificial' vessels microfabricated to possess topographies and chemistries similar to 'real' *in planta* vessels. We will be able to determine how physical and in some cases biological parameters affect biofilm formation and plugging induced by virulent and avirulent or weakly virulent strains. Furthermore, it will be possible to differentiate between plant-induced responses and those induced specifically by the pathogen.

OBJECTIVES

1. Understand how the physical parameters of xylem tracheae and vessels influence *Xylella fastidiosa* colonization. Toward this, we will evaluate colony formation, mucilage production, biofilm development and flow rate during and following colonization
2. Determine whether *X. fastidiosa* produces acyl-homoserine lactone autoinducer molecules that are involved in regulation of genes associated with ability to cause Pierce's disease.

RESULTS AND CONCLUSIONS

We have just received the funding for this project (October 2002).

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FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

MANAGEMENT OF PIERCE'S DISEASE OF GRAPE BY INTERFERING WITH CELL-CELL COMMUNICATION IN *XYLELLA FASTIDIOSA*

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Reporting Period: The results reported here are from work conducted from December 15, 2001 to October 15, 2002.

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. 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. We expect activities required for movement 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. Cells assess the size of their local population via cell-cell communication and coordinately regulate the expression of genes required for such processes. Our study aims to investigate cell-cell communication in *Xf* to determine its role in colonization and pathogenicity in grapevines.

Xf shares sequence similarity with the plant pathogen *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). Two of the Rpf proteins, RpfB and RpfF, 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 (Dow and Daniels 2000). Thus *Xf* likely 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.

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. Characterize cell-cell signaling factors in *Xylella fastidiosa*.
2. Determine role of signaling factors on virulence and transmissibility of *Xylella fastidiosa*.
3. Identify degraders of signaling factors of *Xylella fastidiosa*.
4. Identify inhibitory analogs of signaling factors of *Xylella fastidiosa*.

RESULTS AND CONCLUSIONS

Objective 1. We have constructed "signal-sensing" strains of *Xcc* to determine whether *Xf* uses the same butyrolactone signal as *Xcc* (Figure 1). These strains carry a green fluorescent protein (*gfp*) gene under the control of a promoter that is up-regulated in response to the signal. We have successfully detected a signal from *Xf* using this system, however the response is much weaker than that of *Xcc* (Figure 2). We conclude that *Xf* may make high concentrations of the signal only under specific conditions, such as *in planta*. A second possibility is that the *Xf* signal differs slightly from the *Xcc* signal and cannot

fully activate the *Xcc* signal sensor except at high concentrations. To distinguish between these hypotheses, we are constructing signal-sensing strains of *Xf* using a *gfp* gene fused to promoters of *Xf* genes we believe should be up-regulated in response to the signal. These strains can be examined *in planta* as well as in culture to sort out the above-mentioned possibilities.



Figure 1. Signal sensor strain overlaid on a wild-type *Xcc* colony (left) or *rpfB* (center) or *rpfF* mutants.

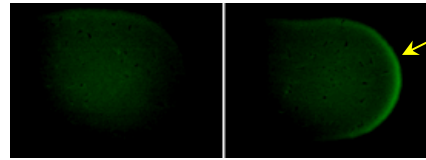


Figure 2. Signal sensor growing to the left of PWG extract (left) or *Xf* extract. Green fluorescence indicates signal presence.

Objective 2. We have constructed strains of *Xf* Temecula in which the *rpfB* and *rpfF* genes, which are each required for production of the signal in *Xcc*, are knocked out. These mutants were constructed using exchange of the wild-type allele for a deleted copy carrying an antibiotic resistance gene on a suicide plasmid (Figure 3). In contrast to other reports of recombination into the *Xf* genome, we obtain almost exclusively double recombinants in the primary transformants after only 7 days of incubation on plates. We are testing *rpfB* and *rpfF* mutant strains for their ability to infect and move within host plants and to cause Pierce's disease symptoms. Our preliminary evidence indicates that neither of these genes is strictly required for virulence as mutant strains cause symptoms similarly to the wild type. However, these genes may play a role in modulating disease progress because the timing of symptom development differs between mutant and wild-type strains. Further characterization of infected plants is underway to investigate the mechanism behind these differences. We are in the process of testing transmissibility of the mutant strains by an insect vector. In addition, we are testing the mutants for signal production using the *Xcc* signal sensor. To better direct our analyses, we have constructed a strain of *Xf* that constitutively expresses *Gfp* in order to bring the *in planta* growth habit of *Xf* during symptom formation into sharper focus (Figure 4). By observing differences in colonization between symptomatic and asymptomatic samples we will have a clearer image of the mechanism of symptom formation and the best strategies for preventing it.

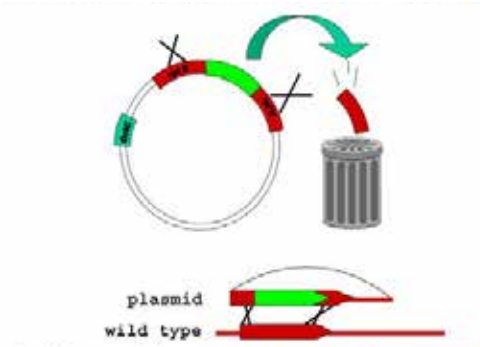


Figure 3. Gene knockout strategy using allelic exchange.

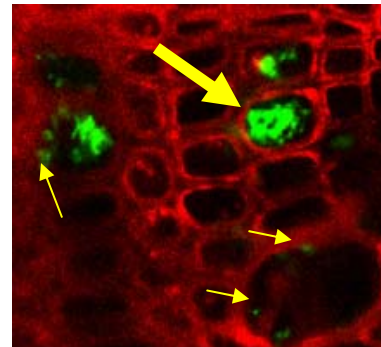


Figure 4. *Gfp*-labeled *Xf* viewed inside the live petiole of a grapevine by confocal microscopy. Large arrow indicates a large aggregate of cells. Small arrows point to individual cells or small groups. Red color is due to auto-fluorescence of the grapevine.

Objectives 3 and 4. We have collected grapevines from vineyards affected by Pierce's disease as well as tomato and cruciferous crop plants infected with the signal-producing pathogens *Xanthomonas campestris* pv *vessicatoria* and *Xcc*, respectively. We have recovered bacteria from inside these samples to generate a comprehensive collection of endophytes that grew in contact with the signal molecule. These endophytes are being tested for the ability to interfere with cell-cell signaling in *Xf* in an assay using the signal-sensing strains from Objective 1. We have thus far isolated several strains that are weakly inhibitory and are through about one-third of our collection.

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FUNDING AGENCIES

Funding for this project was provided by the American Vineyard Foundation, the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board, and the California Competitive Grant Program for Research in Viticulture and Enology, as well as by a National Science Foundation Postdoctoral Fellowship in Microbial Biology to K.L.N.

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

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Reporting Period: The results reported here are from work conducted from July 1, 2001 to June 30, 2002.

INTRODUCTION

Competitive exclusion of plant pathogenic bacteria with nonpathogenic strains has been demonstrated in other systems where pathogens were excluded from either plant surfaces or vascular tissues. The ideal situation is where the nonpathogenic bacteria are stable derivatives of the pathogen itself, so that nutritional and other growth requirements are identical and thus facilitate successful competition for colonization. Our project is to construct such mutants from the Pierce's disease (PD) pathogen, *Xylella fastidiosa*, through a systematic process of identifying which virulence genes are important for disease expression but not essential for colonization of plants in a nonpathogenic state. We are utilizing knowledge from comparative genomic sequence analysis with mutational studies to identify important virulence genes. This year, we have begun to employ an additional strategy (DNA macro/microarrays) based on analysis of differential gene expression between the bacterium grown in culture vs. during infection of plants.

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

Macro/microarray analysis of the expression profile of select candidate pathogenicity genes in Xf:

A procedure for macroarray analysis of about 100 genes selected as possible virulence genes based on comparative sequence analysis was developed, and this was reported at the Annual Meeting of the American Phytopathological Society in July 2002 (Hernandez-Martinez et al., 2002). To identify genes involved in pathogenicity in the PD strain, the sequence of the CVC strain was used to select open reading frames specifying putative pathogenicity and virulence factors. DNA fragments of these genes were obtained by PCR amplification from the genome of the PD strain I03. In this preliminary study, we constructed macroarrays for the analysis of the expression profile of select candidate pathogenicity genes of *Xf* and to study their expression in PD3 medium. We have shown that these genes are expressed to varying degrees ranging from none to very high. These arrays are being used to analyze the gene expression profile of different *Xylella* strains *in planta* and *in vitro*. This work is following the hypothesis that genes important in virulence and symptom expression are up-regulated in the plant. This will help us to refine the potential target genes for construction of non-pathogenic derivatives for biological control.



Figure 1. Autoradiograph of nylon filter macroarray probed with label cDNA from *Xylella fastidiosa*.

Mutational analysis of virulence genes:

Construction of several virulence gene mutants of *X. fastidiosa* has been done using the EZTN transposon or by insertional cloning of antibiotic resistance cassettes to create disruptive insertions into cloned genes that were amplified by PCR based on genomic sequence. The mutated clones have been subcloned into pUC129 for gene knockout experiments. Among

virulence genes included in mutational studies are those of the *gum* operon, for which we have recently constructed successful knockout mutations in *Xylella*. Other genes we are manipulating include a number of regulatory genes that likely control other virulence factors, such as RsmA.

We are also working to develop a more efficient transposon delivery system for *Xylella* especially for the analysis of the genes of unknown functions. Because of the high price of the EZTN system, we have cloned the 16S rRNA promoter of *Xf* to drive the transposase gene in a self-cloning modular transposon, pTnModOKm (Dennis and Zylstra, 1998). We expect this to significantly increase the transposition efficiency, since the low level of transposase expression has often been given as the reason for the low efficiency. If this improves the efficiency of the transposon, we will clone a promoter-less reporter within the transposon to be used as a measure of gene expression level of the genes to be inactivated.

Tissue culture of grape to develop in vitro inoculation system for Xylella pathogenesis:

We are working with grape tissue cultures, as well as other potential host plants that would show rapid symptoms, such as mustard, to develop rapid assays for analysis of virulence. The tissue culture system should also be useful in our macro/microarray work, where gene expression profiles of the bacterium with and without contact with grape cell cultures can be accomplished in a more controlled and sterile environment than whole plants.

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FUNDING AGENCIES

Funding for this project was provided by the California Department of Food and Agriculture.

CONTROL OF PIERCE'S DISEASE THROUGH DEGRADATION OF XANTHAN GUM

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Reporting Period: The results reported here are from work conducted from January 1, 2002 to December 31, 2002.

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 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

Production of xanthan gum for enrichment of xanthan-degrading bacteria:

The sequence of the xanthan gum biosynthetic operon in the genome sequence of the agent of Pierce's disease, *Xylella fastidiosa*, is different than the bacterium from which commercial xanthan gum is prepared, *Xanthomonas campestris*. This suggests that *Xylella* xanthan gum is chemically different, and we therefore wished to produce xanthan gum from *Xylella* for our enrichment studies. However, as described in our original proposal, it is not feasible to produce enough xanthan gum for our studies from the slow-growing *Xylella fastidiosa*. As proposed, we instead genetically modified a strain of the fast-growing *Xanthomonas campestris* to produce xanthan gum with the same chemical structure as that from *Xylella*. This was accomplished by deleting the *gumI* gene from the biosynthetic operon. Our genetic construction was confirmed, and we have produced significant quantities of xanthan gum from this mutant strain. The modified gum is still viscous, but has a measurable decrease in viscosity compared with gum isolated from the wild-type strain of *Xanthomonas*.

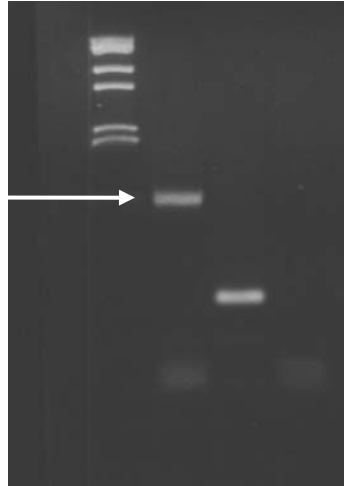
Enrichment for bacteria that degrade xanthan gum:

We used the modified xanthan gum from the *Xanthomonas* mutant described above as the sole carbon source for enrichment culture from Pierce's disease infected grapevines. To isolate the endophytic bacteria, we collected 200 grapevine samples infected with Pierce's disease in Temecula and Bakersfield and 100 oleander samples infected with leaf scorch disease in Riverside. Individual tissue segments were placed into sterile test tubes with 10 ml of 1% NaOCl solution with 0.1% tween 20. Surface-disinfected pieces were aseptically transferred through three washes of 10 ml of sterile PBS (phosphate buffered saline). To check for surface contamination, 0.1ml of the third wash for each sample is transferred to 5ml of Tryptic soy broth medium and incubated at room temperature on a rotary shaker for 2 days. Surface-disinfected pieces were macerated with PBS with 0.1% tween 20 using mortars and pestles. Suspensions were transferred to minimal media with xanthan gum as the sole carbon source and incubated at room temperature on rotary shaker for 7 days. Cultures were centrifuged, and the viscosity of their supernatant was measured. Cultures that had a decreased viscosity were transferred to fresh media and incubated for 3 days. This enrichment step was repeated twice. Cultures were finally spread on solid media with xanthan gum as the sole carbon source, and individual colonies were streaked to purity on fresh plates. Pure cultures were tested for reduction of viscosity of xanthan gum as measured with an Ostwald capillary viscosimeter. Over 100 bacterial strains were initially recovered from these enrichment experiments, and 11 were subsequently confirmed to effectively degrade xanthan gum. These strains were then tested for cellulase activity. Degradation of the cellulosic backbone of the xanthan polymer would be desirable, but we do not want enzymes that recognize and degrade plant cellulose. Six of the strains had low or non-detectable cellulase activity and will be further tested for biological control efficacy in plants.

Expression of the xanthan gum operon in Xylella fastidiosa:

To support our hypothesis that xanthan gum is produced in infected plants, we have initially tested whether the xanthan gum operon is expressed. RT-PCR was performed with primers directed toward the first gene of the xanthan gum biosynthetic operon, *gumB*, with RNA extracted from different strains of *Xylella fastidiosa*. *gumB* mRNA was detected from some, but not all, strains of *X. fastidiosa* grown *in vitro*. Xanthan gum and other virulence factors may be produced at high levels only in plants when bacterial populations have reached a high density. Extraction of RNA from infected plants is in progress to study expression of the xanthan gum operon *in planta*.

gumB mRNA detected by RT-PCR
from *Xylella fastidiosa* culture



FUNDING AGENCIES

Funding for this project was provided by the USDA Animal and Plant Health Inspection Service.

PARATRANSGENESIS FOR CONTROL OF PIERCE'S DISEASE: MANIPULATION OF ENDOPHYTIC BACTERIA FOR PARATRANGENIC CONTROL OF PIERCE'S DISEASE

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Reporting Period: The results reported here are from work conducted from August, 2001 to November, 2002.

INTRODUCTION

The Glassy-Winged Sharpshooter (GWSS) is the principal vector of *Xylella fastidiosa*, the causative agent of Pierce's disease, in the California wine country. One strategy to control the spread of *Xylella* by the GWSS is to make the insect refractory to transmission of the bacterium. One can imagine at least two ways this can be done. The first is to genetically engineer the population of sharpshooters directly to become refractory. This transgenic method is being seriously discussed by biologists wishing to halt the spread of malaria in Africa, via the direct genetic manipulation of *Anopheles gambiae* populations (Kiszewski and Spielman 1998). This method is fraught with potential difficulties of many sorts and, in any case, has not been tried to date.

The second method is to manipulate the insect vector indirectly by manipulating its gut flora. This technique is termed paratransgenesis and has many potential advantages over the direct transgenic approach. First, bacteria are far easier to manipulate genetically. Secondly, bacteria can be made to secrete or carry very specific agents of control, like single chain antibodies. Paratransgenesis has been attempted to control Chagas' disease in S. America (Beard et al. 2001) and a form of it is being developed to deliver therapeutic agents in mouse models of human disease, for eventual applications for humans (Beninati et al. 2000).

In attempting to create transgenic gut symbionts of the GWSS several problems present themselves immediately. The final transgenic strain will need to be stable (i.e., the exogenous DNA not contained in a virus or bacterial transposon), the exogenous DNA should be incorporated into the chromosome and not borne on a plasmid, no drug markers should be left in the strain, and as little exogenous DNA should be transferred as possible. We have developed a genetic modification system that meets those requirements based on the *mariner* family of eukaryotic transposable elements. These elements are active in all domains of life when appropriately manipulated, but do not occur naturally in prokaryotes. Thus stable strains of GWSS gut symbionts can be created that should be suitable for release into the wild for the control of *X. fastidiosa*.

OBJECTIVES

1. Construct a genetic DNA insertion system for *Alcaligenes* sp. and *Chryseomonas luteola* based on *mariner* family transposable elements.
2. Identify single chain antibodies that bind specifically to the surface of *Xylella fastidiosa* and express these on the surface of one or more gut symbiotic bacteria of the GWSS as agents of control.

RESULTS AND CONCLUSIONS

A genetic manipulation system for GWSS endosymbionts:

We have constructed a matable transformation system for two GWSS bacterial symbionts based on the *mariner* transposable element, *Himar1*. Below is a figure illustrating the features of the system that help it fulfill the requirements set out in the introduction. All of the necessary requirements are borne on a single suicide plasmid. The plasmid has an RP4 origin of transfer so it can be mated from *E. coli* to *Alcaligenes* or *Chryseomonas*. It also contains a R6K origin of replication so that it 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 transposon are obtained, the drug marker can be removed by recombination. Since the transposase gene lies outside the inverted terminal repeats (ITRs) the insertions are stable after the plasmids are lost.

Using this system we obtained insertions at random positions in the chromosome of *Alcaligenes* and *Chryseomonas*. Genetic and Southern evidence showed that only the transposon inserted and not any part of the plasmid backbone. Furthermore, we

were able to remove the drug marker by FLP-mediated excision. These systems were used to introduce fluorescent protein genes into each of these species for microbial ecology studies.

Specific modifications to gut symbionts:

The goal of paratransgenesis is to indirectly modify the phenotype of the vector (or host plant) through the modification of a symbiotic organism. There are many traits that could be added to the symbiont, including secreted enzymes, toxins, antibacterial peptides, or single chain antibodies (scFv's). An alternative to secreting a factor is to express it in the outer membrane. We are currently screening phage display scFv libraries to identify scFv's that can bind specifically to the surface of *Xylella fastidiosa* with high affinity. Candidate scFv's will be expressed as OmpA-scFv fusions (inserted into the chromosome with the *mariner* transformation system described above) that will allow the transgenic bacteria to adhere tightly to the surface of *Xylella*, either preventing infection or slowing its spread. Although we have targeted the entire surface of *Xylella* initially, specific outer membrane targets identified from *Xylella* genome project can also be targeted individually, particularly those that are associated with pathogenesis.

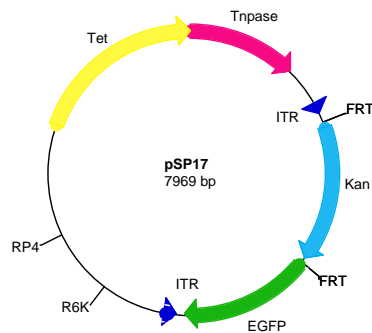


Figure 1. pSP17, a matable suicide plasmid designed to create stable transgenic gut symbiotic bacteria from the glassy-winged sharpshooter.

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FUNDING AGENCIES

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**INSECT-SYMBIOTIC BACTERIA INHIBITORY TO *XYLELLA FASTIDIOSA* IN SHARPSHOOTERS:
PRESSURE BOMB EXTRACTION OF XYLEM FLUID TO IMPROVE BACTERIAL DETECTION
OF *XYLELLA* IN PLANTS**

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Reporting Period: The results reported here are from work conducted from December 1, 2001 to December 1, 2002.

INTRODUCTION

The glassy-winged sharpshooter (GWSS) is the principal vector of the xylem-limited bacterium *Xylella fastidiosa* (*Xf*), which causes Pierce's disease (PD) in grapes and oleander leaf scorch. Limiting the spread of this pathogen by rendering GWSS incapable of pathogen transmission is a promising method of pathogen control.

Paratransgenic approaches to pathogen control are currently being developed to deliver single-stranded antibodies to disrupt Triatomid transmission of *Trypanosoma cruzi* (Beard et al. 2002), and to prevent colitis in mammals (Beninati 2000; Steidler, 2002). Candidate paratransgenic bacterial agents must thrive in the cibarial/foregut area where they will be in close proximity to the pathogen, *Xf* for a paratransgenic strategy to be applicable to the *Xf*/GWSS system.

Alcaligenes xylosoxidans denitrificans (*Ax*) was cultured and identified from the cibarium and foregut regions of GWSS's alimentary canal several times throughout the growing season indicating that it is a consistent symbiotic organism (Lauzon, in preparation). *Ax* has been described as a non-pathogenic plant endophyte and a non-pathogenic soil-borne microbe (Meade et al. 2001, Yang et al. 1999). This bacterium was genetically transformed with exogenous DNA being incorporated into the chromosome to express fluorescent markers (Lampe, in preparation). My project was to set up a disease cycle, which would offer evaluation of paratransgenic delivery. To do this it was important to improve detection of *Xf*.

OBJECTIVES

1. Develop a delivery system to re-introduce paratransgenic bacteria to GWSS and introduce *Xf* to GWSS to improve the disease cycle in the laboratory.
2. Improve detection of *Xf* in plants and insect vectors.

RESULTS AND CONCLUSIONS

Cycling of dsRed Alcaligenes xylosoxidans denitrificans in GWSS:

I have developed a plant-based bacterial delivery system for GWSS that allows bacteria to be available for consumption by the insect vector. Earlier attempts to feed GWSS on varied sucrose solutions from membrane sachets were unsuccessful because GWSS did not probe and died within 24 hr. GWSS probed from a flowing feeding system but would not sustain feeding and died within 24 hr. The manipulation of the chrysanthemum xylem fluid by forcing a bacterial suspension through a cut stem allowed a GWSS to feed on a concentrated bacterial suspension and was successful in maintaining GWSS for up to 5 days.

GWSS were exposed to the bacterial delivery system that contained dsRed *Ax* (OD₆₀₀=2.85) for 48 hr. then removed and placed on "clean" chrysanthemum plants for an indefinite period of time. At 0, 7, 14, 21, 28, and 35 days post-exposure, GWSS were collected and inspected by fluorescence microscopy for presence of dsRed *Ax*. Samples from day 0 had individual *Ax* in the cibarium and foregut. GWSS sampled at 7 days and beyond had "clumps" of *Ax*, indicating colonization of the cibarium and foregut. *Ax* was present on all dates sampled and was independent of sex.

Evidence of horizontal transmission was also collected by introducing a single dsRed *Ax*-fed GWSS into a population of 10 "clean" GWSS for 14 days caged on a single chrysanthemum. *Ax* was detected in the cibarium or foregut of 17 of 42 GWSS that survived to the end of the trial.

Creating the PD disease cycle in the lab: bacterial delivery system for introduction of *Xf* to GWSS:

After 48 hours exposure to *Xf* offered through the bacterial delivery system, 100% of GWSS heads from exposed insects tested positive by PCR for the presence of *Xf*.

The Scholander pressure bomb is used to extract xylem fluid from a plant (Hallmann et al. 1997), allowing collection large amounts of fluid (Guo et al. 2001). The use of the pressure bomb technique for detection of *Xf* in oleander was less sensitive than traditional sample collection when used in conjunction with ELISA and PCR. Attempts at culturing *Xf* from xylem fluid collected using the pressure bomb was more prone to contamination than from traditionally collected samples. The consistency of the oleander pressure bomb xylem fluid was semi-solid making use with DNA extraction for PCR, ELISA, and culturing difficult. Therefore, pressure bomb collection of xylem fluid as a technique for early detection of *Xf* was discontinued.

Use of the pressure bomb for improving *Xf* detection in grape plants was more successful. By ELISA and PCR, pressure bomb fluid collections almost doubled the level of detection when compared to traditional sample collection. However, as with oleander culturing of *Xf* from xylem fluid collected with the pressure bomb was prone to contamination.

Development of the Pierce's disease cycle in the laboratory and the improvements in *Xf* detection provides a dependable system to test candidate paratransgenic bacteria identified by Carol Lauzon and genetically altered by David Lampe with toxins provided by Don Cooksey.

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FUNDING AGENCIES

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INSECT-SYMBIOTIC BACTERIA INHIBITORY TO *XYLELLA FASTIDIOSA* (PARATRANSGENESIS FOR CONTROL OF PIERCE'S DISEASE): IDENTIFICATION OF ENDOPHYTIC BACTERIA CYCLED BY GLASSY-WINGED SHARPSHOOTERS TO HOST PLANTS

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Reporting Period: The results reported here are from work conducted from April 1, 2001 to October 31, 2002.

INTRODUCTION

Homalodisca coagulata Say, the glassy-winged sharpshooter (GWSS) is known to transmit the etiological agent of Pierce's disease, *Xylella fastidiosa*. A paratransgenic approach designed to disrupt the ability of the insect vector to transmit the pathogen involves finding bacterial candidates that possess some degree of intimacy either within the insect and/or host plant xylem. Once a candidate(s) is/are found, then avenues open for finding strategies aimed at controlling *X. fastidiosa* infection and/or transmission, such as paratransgenesis. This report details the survey for, and isolation and identify of candidate bacteria for use in a paratransgenic strategy to control Pierce's disease. It also includes information about the possible relationship internal extracellular bacteria have with GWSS.

OBJECTIVES

1. Identify bacterial candidates for use in a paratransgenic strategy for control of Pierce's disease.
2. Understand the relationship(s) between and among bacteria in sharpshooters and their host plant xylem.

RESULTS AND CONCLUSIONS

Glassy-winged sharpshooters, captured in nature, were aseptically dissected for their alimentary canal organs, particularly, cibarial pumps, fore- and midguts. Bacterial inhabitants were retrieved using dilute nutrient media held at 22-24°C. The lower concentration of nutrients in bacteriological media mitigated the typical problems associated with growing endophytic bacteria under laboratory conditions (Bell et al. 1995). Isolates were identified using standard biochemical tests and morphological methods. Three primary bacterial species were isolated and identified from the cibarial region and fore-and midguts as (in order of dominance): *Alcaligenes xylosoxidans denitrificans*, *Chryseomonas luteola*, and *Ralstonia pickettii*. These bacteria are typical of plants (endophytes), soil, and water (Holt and Krieg, 1992). Two *Bacillus* spp., *Bacillus coagulans* and *Bacillus brevis* were infrequently isolated from midgut samples. Another isolate, tentatively identified as *Sporosarcina* sp., and a yeast-like organism were also infrequently isolated from pump samples.

Twenty-four biochemical tests were performed on the three primary isolates. The data suggest that these bacteria may be participating in nitrogen and hydrogen cycling within GWSS. To begin to determine the extent of nitrogen and hydrogen activity within the gut of GWSS and the possible contribution of bacteria with these activities, we performed a cytochemical assay using transmission electron microscopy (McLean et al. 1985). We found that nitrogenous compounds are concentrated within the midgut of GWSS (Figure 1) and that where bacteria were present, they participated in nitrogen catabolism within the insect gut (Figure 2). Metabolism of nitrogenous and/or other organic compounds by sharpshooters has been examined (i.e. Anderson et al. 1989; Brodbeck et al. 1993, 1995, 1996, 1999), however, the contribution of bacteria in xylem and/or in sharpshooters to these processes has not been addressed.

The mouthparts and guts of wild-captured GWSS were also examined for microorganisms using fluorescent techniques. Dissection and transection of GWSS tissues were stained using the ViaGram™ Red+ Bacterial Gram Stain and Viability Kit (Molecular Probes Inc., Eugene, OR). Images were obtained using fluorescent and confocal laser scanning microscopes and revealed the presence of stationary and motile viable bacteria within the mouthparts and gut regions of GWSS samples. In addition, the yeast-like microorganism was found within mouthpart samples. Similar images were acquired using scanning electron microscopy of GWSS samples.

The three primary bacterial isolates, *Alcaligenes* sp., *Chryseomonas* sp., and *Ralstonia* sp., were screened for their response to a battery of antibiotics in preparation for genetic manipulation experiments performed by Dr. D. Lampe (the reader is referred to the report presented by D. Lampe for details). Subsequently, we received numerous GWSS from Dr. B. Bextine.

Dr. Bextine used his plant-based delivery system (the reader is referred to the report presented by B. Bextine for details) for the introduction of DsRed *Alcaligenes xylosoxidans denitrificans* (RAX) to both GWSS and plants. In both cases, we often detected (typically in 40% of samples) RAX in treated samples with no detection of RAX in controls. Therefore, RAX was found to cycle from plants to GWSS and additionally, from GWSS containing RAX to plants.

We are currently engaged in experimentation designed to determine the physiological parameters that facilitate optimal establishment and cycling of RAX in plant and insect samples. We are working toward determining the accuracy of our detection level of RAX in insect and plants, i.e. is our current detection methodology using fluorescent microscopy an underestimate of the actual presence of cells within the samples? Concurrently, we are attempting to determine the optimal delivery dose and physiological state of RAX necessary for use in an effective paratransgenic strategy. We aim to conduct similar experiments using the transformed *Chryseomonas* sp. in the near future.



Figure 1. Midgut from a wild GWSS after partial treatment for detection of nitrogenous compounds. Dark areas reveal the presence of localized nitrogen.

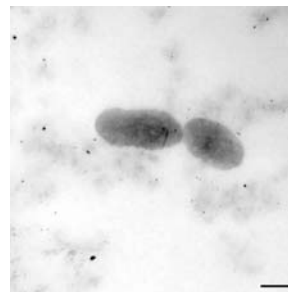


Figure 2. Transmission Electron micrograph showing bacteria in the same wild GWSS midgut participating in nitrogen catabolism. Dark areas are indicative of nitrogen catabolism and nitrogenous products.

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FUNDING AGENCIES

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*Genetics of
Xylella
fastidiosa /
Miscellaneous*



SURROGATE GENETICS FOR *XYLELLA FASTIDIOSA*

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Reporting Period: The results reported here are from work conducted from July 1, 2002 to October 31, 2002.

INTRODUCTION

Xylella fastidiosa presents a formidable challenge to the molecular geneticist. Methods for the basic operations of genetic exchange, mutant isolation, and complementation are in early stages of development. The slow generation time, poor plating efficiency and requirement for complex culture media are further complications. Surrogate genetics (Maloy and 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., *Xylella 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., *pil* genes, encoding type IV pili) as well as "housekeeping" genes involved in central metabolism (e.g., amino acid biosynthesis).

OBJECTIVES

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

RESULTS AND CONCLUSIONS

A first approach to defining transcriptional regulatory mechanisms in *X. fastidiosa* is to examine visually the upstream nucleotide sequences of genes whose regulation has been well-studied in other organisms. Common regulatory strategies will be revealed by common features in the sequences. 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, as revealed by the genome sequence of *X. fastidiosa* strain 9a5c (Simpson et al. 2000), 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 (Rodrigues da Silva et al. 2001). 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 initially were thought most likely to 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 $\Phi(gumB-lacZ)$ 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 therefore chose to study the regulation of *pil* gene expression. These genes control the formation of type IV pili in a variety of organisms, and are required for gliding motility, adhesion and pathogenesis (Winther-Larsen and Koomey 2002; Shi and Sun, 2002). 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 $\Phi(pilA-lacZ)$ operon fusion in *E. coli*, and observed that it expressed detectable levels of LacZ enzyme. We also cloned the regulatory *pilSR* genes (XF2546 and XF2545) from *X. fastidiosa*. However, we have not yet observed a *pilR*-dependent increase in LacZ synthesis, indicating that the PilSR regulators may not function well in *E. coli*.

Unpublished work of others indicates that σ^{54} -dependent activators from other species do not function well with *E. coli* RNA polymerase. To approach this question directly, we are currently studying expression of the *glnA* gene encoding glutamine synthetase (XF1842). This is the best-studied σ^{54} -dependent gene in *E. coli*, and the *X. fastidiosa glnA* upstream regulatory region is similar to that of *E. coli*. Furthermore, *X. fastidiosa* encodes the NtrB-NtrC sensor-regulator system for controlling *glnA* gene expression (XF1849 and XF1848). Because *E. coli* also encodes NtrB-NtrC, we will be able to evaluate *glnA* expression in response to both the *X. fastidiosa* and the *E. coli* regulatory proteins.

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FUNDING AGENCIES

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DIRECTING POTENTIAL ANTI-XYLELLA GENE PRODUCTS TO THE XYLEM OF TRANSGENIC GRAPEVINES

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Reporting Period: The results reported here are from work conducted from November 1, 2001 to November 1, 2002.

INTRODUCTION

Genetic engineering offers the possibility of introducing genes that will improve tolerance to Pierce's disease in existing grape varieties without otherwise changing their viticultural or enological characteristics.

One of our target genes is a pear *pgip* cloned in the Labavitch lab (Stotz et al. 1993). PGIPs are proteins containing a leucine-rich repeat domain that are targeted to the plant cell wall and that specifically inhibit fungal polygalacturonases (PGs). By inhibiting PGs, PGIPs directly interfere with host cell wall degradation and may thus prevent degradation of pectic oligomeric elicitors that are inducers of the plant defense response. Their role in plant defense response suggests that they may be useful for engineering transgenic plants resistant to pathogen infection. Powell et al. (2000) showed that transgenic tomato plants transformed with the pear *pgip* gene exhibited reduced susceptibility to infection with *Botrytis cinerea*. The fact that *Xylella fastidiosa*, the causal agent of Pierce's disease (PD) in grapevines, has genes putatively encoding PG and other cell wall-degrading enzymes (Simpson et al. 2000) led us to hypothesize that PGIP could confer tolerance against *Xylella* in grapes. In order to test this hypothesis, proembryogenic calluses originating from anthers of *Vitis vinifera* cvs. Thompson Seedless and Chardonnay were co-cultivated with *Agrobacterium tumefaciens* strain EHA 101 harboring binary plasmid pDU94.0928 that contains the pear *pgip* gene under the control of the CaMV 35S promoter.

We are also investigating the targeting of transgene products to xylem tissue. Because *X. fastidiosa* is xylem limited, it will be essential that any anti-*Xylella* gene product be present in the xylem in an effective concentration. We have obtained a xylem-specific gene from cucumber, XSP30, from colleagues in Japan (Masuda et al. 1999). We have fused its leader sequence to a GFP marker gene, the expression of which is readily detectable in the laboratory (Maximova et al. 1998), in order to study its ability to target the expression of marker gene products to the xylem stream of grapevines.

OBJECTIVES

1. Evaluate the effect of PGIPs on the development of Pierce's disease in transgenic grapevines.
2. Evaluate the effect of several signal sequences on the targeting of transgene products to the xylem.

RESULTS AND CONCLUSIONS

Effect of PGIPs on the development of Pierce's disease in transgenic grapevines

We have produced 50 transgenic lines that have been transferred successfully to the greenhouse, all from independent transformation events. The presence of the gene and the protein has been confirmed by PCR and Western blots respectively, and high levels of enzyme activity have been found in crude extracts from leaves.

A group of lines has been tested against *X. fastidiosa*. Five to seven plants of each line were mechanically inoculated with the Temecula strain of this pathogen. Additional plants were inoculated with *Xylella*-free buffer or left untreated. Untransformed plants were subjected to the same treatments. The development of Pierce's disease symptoms was delayed in some lines by several weeks (Figure 1). These lines are currently being evaluated for bacterial growth by ELISA and hydraulic conductance. Infection experiments on the rest of the lines are underway.

In addition we have found PGIP activity in the xylem sap of the transgenic plants but not in untransformed controls. These results suggest that the presence of PGIP in the xylem might interfere with cell wall degradation, preventing vascular occlusion and bacteria movement and/or favoring the accumulation of elicitor-active molecules. We will carry out grafting experiments to determine the transmissibility of the gene product in scion xylem sap.

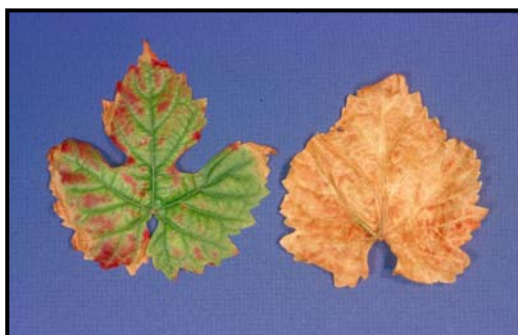


Figure 1a (left). Thompson Seedless vines 3 months after inoculation with *Xylella fastidiosa*. Vine on right is untransformed and has lost all of its leaves. Center vine is a transgenic vine expressing pear PGIP. Vine on left is untransformed and was inoculated with buffer only.

Figure 1b (top). Thompson Seedless leaves 3 months after inoculation with *Xylella fastidiosa*. Leaf on right is from an untransformed control vine. Leaf on left is from a transgenic vine expressing pear PGIP.

Effect of several signal sequences on the targeting of transgene products to the xylem:

A fusion between the leader sequence of a *Cucumis sativus* xylem sap protein (XSP30) and GFP was done in the Dandekar lab. Proembryogenic calluses of cvs. Chardonnay and Thompson Seedless were transformed in May 2002 and are being cultured in germination medium. The strong fluorescence detected in germinating embryos and their periphery (Figure 2) indicates high levels of enzyme synthesis and suggests that GFP is being secreted but additional analysis is needed to determine its subcellular localization.

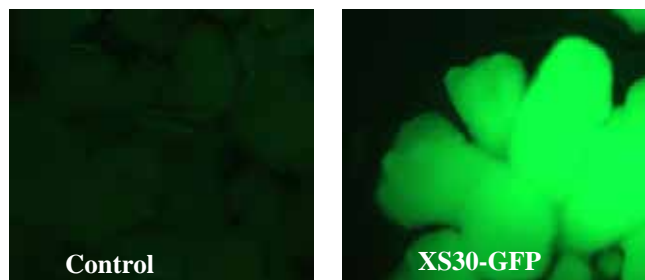


Figure 2. GFP fluorescence of embryos of 'Thompson Seedless' transformed with 35S-XS30-gfp.

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FUNDING AGENCIES

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BIOLOGICAL, CULTURAL, GENETIC, AND CHEMICAL CONTROL OF PIERCE'S DISEASE: PRODUCTION AND SCREENING OF *XYLELLA FASTIDIOSA* TRANSPOSON PATHOGENICITY AND ATTACHMENT MUTANTS

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Reporting Period:

INTRODUCTION

One of our projects involved the development of a transformation and transposon mutagenesis systems for the bacterium that causes Pierce's disease (PD), *Xylella fastidiosa* (Xf). We now have a random transposon mutagenesis system working for Xf (Guilhabert, et al. 2001) and recently we have developed an *E. coli*/Xf plasmid shuttle vector. We are currently assessing the stability of the *E. coli*/Xf plasmid shuttle vector in Xf without antibiotic selection. The results of these experiments will let us know whether this plasmid will be stably maintained in Xf cells that are inoculated in plants, something that will be essential for evaluating genes *in planta*.

Understanding the complex interactions between the plant, pathogen, and insect vector is imperative for the development of effective disease controls. Recently, the complete genome sequence of a citrus strain of Xf was determined (Simpson et al., 2000) and the complete sequence of a grape-infecting Xf strain (Temecula) is nearly complete. Earlier analysis of the CVC genome revealed important information on potential plant pathogenicity and insect transmission genes. However, more than half (53%) of the identified ORFs in Xf CVC encode proteins with no assignable function. In addition, some of the putative gene functions assigned on the basis of sequence homology with other prokaryotes may be incorrect. In order to identify and understand the function of Xf genes, it is imperative to develop techniques to knock out and complement putative pathogenicity or transmission genes.

OBJECTIVES

1. Development of transformation and marker-exchange systems for *Xylella fastidiosa*.
2. Screen *Xylella fastidiosa* Tn5 mutants for their ability to move and cause Pierce's disease in chardonnay grapevines

RESULTS AND CONCLUSIONS

Development of a transformation system for Xf with plasmid DNA:

The Tn903 kan-2 cassette, which we know is expressed in Xf (Guilhabert et al. 2001), was cloned in four broad host range plasmids, pUFR027, pLAFR3, RSF1010 and pMMB622 a derivative of RSF1010, forming the plasmids pXF002, pXF003, pXF004 and pXF005, respectively. Plasmids pXF002 and pXF003 failed to transform the Temecula strain of Xf. However, electroporation of Xf cells with 500 ng of pXF004 and pXF005 plasmid DNA produced an average of 131 and 208 Xf Kan^R clones respectively when selected on PD3 plates supplemented with 5 µg/mL of kanamycin. Plasmids pXF004 and pXF005 were found to be present as autonomous, structurally unchanged DNA molecules when propagated in Xf. However, neither pXF004 nor pXF005 were stably maintained in Xf after 5 passages without antibiotic selection. We are currently in the process of reproducing the same experiment with one, two and three passages without antibiotic pressure. When plasmid DNAs were isolated from Xf or plasmid DNAs isolated from *E. coli* were supplemented with a TypeOne™ Inhibitor, TRI, the frequency of transformation was increased by 13 or 5 fold, respectively. Plasmid pXF005 was also used to transform an additional grapevine strain of Xf.

Development of a marker exchange system for Xf:

The gene *rpjF* is required, together with the *rpjB* gene, for the production of a diffusible molecule, termed DSF, that may represent a novel cell density-dependent signaling factor in *Xanthomonas campestris* pv. *campestris* (Barber et al. 1997). The *rpjF* gene was PCR amplified from the Temecula strain, cloned and disrupted with the Tn903 Kan-2 cassette. Replacement of the wild-type gene in the genome of the Temecula strain by the disrupted *rpjF* gene was accomplished by a double crossing over event. The disrupted *rpjF* Xf mutant was inoculated into Chardonnay grapevines using a pinprick method (Hill and Purcell 1995; Purcell and Saunders 1999). The parental Temecula wild type strain served as a positive

control, and a water inoculation served as a negative control. The vines were observed for symptom development 16 weeks after inoculation. No difference was noted in the symptom development of vines inoculated with the *rpfF* *Xf* mutant compared to the plants inoculated with the parental Temecula wild strain. The presence of the *rpfF* *Xf* mutant in the symptomatic tissues was confirmed by immunocapture PCR (Smart et al. 1997) using the oligonucleotide primers used to amplify the *rpfF* gene. The population, systemic colonization, as well as the insect transmissibility of the *rpfF* *Xf* mutant, is currently being determined.

Testing of Tn5 mutant strain:

The bacterial cultures were inoculated into PD3 medium and adjusted to a concentration of 10^8 cells/mL. Approximately 1,000 random Tn5 mutants were inoculated each into two canes of Chardonnay grapevines using a standard pinprick method using 20 μ l of the adjusted bacterial culture (Hill and Purcell 1995; Purcell and Saunders 1999). The parental wild type strain served as a positive control, and a buffer inoculation served as a negative control. The vines were observed for symptoms development for 16 weeks. After 16 weeks, each inoculated grapevine was sampled (0.5 g of cane tissue) 10 inches above the point of inoculation. *Xf*-specific IgG was purified from *Xf* antiserum and conjugated with peroxidase using procedures developed in the Walker lab. The same ELISA procedure used by the Walker laboratory has been used to analyze each inoculated grapevine. The tissue was ground in ELISA buffer and approximately 300 inoculated grapevine samples have been sampled and frozen to date. Any mutants that seems to show altered virulence, multiplication, survival or movement will be retested in a similar manner on 6 canes growing on 3 separate grapevines.

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FUNDING AGENCIES

Funding for this project was provided by the American Vineyard Foundation, the California Department of Food and Agriculture, the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board, the California Raisin Marketing Board, the California Table Grape Commission, the USDA Animal and Plant Health Inspection Service, the University of California Pierce's Disease Grant Program, and the Viticulture Consortium.

SEQUENCE OF THE GENOME OF *XYLELLA FASTIDIOSA* CAUSING PIERCE'S DISEASE IN CALIFORNIA

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Reporting Period: The results reported are from work conducted from October 1, 2001 to September 30, 2002.

INTRODUCTION

Xylella fastidiosa (*Xf*) causes economically important diseases of agronomic, horticultural and landscape plants (Freitag 1951; Hopkins 1989; Purcell and Hopkins 1996). In addition to a wide diversity of *Xf*-host plant relationships, diseases such as Pierce's disease (PD) of grapevines and citrus variegated chlorosis (CVC) exhibit distinct symptoms and have different geographical distributions. In the previous reporting period, essentially the complete genomic sequence of an *Xf* strain associated with Pierce's disease in California was determined to help elucidate the molecular basis of *Xf* pathogenicity. Here we report the comparative analyses of the complete genome sequences and annotations of *Xf*-PD and *Xf*-CVC to provide further insight into *Xf*-plant host interactions and the relationships among *Xf* strains.

OBJECTIVES

1. Complete the sequencing of the genome of a *Xylella fastidiosa* strain associated with Pierce's disease (PD) in California.
2. Comparatively analyze the genome sequences and annotations of *Xylella fastidiosa* strains associated with PD in California and CVC in Brazil.

RESULTS AND CONCLUSIONS

The *Xf*-PD genome is composed of a single circular chromosome (2,519,802 bp) and a small plasmid (1,345 bp) similar to that reported in other *Xf* strains (Hendson et al. 2001). The major differences between the genomes of *Xf*-PD and *Xf*-CVC strains are the (1) 159,503 bp smaller size of the *Xf*-PD chromosome and (2) absence of the large pXF51 plasmid in the *Xf*-PD strain. Of the 2,066 protein coding genes annotated in *Xf*-PD, 2025 (98%) are also present in the *Xf*-CVC strain. The average amino acid identity of the ORF's in both strains is 95.7%. The most conserved *Xf*-PD genes include those that determine the basic metabolism and cellular functions of the bacterium, and, we conclude, are mostly identical to those of the *Xf*-CVC described previously (Simpson et al. 2000). Genomic structural/organizational differences between these two strains are associated with phage-mediated chromosomal rearrangements and deletions that also account for strain specific genes present in each genome (Figure 1). All of the rearrangements are flanked at one border by a putative phage-related integrase. Two genomic islands (gi), one specific to each genome, are characterized by regions with marked decreases in protein identities, different GC content and codon bias. In *Xf*-PD, giPD1 is 15.7 kb long with 61.2% GC content and harbors an extra copy of a hemagglutinin gene with a phage related integrase at one end. In *Xf*-CVC, giCVC1 is 67 kb long with 63.3% GC content and is inserted with tRNA Gly-2. The presence or absence of giPD1 and giCVC1 was associated with different groups of *Xf* strains. Essentially, all of the differences between the genomes of these two strains can be accounted for by the number and relative position of clusters of phage-related genes and insertion/deletion events, including giPD1 and giCVC1. We propose that the evolutionary divergence of these two *Xf* strains is due mainly to the lateral gene transfer mediated mostly by phage. Despite the genome rearrangements, most of the genes in these two strains are highly conserved including not only those concerned with basic cellular house keeping but also those likely to have a direct role in pathogenicity. This suggests that diseases caused by different *Xf* pathotypes most probably rely on the expression of a common set of bacterial genes to become established *in planta* (i.e., plant colonization, pathogenesis) permitting convergence of functional genomic strategies. Knowledge acquired from the comparison of the complete genomes of both *Xf*-PD and *Xf*-CVC strains has numerous applications, including designing strain specific primers for *Xf* detection and differentiation to screen germplasm and in clinical field samples to control pathogen dissemination.

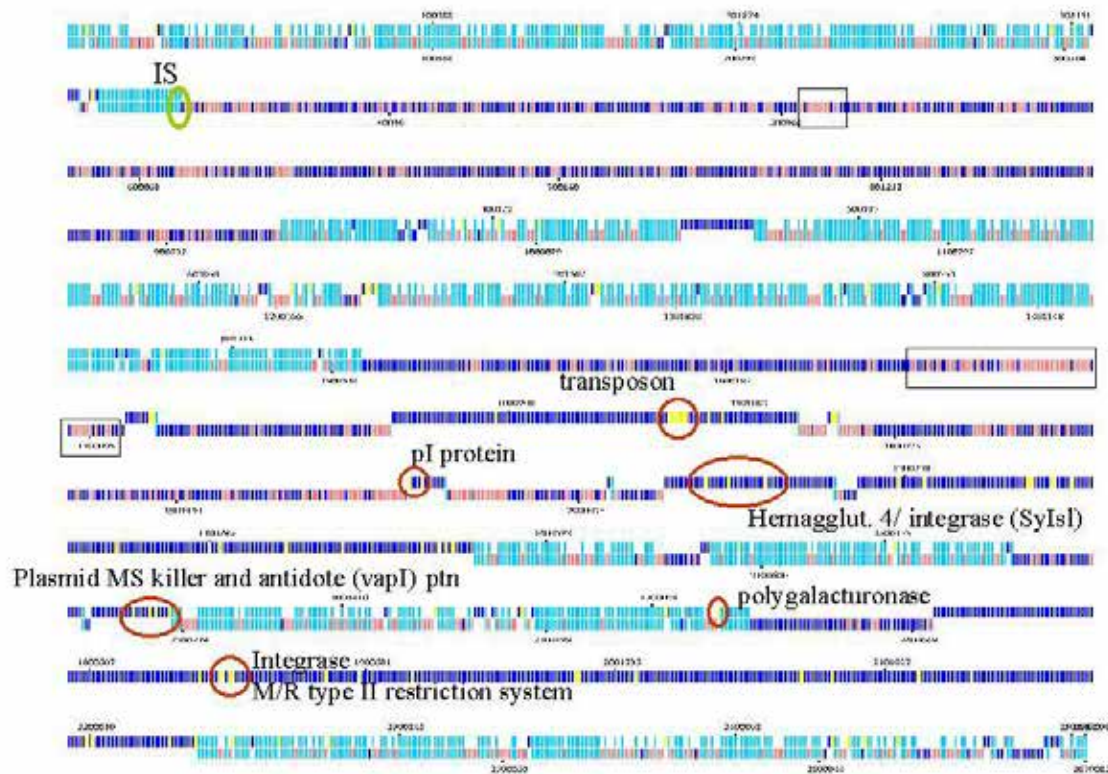


Figure 1. Chromosome alignment between *Xf*-PD Temecula and *Xf*-CVC 9a5c. Light blue dash represents colinear genes and dark blue dash represents rearranged genes in *Xf*-PD and *Xf*-CVC genome while yellow and red dashes represent strain specific genes some of which are highlighted.

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FUNDING AGENCIES

Funding for this project was provided by the USDA Agricultural Research Service, the American Vineyard Foundation, the California Department of Food and Agriculture, the State of Sao Paulo Research Foundation (FAPESP), and the Brazilian National Council of Scientific and Technological Development (CNPq).

BIOLOGICAL, CULTURAL, GENETIC, AND CHEMICAL CONTROL OF PIERCES DISEASE: XYLEM FLUID CHEMISTRY MEDIATION OF RESISTANCE TO PIERCE'S DISEASE

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Reporting Period: The results reported here are from work conducted from October 31, 2001 to November 1, 2002.

INTRODUCTION

The influence of xylem chemistry on the establishment, colonization and movement of *Xylella fastidiosa* (*Xf*) can be tested at various levels. The chemistry of xylem fluid is relatively simple compared to other plant tissues. Xylem fluid consists of over 98% water, and the major chemical entities monomeric (amino acids, organic acids and sugars) and inorganic ions. Few secondary compounds are present in appreciable quantities in xylem fluid, although peroxidases are often detected in low concentration. We feel the contribution of plant nutrient status is an undervalued component of plant resistance.

Some research areas that we hope to make contributions including: 1) to define the chemical basis of establishment, multiplication and spread of *Xf* in grape genotypes and other plant species; 2) the creation the development of a vastly simplified chemically defined medium; 3) the elucidation of factors that promote *Xf* aggregation and biofilm formation; 4) the determination of the antibacterial properties of lytic peptides; 5) the determination of the presence of peroxidases in xylem fluid and to establish the promotion/inhibition of *Xf in vitro*.

OBJECTIVES

The all-encompassing objective was to establish the role of xylem chemistry on resistance/susceptibility of *Vitis* genotypes to *Xylella fastidiosa* (*Xf*) and Pierce's disease (PD). The objectives are to determine:

1. the resistance of 10 grape genotypes to PD after mechanical inoculation with *Xf* and discern the relationship between chemical profiles of xylem fluid and resistance. (Dr. Andrew Walker, cooperator);
2. the mechanism of resistance to PD of host plants that are common in riparian habitats in California. (Dr. Alexander Purcell, cooperator);
3. validate the influence of specific chemical profiles on the growth and survival of *Xf* by tests *in-vitro* culture;
4. the naturally occurring antimicrobial peptides in *in vitro* experiments for efficacy against *Xf* and study the stability of these peptide compounds in buffer and in xylem fluid, and;
5. the concentrations of peroxidases in xylem fluid of 10 grape genotypes.

RESULTS AND CONCLUSIONS

In 2002, we have analyzed the xylem chemistry of 10 *Vitis* genotypes that expressed differential rates of *Xf* susceptibility. The primary organic compounds (amino acids, organic acids and sugars) and inorganic ions were genotype dependent. Xylem chemistry was influenced by geographic location (California and Florida) and season of the year (dormant and growing season). Very unbalanced chemical profiles occurred. For example, in one study the concentration of glutamine varied between 46% (Chardonnay) and 70% (Dogridge) of the total amino acids in xylem fluid. Chemical profiles among *Vitis* genotypes varied greatly when xylem fluid was collected during the dormant season. Biofilm formation is considered an important component of colony formation of *Xf* and is likely involved in pathogenesis. The relationships of chemical profiles and specific chemical entities to *Xf* colonization, spread and biofilm formation are being investigated. (Biofilm was quantified *in vitro* by the crystal violet/ethanol elution method).

A short-term exposure to xylem fluid from grapevine genotypes caused the development of differential colony numbers and colony size of *Xf* UCLA PD strain when grown on agar culture. In most cases the effect of xylem fluid on colony number was not greatly altered by increasing incubation time from 1 to 24 hours suggesting that the effects on *Xf* are rapid. Anomalous results were obtained showing that colony number decreased with exposure to xylem fluid from PD-susceptible genotypes of *V. vinifera* (Chenin blanc and Chardonnay); however these genotypes formed significantly larger colonies than PD-resistant genotypes. The formation of large colonies may be critical to expression of *Xf* virulence *in-planta*, in that *Xf* may typically survive and persist in PD-resistant *Vitis* genotypes; colonies simply do not form that adhere to xylem walls and occlude vessels. We investigated this phenomenon again in an effort to quantify biofilm formation using *Xf* UCLA and STL strains in liquid culture. *Xf* was incubated for 96 hours in xylem fluid of *V. rotundifolia* Noble and *V. vinifera* Chardonnay. Xylem fluid was collected from dormant, field-grown and screen house grown vines. *Xf* strain and xylem fluid treatment had a highly significant effect on subsequent colony numbers. Biofilm formation varied greatly with xylem fluid treatment. The highest amount of biofilm and the highest ratio of biofilm to colony numbers in solution occurred for *V. vinifera* Chardonnay. These data taken collectively show that the chemistry of xylem fluid can have a profound effect on *Xf* colony number and biofilm formation, and the highest tendency toward biofilm formation occurred for PD-susceptible species.

We examined xylem chemistry throughout the year on a large variety of alternative (non-*Vitis*) host plants and compared these to rates of *Xf* infection. The best statistical correlation was found for percentage of plants infected and the concentration of total amino acids when sampled during the dormant season. This will further our knowledge of resistance mechanisms are the same for other host species as for *Vitis*, and to further our knowledge of alternative hosts that may be important in the spread of *Xf*.

In 2002, we completed the formulated of new chemically-defined media for *Xf*. Several aspects contributed to the completion of this phase. Several alternative methodologies were implemented to assure the complete evaluation of these new formulated diets. New media were evaluated on the basis of agar culture, liquid culture and biofilm formation. The most simple medium that was successful consisted of 4 organic compounds and 3 inorganic salts. Other chemically defined media were based on the chemistry of Chardonnay (a susceptible grape genotype to *Xf*). The performance of *Xf* in different media was dependent on the strain, the media composition and the strain X media interactions. These results support the contention that xylem chemistry may be critical in determining pathogenesis.

The antimicrobial activity of naturally occurring lytic peptides (cecropin A, cecropin B, magainin I, magainin II, indolicidin, lysozyme) has been investigated. The cecropins were the most lethal to *Xf*. The minimum inhibitory concentration for 100% *Xf* mortality in PW⁺ medium was as follows: cecropin A 1 μ M, cecropin B 1 μ M, indolicidin 10 μ M, magainin II 80 μ M, magainin I 80 μ M, tetracycline 100 μ M, lysozyme > 1000 μ M. The persistence of lytic peptides in xylem fluid *V. rotundifolia* was investigated. Xylem fluid plus cecropin A (10 or 20 μ M) and cecropin B (2, 10, 20 μ M) resulted in 100% *Xf* (UCLA strain) mortality for 5 hours or less of incubation. Xylem fluid of *V. rotundifolia* Noble and *V. vinifera* Chardonnay incubated with *Xf* UCLA strain plus cecropin B (1 μ M) resulted in high colony counts and low biofilm production for Noble, but low colony counts and high biofilm production for Chardonnay. A timecourse of cecropin B (1, 10, 50 and 100 μ M) activity in xylem fluid of *V. rotundifolia* Noble and *V. vinifera* Chardonnay was followed by SDS Page gel electrophoresis. Cecropin B showed reduced activity from 1 to 96 hours, although at a concentration of 100 μ M the cecropin B band did not disappear entirely. The cecropin bands disappeared at lower concentrations of cecropin B indicating a loss of stability probably as a result of proteolytic breakdown. Lytic peptides may eventually be incorporated in control strategies for *Xf* via genetic engineering or direct application of compounds into xylem fluid.

Proteins and specifically peroxidases were detected in low concentrations in xylem fluid of all 10 grape genotypes. Protein content and total peroxidase activities varied with genotype. SDS-PAGE gels of peroxidases from the 10 grape genotypes show a different banding pattern and banding intensities indicating that different isozyme exist in different genotypes and also that concentrations vary with genotype. There is ample justification for continuing this work as we feel that proteins, and specifically peroxidase activity is important to redox reactions at the xylem vessel/bacterium interface, and as such may be involved in some of the earlier responses of plants to PD-infection. In addition, we feel that enzymes are involved in the proteolytic breakdown of lytic peptides.

In conclusion, we have found that xylem fluid chemistry varies greatly with genotype, location (i.e. edaphic conditions), and season of the year. Even a short-term exposure to *Xf* to different growth media altered *Xf* colony number. Exposure of *Xf* to different xylem fluid or to chemically defined media can also induce biofilm formation. The development of a 4 organic compound based chemically defined media will now allow us to delineate the role of specific compounds in *Xf* colony formation and biofilm formation. The biological parameters of naturally occurring lytic peptides have been quantified. We have detected the presence of proteins (in very low concentration) in xylem fluid of all grape genotypes. We have also quantified peroxidase isoenzymes in xylem fluid of all genotypes by gel electrophoresis. The role of peroxidase in early stages of *Xf* colonization *in planta* needs to be evaluated.

FUNDING AGENCIES

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GENOME-WIDE IDENTIFICATION OF RAPIDLY EVOLVING GENES IN *XYLELLA FASTIDIOSA*: KEY ELEMENTS IN THE SYSTEMATIC IDENTIFICATION OF HOST STRAINS, AND IN THE SEARCH FOR PLANT-HOST PATHOGENICITY CANDIDATE GENES

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Reporting Period: The results reported here are from work conducted from September 1, 2002 (start of grant) through November 1, 2002.

INTRODUCTION

Xylella fastidiosa is not only a bacterium that causes Pierce's disease (PD) of grapevines. It also has a number of genetically distinct host strains (see Hopkins 1989). These strains show varying levels of cross-host pathogenicity. For example, PD strains do not infect peach, and phony peach disease strains do not infect grapevines. Similarly, the strains causing oleander leaf scorch (OLS) do not cause PD in grapevines or cause infectious symptoms in a number of other species (Purcell et al. 1999). The lack of cross-host infection means that genetic differences among the strains must encode the causes of host-specific pathogenicity. Identifying the genes responsible for host-specific effects is an important step in understanding how infection might be controlled.

The genetic differences determining host-specific adaptations are probably only a small fraction of the total genetic differences between the strains. To facilitate identifying host-specificity candidate genes, we need some initial filter that selects those genes most likely to be involved in host adaptation from among the approximately 2700 genes carried by *X. fastidiosa*. To this end, genomic research provides us with some extraordinarily powerful new tools for solving this kind of applied problem.

It is self-evident that adaptive evolution depends upon changes in specific genes. In some cases, a single base substitution in a gene may be sufficient; however, such simple changes recur repeatedly in bacterial populations. The apparent separation of *X. fastidiosa* into stable host strains suggests host adaptations involve more complex changes. For this reason, we believe that the genes involved in host adaptation will be among those exhibiting the most rapid evolutionary change.

OBJECTIVES

The identification of the rapidly evolving genes in the *Xylella fastidiosa* genome. This is the first step towards achieving our four primary objectives. These are:

1. Develop a systematic multigenic method for identifying host strains of *X. fastidiosa*. Our objective is to develop a method that unambiguously identifies the known host strains, and that allows researchers to efficiently recognize the invasion of new strains.
2. Identify plant-host specificity candidate genes. We will use our database of rapidly evolving proteins to test for evidence of strong natural selection and for statistical links between the rapid genetic divergence of host strains and specific biochemical functions.
3. Measurement of clonal variation within host strains. Our objective is to assess within-strain genetic variability at rapidly evolving gene loci and to use these results to assess the evidence that all members of a given host strain share common ancestry.
4. Estimate the frequency of recombination. Our objective is to look for evidence of both within- and between-strain genetic transfer. Genetic transfer can dramatically increase the rate of evolution, and potentially can increase the rate at which new host strains arise.

RESULTS AND CONCLUSIONS

There are estimated to be about 2700 genes in the genome of the CVC (citrus) strain of *X. fastidiosa*; however, many of these genes are of unknown function. In the first phase of this project, we have screened just over 1000 genes of known function from the genomes of the OLS (oleander), ALS (almond) and CVC (citrus) strains. Using a modified relative rate test (with CVC as the outgroup), we have examined the evolutionary pattern of these genes in the OLS and ALS lineage.

Results so far suggest that the rate of gene evolution is generally higher in the OLS strain, with 15% of the genes showing significantly higher rates of change in OLS and 5% in the ALS strain. Since this includes synonymous (silent) and non-synonymous (replacement) base pair substitutions, it is possible (and probable) that the bias is due to a shorter generation time in the OLS strain. On the other hand, we have found a slight trend for faster protein evolution in the ALS strain. After correcting for the underlying rate differences, we find that almost exactly 10% of the 1070 genes screened show unequal rates of protein evolution between OLS and ALS. Of these 106 genes, 75% show faster evolution in ALS, and 25% in OLS. These genes are the initial candidates for being involved in host adaptation. However, since we have done 1070 tests, we can expect about 53 type 1 statistical errors (i.e. 5% of 1070), so that a conservative estimate is that only about 50% of our candidate genes are "real". However, even with this correction, it appears that more than 50 genes in our sample are likely to be involved in adaptive differences between the OLS and ALS strains.

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FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

FATE OF *XYLELLA FASTIDIOSA* IN ALTERNATE HOSTS

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Reporting Period: The results reported here are from work conducted from November 2001 through October 2002.

INTRODUCTION

This project investigated the fate of the Pierce's disease bacterium *Xylella fastidiosa* (*Xf*) in alternate hosts from which sharpshooters might acquire *Xf* by feeding. We identified additional bacterial hosts among vineyard weeds, cover crops, field crops and adjacent vegetation common to vineyards in California's San Joaquin Valley. Field studies conducted at the Kern County Agricultural Center in Bakersfield tested the survival of *Xf* in field conditions for five weed and cover crop species known to be systemic hosts of *Xf*.

The rapid and striking emergence of Pierce's disease of grape in the General Beale Road project area in Bakersfield during summer 2001 showed the damage that can be done by the glassy-winged sharpshooter (GWSS). Identification and eradication of plants that are bacterial hosts is important where the insect vector has large populations and feeds on many different plants. *Xf* survives and multiplies in an unusually large number of plants (Freitag 1951; Hopkins 1988), and sharpshooters collected distant from agricultural habitats can be infectious with *Xf* (Freitag and Frazier 1954). Previous studies of *Xf* in four plant species established that *Xf* multiplies in plants at the inoculation site but moves systemically within the plant in only some plant species (Hill and Purcell 1995b). Lab and field studies of *Xf* in 33 species of riparian plants commonly found in Napa Valley revealed that most plants were propagative but non-systemic hosts of the bacterium and suggested that *Xylella* eventually disappears from non-systemic hosts (Purcell and Saunders 1999).

Research during 2000 and 2001 (funded by Kern-Tulare Glassy-winged Sharpshooter-Pierce's Disease Task Force), identified 7 species of weeds as systemic high- and mid-population hosts of *Xf* and that 12 other weed species were infrequently infected, supported low *Xf* populations, or had limited bacterial movement beyond the site of insect feeding. We tested 13 additional plant species as hosts of *Xf* this year. Recent studies of the effects of temperature on *Xf* growth in culture or in grapevines indicated that *Xf* slowly dies instead of multiplying at temperatures below 10°C or above 34°C (Feil and Purcell 2001). To determine how well field plants support the growth of *Xf* during winter and summer, we followed the population changes of *Xf* in systemic weed or cover crop hosts of *Xf* grown in Kern County in a protective cage (to exclude vector transmission) in two cool season and two warm season trials.

OBJECTIVES

Evaluate the fate of *Xylella fastidiosa* in Central Valley weeds.

RESULTS AND CONCLUSIONS

We continued investigations into the fate of *Xf* in 13 previously untested species of weeds, field and cover crops, and vegetation commonly found adjacent to San Joaquin valley vineyards. We inoculated plants with blue-green sharpshooters (BGSS) or mechanically, and tested for the presence of *Xf* at 1, 3 and 9 weeks after inoculation. Culture on semi-selective medium (PWG) estimated bacterial populations (\log_{10} colony-forming-units [cfu] per gram) and systemic movement of the bacteria throughout the plant beyond the inoculation site.

Recent tests showed that 'Ace' tomato, 'Violeta lunga' eggplant, black nightshade and red gum (mechanical inoculation only) consistently developed *Xf* infections with populations over $\log_{10}6$ /cfu/g. Quinoa, field bindweed, yellow nutsedge, and blue gum had high *Xf* populations (between $\log_{10}5$ and $\log_{10}7$) in plant tissue at the inoculation site but rarely developed systemic infections in the greenhouse. Plants with fewer than 10% of their sites infected, or that supported populations at or below $\log_{10}3$ cfu/g were: johnsongrass, jojoba, prostrate pigweed, annual sowthistle, southwestern cupgrass, whitestem filaree, and watergrass. These plants also had systemic infections at less than 10% of their inoculation sites. Three species of recently tested weeds: cheeseweed, sacred datura, and red gum, frequently developed infections when mechanically inoculated but not when inoculated by insects. Jojoba developed infections after exposure to BGSS but not after needle inoculation. Systemic populations of *Xf* over \log_5 and especially over \log_6 are most likely to be significant sources of *Xf* for sharpshooters that feed on them, but this needs to be tested for representative weed species.

Bacterial survival in field conditions was tested with five species of common vineyard weeds that previously had been identified as systemic hosts of *Xf*. Cocklebur, wild sunflower, and prickly lettuce were tested from July to November 2002. Prickly lettuce, poison hemlock, and 'Aquadulce' fava bean (used in cover crops) were grown from November 2001 to

March 2002. After inoculation in the greenhouse, half the plants remained in the greenhouse, and half were planted in a vector-proof cage outside at the Kern County Cooperative Extension office. We sampled plants by culturing 1, 3, and 9 weeks after inoculation to estimate bacterial populations and systemic movement.

We recovered *Xf* less frequently and in lower populations from field-grown plants when compared to greenhouse-grown plants during the first three weeks. In all four trials comparing *Xf* infections in the field and greenhouse, fewer infections became established in field-grown plants, bacterial populations were lower, and fewer infections moved systemically beyond the inoculation site to colonize the entire plant. For tests done in cool weather from November 2001 to March 2002, *Xf* was recovered from 26% of field-grown plants (31 of 134) and 46% of greenhouse-grown plants (50 of 109 inoculation sites). For tests with summer weeds from July to November 2002, we recovered *Xf* from 35% of greenhouse-grown weeds (56 of 158 sites) and 21% (27 of 127) of field-grown weeds. Field-grown plants also had fewer systemic infections and lower bacterial populations. We conclude that *Xf* multiplies and survives more poorly under field conditions than in ideal growth temperatures (26-29 °C) maintained in greenhouse studies. This confirms our initial assumption that lab tests were more suitable for initial screens of plants' abilities to support the multiplication of *Xf*, and is consistent with our predictions that *Xf* would grow more slowly under fluctuating field temperatures that exceed or fall below permissive growth temperatures.

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FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.

TRAP CROPS FOR REDUCING SPREAD OF PIERCE'S DISEASE

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Reporting Period: The results reported here are from work conducted from May 1999 to September 2002.

INTRODUCTION

Where the blue-green sharpshooter (BGSS), *Graphocephala atropunctata*, is the primary vector of *Xylella fastidiosa* (Xf), Pierce's disease (PD) generally occurs near the edges of vineyards. The distribution of diseased vines matches the springtime movement of sharpshooters from overwintering habitats into vineyards (Varela, Smith and Philips 2001). Attempts to manage PD often include late winter or early spring insecticide applications to the edge of the overwintering habitat in order to limit the springtime movement of sharpshooters. This practice may reduce PD incidence, but often fails due to limitations in available insecticides, difficulty in timing sprays to coincide with vector movement, differences in PD susceptibility due to variety (Purcell 1979) or vine age, and regulatory issues that prevent treatment of the overwintering habitat.

Another possible control strategy is growing a buffer of plants that are not damaged by Xf and treating them with persistent, systemic insecticides to impede the movement of BGSS vectors into the vineyard.

We initiated this project in 1999 to examine the effects of an insecticide-treated grapevine trap crop on the incidence of PD. We selected St. George rootstock (*Vitis rupestris*) for the trap crop because it buds out early in the spring, is attractive to BGSS and is not killed by PD. We planned to treat trap crop vines during fall or winter with soil-applied imidacloprid (Admire, Bayer Corporation). At the time, we believed that adult BGSSs feeding on treated trap crop vines would quickly acquire a lethal dose of imidacloprid.

OBJECTIVES

1. Determine if insecticide treated trap crops at the ends of rows can reduce the incidence of PD.

RESULTS AND CONCLUSIONS

We established two trap crop trials in 1999 on opposite sides of a large vineyard. Each side is bordered by riparian habitat and has a history of PD. One trial borders the Napa River, the other Milliken Creek. The entire vineyard was planted in 1999, so our trap crop vines developed at a similar pace to the producing vines.

In each trial, there are three replications of trap crop plantings and controls. Vine spacing is 9 feet between rows and 5 feet between vines. St. George vines are planted at the ends of adjacent rows to create the trap crop treatments. Each replicate trap crop planting includes the first 6 vines in 12 adjacent rows (approximately 30 feet deep by 108 feet wide). In the control treatments, Chardonnay or Pinot Noir vines extend to the end of the rows. Trap crop vines have been trained up into the trellis to produce large "hedges" in the vine row.

We selected St. George for the trap crop plantings in part because we believed it would begin to grow earlier in the spring than the rest of the vineyard. If it did, it would be a more effective trap crop because BGSS would likely move to it to feed while the other vines were still dormant or just budding out. In these trials, this has not been the case. The St. George vines initiate growth at about the same time as the rest of the vineyard, which is planted to Chardonnay and Pinot Noir – both early-growing varieties. The entire vineyard is pruned early each winter (December), which further hastens budbreak.

Admire was applied to trap crop vines in early November 2000. Subsequent studies showed that BGSS do not readily acquire lethal doses of imidacloprid from treated vines. In cage studies (Purcell 2000, unpublished), BGSS greatly reduced their feeding rate and lived for a considerable time period. There was no rapid kill. Therefore, our treated trap crops were not likely to kill insects landing upon them. We hypothesized that Admire-treated trap crops could actually increase dispersion of BGSS if after initial probing they flew away from that vine rather than continuing to feed. We therefore abandoned plans for further Admire treatments. In 2001 and 2002, trap crops vines were sprayed with imidacloprid (Provado, Bayer Corporation) in April and May, respectively. We recognize that foliar treatments are likely to have limited

effectiveness for a trap crop program because they are not fully systemic and actively growing vines will have untreated tissue much of the time.

BGSS activity was monitored from 2000-2002 using yellow sticky cards placed between the riparian habitat and the end vine of the vineyard. Two cards were used in each replicate and were monitored weekly from March-October. BGSS were present in all replicates each year.

PD incidence was determined by visual assessment in September or October 2000-2002. The first 30-40 vines in each row were rated on a 1-3 scale, with 3 being the highest severity. In control treatments, diseased vines in the first six positions are not considered, as these positions correlate to the trap crop vines. In the Milliken Creek trial, only 1 vine has displayed PD symptoms to date. In the Napa River trial, PD has appeared only in the third replicate, in both control and trap crop treatments. There were more PD vines in the control treatment (Table 1), however, that plot also had more BGSS, as determined by trap catches (Table 2).

Table 1: PD vines in Napa River trial (rep 3)

	2000	2001	2002
Trap Crop	0	1	6
Control	0	3	11

Table 2: BGSS trap counts* in Napa River trial (rep 3)

	2000	2001	2002
Trap Crop	3	6	1
Control	14	23	5

* March - June

Due to the small number of diseased vines, no conclusions can be drawn regarding the effectiveness of treated trap crops at this time. However, the lack of an effective systemic insecticide that will kill BGSS immediately upon feeding makes the outlook for this being a successful control strategy much less likely.

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FUNDING AGENCIES

Funding for this project was provided by the American Vineyard Foundation (2000-2001) and the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board (2002).

BIOLOGICAL, CULTURAL, GENETIC, AND CHEMICAL CONTROL OF PIERCES DISEASE: SIGNIFICANCE OF RIPARIAN PLANTS IN THE EPIDEMIOLOGY OF PIERCE'S DISEASE

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Reporting Period: The results reported are from work conducted from April 1, 2001 to March 31, 2002

INTRODUCTION

Past research (Purcell, 1976, 1981) has demonstrated the direct relationship between incidence of Pierce's disease (PD) in grapevines and proximity to riparian plants bordering vineyards in the North Coastal grape-growing region of California. Vineyard rows closest to riparian plants experience the heaviest losses, but the concentration of diseased vines decreases with increasing distance away from riparian plants. Riparian habitats adjacent to vineyards contain host plants that serve as feeding and breeding hosts for *Graphocephala atropunctata* (blue-green sharpshooter, BGSS), the most efficient vector of PD in the Napa Valley (Hewitt et al. 1949; Purcell 1975). Not only do many riparian plant species provide habitat for BGSS, but some also serve as reservoir hosts of the causal agent of PD, *Xylella fastidiosa* (Xf) (Freitag 1951). A variety of common riparian plants, including native and non-native trees, shrubs, and herbaceous annuals, are capable of maintaining Xf infections without expressing disease symptoms. The ability of Xf to multiply and spread within a plant host, once it has been infected, varies from species to species. The efficiency of Xf acquisition and transmission by vectors is influenced by the concentration of Xf in the plant host during feeding; the higher the concentration of Xf in a host plant, the higher the probability of BGSS acquiring Xf (Hill and Purcell 1997). Purcell and Saunders (1999) found that Xf populations are, generally, lower in riparian hosts than in grape. After screening several breeding hosts of BGSS for systemic movement of Xf, Hill and Purcell (1995) found that only two, *Rubus discolor* (Himalayan blackberry) and *Vitis vinifera* (grapevine), supported systemic Xf populations. These results imply that some riparian plant species are likely more important than others as reservoirs for the spread of Xf to grapevines.

A replicated field experiment was initiated at three commercial vineyards in Napa County, CA, to measure Xf populations in five riparian plant species: *Vitis californica* (California grape), *Rubus ursinus* (California blackberry), *Rubus discolor* (Himalayan blackberry), *Sambucus mexicana* (blue elderberry), and *Vinca major* (periwinkle). All five species are breeding hosts of BGSS and systemic hosts of Xf (Purcell and Saunders 1999). Xf could potentially overwinter in systemic hosts. Overwintering hosts of Xf likely play an important role in the epidemiology of PD in providing a source of bacteria for spring infections, especially near vineyards where infective adult BGSS do not survive the winter. BGSS transmission of Xf from riparian plants to grapevines in spring is more likely than mid- or late-season infections to result in chronic disease (Purcell 1981). By measuring seasonal populations of Xf in riparian plants adjacent to vineyards, we will determine if and when concentrations are high enough for acquisition by BGSS.

OBJECTIVES

Determine the epidemiological role of seasonal fluctuations of *Xylella fastidiosa* populations in riparian host plants of North Coastal California.

RESULTS AND CONCLUSIONS

Populations of Xf reached detectable levels in California blackberry, blue elderberry, and California grape by mid summer and increased by early fall (Table 1). Xf was not detected in periwinkle until early fall, when populations were found to be as high as that of California blackberry, blue elderberry, and California grape (10^5 - 10^6 CFU/g of petiole tissue). Xf populations of at least 10^4 - 10^5 CFU/g of plant tissue are required for acquisition by BGSS (Hill and Purcell 1997). Estimated Xf populations in California blackberry and California grape in mid summer and blue elderberry and periwinkle in early fall are sufficient for acquisition by BGSS. Our two culture attempts coincided with the emergence and increased flight activity of young adult BGSS, which peaks in mid summer and remains high through early fall (Feil et al. 2000). Assuming BGSS feeds on California blackberry, California grape, blue elderberry, and periwinkle in early fall, Xf may be transmitted from infected riparian plants to adjacent vineyards before the end of the growing season. Late season infections of grapevines are unlikely to result in chronic disease and infected canes are pruned out during the winter (Purcell 1981). However, young

adult BGSSs that acquire *Xf* in mid summer to early fall and survive the winter are still capable of transmitting *Xf* the following spring after budbreak.

Our inoculations resulted in a lower then expected number of infected plants. Past research (Hill and Purcell 1995, 1997; Purcell and Saunders 1999) on populations of *Xf* in four of the five riparian species we inoculated showed higher inoculation success (higher number of plants that developed infections out of total plants inoculated). Differences in inoculation method (insect versus mechanical), *Xf* strain (YVPD versus STL), and/or environment (greenhouse versus field) may explain differences in inoculation success.

None of the inoculated Himalayan blackberry individuals developed infections. Insect inoculation of Himalayan blackberry with the YVPD strain of *Xf* in the greenhouse showed that *Xf* populations can reach 10^7 CFU/g of plant tissue at 32 days after inoculation (Hill and Purcell 1995, 1997). Again, this difference may be due to our inoculation method, the strain of *Xf* we used, and/or the fact that our experiment was carried out in the field.

Table 1. Culture of *Xylella fastidiosa* from riparian plants in the field following mechanical inoculation.

Species	Number Inoculated ^a	Number Infected	Number Not infected	Number Contaminated	CFU/g ^b	Incubation (days) ^c
Himalayan blackberry	29	0	11	18	0	41-54
Himalayan blackberry	26	0	4	22	0	119-124
California blackberry	35	2	11	22	log 3-4	41-54
California blackberry	35	8	5	22	log 4-6	119-124
Blue elderberry	30	1	11	18	log 2	41-54
Blue elderberry	18	1	1	16	log 6	119-124
Periwinkle	31	0	8	23	0	41-54
Periwinkle	30	1	2	27	log 5	119-124
California grape	27	2	11	14	log 2-5	41-54
California grape	25	1	3	21	log 6	119-124

^a Plants were mechanically inoculated with STL strain of *X. fastidiosa* on 7, 13, and 18 June 2001.

^b Colony forming units per gram of plant tissue (log scale).

^c Number of days between inoculation and two culture attempts. The first culture attempt was at 41 to 54 days after inoculation (July 24 to August 8, 2001). The second attempt was from the same plants, but from different petioles, at 119-124 days after inoculation (October 9 to October 15, 2001).

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FUNDING AGENCIES

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BREEDING PIERCE'S DISEASE RESISTANT TABLE AND RAISIN GRAPES

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Reporting Period: The results reported here are from work conducted from December 31, 2001 to November 1, 2002.

INTRODUCTION

This project 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. The project also integrates efforts to develop genetic maps for resistance to *Xylella fastidiosa* (Xf) in segregating populations containing resistance from *Muscadinia rotundifolia* and from southeastern US (SEUS) *Vitis* species. The preliminary goal of these mapping efforts is the development of strongly linked DNA markers to expedite breeding and the eventual goal is characterization and location of Xf resistance genes leading to genetic transformation efforts.

OBJECTIVES

1. Develop PD 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

Greenhouse Evaluations of Xf resistance:

There are now 60 PD resistant cultivars from the southeastern US (SEUS) at UCD and 13 at the USDA-Fresno. We tested and added 10 new accessions this year after finding them to be highly resistant to Xf. Sixty-five F1 progeny from SEUS resistance sources by Ramming advanced seedless *vinifera* were screened. Seven of these seedlings were backcrossed to advanced seedless table grapes in 2002. This winter we will be testing SEUS germplasm from Fresno and include promising selections from their disease resistant table grape program (focused on powdery mildew) with potentially PD resistant parentage.

During summer 2001, replicated cuttings from 130 seedlings were made from a 2000 cross of a female Xf resistant *V. rupestris* x *M. rotundifolia* (8909-15) x B90-116 (Ramming advanced seedless selection) – population 0023. This population is being used for the mapping of both genetic markers and phenotypic traits. The most resistant of these F1 progeny were used in the crosses this year described below.

2002 Crosses:

UCD – Twelve crosses were made with 4 F1 *V. rupestris* x *M. rotundifolia* (rup-rot) selections with excellent Xf resistance by 5 USDA table and raisin parents to produce 1,613 seeds. Twenty-five crosses were made using 10 SEUS (5 newly evaluated) Xf resistant parents x advanced Xf resistant and *vinifera* parents to produce 33,306 seeds. Two other crosses were made to expand the two mapping populations that are based on a seedless female parent – one from a SEUS resistance source (5025), and the other on rup-rot (5014). Embryo rescue techniques at Fresno obtained 92 and 68 ovules respectively.

USDA-Fresno – Crosses were made to seedless *vinifera* parents in 2002. Twenty-nine crosses were made with 19 advanced seedless table and raisin grape selections using 16 Xf resistance sources, most of which were F1 selections from advanced seedless *vinifera* x rup-rot selections. 6,171 ovules were extracted from these crosses and 1,198 embryos are establishing. This material will make great advances towards commercial quality.

2002 Plantings and Evaluations:

UCD – 2,145 seedlings were planted from crosses made in 2001. These seedlings were produced by crossing 6 SEUS resistance sources with 9 advanced USDA seedless table and raisin grape selections. Based on the early planting date, excellent growth (virtually all have produced a short 75 cm cordon on the wire), and the success we had this year pushing the 2000 seedlings, we expect many of these to flower in 2003.

About 20% of the 1,150 seedlings from SEUS resistance sources produced from the 2000 crosses and planted in 2001 were evaluated for fruit quality and 15 with good quality were greenhouse screened for PD resistance. The best of these will be crossed to advanced *vinifera* selections in 2003. The quality of the 2000 seedlings was better than expected. Some of the seedlings have fruit that is partially seedless, with firm flesh, and markedly improved berry size and skin thickness.

USDA-Fresno:

In 2001, 14 crosses were made with 3 seeded female parents (2 *vinifera* and 1 SEUS PD resistant) by 10 males (most SEUS PD resistant selections). Fifty-eight seedlings were planted. The majority of the 2001 crosses were to seedless female parents and the progeny were embryo rescued. There were 27 crosses with 9 advanced seedless females x 17 different sources of PD resistance from the SEUS. About 3,345 ovules were cultured, 1,220 embryos were rescued and 415 seedlings were planted in the field. These progeny are expected to start blooming in 2003 and should make excellent progress towards our goal of PD resistance in a high quality *vinifera* table and raisin grape background.

PD Field Trial:

In 2001, we established a replicated field trial at a PD infected vineyard in Yountville using 13 SEUS PD resistant selections and 9 resistant and 7 susceptible rup-rot selections. Each plant was inoculated in May and June 2002 by needle inoculation. The SEUS selections were chosen because most displayed severe PD symptoms after greenhouse testing, although they are considered highly resistant in the SEUS. Observations of leaf scorch, cane lignification and impact on vigor were made in Fall 2002. The observed range of responses correlated well with *Xf* titer results from previous greenhouse testing. The plants will be evaluated Spring 2003, to determine whether *Xf* is lost over winter due to lack of downward spread followed by pruning, and to determine the extent of PD symptoms.

FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board, the USDA Animal and Plant Health Inspection Service, the California Raisin Marketing Board, and the California Table Grape Commission.



*Epidemiology
of
Pierce's
Disease*



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

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Reporting Period: The results reported here are from work conducted from November 30, 2001 to October 30, 2002.

INTRODUCTION

Current attempts to reduce the economic impact of the glassy-winged sharpshooter, *Homalodisca coagulata*, (GWSS) to important crops in California have focused on GWSS or the causal pathogen, *Xylella fastidiosa*, (*Xf*), but little attention has been given to an essential step of *Xf*-diseases: the transmission of the pathogen by its insect vector. *Xf* has been causing diseases in California for a long time, but GWSS is apparently a more effective vector than other sharpshooters previously involved in California. Our objectives are to characterize *Xf* transmission by GWSS to grapevines, since this is a vital but understudied area of PD. Because of space limits, this report emphasizes objective #1.

OBJECTIVES

1. Characterize the transmission of *Xylella fastidiosa* to grapes by the glassy-winged sharpshooter.
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.

RESULTS AND CONCLUSIONS

GWSS transmission of *Xf* to grapes:

The basic characteristics for *Xf* transmission determined for other vectors were also found for GWSS. Transmission i) occurred without latent period, ii) was persistent over time, iii) unless molting occurred (no transtadial transmission), iv) nymphs and adults were vectors. GWSS transmitted *Xf* to 2-year-old woody tissue of grapevine cuttings with similar efficiency as that to green shoots. Transmission by nymphs had efficiency of approximately 70% (2 days inoculation access period - IAP). Inoculation efficiency increased with longer IAP, but even with 96h IAP efficiency was approximately 35%, a value lower than that obtained for an efficient *Xf* vector, the blue green sharpshooter (BGSS, *Graphocephala atropunctata*). Acquisition efficiency did not increase with longer acquisition access periods (AAP) after 6 hours. Overall transmission efficiency was 15-20% per insect per day, with large variability in transmission rates among experimental repetitions. Comparable transmission efficiency by BGSS is over 90% (Hill and Purcell 1997). Using the culture detection method for *Xf*, we found no association between *Xf* detection in the heads of GWSS and individual transmission of the pathogen to plants, similar to the findings of Hill and Purcell (1997) for BGSS. Further studies using other detection methods based on PCR may prove to be better predictors of vector infectiousness. In general, GWSS transmission of *Xf* had the same characteristics observed for other vector species, but had much lower and more variable transmission efficiency among experiments. GWSS inoculation of 2-year-old wood of grapevines in the lab suggested that summer and fall inoculations in the field may occur, and that these infections may become chronic disease because plant tissues where inoculation occurred will not be removed during regular winter pruning.

GWSS transmission of *Xf* to dormant grapes in the field:

Because GWSS has been found to feed on dormant vines during the winter, we tested the possibility of GWSS inoculating *Xf* into dormant vines in the field. We previously reported that GWSS transmitted *Xf* to dormant grapes under laboratory. Our field experiment with dormant plants was done in a screen cage built in Bakersfield, Kern Co. Grape 'Pinot noir' cuttings were planted within the cage in September 2001, and standard cultural practices used for the plants. In February 2002, 3 sets of inoculations were done with groups of GWSS taken on plants to Bakersfield. Briefly, adults had 4 d AAP on source plant,; we later transferred these GWSS (groups of 4) in the greenhouse to green seedlings for 4 d IAP, which served as indicators of group infectivity. Plants with insects were taken to Bakersfield and transferred to dormant plants for a 1 week IAP, seedlings returned to Berkeley for symptom development. One inoculation was done in May, as a positive control to test GWSS survival under similar conditions and transmission to green plants growing in the field. We inoculated 64 dormant plants during three dates in February, 13 on May, and left 16 un-inoculated. We verified transmission to dormant plants in the field with efficiency not much lower than that to green seedlings in the greenhouse. No negative control plants were positive for *Xf* or showed any PD symptoms. Survival of insects in the field was high (47-90% for all insects in different dates). Figure 1 summarizes the results obtained.

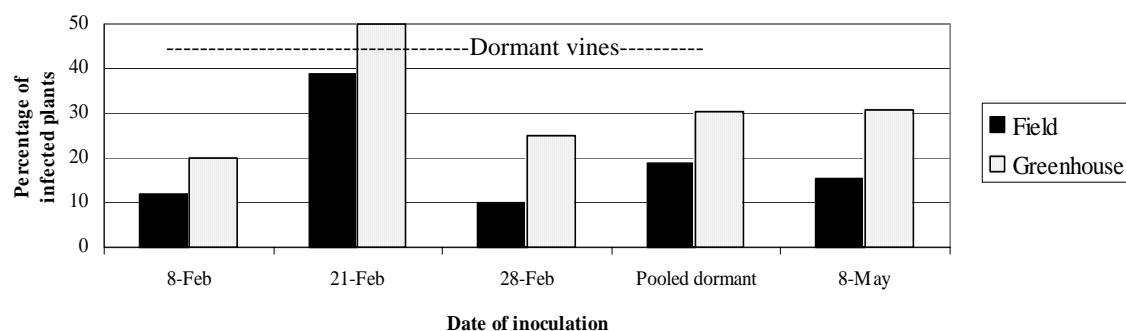


Figure 1. Percentages of dormant (in the field) and green (in the greenhouse) vines infected with *Xf* by GWSS.

Association of *Xf* in vectors' foregut and its transmission to grapes:

We used scanning electron microscopy to observe *Xf* cells attached to the foregut of GWSS and BGSS. We found the expected structures in the pre-cibarium (sensilla, pre-cibarial valve). Even though GWSS has low and variable transmission efficiency (see above), we tested if *Xf* could be found in the foregut of insects that had 4 d AAP followed by ~1 week of incubation period. We found *Xf* cells in only 1 out of 35 insects, and then decided to do a similar test with an efficient vector (BGSS). In this test, 14 BGSS had 4 d AAP on source plants, ~2 weeks on mugwort and 4 d IAP on healthy grape. All insects that transmitted to plants had large amounts of *Xf* in the pre-cibarium. Similar pictures have already been reported (Purcell et al. 1979 and Brlansky et al. 1983). In vitro assays of sharpshooter feeding through a membrane on suspensions of *Xf* in sterile xylem sap revealed that sharpshooters picked up *Xf* in larger numbers than from *Xf*-infected plants but did not subsequently transmit the bacterium to grape. We have isolated numerous bacteria from the surface-sterilized heads of GWSS fed on PD-grape but that failed to transmit *Xf*. Continuing studies will attempt to assess these bacteria as possible antagonists to GWSS transmission of *Xf* to grape. So far have not recovered any of the isolates that we sprayed onto foliage in greenhouse experiments.

Electronic monitoring of BGSS:

Because we found that GWSS general *Xf* characteristics are the same as those for other vector species, and that it has lower and more variable transmission efficiency than BGSS, we have used BGSS as a model vector to start a study on feeding behavior and *Xf* transmission. This work was done in cooperation with Dr. Elaine Backus (University of Missouri, Columbia). Although BGSS probing behavior has already been studied electronically (Crane 1970), we found that waveforms previously observed were not comparable to the system we used, mostly due to technological advances. We characterized various waveforms for BGSS probing behavior, and found that insects feed on xylem and mesophyll (may be phloem too, but data inconclusive).

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FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

MECHANISMS OF PIERCE'S DISEASE TRANSMISSION IN GRAPEVINES: AN ANALYSIS OF THE MOVEMENT OF *XYLELLA FASTIDIOSA* IN XYLEM PATHWAYS

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Reporting Period: The results reported here are from work conducted from June 15, 2002 to October 15, 2002.

INTRODUCTION

Pierce's disease (PD) is a consequence of the spread of xylem-limited bacteria, bacterial products, or plant responses to either leading to blockage of water movement within the grape's hydraulic network (Hopkins and Mollenhauer 1973). The progression of symptoms and movement of PD pathogens from the point of inoculation into the hydraulic network is poorly understood. The development of xylem blockage from the inoculation point to distal or basal organs and the pathways for movement of bacteria within grapevines needs to be determined.

The general vegetative anatomy and the primary vascularization of grapevine have been summarized (Pratt 1974, Mullins et al. 1992, Fournioux 1982) and anatomical symptoms of PD have been documented (Esau 1948, Tyson et al. 1985). Although a general pattern of grapevine hydraulic architecture has been proposed, the vascular arrangement within grapevine must be studied in the context of the spread of PD within the plant from the site of inoculation to a systemic presence. It is unknown whether the mechanisms of pathogenesis of PD are a direct result of xylem blockage by the bacteria (Hopkins 1981), phytotoxins produced by the bacteria (Lee et al. 1982), resultant gums and tyloses produced by the plant (Esau 1948), or a combination of these factors.

OBJECTIVES

Through an analysis of the vascular system of grape shoots correlate the progression of PD from inoculation to infected organs with the movement of *Xylella* bacteria, the development of tyloses and gums, and the loss of water transport.

RESULTS AND CONCLUSIONS

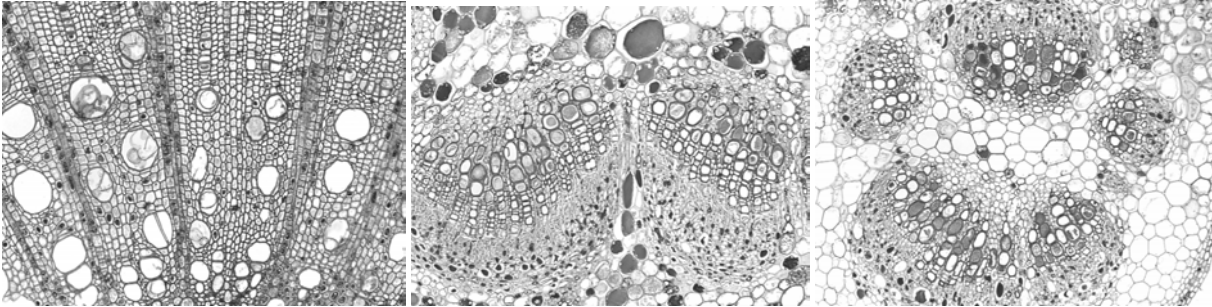
Grapevines were inoculated with *Xylella* in one of the two shoots per plant. Six weeks following inoculation, symptoms consistent with PD were observed on the leaves (marginal leaf necrosis) and the stem (incomplete cork formation on stem) of the inoculated shoot, but the opposite, non-inoculated shoot was asymptomatic. Anatomical examination of stem, petiole, and midrib xylem with both light and electron microscopy revealed the internal progression of PD corresponding to external symptoms. The petioles and midribs of leaves displaying external PD symptoms contained tracheary elements with abundant gummosis and accumulation of bacteria, but few tyloses (Figures 1-3). Bacteria observed in symptomatic leaf midrib xylem, and to a lesser extent in petiole xylem, were embedded within a globular matrix (Figures 5-6). Stem xylem proximal to leaves showing PD symptoms included tracheary elements with abundant tyloses, but little gum formation (Figure 4). Bacteria were rare in the proximal stem and bacterial cells were not contained within a matrix. Observations of fully expanded asymptomatic leaves and stem tissue distal to the inoculation site showed similar anatomical pathology to nearby symptomatic leaves. Consequently, internal progression of PD appears to precede external symptoms. Six weeks following inoculation, no anatomical symptoms of PD were found in the basal main shoot subtending the inoculated shoot, nor in the opposite non-inoculated shoot. Eight weeks following inoculation, PD symptoms manifested in the opposite, non-inoculated, shoots. Anatomical examination showed the same pathology as was observed two weeks earlier in the inoculated shoot, including the accumulation of bacteria and embedding matrix in the xylem vessel members of leaf midribs and petioles.

Following these observations, a working hypothesis of the progression of PD within a young grapevine shoot can be proposed. *Xylella* inoculation of stem xylem precedes a relatively rapid movement of bacteria through the hydraulic network to distal stem regions, petioles, and leaf vascular tissue. The rapid movement is potentially facilitated by one, or a combination, of three mechanisms:

1. Grapevine vessels are long and few vessel-vessel transitions are needed to reach distal tissues,
2. Pit membranes of grapevine are frequently damaged, either in development, or as a result of frequent cavitation/refilling cycles, or
3. Bacteria are able to quickly digest pit membranes of terminal vessel elements.

Once bacteria moving in the transpirational stream enter regions of the hydraulic network that contain many narrow tracheary elements and more frequent terminal tracheary elements (i.e. shorter vessels in petioles and leaves), bacteria are 'filtered out' and accumulate, and become embedded in a surrounding matrix which effectively blocks water flow in that conduit. It is unknown whether this matrix is secreted from the bacteria itself, from the plant either as a defense reaction or responding to bacterial stimulus, or a combination of the two. Tylose formation in the stem coincides with bacterial infection, but at least

initially, is not present to such a degree that bacterial movement is prevented or that the water supply to distal tissues is restricted to levels causing visual symptoms. Additionally, bacteria can move from an inoculated shoot to another shoot via the subtending trunk relatively quickly. Consequently, it can be proposed that the PD symptoms observed in multiple shoots of a grapevine are not a symptom of a whole-plant response to a localized infection, but rather are an indication of a systemic *Xylella* presence. For this to occur, bacteria must move basipetally from the site of inoculation, into the basal stem and then acropetally into the opposite shoot. Whether bacteria are moving against the transpirational stream in an intact water column, or whether the downward bacterial movement is facilitated by the release of tension in a cavitated water column is unknown.



Figures 1-3. Light micrographs of grapevine stem, petiole, and leaf midrib (l-r) six weeks after inoculation. Tyloses are frequent in vessels of stem sections, whereas xylem is occluded by gums in petioles and midribs.

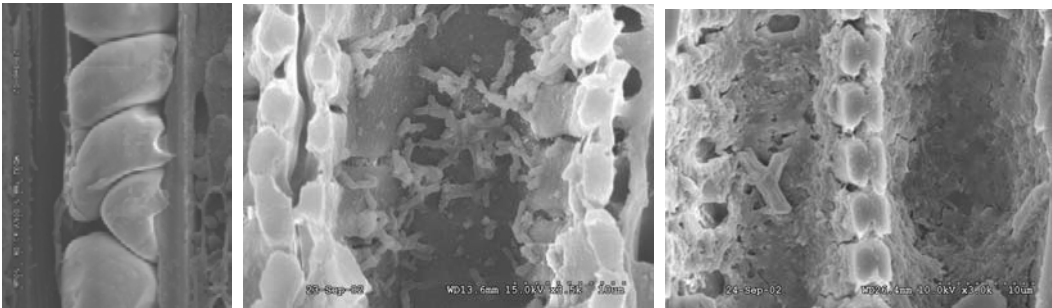


Figure 4-6. Scanning electron micrographs of grapevine stem, petiole, and leaf midrib (l-r) six weeks after inoculation. Symptoms of PD in stem xylem were typically tyloses, whereas petioles and midrib symptoms were associated with an abundance of bacteria and surrounding matrix increasing from petiole to leaf.

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FUNDING AGENCIES

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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 through and between vessels, whether vessels cavitate upon introduction of the bacterium by the insect vector or artificial inoculation, and whether PD symptoms can be found in tissues at a distance from local concentrations of bacteria. 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. The studies in our project are designed to test the following hypothetical "model" of the events contributing to the development of PD.

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

OBJECTIVES

For this research period, the work in our project has been closely coordinated with work in the new project led by Rost and Matthews. Our project follows several aspects of PD development following introduction of *Xylella fastidiosa* (Xf) to grapevines by hypodermic injection to basal stem internodes. The focus of our effort in this period has been on factors that limit systemic spread of the Xf population and contribute to reduced water movement in the xylem.

1. Determine the "porosity" of the pit membranes that regulate movement from one xylem vessel to the next.
2. Determine whether digestion of cell wall polysaccharides in the pit membrane is required for passage of Xf through the xylem.
3. Determine how quickly, post-inoculation with Xf obstructions occur in the xylem.

RESULTS AND CONCLUSIONS

Factors limiting movement of bacteria and water in grapevines:

Using PCR-based detection of bacterial DNA sequences, we observed the rapid (within 14 days) spread of Xf up inoculated shoots of small grapevines (30 – 40 cm in height). This raised the possibility that the bacteria were moving unimpeded through the xylem. This could occur because a significant population of the vessels was sufficiently long that bacteria could be swept along in the transpirational stream without encountering a vessel end and, hence bordered pit. Alternatively, the pits might not offer the expected restriction to bacterial movement among vessels. Analysis to determine the length of the longest vessels in shoots of other experimental material (80 - 100 cm shoots) has indicated a 30 - 50 cm maximum, with a mean of 34 ±12 cm. By comparing the air flow rates through stems from which apical segments were repeatedly excised, it is possible to observe the relative distribution of vessel lengths. This analysis indicated that most vessels were less than 15 cm long (Figure 1). Thus, there appear to be very few paths longer than about 10 – 15 cm that do not include a vessel end. However, we have not performed the same analysis on the shorter stems used in the experiment showing rapid systemic spread of Xf (above).

Electron micrographs published by others have given the impression that Xf size is too large to pass through the cell wall meshwork in the xylem "pit membranes" that fill the pit passageway from one water-conducting xylem element to the next. Xf dimensions appear to be ca. 0.5 by 1.5 µm while the gaps between the cell wall elements that comprise the pit membrane appear to be no larger than 0.3 µm in size. It was important that we be certain that the pit membrane "pores" would block bacterial passage. Our hypothesis is that passage through pits could occur only if bacterial wall-degrading enzymes were able

to digest a pathway through the polysaccharides of the pit membrane. That would be unnecessary if its normal pore size did not limit *Xf* movement.

The distal cut end of an explanted stem of a healthy vine was attached to a vacuum pump that was adjusted to apply a negative pressure of 0.5 atmospheres. The proximal cut end was then placed in a flask containing water plus various test materials. We first followed water movement through the stem for a set period of time by measuring the volume of water that exited the distal end. After drawing water for a time, the proximal end was placed in 10 mM KCl in water. When a steady state of flow was reached, the water movement was measured again. The test was repeated using a 50 mM KCl solution. With each increase in salt concentration, the volume of water moved increased. This result reflects earlier reports that concluded that the increasing ion concentration had reduced the water shell around the polysaccharides in the pit membrane and this, in turn, had decreased the resistance of the membrane to water flow.

The experiment was repeated, this time with red-stained polystyrene beads of defined dimensions. The idea was that the beads would serve as useful surrogates for the non-motile *Xf* cells that had been introduced to grapevines by the glassy-winged sharpshooter or the "needle stab" inoculation technique we were using. We used beads of 1.0, 0.5, 0.3 and 0.029 μm average diameter. No beads of any size were moved the length of the stem segments tested, no matter which test solution was used (Figure 2). These experiments were conducted with shoots that were longer than the longest vessel in the test shoots, so a bead would have had to pass through at least one pit membrane on its path from one end of the stem explant to the other. This test was repeated using soluble, naturally colored proteins of known molecular weight and predictable, average molecular diameters. Cytochrome c (MWt of 14.8 kD, diameter of 0.005 μm) was not drawn up the stems when it was dissolved in water; it did move slowly when it was in 10 mM KCl and more quickly when it was in 50 mM KCl. Hemoglobin (MWt of 64 kD, diameter of 0.088 μm) was drawn up the xylem only when it was dissolved in 50 mM KCl and the rate of movement was quite slow compared to that for cytochrome c.

The beads of 0.029 μm diameter represent less than 2% of the estimated volume of a *Xf* cell and have a diameter that is less than 10% of the bacterial "width." Therefore, these experiments confirm that the cell wall mesh of the pit membrane represents a substantial barrier to the movement of *Xf* from one vessel to the next as long as it is intact. The experiments also indicate that the pit membrane mesh provides much smaller pores than had been suggested in some earlier reports and that the chemistry of the xylem fluid can have an effect on the resistance to water flow in the xylem.

Do bacterial populations spread systemically because they produce enzymes that digest pit membranes?

The *Xf* genome contains DNA sequences that are predicted to encode cell wall-degrading enzymes like polygalacturonase (PG, a cell wall pectin-digesting enzyme) and endo- β -1,4-glucanase (EGase, sometimes called cellulase). Graduate student Caroline Roper has cloned the PG-like sequence of the PD-causing bacterium and is now attempting to get the cloned gene to be expressed in *E. coli*. Once the protein is isolated, we will confirm its activity and then introduce it into explanted stems to see if it opens the xylem to passage of beads or killed *Xf* cells, presumably by breaking the pit membrane cell wall mesh. Currently we have a few non-*Xf* PGs to use in the same sort of test. The experiment is made more complicated because the PG protein is too large to pass the intact pit membrane "barrier." The result of this test may be available by the time of the Symposium.

Xf-induced xylem obstructions:

Last year, we reported on microscope-assisted observations that showed both tyloses and plant cell wall-derived "gels" obstructing many of the vessels in PD-infected grapevines. We have observed tyloses in vessels of *Xf*-inoculated grapevines as early as 4 weeks after introduction of an aliquot of bacterial suspension (Figure 3). Dr. Josh Stevenson (in the Rost/Matthews project) has developed these investigations further and will report additional details about observations of tyloses and gels in the vessels of stems and leaf petioles and midribs of PD-infected vines. Briefly, reduced hydraulic conductance in petioles is correlated with the accumulation of bacteria and gums in the petiole. Data in Table 1 indicate the correlation of PD leaf symptoms with reduced hydraulic conductance following stem inoculation with *Xf*. A related study, focusing on *Xf*'s impact on grapevine water movement is focusing on the hypothetical production, by *Xf*, of exopolysaccharides in xylem vessels. Based on Dr. Stevenson's observations, we are now using a direct extraction and chemical analysis approach to determine whether the amorphous gels occluding vessels in petioles and leaves of infected vines contain bacterial polysaccharides like those predicted to be produced by *Xf*, based on gene sequences identified in its genome. Our interest is in coincident microscopic observation of gels and chemical measurement of bacterial polysaccharide component sugars.

In stems, few bacteria and little gum has been found to accumulate. There is an increased frequency of tyloses in stems of PD-infected and symptomatic vines. Our hydraulic conductance measurements have seldom revealed reduced water transport in stem segments. However, our samples may have been of young tissue not yet competent for extensive tylose development. Also, our hydraulic assay methods could have repaired cavitated vessels. Therefore we are developing other approaches to quantify cavitation, including a pneumatic assay that should prevent refilling, ultrasonic acoustic emissions, and the imaging technique described below.

Attempts to "see" points of reduced water flow in intact grapevines:

Typical tests of grapevine water-conducting capacity require that the stem be explanted and then tested. The decommissioned McClellan Air Force base in Sacramento houses a nuclear reactor that is a source of fast neutrons. This source is being made available for use in fundamental research. It may provide an opportunity for visualizing water flow through intact grapevines. Preliminary tests using well-watered and water-stressed vines confirmed that images (analogous to X-rays) can show differences in water moving through the stems. We are continuing pilot studies, working with the very cooperative McClellan staff scientists in attempts to enhance contrast in the photographic images that constitute the data record of these experiments. Our hope is to use the observation of points of reduced water flow in intact healthy and diseased vines to guide us in the "destructive" sampling for bacterial presence and observations of xylem obstructions. Consistent observations of obstructions in regions of reduced water transport, whether or not significant populations of *Xf* are co-localized, will be of importance in developing our ideas about progression of PD symptoms and *Xf* populations in the xylem.

Preliminary conclusions:

The picture that is emerging is that PD leaf symptoms are seen in inoculated vines at times when bacterial populations are small and that reductions in water flow may occur when no bacteria are detected. This seems to suggest that PD symptoms can develop in advance of the systemic spread of *Xf* because of the acropetal movement of thus far undefined signals that trigger responses in the xylem. We continue to address questions that are relevant to this preliminary conclusion, a conclusion that is at the center of the hypothetical PD "model" that formed the core of our proposal.

Table 1. Estimated hydraulic conductance (Kh), presence or absence of PD symptoms, and presence or absence of *Xf* for petioles of leaves from PD-infected and control plants. Kh was determined as flow rate through excised petioles at pressures of 0.8 – 1.6 bars. Presence of bacteria was determined from SEM micrographs of similar and adjacent leaves.

Treatment	Kh (ml/bar/sec) n = 3 or 4	+/- presence of <i>Xf</i> .	+/- leaf chlorosis symptoms
- PD Control	0.53 – 0.83	-	-
+ PD Inoculated*	0.74 – 1.53	not assayed	-
+ PD Inoculated**	0.13 – 0.17	+	-
+ PD Inoculated	0.01 – 0.1	+	+

*These leaves were located (6 – 14) nodes acropetal to the symptomatic leaves.

**These leaves were located 1 or 2 nodes acropetal to the symptomatic leaves.

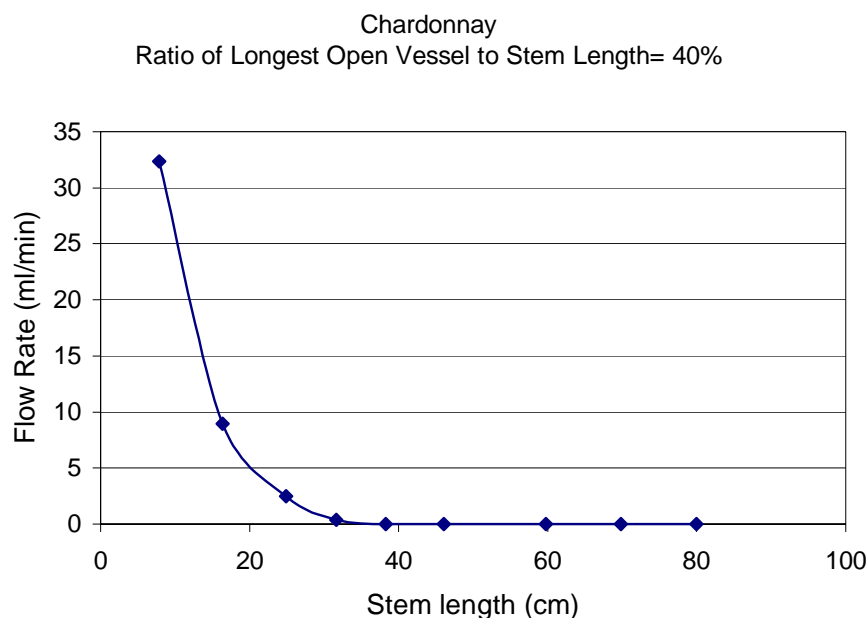

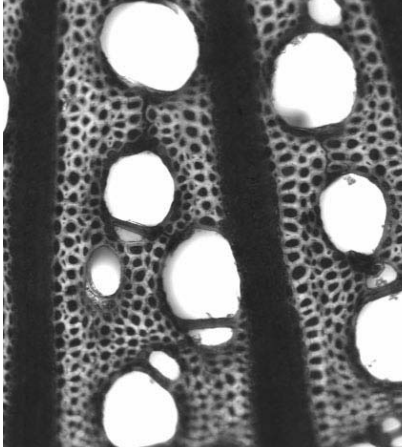
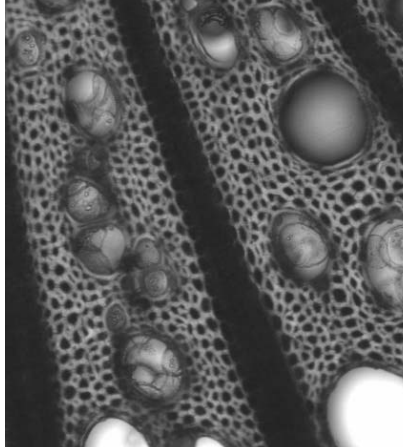


Figure 1. Rate of air flow through stem segments of varying length. Air was supplied to basal internode at pressures of 0.4 – 0.6 bar, and its rate of escape from the apical end was recorded after repeated excision of stem segments from the apical end of the shoot. The low pressure should not have passed pit pore membranes. Therefore, the flow rate should reflect the number of open vessels present.

<p>Figure 2. Polystyrene beads in a grapevine xylem vessel.</p>	<p>Figure 3. Sections through the stems of young grapevines reveal extensive xylem blockage in vessels of all developmental ages. While tyloses are occasionally seen in uninoculated vines, introduction of <i>Xf</i> substantially increases their appearance.</p>	
		
<p>Red polystyrene beads (0.3 μm diam.) filling one vessel in this light microscope view of a cross-section of a grapevine stem. Note that there are many other “open” vessels which contain no beads. This suggests that the other vessels in this view did not extend all the way to the basal, cut end of the stem where beads were introduced.</p>	<p>Light microscopy examination of a section through the stem of an uninoculated grapevine. Vessels are seen to be unobstructed.</p>	<p>Light microscopy examination of a section through the stem of a grapevine 4 weeks after inoculation with <i>Xf</i>. Most of the vessels are obstructed by one or many tyloses. PCR analysis of other individuals in this set of test plants showed <i>Xf</i> throughout the vine. Typical PD leaf symptoms did not appear in these vines for 4 more weeks.</p>

FUNDING AGENCIES

Funding for this project was provided by the USDA Animal and Plant Health Inspection Service.

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

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Reporting Period: The results reported here are from work conducted from November 1, 2001 to October 31, 2002.

INTRODUCTION

Knowledge of the source of disease inoculum from vectors, whether from inside or outside the vineyard, is critical to development of management strategies for disease control, such as the choice and management of plant species surrounding vineyards. In addition, 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.

OBJECTIVES

1. Determine which plant species near vineyards harbor *Xylella fastidiosa* 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 *Xylella fastidiosa* 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:

We are completing our third and final season of plant host sampling. We are still consistently getting positive detection of *X. fastidiosa* in several plant species in Temecula, including grapevine, oleander, Spanish broom and the few almond trees that remain. We also detected the presence of *X. fastidiosa* in *Brassica nigra* (wild mustard) by ELISA and PCR, but have not yet been able to culture it from this host. We increased the sampling of *Brassica nigra*, coyote brush, and elderberry and other weed and ornamental hosts that either appear symptomatic, or that have occasionally tested weakly positive with ELISA in previous years. We were never able to confirm positive results for coyote brush or elderberry with other methods, suggesting that they could have been false positives.

In other areas of Riverside, San Bernardino and Orange Counties some symptomatic landscape plants have tested positive for *X. fastidiosa*. Thus far, liquidamber, olive, mirror plant, and ornamental plum all tested positive by ELISA and PCR. We have also obtained cultures of *X. fastidiosa* from several samples of ornamental plum, but so far, have only been able to obtain one culture from olive samples. Several landscape plants, including olive and liquidamber, were repeatedly tested in the Temecula valley, but thus far, these species have not tested positive for *X. fastidiosa* in that area. The detection of *X. fastidiosa* does not necessarily mean that the bacterium is causing disease in these hosts; other pathogens or abiotic factors may be causing the observed symptoms. Additional studies will need to be conducted to determine if *X. fastidiosa* alone can cause disease in these species.

We are in the process of sequencing amplification products to identify the strains of *X. fastidiosa* that are infecting these new hosts.

Transmission studies:

Studies were initiated last year to test the ability of GWSS to transmit *Xylella* from infected grape to several species of host plants including: grape, lemon, grapefruit, orange, almond, oleander, blackberry, bougainvillea, toyon, coyote brush, *B. nigra*, brittlebush, mule fat, sage, California buckwheat, sugar bush, laurel sumac, tree tobacco, elderberry, alfalfa, peach, and coast live oak. One year after inoculation, sampling of test plants with ELISA and PCR found that transmission occurred only from infected grape to grape, and from infected grape to *B. nigra* plants. None of the other hosts have been confirmed positive thus far. Transmission experiments were also conducted to see if GWSS could transmit the pathogen from field-infected Spanish broom into grape test plants. In that study, 9/26 grape plants tested positive for the pathogen, indicating that Spanish broom may serve as a source of inoculum for Pierce's disease. Similar studies testing GWSS from greenhouse-infected *B. nigra* plants to grape found 1/9 grape plants became infected. This year, addition replicates of these species and

11 additional species (including Spanish broom) were initiated. Sampling two months after inoculation found only 1 grape plant tested positive so far.

Evaluation of detection methods:

We are continuing to evaluate the effectiveness of various methods for detecting *X. fastidiosa* in plants and in the insect vector. Both ELISA and immunocapture-PCR methods work well for plant samples. An additional method of extracting bacterial DNA from plants and insects using a commercially available kit was successful. This type of extraction can provide enough material for multiple PCR reactions to allow sequencing of DNA products. Strain specific primers have also been identified that can detect the OLS and PD strains of the pathogen. One primer set amplifies the PD but not the OLS strain, the other amplifies the OLS but not the PD strain. Although these primers pairs can be used to distinguish between these two strains, these primer pairs alone cannot necessarily distinguish these strains from all other strains that might be present in the environment.

FUNDING AGENCIES

Funding for this project was provided by the California Department of Food and Agriculture and the American Vineyard Foundation.

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

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Reporting Period: The results reported here are from work conducted from November 1, 2001 to October 31, 2002.

INTRODUCTION

Almost nothing is known of the stylet penetration (probing) behaviors of the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*, and how they interact with populations of Pierce's disease (PD) bacterium, *Xylella fastidiosa*, to facilitate transmission to grapevine. The Backus project is combining the three most successful methods of studying leafhopper feeding (i.e. histology of fed-upon plant tissues, videotaping of feeding on transparent diets, and electropenetration graph [EPG] monitoring) to definitively identify all details of feeding. Both AC and DC EPG monitoring are being performed. All recorded waveforms will be 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. This research will provide crucial baseline information for the present projects of collaborators, as well as the future development of a Stylet Penetration Index for PD inoculation behavior, for screening differences among grapevine varieties and other uses.

OBJECTIVES

1. 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 *Xylella fastidiosa* in the foregut.
2. Identify the role of specific stylet activities in *Xylella fastidiosa* transmission, including both the mechanisms of acquisition and inoculation, and their efficiency. Emphasis is on inoculation.
3. Develop a simple, rapid method to assess feeding, or detect the likelihood of *Xylella fastidiosa* transmission (an "inoculation-behavior detection method"), for future studies.

RESULTS AND CONCLUSIONS

We spent the first 4 months of this year completing the purchase of equipment, upgrading facilities, establishing plantings, and hiring personnel, as described in last year's progress report. Unforeseen delays in acquiring a post-doc visa were finally overcome in early February 2003, when Dr. Fengming Yan, Associate Professor, Peking University, arrived. Research was begun in March 2003, and has continued for 8 months. Work this year supported Objectives 1 and 2.

Objective 1

A. Adult GWSS were collected on citrus in Riverside, California by Cooperator Matt Blua, who express-mailed them to Missouri every 2-4 weeks from mid-February to mid-October 2002. Sharpshooters were maintained on chrysanthemum and basil under quarantine, but conditioned for 48 hours on grapevine, cv. 'Cabernet Sauvignon' (from FPMS, UC Davis) prior to testing on grape.

B. For Experiment 1, Yan and Backus EPG-monitored a total of 242 male and female sharpshooters feeding on chrysanthemum or grapevine, for access periods ranging from 4 to 20 hrs. Both AC and DC EPG monitors were used, each with separate insects. We used these results to identify, characterize and label waveform phases, families and types, after the now-standard conventions used for EPG (Reese et al. 2000, Cline and Backus 2002). The most common categories of AC waveforms and their characterizations are described in Table 1; representative appearances are pictured in Figure 1A and B. In general, AC and DC recordings looked quite different, but were dividable into the same 3 phases, designated: pathway, ingestion and interruption (Figure 1A). Both AC and DC waveforms were very complicated at the most expanded (fine-structure) level of characterization (termed waveform types) (Figure 1B). In the interest of time, we characterized the DC waveforms only to phase, while concentrating on more in-depth characterization of the AC waveform types, until we perfect the AC-DC correlation monitor (see D below). Preliminary analysis suggests that there are no differences in AC waveform types between males and females, or among insects feeding on chrysanthemum or grapevine. Selected traces will be quantified and descriptive statistics applied for a preliminary publication.

C. In September 2002, Bennett completed the building and, with Backus, the testing of a prototype AC-DC correlation monitor, whose design was based on suggestions kindly provided by W.F. Tjallingii (of Wageningen University, The Netherlands; pers. comm. and Tjallingii 2000), with modifications by Bennett (ms. in prep). This monitor allowed, for the first time ever, display of two simultaneous signals from the same feeding insect, one AC and the other DC. Its only drawback was that the two views were not absolutely identical to those of normal AC and DC monitors. However, they were very similar and interpretable, and further minor adjustments may make them closer to normal. This new monitor will facilitate future correlation of DC waveforms with existing AC waveform categories.

D. For Experiment 2, Yan and Backus developed protocols to: 1) rapidly terminate the feeding of wired sharpshooters, to produce short EPG excerpts ending in a certain waveform type, and 2) mark the feeding site on grapevine petiole, for excision of the plant tissue containing the salivary sheath. Yan then performed 98 such waveform terminations, with matching excised petioles fixed for histological examination of sheaths. Habibi is preparing, sectioning and examining these tissues, locating salivary sheaths and producing digital micrographs. To date, a total of 36 salivary sheaths have been correlated with the 6 presently identified AC waveform types (Table 1), i.e. 4 to 7 for each type. Results from preliminary analysis by Backus and Yan are summarized in Table 1. In short, we found that sheaths from pathway waveforms indeed lie along a path to the xylem. However, not all sheaths from ingestion-containing excerpts terminate in mature xylem elements. Some also terminate in proto-xylem or xylem sclerenchyma, as well as pith cells or interfascicular bundle sheath cells. In several cases of multi-branched sheaths, we could assign some branches to a certain waveform event by comparing degree of hollowness of each branch. Preliminary analysis suggests that the shortest-duration events occur in immature or non-xylem cells, while the longest-duration events occur in mature tracheary elements. However, this conclusion must be verified.

E. For Experiment 3, Yan used AC EPG and videomicrography to record sharpshooter feeding on Parafilm sachets containing expressed grape xylem sap (provided by Collaborator Purcell). Preliminary analysis shows that sharpshooters performed all of the pathway waveform types on such diet. However, ingestion waveforms were abnormal and their duration was very brief; even the hungriest insects terminated probing after only a few minutes. Sheath salivation was easily visible, although protocol modification will be necessary before watery salivation can be detected. Further recordings and frame-by-frame analysis will allow many correlations of waveform fine structure with stylet activities.

Objective 2

A. For Experiment 4, Yan and Habibi used the same waveform excerpting and plant techniques for a study correlating waveforms and salivary sheaths with inoculation of *Xylella* to healthy grapevine. In addition, all sharpshooter heads were excised and fixed for later scanning EM, to allow additional correlation with size and appearance of *Xylella* colonies inside the precibarium and cibarium. Eight treatments were performed, using a 2x4 factorial, randomized complete block design with 10 replicates of each treatment. The treatments were composed of two waveform excerpt treatments ([1] pathway only, or [2] pathway + 1 hr of ingestion [including any interruptions]) and four *Xylella* detection methods ([1 and 2] plants held in the greenhouse for 6 weeks, then fed-upon tissues tested via PCR [by Collaborator Civerolo] or bacterial culturing [by Collaborator Purcell]; [3] plants held for three months, then assessed for PD symptom development; or [4] plants held for 5 d, then the fed-upon petiole histologically prepared for immunocytochemical detection of both salivary sheaths and *Xylella*). This experiment is still in progress.

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FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

Table 1. Proposed categories, their characteristics and meanings, for the most common AC waveforms of the GWSS.

Phase Name	Waveform Name	Waveform Characteristics	Proposed Biological Meaning	
			Plant Tissue/Cell	Insect Activity
Non-probing	Z	Irregular, small waveforms; amplitude and frequency vary	Plant surface	Walking on plant surface, moving around, labial dabbing
Pathway	A1	Highest amplitude, ascending waveform at beginning of probe w/ or w/o spikes at the top	Parenchyma or bundle sheath	Breakage of plant surface, secretion of salivary sheath and/or watery saliva
	A2	Medium amplitude, declining slope; irregular high frequency	Parenchyma or bundle sheath	Lengthening and/or hardening of salivary sheath
	A3	Medium amplitude, relatively flat irregular high frequency	Parench., bundle sheath or xylem	Further sheath salivation
	B	Regular, high frequency, short (4~5 s), with distinct phrases	Vascular or inter-fascicular tissue	Stylet tip fluttering? possibly w/ sheath salivation?
Ingestion	C (to be subdivided)	Regular, low frequency with distinct phrases	Usually xylem, but sometimes pith	Ingestion (watery excretory droplets correlated)
Interruption	N	Irregular, appears A-like, but occurs during C; ave. duration 16 sec	Vascular or inter-fascicular tissue	Salivary sheath extension or branching

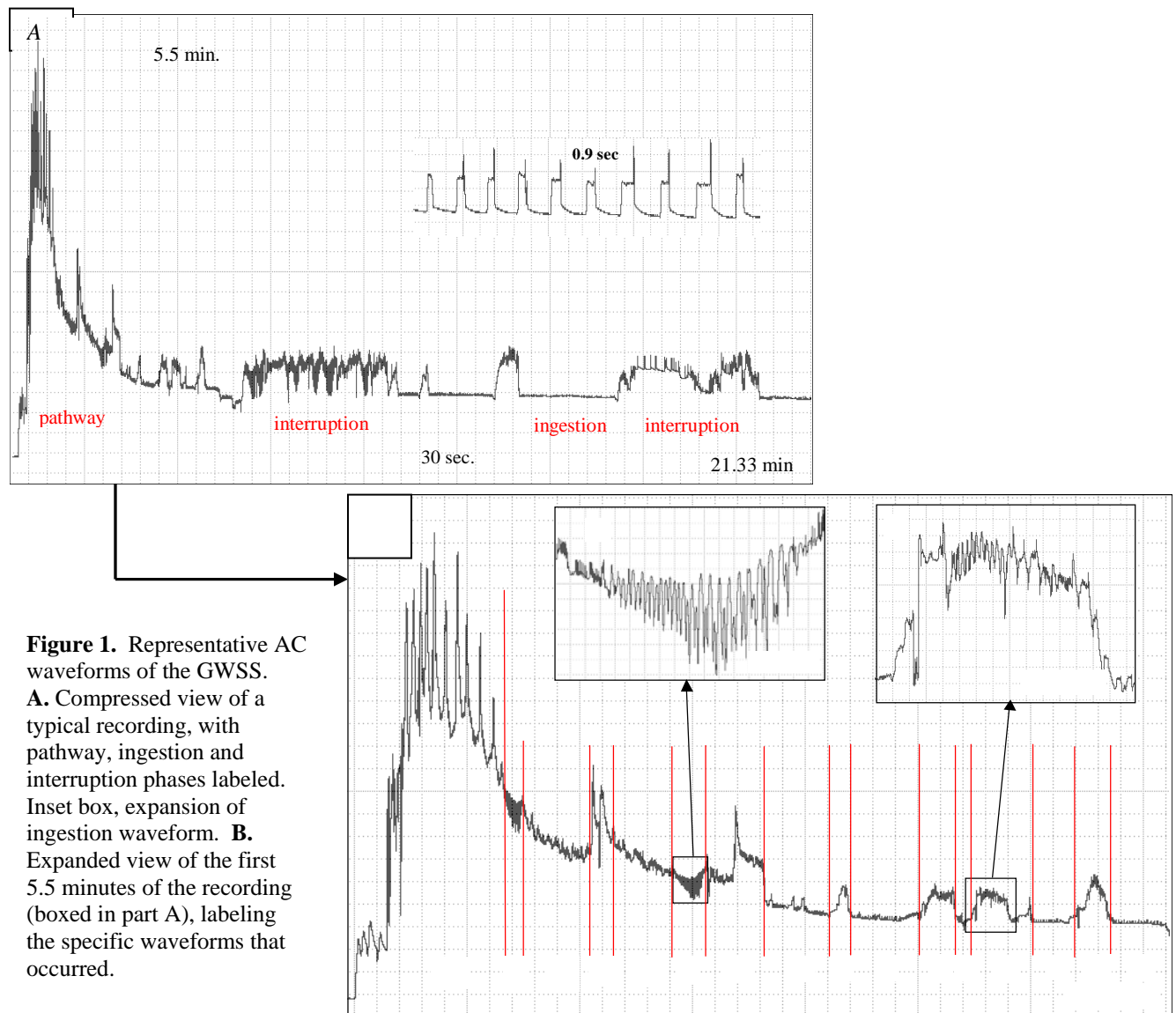


Figure 1. Representative AC waveforms of the GWSS. **A.** Compressed view of a typical recording, with pathway, ingestion and interruption phases labeled. Inset box, expansion of ingestion waveform. **B.** Expanded view of the first 5.5 minutes of the recording (boxed in part A), labeling the specific waveforms that occurred.

EPIDEMIOLOGY OF PIERCE'S DISEASE IN THE COACHELLA VALLEY

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Reporting Period: The results reported here are from work conducted from May 1, 2001 through September 30, 2002.

INTRODUCTION

The table grape industry in the Coachella Valley is represented by 10,465 acres of producing vines, which generated grapes valued at \$108.5 million in 2001 (Riverside County Agricultural Commissioner, 2001). The glassy-winged sharpshooter was identified in the Coachella Valley in the early 1990's (Blua et al. 1999), and we have documented increases in the numbers of this efficient PD vector over the past 17 months. In July 2002, we confirmed the occurrence of *X. fastidiosa* (PD strain) in 13 vines from 2 adjacent vineyards in the southeastern part of the Valley. With this discovery, and the increasing numbers of GWSS, the CDFA Pierce's Disease Program in concert with the Riverside County Agricultural Commissioner's Office is developing an area-wide vector suppression program. The data gathered in our epidemiological studies provide fundamental information that are valuable for this program.

OBJECTIVES

The goal of our epidemiological studies in the Coachella Valley is to discover characteristics that are unique to geographic areas with and without PD, and to use this information to design management strategies to reduce disease spread.

Two objectives are pertinent to this report:

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 contribute to GWSS abundance.

RESULTS AND CONCLUSIONS

PD incidence and distribution:

For the past 2 grape growing seasons, we have surveyed the Coachella Valley in search for PD. In the summer of 2001, we visually inspected 300 plants in each of 25 vineyards and all vines in a 60-acre vineyard proximal to an area that had PD in 1985. We collected 233 suspected samples and analyzed them with ELISA. None of these plants were positive for *X. fastidiosa*. In 2002, we visually sampled 300 plants in each of 25 vineyards, and visually inspected 35,000 vines randomly distributed throughout the Valley. We analyzed (by ELISA) 268 plants from these surveys and found 13 vines with *X. fastidiosa*. Bacteria were confirmed in these plants with selective-media plating and PCR, amplifying for PD-specific DNA. These 13 vines were in 2 consecutive vineyards, located in the southeast part of the Coachella Valley. The vines were removed and the fields were treated with Admire. Several surrounding vineyards also were treated with Admire.

Spatial and temporal abundance of GWSS:

We used yellow sticky traps distributed uniformly at one-mile intervals throughout the Coachella Valley to monitor the seasonal cycle of adult sharpshooter activity. GWSS catches rose into the summer of 2001, were depressed for 3 weeks in late July, peaked again in mid-August, and then declined into the fall (Figure 1). Numbers were extremely low until a period of increased activity, presumably by overwintering adults, in January and February, after which counts declined again until May 2002. Average counts in the summer of 2002 were higher than in 2001, suggesting a trend toward generally higher levels in the Valley.

Our project is particularly interested in the effect of the presence of citrus on sharpshooter numbers. Among the area-wide traps, those adjacent to citrus caught more GWSS than those not adjacent to citrus (Figure 2). However, the presence of citrus did not always result in elevated GWSS catches; fewer than 35 percent of the traps adjacent to citrus caught GWSS on any given week (Figure 3). This indicates that vector control strategies should be targeted at citrus, but all citrus groves in the Coachella Valley do not need treatment at this time. We also conducted extensive studies at 25 citrus/grape interface study sites. At each site, traps were placed in 4 plots: along the citrus border, within the vineyard adjacent to the citrus (designated "Grapes-Near," Figure 4), 500 ft from the citrus (Grapes-Medium), and 1000 ft from the citrus (Grapes-Far).

Traps near citrus consistently caught more GWSS than traps within the vineyards, and there were significant differences in GWSS catches among the plots on 27 of the 42 trapping dates ($P < 0.05$, Tukey-Kramer). During each of the 6 weeks of GWSS catches in January/February (data not shown), traps along the citrus border caught significantly more GWSS than did those within the vineyards, and there were no significant differences in catches among the traps within the vineyard ($P > 0.05$, Tukey-Kramer). In the most recent 7 trapping dates, GWSS catches in citrus were significantly higher than catches on the Medium and Far vineyard traps, however there were no differences between catches within citrus and catches on the Near vineyard traps ($P < 0.05$, Tukey-Kramer). The effect on PD epidemiology of these decreases in GWSS with distance from citrus are not clear, but practices to reduce vector pressure should be focussed on the citrus and the grapes immediately adjacent to the citrus. Our data suggest that area-wide insecticide applications in vineyards that are not close to citrus are unwarranted. We shall continue to monitor this relationship to get a clearer picture of GWSS activity in the vicinity of its reproductive hosts.

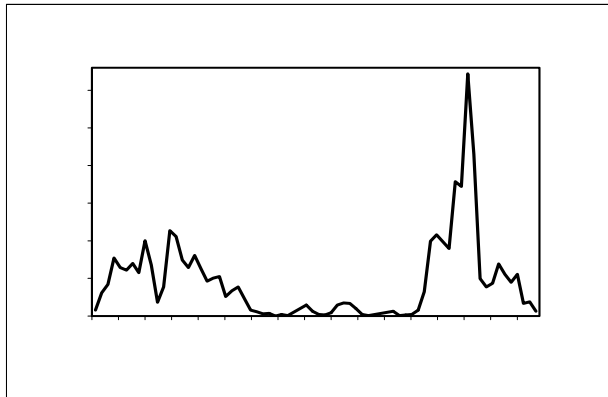


Figure 1.

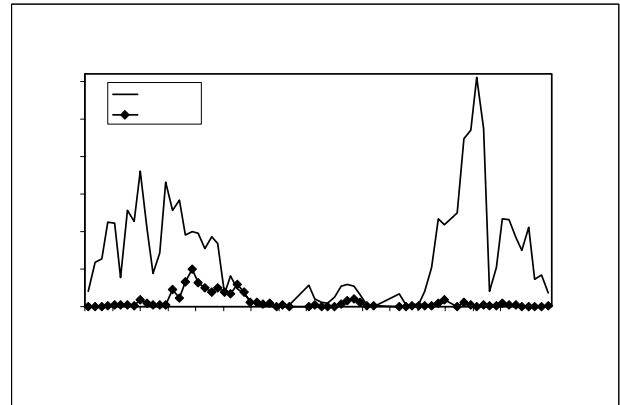


Figure 2.

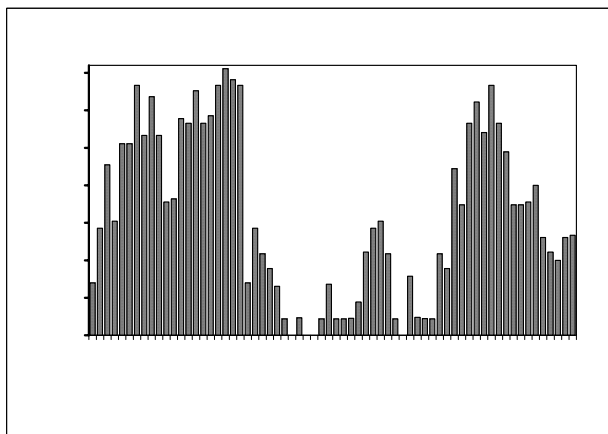


Figure 3.

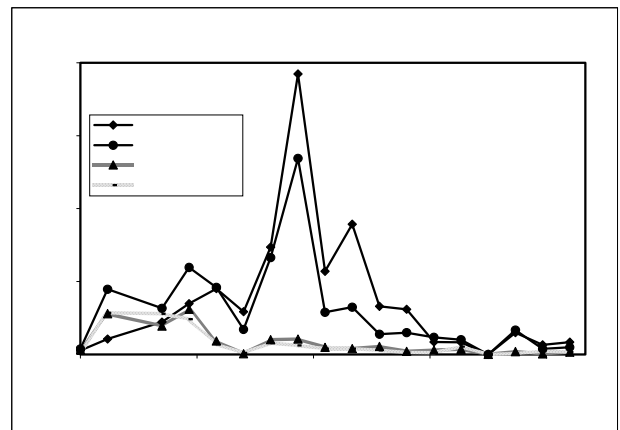


Figure 4.

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FUNDING AGENCIES

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ROLE OF TYPE I SECRETION IN PIERCE'S DISEASE

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Reporting Period: The results reported here are from work conducted from November 8, 2001 to October 31, 2002.

INTRODUCTION

Xylella fastidiosa (*Xf*) is a xylem-inhabiting Gram-negative bacterium that causes serious diseases in a wide range of plant species (Purcell and Hopkins 1996). Two of the most serious of these are Pierce's disease (PD) of grape and Citrus Variegated Chlorosis (CVC). The entire genomes of both PD and CVC have been sequenced (Simpson et al. 2000). Availability of the complete genomic DNA sequence of both a PD and a CVC strain of *Xf* should allow rapid determination of the roles played by genes suspected of conditioning pathogenicity of CVC and/or PD. For example, analyses of the CVC and PD genomes showed that there was no type III secretion system, but there were at least two complete type I secretion systems present, together with multiple genes encoding type I effectors in the RTX (repeats in toxin) family of protein toxins, including bacteriocins and hemolysins. RTX proteins form pores in lipid bilayers of many prokaryotic and eukaryotic species and cell types; at least one is associated with pathogenicity in plants. However, lack of useful DNA cloning vectors and/or techniques for working with either CVC or PD strains have impeded progress in functional genomics analyses.

There have been only three reports of transformation of *Xf*; one with a commercial transposase-transposon complex (Guilhabert et al. 2002) and two others with narrow host range plasmids that utilize origins of replication derived from *Xf*. One plasmid is an integrative vector that carries the CVC chromosomal origin of replication that provides a brief period of unstable replication in *Xf* (Monteiro et al. 2001). The second is a replicative shuttle vector that carries the pUC origin for replication in *Escherichia coli* and a rolling circle replicon derived from a cryptic CVC plasmid (Quin and Hartung 2001). However, this plasmid proved unstable in the absence of antibiotic selection.

We describe here the transformation of two *Xf*/PD strains using the small, stable, broad host range shuttle vector, pUFR047 (De Feyter et al. 1993). This vector is one of a series of well characterized conjugational shuttle vectors based on repW and is widely used to shuttle DNA fragments from *E. coli* to various species and strains of *Xanthomonas*, where the vector is stabilized in the absence of antibiotic selection by the parA locus (De Feyter et al. 1990). This is the first report of stable transformation of any *Xf* strain using a broad host range cloning vector.

OBJECTIVES

This is a two year proposal with three objectives: 1) develop an effective functional genomics tool kit for efficient transformation and gene knock-out experiments in a PD strain (Year 1); 2) determine culture conditions for activation of type I secretion (Year 2), and 3) determine the effect of type I secretion gene knockout experiments on pathogenicity of a PD strain on grape (Year 2).

RESULTS AND CONCLUSIONS

PD strains of *X. fastidiosa*, PD-A (Hopkins 1985) and Temecula, (Guilhabert et al. 2001), were grown in PD3 (Davis et al. 1981) medium supplemented with MOPS (3-4[morpholino] propane sulfonic acid), (Gabriel et al. 1989). Both strains were confirmed to be pathogenic on Madagascar periwinkle. Symptoms appeared after 3 months. pUFR047 was transferred from *E. coli* DH5 to the spontaneous Rif resistant PD-1R strain by triparental conjugation. Selection was on PW-H containing gentamycin (Gm), 1.5 mg/L, and Rif, 75 mg/L. Transfer by conjugation was very inefficient and difficult to reproduce due to overgrowth of *E. coli* donor and/or helper colonies resistant to rifamycin; only a few PD-1 exconjugants were rescued from the selection plates. Presence of pUFR047 in the transformants was confirmed by agarose gel electrophoresis of alkaline lysis minipreps of the PD-1 exconjugants, transformation of *E. coli* with these minipreps followed by detection of pUFR047 in the transformants, and PCR using IncW repA-specific primers and miniprep DNA. In Figure 1A is shown the results of an alkaline lysis miniprep of a PD-1 transformant. By contrast with conjugal transfer, pUFR047, with and without a 3 kb DNA insert, was readily transferred by electroporation into both the PD-1 and Temecula at a frequency of ca. 50 transformants/microgram DNA. PD cells were harvested by centrifugation, washed twice and resuspended in 0.3 ml of 10% glycerol. The cells were electroporated with 0.5-1 g of plasmid DNA at 1.8 kV to generate a pulse of 5.8 to 6.0 ms. Cells were then incubated at 28°C for 24 h with constant shaking at 100 rpm and then selected by plating on PD3 agar medium supplemented with 2 mg/L Gm.

Maintenance of pUFR047 was measured in the absence of antibiotic selection for 30 generations. Cultures sampled at the beginning of each cycle were plated on both selective and nonselective medium by serial 10-fold dilutions in MOPS buffer, pH 6.2, containing 0.001% Silwet L-77 to disperse clumps. Use of Silwet L-77 greatly facilitated reproducibility in the cell counts, and did not appear to be toxic to either PD strain at concentrations used (data not shown). After 30 generations of growth, 48% of the cells retained the plasmid in the absence of antibiotic selection (Figure 2).

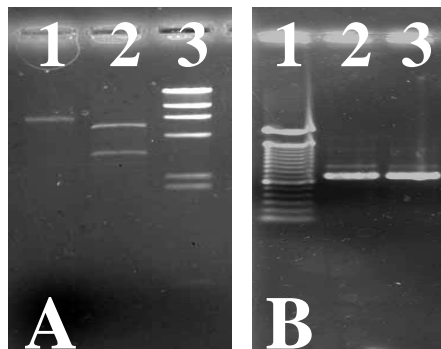


Figure 1. Transformation of PD-1 using pUFR047. A. Plasmid DNA extracted from a single colony of PD-1 after transformation with pUFR047. Lanes: 1) undigested; 2) digested with *Bgl*III, 3) Lambda digested with *Hind*III. B. PCR product amplified by *X. fastidiosa*-specific primers RST31 and RST33 1) 100 bp DNA ladder; 2) PD-A; 3) PD-A/pUFR047.

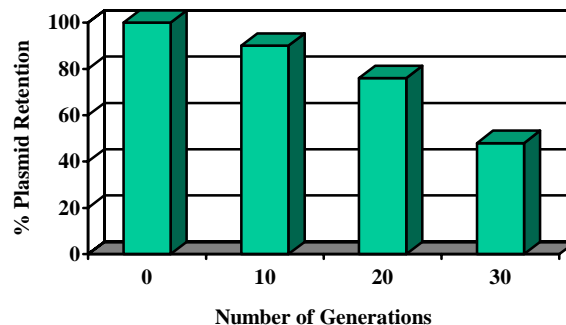


Figure 2. Plasmid maintenance in PD-1 in broth culture. Cultures were grown with antibiotic to late-exponential-growth phase, and diluted 1/1000 into fresh broth in the absence of antibiotic. Growth was continued to late-exponential phase, and the dilution growth procedure repeated for three cycles.

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FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

THE EPIDEMIOLOGY OF PIERCE'S DISEASE

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Reporting Period: The results reported here are from work conducted from July 2002 through November 2002.

INTRODUCTION

The epidemiology of Pierce's disease (PD) changed dramatically in California with the arrival of the glassy-winged sharpshooter (GWSS) about 15 years ago. Before that time infections that persisted and resulted in vine death were the result of primary spread, i.e. from inoculum sources outside the vineyard. The disease caused losses, but the spread was linear, not logarithmic, and the damage was a gradual linear accumulation resulting in the loss of a small percentage of vines. With the exception of some traditional "hotspot" areas, losses from PD were important but not severe enough to preclude grape production. With the arrival of the GWSS, however, the transmission of the causal bacterium appears to be both primary and secondary (from vine to vine) and subsequent disease spread has become logarithmic, such that entire vineyards can be destroyed in as little as 3 to 5 years (Perring et al. 2001; Blua, Phillips et al. 1999; Purcell and Saunders 1999). To cope with this development there have been extensive field studies to determine methods to control the glassy-winged sharpshooter. However characterization of the changes in the epidemiology of PD when the causal bacterium is transmitted by GWSS has been based largely on anecdotal information and general observations with limited actual field data. These two coordinated projects propose to use field data from large numbers of vineyards to assess the impact of the glassy-winged sharpshooter on the epidemiology of Pierce's disease. This is the first year of a proposed 5 year project. The resulting improved understanding of PD epidemiology may also enable UC Cooperative Extension to propose some preliminary recommendations for disease-based control strategies that growers can implement.

Two critical issues are how much economic loss can be expected where GWSS occurs or when the insect moves into new viticulture areas, and what disease-based control methods can be employed in areas already infested with GWSS. The current economic loss models for GWSS are not based on empirical data but on arbitrary projections. Empirical mapping and disease tracking data that enables the comparison of various epidemiological factors (such as cultivar and susceptibility, vineyard age, proximity to GWSS hosts, cultural and control practices in grapes and other crops, etc.) are needed to make better informed projections. Current epidemiological models based on other native sharpshooter vectors (Purcell 1981) are not adequate to account for vine-to-vine spread when GWSS is the vector. Historically, mapping the incidence and vine locations of PD and tracking the spread over a few consecutive years has led to key conclusions regarding the sources of PD spread (Hewitt and Houston 1941, Purcell 1974) and the effectiveness of various control methods (Purcell 1979, Hewitt, Frazier et al. 1949). For example, these previous efforts paid off in identifying the highest risk areas to be avoided with new grape plantings.

OBJECTIVES

1. Develop a model for PD epidemiology when *Xylella fastidiosa* is vectored by GWSS, that evaluates the importance of epidemiological factors such as GWSS population size, vine age, cultivar susceptibility, control practices, and GWSS control treatments in vineyards and nearby GWSS hosts or habitat.
2. Develop PD identification and management strategies for use by growers to reduce risk and damage. Update and provide educational materials to assist vineyard managers, pest control advisors, and county, state, and federal staff involved in advising growers and area-wide management plans.
3. Create a central data processing facility to compile the data from these projects in a GIS format. Share the resulting data, maps, and information with collaborating plant pathologists, statistical analysts, agricultural economists, and other legitimate researchers.

RESULTS AND CONCLUSIONS

Two projects with shared methods and objectives were pursued cooperatively to avoid duplication and make the most efficient use of management and field personnel, equipment and other resources. Field surveys were conducted between early August and November 18, 2002, after which the data compilation began. A field crew composed of CDFA and UC people was trained, and the surveys were done using all terrain vehicles. Another project using identical methods and funded by private sources was conducted by Gisela Wittenborn, and her data were made available to the overall project. Every vine

displaying possible PD symptoms was identified, tagged, mapped, and a sample was taken and sent to the CDFA diagnostic laboratory in Sacramento and tested by ELISA for *X. fastidiosa*. In all more than 250 blocks (> 6000 acres total) in Kern County and more than 60 blocks (>3000 acres total) in Tulare County were surveyed and mapped. More than 30 growers participated in the project. As the data are compiled these participants will be provided with mapped survey results for their vineyards to assist in disease control. The following cultivars were included in the study: Red varieties include Christmas Rose, Crimson Seedless, Flame Seedless, Redglobe, Ruby Seedless. White varieties include Calmeria, French Columbard (wine), Jade Seedless, Muscat, Perlette, Thompson Seedless, Superior Seedless. Purple varieties include Autumn Royal, Black Emerald, Fantasy Seedless. A data center at the Center for the Assessment and Monitoring of Forest and Environmental Resources (CAMFER) at University of California, Berkeley is beginning to compile the data and create a GIS based data set that will be used in these projects and made available to other legitimate researchers. The sites that were surveyed were selected to enable a wide range of comparisons within the data set to enable the evaluation of epidemiological variables, projection of disease progression over time, and the effectiveness of disease control practices.

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FUNDING AGENCIES

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*Biological Control
of the
Glassy-winged
Sharpshooter*



PROGRESS ON THE DEVELOPMENT OF 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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Reporting Period: The results reported here are from work conducted from January 1, 2002 to November 1, 2002.

INTRODUCTION

Effective control of glassy-winged sharpshooter (GWSS) 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 also increase the effectiveness of chemical, mechanical, and cultural control. Unfortunately, very little information exists on predaceous enemies of GWSS. Evidence of predation on GWSS has been observed in the field (JRH, pers. obs.); however, the GWSS predator complex and its impact on GWSS mortality are unknown. A useful technique for identifying a pest's natural enemy complex is through the use of predator gut content immunoassays employing pest-specific antibodies (Greenstone 1996).

Over the past decade we have developed a library of MAbs specific to the egg stage of *Lygus hesperus*, *Pectinophora gossypiella*, and *Bemisia argentifolii* (Hagler et al. 1991, 1993, 1994) for use in studying egg and adult female predation in the field (Hagler et al. 1992; Hagler and Naranjo 1994a,b). Our MAb library provided an avenue to qualitatively identify and assess the impact of over a dozen predator species on populations of key insect pests; provided a quick, efficient, and cost effective technique for screening numerous predators in a conservation biological control program (Hagler & Naranjo, 1994a,b; Hagler, 2002); and provided a method to compare the efficacy of *in vitro*-reared predators with that of their wild counterparts in an augmentative biological control program (Hagler and 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 (STSS), *Homalodisca lacerta*. The eggs of this species are virtually indistinguishable by the naked eye from GWSS eggs. Thus it is difficult to separate the relative rates of predation and parasitism of GWSS and STSS 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 visually. For example, Greenstone (1995) developed an egg-specific MAb diagnostic test that differentiates *Heliothis virescens* from *H. zea*. Pest control advisors have used this MAb in a squashblot immunoassay to rapidly and positively screen field collected eggs. Early detection of *H. virescens* infestations is critical for effective and environmentally sound pest management. A MAb specific to GWSS egg would be an invaluable tool for early monitoring of pest infestation and decision-making in pesticide application. To date, we have developed a series of antibodies specific to GWSS. In this report we describe the antibodies that are currently available for mass screening the GWSS predator complex.

OBJECTIVES

1. Develop a GWSS monoclonal antibody based enzyme-linked immunosorbent assay (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

Parental Hybridoma Cell Lines:

Over a dozen parental GWSS hybridoma cell lines were screened by ELISA for reactivity against GWSS and STSS eggs, nymphs and adults as well as the adult or larval (lepidopterans) stage of 15 other insect species. The majority of cell lines were reactive to the GWSS and STSS egg stage. Additionally, 3 of the cell lines showed reactivity to the GWSS and STSS adult female lifestage. None of the hybridoma cell lines reacted to the other 15 insect species tested (Figure 1).

From the original GWSS hybridoma cell lines examined, 3 hybridomas were selected for additional cloning. The cell lines selected were 1D4, 6C4, and 6D5. These 3 cell lines were selected because: 1D4 only responded to the GWSS and STSS egg stage; 6C4 only responded to the GWSS and STSS egg and adult female stages; and 6D5 had a stronger reaction to the STSS egg stage than the GWSS egg stage (Figure 1). Additionally, each cell line yielded a weak response to the other insects tested. Sub-cloned cell lines 1D4-1D8 and 6D5-2H1 have been mass-produced and are now ready for use for screening potential predators of GWSS and STSS. We collected predators every other week (June through October) from three different locations in California. This winter we will assay them by sandwich ELISA for the presence of GWSS egg antigen.

The fact that these antibodies react to the egg stage of both species should not affect our predator evaluations because the sites we collected from did not contain STSS (see Objective 1). However, these antibodies will help us fulfill our second goal, that is, a MAb capable of differentiating GWSS from STSS. Next year we will select other potential parental cell lines (Figure 1) and clone them to try to obtain an antibody specific only to GWSS.

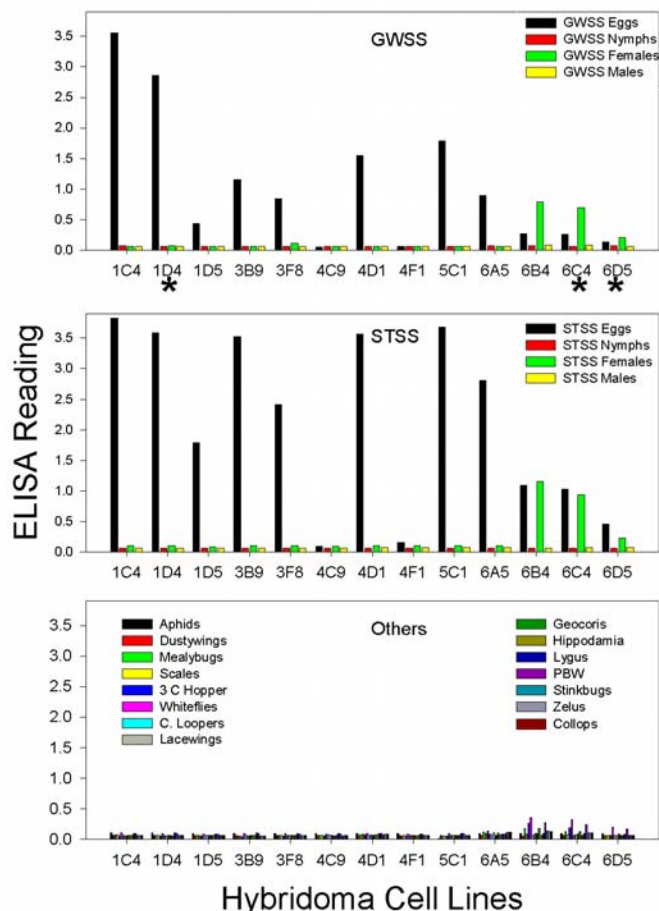


Figure 1. Parental hybridoma cell lines screened for reactivity against GWSS (top), STSS (middle), and other insect species (bottom). Those cell lines marked with an asterisk below them have been sub-cloned, screened for reactivity, and mass-produced.

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FUNDING AGENCIES

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BIOLOGICAL CONTROL OF THE GLASSY-WINGED SHARPSHOOTER IN KERN COUNTY, CALIFORNIA

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Reporting Period: The results reported here are from work conducted from May 2002 through December 2002.

INTRODUCTION

Throughout 2001, a technique to rear the glassy-winged sharpshooter was developed at the USDA-APHIS, GWSS facility located in Mission, Texas. Series of observations and experiments were carried out, in particular to select suitable host plants for feeding and oviposition and determine optimum environmental conditions. Difficulties experienced during the fall and winter of 2000 related to this insect's development and reproductive behavior were largely overcome and a self-sustaining glassy-winged sharpshooter colony with continuous egg production was finally achieved. We now find ourselves nearly independent of having to perform repeated field collections, with the exception of limited collections intended to increase the genetic diversity of our colony. From March 2001 to present, rearing personnel in Mission have produced 11 consecutive generations of glassy-winged sharpshooters, about 55,000 insects in 2001 and three times that many in 2002. This has allowed us to start rearing and studying several glassy-winged sharpshooter parasitoids during 2002. Since April, 20 generations of 3 parasitoid species, *Gonatocerus ashmeadi*, *G. triguttatus* and *G. morrilli* (Hymenoptera: Mymaridae) isolated from 7 geographically diverse sites have been produced at our laboratory, with a total production of over 50,000 parasitoids to date. In addition, several cultures of non-target sharpshooter species are being maintained for parasitoid host range studies (*Homalodisca insolita*, *H. lacerta* and *Oncometopia* sp.). Finally, several species of exotic parasitoids from Argentina are maintained within the USDA-APHIS quarantine facility and studied in a collaborative effort with Walker Jones (USDA-ARS).

Concomitantly, the Kern County Glassy-winged Sharpshooter Pilot Project developed several chemical-based management strategies to control this insect pest in an area-wide fashion (see report by L. Wendel and M. Ciomperlik). Initial goals established in this program called for the testing and integration of biological control methods with those chemical control methods that would be shown effective. Information gathered from laboratory observations, field testing and improvements to mass rearing of parasitoids indicate that area-wide integrated pest management for glassy-winged sharpshooter may be feasible in the immediate future. Classical biological control of glassy-winged sharpshooter may ultimately prove successful in the long term; however, augmentative approaches that follow area-wide population control programs, such as those in Bakersfield and Temecula, warrant further study. Large scale field testing in the 3700 acres of citrus in the Kern Pilot Project, comparing the efficacy of 3-4 species of *Gonatocerus* is planned in 2003.

Although very reliable, current rearing techniques must be further improved to where they become highly efficient and economical and allow to produce high numbers of natural enemies for field releases in the biological control and area-wide integrated pest management programs. The challenge: the glassy-winged sharpshooter develops rather slowly to adulthood, its development rate being dependent upon plant quality and host species selected for rearing, exhibits a reproductive diapause under unsuitable natural environmental conditions, a moderate fecundity otherwise, and usually, high mortality rates in captivity. Based on our current knowledge of GWSS insect biology, an experiment was designed to study the effect of rearing densities on the development and reproductive biology of GWSS under greenhouse conditions, using conditions matching as much as possible with its rearing activities.

OBJECTIVES

1. Study the development and reproductive biology of the glassy-winged sharpshooter under semi-controlled conditions.
2. Determine the effect of increasing the density of glassy-winged sharpshooters per plant on its reproductive potential.
3. Optimize current rearing techniques accordingly. Increase current glassy-winged sharpshooter egg production by a factor of 10-15.
4. Continue studying the biology and behavior of several glassy-winged sharpshooter parasitoid species under laboratory, semi-controlled and field conditions.
5. Evaluate field efficacy of 3-4 *Gonatocerus* species in citrus using parasitoid inoculated plants.
6. Participate in collaborative studies relating to chemical control, classical biological control (exotic parasitoids), DNA analyses, cold storage and development of an artificial diet.

RESULTS AND CONCLUSIONS

In an ongoing experiment initiated in May 2002, four densities were tested: 50, 100, 150 and 200 first instar nymphs per cage, equivalent to 2, 4, 6 and 8 nymphs per plant, respectively. Each density was replicated 16 times. Glassy-winged sharpshooter nymphs were provided 25 potted pea plants replaced twice a month and nymphs were monitored daily for

development to adulthood. Slightly different methodologies were used to handle the cages to allow for determination of development time (8 replicates), size of resulting adults (4 replicates and partial data), total egg production (12 replicates), nymphal mortality (8 replicates and partial data) and, as precisely as possible, adult mortality over time (12 replicates).

Preliminary observations and analyses showed a significant effect of density on the growth and development of the glassy-winged sharpshooter. At the two lowest densities tested, individuals developed in 34 to 36 days as compared to 38 to 41 days at the highest densities. The size of resulting adults decreased significantly when reared at 200 nymphs per cage. Males were significantly smaller than females at all densities. Nymphal mortality averaged 35% and did not vary significantly with increasing density. Total egg production did not vary significantly with increasing rearing density. This indicates an indirect negative impact of high rearing densities on glassy-winged sharpshooter females' reproductive potential, possibly due to nutritional requirements. In addition, significantly higher premature adult mortality was recorded at the highest densities studied. Females produced an average of 1800 eggs per cage. Finally, it appears that the optimum rearing density is no more than 5-6 glassy-winged sharpshooter per plant, given the type of plants selected for this experiment. Based on these observations, multiple steps are being taken to modify all current production activities in such a way that glassy-winged sharpshooter production per space unit increases consistently.

FUNDING AGENCIES

Funding for this project was provided by the USDA Animal and Plant Health Inspection Service.

DEVELOPMENT OF AN ARTIFICIAL DIET FOR THE GLASSY-WINGED SHARPSHOOTER

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Reporting period: The results reported here are from work conducted from October 1, 2001 through October 1, 2002

INTRODUCTION

This work is directed at the development of an artificial diet and a diet-based rearing system for *Homalodisca coagulata*. The approach taken to this work is to analyze the feeding mechanism and feeding dynamics of *H. coagulata* in order to use this information as a guide to the feeding needs of this insect. The assumptions behind this work are that the profile of components in xylem sap used by *H. coagulata* will be an optimal or at least suitable diet for this species, and details of the feeding biology of this insect (such as knowledge of specific feeding strategies and digestive enzymes) will help identify its dietary needs (Cohen 2003). Towards this end, studies were undertaken to pinpoint the details of feeding biology of this species, including how the insect may impact changes in the plant's sap profile.

OBJECTIVES

1. Develop an artificial diet for *H. coagulata*.
2. Develop an understanding of the morphology of the feeding system of this insect.
3. Develop an understanding of the digestive processes used by *H. coagulata* in handling its food.
4. Develop an understanding of the interactions between *H. coagulata* and its host plants to determine whether or not the insect is a passive feeder that simply ingests whatever the plant offers or an active feeder that manipulates or affects the plant's xylem sap composition.

RESULTS AND CONCLUSIONS

A series of diets has been formulated, and tests of these diets are underway. It has been demonstrated that the insects will feed on artificial diet presented through a membrane. Currently, feeding stimulants and profiles are being refined to maximize feeding rates. Mixtures that contain combinations of free amino acids and peptides are being tested now in light of the findings on the digestive physiology and biochemistry of *H. coagulata*.

The gross anatomy, fine anatomy, and ultrastructure of the feeding system of *Homalodisca coagulata* (Say) (Homoptera Cicadellidae) were studied with light (Figures 1-a, 1-b) (bright field, differential interference, fluorescence) and electron microscopy (cryofracture-based scanning and transmission). The mouthparts of *H. coagulata* (including the labium, labrum, and stylets) are relatively short in comparison with those of other Homoptera in relation to the ratio of these structures and the insect's body length. The bristles herein referred to as stylets, contain lateral, paired mandibular stylets, which have a dentition consistent with plant penetrating function. Typical of the Homoptera, *H. coagulata* produces a salivary sheath that extends from the exterior of the plant surface into the stem tissues terminating in the xylem elements. The sheath substance is produced by the paired salivary glands, which lie ventrally between the head and the prothorax. The sheath material fluoresces (Figure 2-a) when excited by various visible and UV wavelengths and can be localized within the plant tissues easily with fluorescence microscopy. Examination of 100 salivary sheaths by light and electron microscopy revealed that these structures are characteristically straight leading directly from the plant surface to the xylem bundles with no evidence of meandering or branching as is seen in aphids and whiteflies. The conspicuous clypeus lies on the anterior and ventral part of the head and marks the region of attachment of the powerful cibarial (sucking) pump muscles, which permit the ingestion of remarkable amounts of xylem sap (which is under negative pressure in the plant's vascular system).

Once xylem sap is ingested, it passes through the food meatus, mouth, and esophagus and empties into the anterior portion of the midgut (mg1). After the sap enters the midgut, it passes through the filter chamber (fc), where it is confined to a tubule that is proximate to a series of four Malpighian tubules and a length of the posterior midgut (MG2). The filter chamber is

extremely active in peristaltic movements that evidently increase the efficiency of concentration of the sap and removal of water to the Malpighian tubules, which remove the water and carry it directly into the hindgut where the water is stored in a bladder-like expansion of the hindgut until it can be discharged. The concentrated sap is processed by the midgut where the final nutrient products are absorbed by microvilli that are on the surface of a highly convoluted series of tubules.

Digestive processes:

We tested for activities of aminopeptidase and general peptidase in the salivary glands (Figure 2-b), filter chamber (Figures 3-a, 3-b), anterior midgut, posterior midgut, and Malpighian tubules of the glassy winged sharpshooter, *Homalodisca coagulata* (Say) (Homoptera Cicadellidae), and of the salivary glands anterior midgut, posterior midgut, and Malpighian tubules of the western tarnished plant bug, *Lygus hesperus* Knight (Heteroptera: Miridae). Both of these are fluid-ingesting species; however, *H. coagulata* is strictly a xylem sap feeder, and *L. hesperus* feeds on slurries of plant materials extracted from protein-rich tissues after pre-digesting plant tissues using extra-oral digestion. As a xylem sap feeder, *H. coagulata* was expected to lack ability to digest peptides because xylem sap is not known to contain substantial amounts of peptides or proteins. However, we found very high activities of aminopeptidase and general peptidase in the midgut of *H. coagulata*. In fact, the aminopeptidase activity from *H. coagulata* exceeded the activity of that enzyme from comparable regions of *L. hesperus* by several fold (Figures 4-a, 4-b). Given the fact that *L. hesperus* is known to ingest protein-rich foods, this finding provides a basis for re-examining our understanding of the *H. coagulata*-plant interaction.

Interactions between H. coagulata and host plants:

The profiles of free amino acids in the xylem sap in infested and uninfested sweet potato plants reflects an increase in the concentrations of most amino acids in the xylem sap from infested plants. Also, the concentration of ninhydrin positive substances was significantly higher in the sap from infested plants than it was for the uninfested counterpart. Most interestingly, when the xylem sap samples were filtered through molecular weight filters of 3 kDa and 30 kDa, there were higher concentrations of ninhydrin positive substances in the samples that ranged from 3-30 kDa in saps from infested plants than from uninfested plants. This supported the hypothesis that the feeding of *H. coagulata* impacted an increase in the available nitrogenous substances. This finding is in accord with the demonstration of an extremely active aminopeptidase in the midgut of this insect.

Artificial Diet: Preliminary Trials:

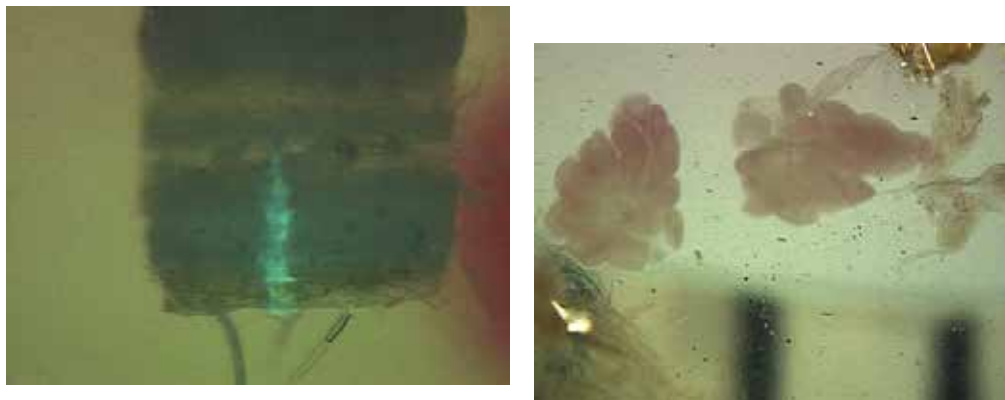
Based on the above findings about complex peptides as part of the feeding profile, a diet was devised to reflect the ability of the *H. coagulata* to use such peptides. The following diet is currently being tested and is stimulating feeding response:

Proteose peptone 1.0 g	Cholesterol 0.0012* (*soluble at 0.0002 g/100 mL water)
Asparagine 0.25 g	β-sitosterol 0.0004
Glucose 0.010 g	Water 200 mL
Fructose 0.025 g	Stir until all components are dissolved.
Citric acid (anhydrous) 0.050 g	Filter through 0.22 μm filter
L-ascorbic acid 0.020 g	Final pH is 4.65.
Wesson salts 0.020 g	

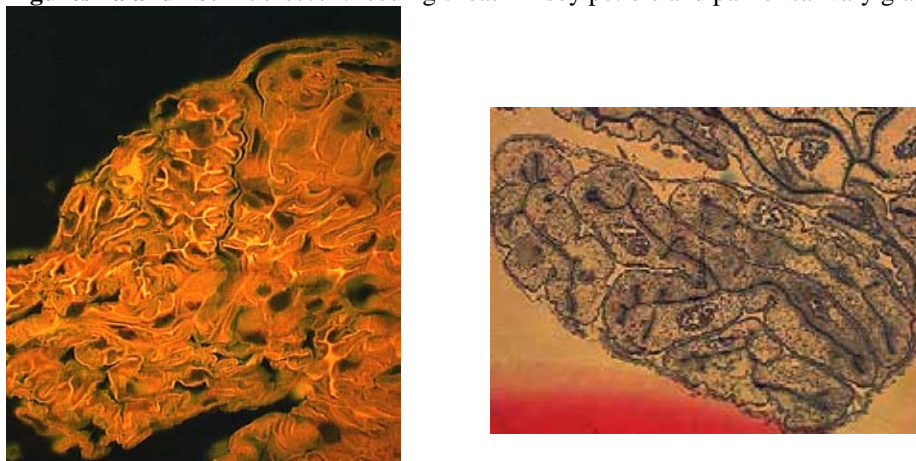
The diet is being presented in small Petri dishes covered with stretched Parafilm, and only a fraction of the nymphs are attempting to feed on the diet in the current presentation format. The mortality is still over 90% of the 1st instar nymphs placed on this diet, but those nymphs that feed last for at least one week and undergo a molt during that time.



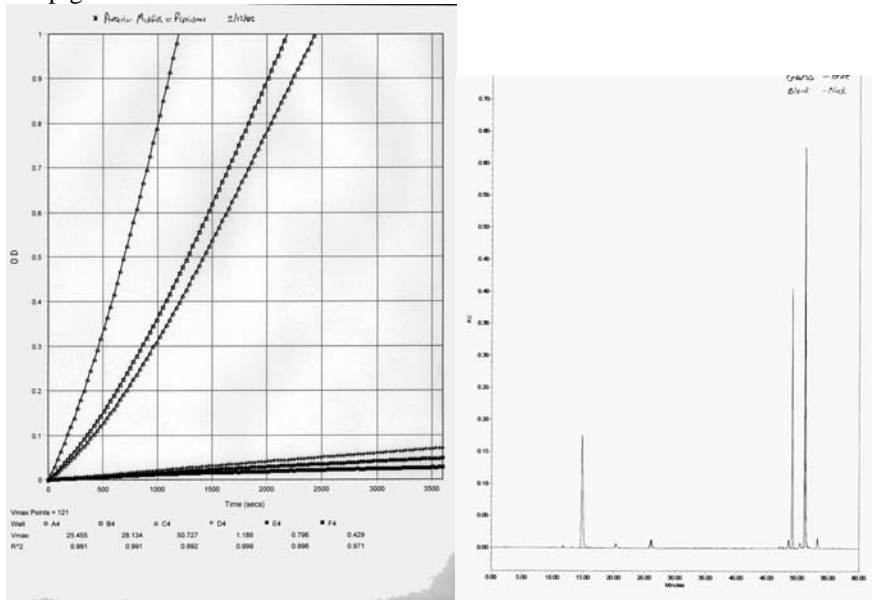
Figures 1-a. GWSS surrounded by dissected gut, **1 b** complete gut, intact.



Figures 2a and 2b: Fluorescent feeding sheath in soy petiole and pair of salivary glands.



Figures 3a and 3b: Fluorescent image of filter chamber (100x) and close-up of filter chamber showing arrangement of Malpighian tubules.



Figures 4a and 4b: Kinetics of amino peptidase activity from GWSS posterior mid-gut (upper three lines) compared to activity from posterior midgut of tarnished plant bug (lower three lines), and chromatogram showing destruction of leucylglycine by GWSS posterior midgut extract.

FUNDING AGENCIES

Funding for this project was provided by the USDA Animal and Plant Health Inspection Service.

INTERSPECIFIC COMPETITION BETWEEN *GONATOCERUS ASHMEADI* AND *G. TRIGUTTATUS* FOR GLASSY-WINGED SHARPSHOOTER EGG MASSES

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Reporting Period: The results reported here are from work conducted from March 2002 to October 2002.

INTRODUCTION

We are currently studying the competitive behavior of two GWSS egg parasitoids, *Gonatocerus ashmeadi* Girault and *G. triguttatus* Girault (both Hymenoptera: Mymaridae), to determine which one shows the most potential as a classical biological control agent for GWSS. A better understanding of the interactions between these two parasitoids may provide an insight into predicting and interpreting field outcomes following the establishment and proliferation of *G. triguttatus* in California as it competes with the precinctive *G. ashmeadi*. Therefore, the following investigations were conducted to investigate which parasitoid species is most competitive using two different experimental designs representing high and low GWSS egg mass densities.

OBJECTIVES

1. To investigate interspecific competition between *G. ashmeadi* and *G. triguttatus* for GWSS egg masses.

MATERIALS AND METHODS

Two experimental designs were used to represent low and high GWSS egg mass densities. The first involved exposing approximately 45 GWSS eggs (egg masses were 1, 3 and 5 days of age) to one mated female *G. ashmeadi* and *G. triguttatus* (~24 hrs of age) for 24 hours in a 3 inch ventilated vial cage at 25°C. The second involved exposing one egg mass (4-8 eggs) to both species (see previous description) for one hour in a 2 inch Petri-dish lined with moist filter paper at 25°C. Visual observations for aggressive behavior were made every 5 minutes during the second experiment and both experiments were replicated 20 times. The number of *G. ashmeadi* and *G. triguttatus* offspring produced per vial or egg mass was recorded.

RESULTS AND CONCLUSIONS

Exposing approximately 45 GWSS eggs (high density situation) to one mated female *G. ashmeadi* and *G. triguttatus* simultaneously produced 45% more *G. ashmeadi* offspring compared to *G. triguttatus* (Figure 1). This may suggest that *G. ashmeadi* is more 'aggressive' than *G. triguttatus* and therefore shows more potential as a biological control agent for GWSS. It also may indicate that *G. ashmeadi* could out compete *G. triguttatus* in the field and prevent its successful establishment and dispersal in California. However, exposing one GWSS egg mass to one mated female *G. ashmeadi* and *G. triguttatus* simultaneously produced 53% more *G. triguttatus* compared to *G. ashmeadi* (Figure 2). The result from the low egg mass density experiment apparently contradicts previous results. This might be due to the differences in GWSS densities between studies and may indicate that *G. ashmeadi* is more efficient at parasitising at high GWSS egg mass densities, whereas when resources are scarce *G. triguttatus* becomes more efficient at excluding competitors. In fact, two observations of *G. triguttatus* aggressively chasing *G. ashmeadi* off the egg mass were recorded during the second study. Furthermore, Lauziere et al. (1999) has shown that at low host densities oogenesis in the parasitoid *Cephalonomia stephanoderis* Betrem (Bethyridae) was delayed and the pre-oviposition phase was extended. Therefore, the lower numbers of *G. ashmeadi* offspring produced in the second study where GWSS egg mass density was low, may be due to *G. ashmeadi* females requiring a longer pre-oviposition period compared to *G. triguttatus* under these conditions.

Alternatively, the difference between results may be a factor of parasitoid age. The first study exposed egg masses to parasitoids that were ~ 24 hrs of age for 24 hours, whereas the second study involved similarly aged parasitoids being exposed to egg masses for just one hour. Therefore, parasitoids were respectively, 48 hours and 25 hours old by the conclusion of the experiments. This may account for differences in competitive behavior between studies because both species may have different pre- oviposition periods or may vary in their response to host interaction and oviposition experience. This may occur because female *G. ashmeadi* and *G. triguttatus* may emerge with complements of undeveloped eggs and, the pre-oviposition period post-emergence during which oocytes develop may differ between these two species. For example, *G. triguttatus* may have a shorter pre-oviposition period allowing superiority at low GWSS density studies; however, it may have a more limiting daily fecundity than *G. ashmeadi*, therefore limiting its efficiency at higher density studies. *Gonatocerus ashmeadi* may require a higher degree of host interaction and oviposition experience compared to *G. triguttatus* to maximize its parasitization efficiency. This would enable *G. ashmeadi* to out compete *G. triguttatus* in the first study because it involved high GWSS densities, a longer exposure time, and a greater potential for host encounters, and subsequent experience. However, to accurately hypothesize about the mechanisms behind why results were contradictory in these two studies, and to determine which species shows more potential as a biological control agent for GWSS, trials

investigating parasitoid pre-oviposition periods, longevity, daily and lifetime fecundity, and field-based competition studies are required.

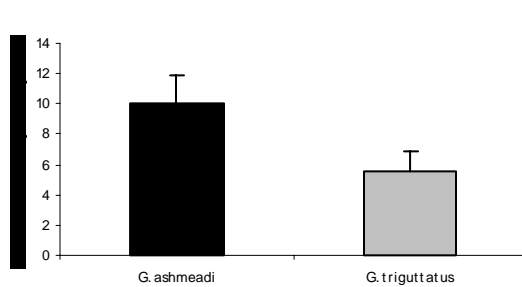


Figure 1.: Mean number of *G. ashmeadi* and *G. triguttatus* offspring produced from exposing 100 GWSS eggs in a vial to one mated female of both species

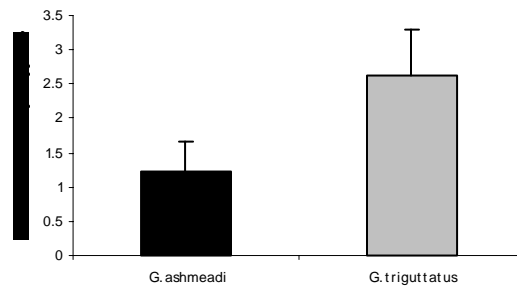


Figure 2.: Mean number of *G. ashmeadi* and *G. triguttatus* offspring produced from exposing one GWSS egg mass to one mated female of both species

Funding for developing mass rearing techniques for biological control programs is limited and results presented here raise the important question: how do we decide which parasitoid species shows the most potential as a biological control agent? Depending on the experimental design used, results can favor a different parasitoid species. It may be beneficial to determine which design is more realistic of a field situation. The first study may be representative of high GWSS egg densities that occur in summer; whereas the second study may be representative of low densities that occur in spring, or could occur year round as a result of a successful biological control program against GWSS. Therefore, experimental designs that simulate low GWSS densities may be more realistic at determining which parasitoid species shows the greatest over all potential for suppressing GWSS population recruitment from the egg stage. Results presented here suggest that as GWSS biological control progresses in California there may be a shift in species dominance from *G. ashmeadi* to *G. triguttatus* as GWSS populations diminish.

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FUNDING AGENCIES

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BIOLOGICAL CONTROL OF *HOMALODISCA COAGULATA*

(revised report; submitted April 15, 2003)

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Reporting Period: The results reported here are from work conducted from October 1, 2000 to October 31, 2002.

INTRODUCTION

Although the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say), occurs throughout the Lower Rio Grande Valley (LRGV) of Texas, it is never abundant and is usually difficult to locate. Although there is an extensive local citrus industry, eggs are only occasionally found in orchards. This insect appears to be most evident in urban areas. Earlier, informal surveys were conducted to collect egg parasitoids for shipment to California for release. Following extensive examination of various plant species, it was found that oviposition occurred during two fairly distinct generations, most abundantly on the native leguminous tree, Texas mountain laurel, *Sophora secundiflora* (Ortega) Lag. ex D.C. (Leguminosae) and varieties of crape myrtle, *Lagerstroemia indica* L. (Lythraceae). Once their favorite oviposition hosts were identified in 2000, a survey was begun to assess the impact of parasitism and predation of egg masses. Also, a qualitative survey of breeding hosts was made.

OBJECTIVES

1. Determine the seasonal impact of egg parasitoids.
2. Determine breeding host plants in the Lower Rio Grande Valley of Texas.
3. Record biological and behavioral attributes of the most important parasitoids.

RESULTS AND CONCLUSIONS

During two years of the survey, 993 and 1,153 egg masses were sampled during 2001 and 2002, respectively. Most were collected from *S. secundiflora* (March-May) and *L. indica* (late May through summer and fall) in Weslaco, TX. All parasitism was by wasps in the genus *Gonatocerus* (Mymaridae). In 2001, 86% of all egg masses on *S. secundiflora* were parasitized, 7% were predated, nymphs emerged from 12%, with 1% unknown (n = 125 masses). On *L. indica*, 85% were parasitized, 8% predated, with 11.4% nymphal emergence (n = 993 masses). During 2002, egg masses on *S. secundiflora* leaves showed 74% of the egg masses were parasitized, 4% incurred some predation and 27% indicated at least some nymphal emergence (n = 285 masses). For *L. indica*, 89% were parasitized, 8% predated, with 8% nymphal emergence (n = 691 masses). Most, but not all parasitized masses were completely parasitized. *Gonatocerus morrilli* Howard, *G. ashmeadi* Girault and *G. triguttatus* Girault were recovered. *G. triguttatus* was the most important species, with the other two species generally only appearing at the very beginning and very end of the seasons.

Mature adult GWSS were seldom seen on trees bearing eggs. Nymphs were observed on a very wide variety of plants, almost always on new growth. Spiders were often observed with captured nymphs and adults.

Observations were made on biological and behavioral attributes of *G. triguttatus*. At 27°C, development from egg to emergence was 12.8 d for males (n = 111); 13.3 d for females (n = 70). Males lived 6.6 d; females lived 6.0 d, when provided pure honey for food. Sibling mating took place on or in the vicinity of the egg mass within confinement of Petri dishes. Unparasitized GWSS eggs eclosed after 7.6 d.

Female parasitoids antennated the egg mass prior to ovipositing but did not oviposit in linear sequence. Nevertheless, they almost always parasitized the complete mass. However, the brochosomes significantly hindered oviposition time. Brochosome particles quickly accumulated on tarsi and antennae, and resisted preening attempts to remove them. Frequently, the parasitoid would leave the mass to spend several minutes preening.

Overall, egg parasitism of GWSS was high throughout the year in the LRGV of Texas, primarily by *G. triguttatus*, but *G. ashmeadi* and *G. morrilli* also occur, though in much lower numbers. A sample of over 50 egg masses in San Antonio, TX during the summer of 2002 yielded only *G. ashmeadi*. Triapitsyn and Phillips (2000) reported *G. triguttatus* from NE Mexico and Weslaco, TX, and although Triapitsyn et al. (1998) didn't report this species in collections from elsewhere, it was recently reported from another sharpshooter host in Apopka, FL (Triapitsyn et al. In press). Thus, the range of *G. triguttatus* may be confined to extremely southern areas only.

The purpose of the white powdery brochosomes that female GWSS place around the egg masses has been a matter of conjecture (Hix 2001, Rakitov 2002). While the material may serve multiple purposes, it clearly slows down the time *G.*

triguttatus is able to complete oviposition of GWSS egg masses. Nevertheless, the high discovery rate of egg masses by parasitoids clearly demonstrates that parasitoids possess efficient host location mechanisms and are important natural enemies of GWSS in south Texas.

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FUNDING AGENCIES

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HOST SELECTION AND LOW TEMPERATURE STORAGE OF THE GLASSY-WINGED SHARPSHOOTER, *HOMALODISCA COAGULATA*

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INTRODUCTION

The egg parasitoid, *Gonatocerus ashmeadi*, is a mymarid wasp that accounts for most of the observed parasitism in California on the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say), a vector for Pierce's disease. In the absence of techniques for propagating the wasp via artificial methods, it is very important to mass-rear the GWSS to provide host eggs for this parasite to be used in bio-control programs. Low temperature storage is an integral part of the process of mass-rearing insects for use in agricultural pest control programs (Leopold 1998). Through cold storage, parasitized and unparasitized GWSS eggs may be accumulated and held for later use in rearing and releasing parasitoids. Although Al-Wahaibi and Morse (2002, submitted) reported that the development of GWSS eggs held at 11.5 °C was retarded and aborted during early stages of eye spot formation, data regarding the effect of low temperature throughout the development of the GWSS and that of the egg parasitoid are lacking.

Further, choosing suitable host plants, which are amenable to cold storage, will be very critical for establishing and maintaining the leafhopper colony and for obtaining large numbers of leafhopper eggs. The sharpshooter is a highly polyphagous leafhopper having over 100 known host plants in Florida (Adlerz 1979). Recent observation shows that the leafhopper can feed on at least 72 plant species in 37 families (Hoddle et al. 2002, submitted), and 73 plant species in 35 families (Blua et al. 1999). Although feeding is apparently limited to xylem vessels on all host plants (Anderson et al. 1989), some studies have shown that the leafhopper exhibits host-plant preference (Adlerz 1979; Mizell and French 1987), and that the amide concentrations in host plants may potentially cause an oviposition preference by the leafhopper (Andersen et al. 1992). Some field observations have indicated the preference for different plant species varied with different times of the year (Adlerz 1979; Mizell and French 1987; Brodbeck et al. 1990). However, little quantitative data are available so far on host plant preference of feeding adult males and females under laboratory or mass-rearing conditions.

OBJECTIVES

1. Examine feeding behavior of GWSS adults on various host plants and determine the effects of cold storage tolerant host plants on size of the egg masses, egg hatch, nymphal development.
2. Determine the cold tolerance of GWSS eggs and parasitized eggs during development.
3. Determine the most effective method for cold storage of GWSS eggs and parasitoids.

RESULTS AND CONCLUSIONS

Host Selection:

The experiments were conducted in USDA-ARS, Biosciences Research Laboratory, Fargo, from July through October 2002. These studies showed that adult female sharpshooters (fourth generation from parents collected in Riverside, California) had a significant host preference when given a choice of 12 plant species at 25°C, RH 65% and L 14: D 10 photoperiod. The test plants included corn (*Zea mays*), sorghum (*Sorghum aethiopicum*), millet (*Milium effusum*), euonymus (*Euonymus spp.*), mums (*Chrysanthemum spp.*), hibiscus (*Hibiscus spp.*), sunflower (*Helianthus annuus*), eggplant (*Solanum spp.*), cantaloupe (*Cucumis melo*), cotton (*Gossypium hirsutum*), wild grape (*Parthenocissus quinquefolia*) and a plant of Lamiaceae family. The percentages of feeding females varied significantly among different plant species at time intervals examined between 6 to 48 hours (Table 1). A majority of the female adults preferred feeding on sunflower while less than 6% females were observed feeding on millet, corn, sorghum, cantaloupe, wild grape and the Lamiaceae plant. For males, no significantly different feeding preferences were observed, although at the end of the test almost 40% of the insects were found on just 3 plants: egg plant, hibiscus and sunflower (Table 1). The sharpshooter deposited eggs on leaves of 7 of the 12 plant species, which were corn, sorghum, millet, euonymus, chrysanthemum, hibiscus and sunflower. Our data indicate that plant species had no significant influence on the size of egg mass (the average number of eggs per mass) but egg hatch was affected (Table 2). Although sunflower was one of preferred host plants, egg hatch was significantly lower on it than the other plants.

Approximately 68% of the sunflower leaves bearing eggs died or wilted from desiccation while still attached to the plant and this consequently caused many of the eggs to die. In addition, our observations indicate that the sharpshooters from the colony shipped to us from the APHIS facility in Texas readily oviposit on eggplant while it is not preferred by our colony females.

Low Temperature Storage:

In a comparison with hibiscus, chrysanthemum and sunflower plants, euonymus cuttings and leaves remain viable the longest in incubators set at either 2, 5, 7, 10, or 12°C. The leaves of hibiscus cuttings or whole plants begin to wilt when stored at 2°C for 24 hr while chrysanthemum remains fresh for 3-4 weeks. Euonymus cuttings placed in nutrient solution remain fresh and viable for rooting after nearly 60 days at 10°C. We collected sharpshooter eggs by using euonymus plants and placed the 0-1 day-old embryos into incubators. After storage at 10°C for 1-6 days, the eggs could hatch in part or all (Figure 1). Furthermore, these embryos had a similar developmental time to hatching as the controls after they were taken out of cold storage (Figure 2). However, no eggs hatched after cold storage for 8 or 10 days at 10°C. The embryos placed at 2°C for 2 or 4 h could hatch within 8-9 days and hatching was 67% and 62%, respectively. After storing 0-1 day-old eggs at 5°C for 6 days, up to 63 % of the embryos emerged as nymphs. No eggs stored at 5EC for more than 8 days would hatch. At 12°C, embryonic development proceeded very slowly and we found that 24% of the eggs could hatch after storage for 20 days. However, because of the low number of egg masses obtained (some of the points on Figures. 1 and 2 had < 10 egg masses), these experiments will be repeated again. Finally, *Gonatocerus ashmeadi* parasitoids within 1-2 day-old sharpshooter eggs on euonymus leaves could completely emerge in the 5°C incubator within 20 days. Furthermore, we found that on the chrysanthemum leaves the parasitoids within 1-2 day-old sharpshooter eggs could emerge after storage at 2°C for 6 days. Further studies are planned to identify the threshold for development for the parasitoid and which developmental stage is the most tolerant to cold storage.

Table 1. Mean (\pm SE)% of total number of *H. coagulata* adults on different host plants at different time intervals

Plants	Sex	Per cent adults on plants at time interval ^a				
		6h	12h	24h	36h	48h
Purple millet	male	4.6 \pm 2.9 b	5.9 \pm 3.2 b	5.0 \pm 0.9 b	6.0 \pm 3.9 b	4.9 \pm 2.9 b
Euonymus		6.4 \pm 3.2 b	9.4 \pm 5.3 b	6.4 \pm 3.2 b	9.4 \pm 5.3 b	9.4 \pm 5.3 b
Egg plant		10.8 \pm 3.5 b	13.8 \pm 1.6 b	12.3 \pm 2.6 b	12.2 \pm 3.4 b	12.2 \pm 4.0 b
Hibiscus		10.3 \pm 4.4 b	9.5 \pm 5.4 b	7.2 \pm 4.3 b	8.4 \pm 5.4 b	10.9 \pm 7.8 b
Cotton		9.1 \pm 2.9 b	9.1 \pm 2.9 b	7.6 \pm 2.0 b	7.5 \pm 3.9 b	9.0 \pm 4.5 b
Corn		4.1 \pm 2.7 b	2.6 \pm 1.4 b	2.6 \pm 1.4 b	2.6 \pm 1.4 b	2.6 \pm 1.4 b
Sunflower		10.7 \pm 6.4 b	12.3 \pm 5.2 b	12.3 \pm 5.2 b	16.1 \pm 5.1 b	16.1 \pm 5.1 b
Sorghum		1.5 \pm 1.5 b	1.5 \pm 1.5 b	1.5 \pm 1.5 b	2.7 \pm 1.4 b	2.8 \pm 1.4 b
Cantaloupe		10.7 \pm 5.5 b	9.2 \pm 5.3 b	10.7 \pm 5.5 b	11.0 \pm 5.6 b	9.5 \pm 4.8 b
Chrysanthemum		5.0 \pm 0.9 b	7.4 \pm 1.9 b	9.0 \pm 1.3 b	9.4 \pm 3.1 b	9.4 \pm 3.1 b
Lamiacea		0 b	2.2 \pm 2.2 b	2.2 \pm 2.2 b	3.5 \pm 1.9 b	3.5 \pm 1.9 b
Wild grape		2.6 \pm 1.4 b	7.6 \pm 2.0 b	7.7 \pm 0.7 b	7.4 \pm 1.9 b	6.2 \pm 0.9 b
Purple millet *	female	1.2 \pm 1.2 c	4.9 \pm 1.3 bc	3.6 \pm 0.1 bcd	2.5 \pm 1.2 b	2.4 \pm 1.1 b
Euonymus*		5.8 \pm 4.1 bc	10.7 \pm 3.9 bc	10.7 \pm 5.4 bcd	11.8 \pm 6.2 b	11.8 \pm 6.2 b
Egg plant		11.0 \pm 4.4 ab	8.6 \pm 3.3 bc	11.0 \pm 4.4 bc	11.0 \pm 4.4 b	11.0 \pm 4.4 b
Hibiscus*		4.9 \pm 3.3 bc	7.2 \pm 2.1 bc	7.2 \pm 2.1 bcd	9.6 \pm 3.2 b	13.0 \pm 5.1 b
Cotton		8.3 \pm 2.9 abc	13.3 \pm 2.6 b	13.3 \pm 2.6 ab	9.8 \pm 3.4 b	8.6 \pm 5.4 b
Corn*		4.8 \pm 2.4 bc	3.6 \pm 2.1 bc	3.5 \pm 2.0 cd	2.5 \pm 2.5 b	2.5 \pm 2.5 b
Sunflower*		15.6 \pm 2.3 a	25.2 \pm 7.1 a	21.5 \pm 4.7 a	22.7 \pm 5.2 a	26.3 \pm 4.6 a
Sorghum*		1.2 \pm 1.2 c	3.7 \pm 2.1 bc	3.7 \pm 2.1 bcd	3.7 \pm 2.1 b	3.7 \pm 3.7 b
Cantaloupe		2.4 \pm 1.2 c	3.6 \pm 1.1 bc	4.9 \pm 1.3 bcd	5.9 \pm 2.2 b	5.9 \pm 2.2 b
Chrysanthemum*		2.4 \pm 1.2 c	4.8 \pm 1.1 bc	6.0 \pm 1.2 bcd	2.5 \pm 1.2 b	2.5 \pm 1.2 b
Lamiacea		0 c	1.1 \pm 1.1 c	1.1 \pm 1.1 d	1.1 \pm 1.1 b	2.3 \pm 1.2 b
Wild grape		2.4 \pm 1.2 c	2.4 \pm 1.2 c	2.4 \pm 1.2 cd	3.5 \pm 2.0 b	2.4 \pm 1.2 b

^a One-way ANOVA – means were separated by Duncan's Multiple Range Test

* Denotes plants on which oviposition occurred.

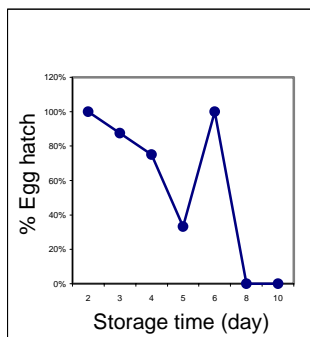


Figure1. Percentage egg hatch at different cold storage times at 10EC

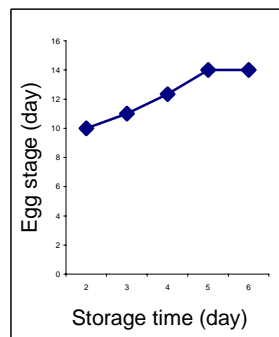


Figure 2. Egg stage of *H. coagulata* eggs stored at 10EC

Table 2. Egg mass size and hatching related to host plants

Plants	No. of eggs/mass (range) ^a	Per cent egg hatch ^a
Euonymus	16.8 ± 1.6 (10~37) b	81.8 ± 9.1 ab
Chrysanthemu m	15.0 ± 3.1 (9~23) b	72.8 ± 16.1 ab
Hibiscus	12.0 ± 2.7 (6~19) b	81.3 ± 12.0 ab
Sunflower	11.1 ± 1.9 (2~31) b	24.7 ± 9.9 c
Sorghum	11.5 ± 1.5 (10~13) b	100.0 ± 0.0 a
Corn	15.0 ± 2.0 (13~17) b	94.1 ± 5.9 a
Purple millet	14.0 ± 3.0 (11~17) b	95.5 ± 4.5 a

^aValues followed by different letters in each column were significantly different (P<0.05)

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FUNDING AGENCIES

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**IS THE GLASSY-WINGED SHARPSHOOTER PARASITOID
GONATOCERUS ASHMEADI (HYMENOPTERA: MYMARIDAE) ONE SPECIES
OR A COMPLEX OF MORPHOLOGICALLY INDISTINGUISHABLE SIBLING SPECIES?**

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Reporting Period: The results reported here are from work conducted from mid-September 2002 to November 1, 2002.

INTRODUCTION

Gonatocerus ashmeadi Girault is a common and seemingly widespread egg parasitoid of glassy-winged sharpshooter (GWSS). Location records for *G. ashmeadi* indicate its natural range to be Florida, Louisiana, northeastern Mexico, Mississippi, North Carolina, eastern Texas (which coincides with the presumed native range of GWSS), and southern and central California (the adventive range of GWSS). *Gonatocerus ashmeadi* was collected from eggs of the native smoke-tree sharpshooter, *Homalodisca lacerta* (Fowler), as well as from GWSS eggs before *G. ashmeadi* releases began as part of an organized biological control program in CA. *Gonatocerus ashmeadi* is currently being imported from different areas within the natural range of GWSS and released in California with the assumption that this is one species and not a complex of morphologically indistinct sibling species. Species identifications have been made using light microscopy to determine the presence of key morphological features for *G. ashmeadi*. Light microscopy has failed to reveal any differences between different *G. ashmeadi* populations except for some specimens from central and southern Tamaulipas and San Luis Potosí, Mexico (Triapitsyn et al. 2002). Due to the minute size of adult *Gonatocerus* parasitoids (1.2-1.7 mm in length), their taxonomic identification is very difficult without careful and costly preparation, which involves mounting on microscopic slides. The morphological characters that are used for differentiating between closely related *Gonatocerus* spp. can be variable and thus species limits are often difficult to assess without supporting data from biological and molecular data. The purpose of work proposed here is to determine whether *G. ashmeadi* in the native range of GWSS is one species or a complex of cryptic species that can't be separated on the basis of currently employed morphological characters. We intend to use three approaches to determine the species identity of different *G. ashmeadi* populations.

OBJECTIVES

1. Reassessment of key morphological features using scanning electron microscopy (SEM) to determine if subtle morphological differences exist between *G. ashmeadi* populations which could possibly indicate species differences.
2. Conduct mating compatibility studies to determine if different populations of *G. ashmeadi* are reproductively isolated, or if mating occurs, whether offspring are viable thereby defining species groups on the basis of successful interbreeding.
3. To determine if molecular differences exist between *G. ashmeadi* populations collected from different regions by comparing mitochondrial and ribosomal DNA sequences. Molecular dissimilarities of key regions could potentially indicate the existence of different species.

RESULTS AND CONCLUSIONS

Results from these three areas (morphology, behavior, and molecular) are currently under investigation and will be evaluated together to determine whether *G. ashmeadi* as it is currently viewed is a valid species or whether it is an aggregate of morphologically indistinguishable cryptic species.

FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

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

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INTRODUCTION

Observations in northeastern Mexico and Texas, USA, during the past three years revealed presence of *Homalodisca coagulata* (Say) (GWSS) there, but in very low densities (Coronado-Blanco et al. 2000, Triapitsyn and Phillips 2000). Almost all egg masses of GWSS and other related sharpshooters, such as *Oncometopia* spp., were heavily parasitized. The climate in the central part of Tamaulipas, Mexico, is very similar to the climate in the valleys of southern California. 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; Triapitsyn in review; Triapitsyn et al. in press) and thus are promising biological control agents. As a result of the collections made in northeastern Mexico during 2000 and 2001, colonies of three species, *Gonatocerus ashmeadi* Girault, *G. morrilli* (Howard), and *G. triguttatus* Girault (Hymenoptera: Mymaridae), were established in UCR quarantine and insectary (Triapitsyn and Hoddle 2001; Triapitsyn et al. 2002) and later propagated and released against GWSS in California by CDFA and USDA researchers.

OBJECTIVES

1. Search for and collect additional egg parasitoids of GWSS, particularly *G. fasciatus* and *Ufens spiritus* Girault (Hymenoptera: 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, establishment of cultures in UCR quarantine, and a following evaluation.
2. Recollect the target species of GWSS egg parasitoids, particularly *G. triguttatus*, in northeastern Mexico and clear them through UCR quarantine to be used for preventing inbreeding in the cultures maintained by our cooperators from the CDFA for a large-scale classical biological control program against GWSS in California.

RESULTS AND CONCLUSIONS

Three exploratory trips were made during 2002: 1) to the States of Cuahuila (Parras and San Lorenzo, where we found numerous egg masses of GWSS which were not parasitized, apparently due to an unusually cold weather), Nuevo León, and Tamaulipas, Mexico, in March 2002 (S. Triapitsyn, V. Berezovskiy, and S. Myartseva); 2) to Cuahuila, Nuevo León, Tamaulipas, San Luis Potosí and Queretaro, Mexico, in April 2002 (D. Yanega and S. Myartseva); and 3) to Louisiana in April 2002 (M. Hoddle and S. Triapitsyn). Material from a trip to Jackson, Mississippi, in March 2002 by D. Morgan was also processed in UC Riverside quarantine as part of this project.

A survey of egg parasitoids of GWSS was undertaken in Baton Rouge, Louisiana, during April and May 2002. It was conducted initially by M. Hoddle and S. Triapitsyn during the first week of April 2002 and was continued after our departure by D. Chouljenko, using sentinel egg masses of GWSS on various plants following Triapitsyn et al. (1998). The fairyfly wasp *G. fasciatus* was reared on numerous occasions from egg masses of GWSS, laid in leaves of several different plants, and shipped under an appropriate permit to UCR quarantine. A colony of *G. fasciatus* was successfully established in quarantine on *H. coagulata* eggs laid in leaves of *Euonymus japonica*. Observations on the biological traits of *G. fasciatus*

revealed that this species has a gregarious habit, with two or more wasps developing per each egg of the host, unlike other common North American parasitoid species of *H. coagulata* from the same genus, such as *G. ashmeadi*, *G. morrilli*, or *G. triguttatus*, which are solitary parasitoids (Triapitsyn et al. in review). Besides the obvious advantages in mass-rearing of a gregarious parasitoid, *G. fasciatus* may be also considered a promising biological control agent for control of GWSS in central and northern California (if GWSS becomes established there) because its native range includes Illinois; thus, *G. fasciatus* must be better adapted to colder climates than any other known mymarid egg parasitoid of GWSS. The species of exotic egg parasitoids collected during 2002 and propagated at UC Riverside (if applicable) are listed in Table 1.

Table 1. The species of exotic egg parasitoids collected during 2002 and propagated at UC Riverside.

Genus and species of egg parasitoid	Originally from: (country and state)	Original host	Propagated on GWSS at UCR quarantine (yes/no)
<i>Gonatocerus ashmeadi</i>	USA: Louisiana	<i>Homalodisca coagulata</i>	Yes
<i>Gonatocerus fasciatus</i>	USA: Louisiana	<i>Homalodisca coagulata</i>	Yes
<i>Gonatocerus triguttatus</i>	Mexico: Tamaulipas	<i>Oncometopia ?clarior</i>	Yes
<i>Ufens spiritus</i> (= <i>Zagella</i>)	USA: Louisiana USA: Mississippi	<i>Homalodisca coagulata</i> <i>Homalodisca coagulata</i>	No (failed) No (failed)
<i>Ufens</i> n. sp.	Mexico: Tamaulipas	<i>Oncometopia ?clarior</i>	No (died)
<i>Ufens</i> sp.	USA: Mississippi	<i>Homalodisca coagulata</i>	No (failed)

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*Biology, Ecology, and
Direct Impact of the
Glassy-winged
Sharpshooter*



BIOLOGY AND ECOLOGY OF GLASSY-WINGED SHARPSHOOTER IN THE SAN JOAQUIN VALLEY

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INTRODUCTION

Table, raisin, and wine grapes grown in the San Joaquin Valley (SJV) comprise some of California's largest and economically productive agricultural commodities. Their commercial existence is now threatened by presence of both the glassy-winged sharpshooters (GWSS), *Homalodisca coagulata*, in the SJV (Phillips 1998, Blua et al. 1999) and the bacterial pathogen, *Xylella fastidiosa*, which is the causal agent of Pierce's disease (PD) (Purcell and Sanders 1999a). GWSS may not be a more "efficient" vector than the native California sharpshooters (Purcell and Saunders 1999a), but it is certainly more important. While initial control efforts will most certainly be directed at chemical suppression or spot eradication, there are a number of questions on GWSS biology and ecology in the SJV that should be addressed in order to improve control programs and/or increase control options. The primary focus of this research is the description of GWSS preference, egg deposition, age structure, population dynamics and levels of natural regulation on different host plants in urban / agricultural interface in the SJV where untreated populations serve as an inoculum source for nearby vineyards and citrus. We will also test sampled GWSS, from selected host plants and ecosystems, for the presence of *X. fastidiosa*.

OBJECTIVES

1. Determine glassy-winged sharpshooter (GWSS) biology and ecology throughout the season, particularly its age structure on and utilization of the different host plants that represent common breeding or dispersion refuges for GWSS in the San Joaquin Valley.
2. Determine the presence of *Xylella fastidiosa* in GWSS collected from different host plant species and in selected ecosystems in the San Joaquin Valley.
3. Begin to evaluate predator release as an additional suppression tactic.

RESULTS AND CONCLUSIONS

We began to categorize GWSS age structure, ecology, and resident natural enemies (particularly predators) on different host plants common in the SJV in spring 2002. Our initial methodology relied on field samples taken over a series of dates and on different host plant in untreated urban and agricultural regions in Fresno County. The search and spray program in that region was so effective that new GWSS sightings were treated within days or, if left untreated, the GWSS population density was too low to sample for our purposes. For these reasons, we adjusted our methodology and region sampled. Studies were moved to Bakersfield and host plant preference studies using potted host plant were included to manipulate the availability of same-aged and same-condition (e.g., irrigation and fertilization amounts) host plants to natural GWSS and natural enemy populations.

Host preference studies were conducted in unsprayed, GWSS infested areas (a citrus orchard and a residential area) in Bakersfield, California. Potted (6.6 liter) ivy, photinia, citrus, gardenia, privet, euonymus, hibiscus, agapanthus (lily of the Nile), grapevine, crape myrtle, eucalyptus, and oleander were set in a randomized block design (3 blocks in the citrus orchard and 4 in residential areas). GWSS eggs, nymphs and adults and GWSS predators and parasitoids were counted weekly from July through October. Initial results confirm field surveys from the Temecula and Riverside infestations that GWSS populations dynamics are influenced by host plants. There was a significant ($P < 0.1$) oviposition preference for some host plants, with more GWSS egg masses on crape myrtle, privet, grape, gardenia, and citrus than other plants (Figure 1). No or few egg masses were found on oleander and ivy. Interestingly, GWSS egg mass density was not related to adults or nymphs density ($F=0.16$, $df=1,82$, $P=0.68$, $r^2=0.002$; $F=0.03$, $df=1,82$, $P=0.86$, $r^2=0.001$, respectively). For example, GWSS nymphs

were significantly more common on oleander, which had no GWSS egg masses, than citrus, which had the most egg masses (Figure 2). There was no treatment difference in the number of adults observed (Figure 3); still, it is difficult to accurately measure adult densities through visual counts. The potted plants were in contact with each other and, therefore nymphs could move between plants, suggesting that oviposition preference may be different from nymph feeding preference.

Another possibility is disparate egg and nymph mortality among treatments, which may be suggested by both a significant treatment difference in the number of predators observed on the potted plants (Figure 4) and significant relationship between predators and GWSS egg masses ($F=8.52$, $df=1,82$, $P=0.005$, $r^2=0.09$). There was no relationship between predator and GWSS nymph densities, although GWSS population dynamics showed a clear reduction in nymph density after oviposition (Figure 5), which may have reduced predators during the very small sampling window. Furthermore, a significant relationship between observed parasitoids foraging on plants and GWSS egg masses ($F=16.2$, $df=1,82$, $P<0.001$, $r^2=0.16$) suggest many nymphs did not emerge (these data are not yet analyzed). In the unsprayed citrus block, we found a season-long “egg mass” parasitism rate of $68.2 \pm 0.02\%$; when an egg mass was attacked most of the eggs were parasitized, resulting in a season-long “egg” parasitism rate of $51.8 \pm 1.8\%$ (there were 11.6 ± 0.2 eggs per egg mass). Late-season parasitism was $>90\%$, as has been reported in previous studies. A subsample of emerged parasitoids has found only *Gonatocerus ashmeadi* present (Triapitsyn et al. 1998). The results from the potted plant experiment suggest GWSS adults have host oviposition preferences that may be different from the nymph feeding preference (see Brodbeck et al. 1995, 1996). Results also suggest that parasitoid and predator densities tract GWSS density. Abiotic and biotic mortality factors accounts for a reduction of ca. 35 eggs per plant to ca. 0.15 large GWSS nymphs per plant.

The experimental use of potted plants presents potential bias. For example, in the citrus block the resident GWSS and parasitoids may have been preconditions to citrus, resulting in both greater GWSS and parasitoid densities on that treatment. Surveys of urban areas were made to determine GWSS and natural enemy host plant relationships. Results are still being processed. Figure 6 provides an example from one survey. The information shows GWSS host plant preference in urban settings. Observations indicate that host plant condition between surveyed regions may be as important as host plant species, with plant vigor (typically fertilization, age or irrigation amounts) being the primary factor. During the GWSS surveys, egg masses are collected to determine parasitoid species composition and activity. Similarly, predator species and density are recorded. Predators are collected and stored at -80°C for later processing by Dr. James Hagler with immunologically-based assays that employ pest-specific monoclonal antibodies (MAbs) that can be used in an ELISA to identify the key predators of GWSS (Hagler et al. 2001). To date, samples have been taken (Bakersfield, Porterville Fresno and Ventura, CA) and will soon be processed.

A description of GWSS biology and ecology on host plants in urban areas of the SJV will help understand GWSS seasonal movement and infestation foci. For example, information on the abundance, host plant use, and seasonal dispersal patterns of resident sharpshooters (e.g., blue-green sharpshooter) (Goodwin and Purcell 1992, Perring et al. 2001). The same critical information for GWSS is lacking for the SJV. This work will provide a needed baseline on resident natural enemies of GWSS in the SJV and their contribution to GWSS mortality. Information on GWSS movement and host plant succession in the SJV may be useful for modification of surrounding vegetation or traps crops can potentially suppress GWSS movement into a vineyard.

Research has not yet begun on identifying the incidence of *X. fastidiosa* in GWSS adults collected from different habitats in different geographic regions, which will aid researchers currently mapping out PD and *X. fastidiosa* sources in the SJV, and on the augmentation of selected natural enemy species.

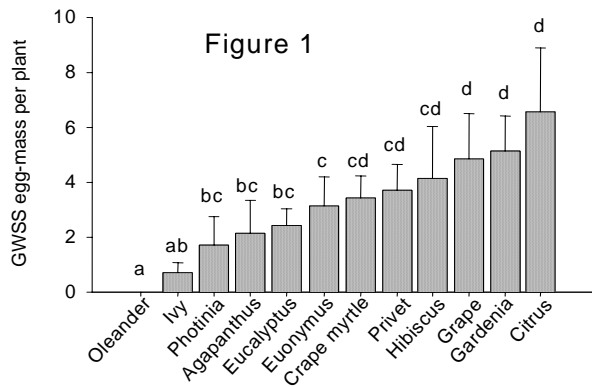


Figure 1. Average densities (sum \pm SEM) of GWSS egg masses on potted plants shows a significant oviposition preference for some host plants or avoidance of other plants. Letters above each mean are significantly different, Fisher's LSD at $P < 0.1$.

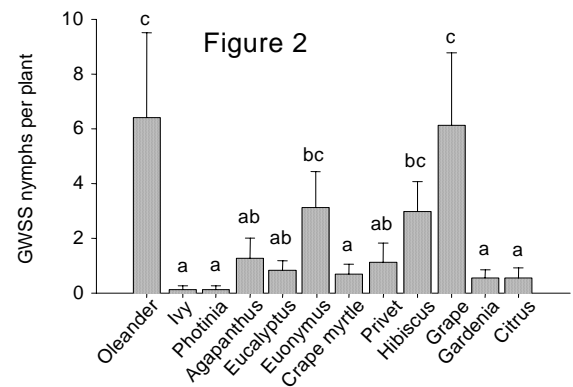


Figure 2. Average densities (sum \pm SEM) of GWSS nymphs on potted plants shows wide discrepancy between egg mass density (Figure 1) and GWSS nymph density. Letters above each mean are significantly different, Fisher's LSD at $P < 0.1$.

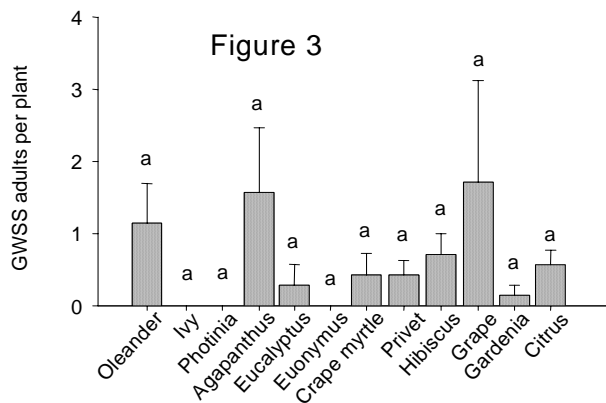


Figure 3. Average densities (sum \pm SEM) of adults observed resting or feeding on potted plants shows no significant different among treatments. These data were collected during last adult flight (July to October 2002). Letters above each mean are significantly different, Fisher's LSD at $P < 0.1$.

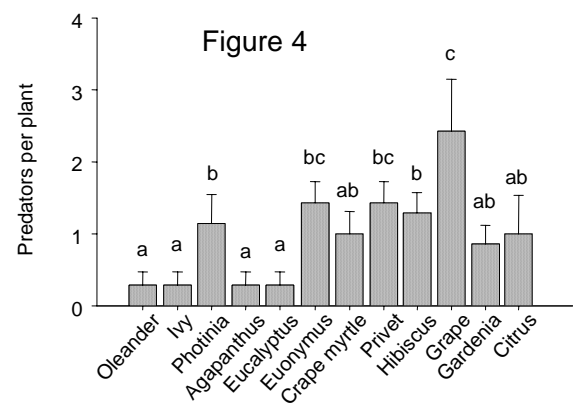


Figure 4. Average densities (sum \pm SEM) of predators observed on potted plants shows a significant different among treatments. Predators were spiders (82.9%), lacewings (11%), preying mantids (3.6%) and assassin bugs (2.4%). Letters above each mean are significantly different, Fisher's LSD at $P < 0.1$.

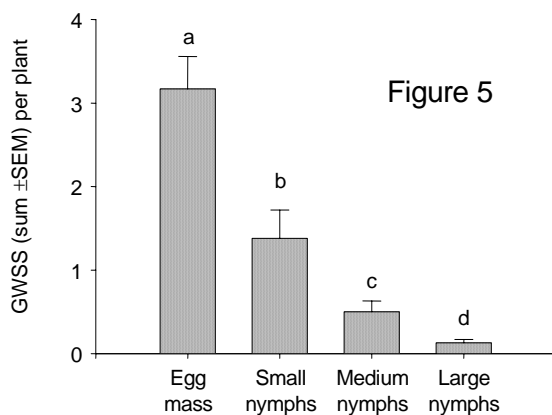


Figure 5. Average densities (sum \pm SEM) of GWSS life stages found on potted plants shows a significant density reduction for each grouping of life stages. These data were collected during last adult flight (July to October 2002). Letters above each mean are significantly different, Tukeys' LSD test at $P < 0.05$.

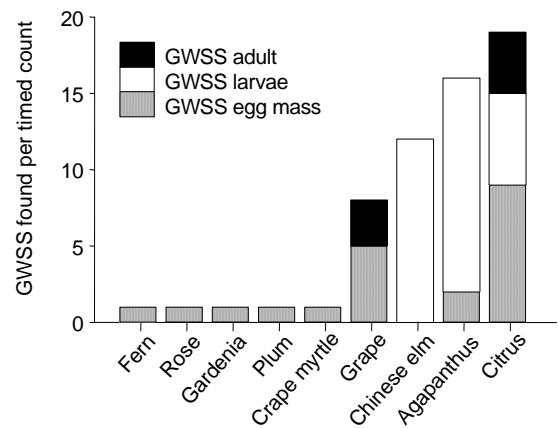


Figure 6. Example of survey information on GWSS life stages found in one sample of host plants on a residential street in Bakersfield, CA (July 10, 2002). During this survey, GWSS were not found on eucalyptus, pecan, pine, ornamental plum, persimmon, oleander, fig, *Opuntia* spp. or acacia.

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FUNDING AGENCIES

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GLASSY-WINGED SHARPSHOOTER IMPACT ON ORANGE YIELD, FRUIT SIZE, AND QUALITY

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INTRODUCTION

We need to know what impact the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*, has on fruit yield, size and quality as well as tree vigor. The goals of this project are to determine the usefulness of management of GWSS to prevent yield loss, fruit size reduction, and degraded fruit quality. This information is paramount before we can even begin to incorporate these into conventional IPM programs. First we have to know what impact GWSS has on citrus, and second we need to know how to use the materials against the GWSS in IPM programs to prevent potential losses. Prior to this study, efforts to manage GWSS in citrus were primarily to suppress populations to limit the spread of *Xylella fastidiosa*.

The primary goal of the first year of this project was to properly set up the three experiments in this project. First, the research sites had to be evaluated for suitability. Second, high and low populations of glassy-winged sharpshooters had to be established at a site with Valencia oranges and a site with Navel oranges.

OBJECTIVES

This research was initiated to:

1. Address the impact of GWSS on fruit yield, and distribution of fruit size when GWSS are controlled compared to untreated blocks of Valencia oranges, 'Washington' navel oranges, and grapefruit
2. Evaluate the effects of high GWSS populations have on fruit quality (sugar/acid ratios, peel thickness, sugar/acid ratio, juice quality, peel texture and firmness, susceptibility to post-harvest disorders) in Valencia and Navel oranges;
3. Evaluate the effects of large GWSS populations have on water stress, nutrient loss (Ca etc.), metabolite loss (amino acids, xylem translocated PGRs) due to xylem feeding and fruit drop and fruit quality, and fruit drop
4. Determine if Admire enhances fruit size, tree health and vigor in the absence of GWSS.

RESULTS AND DISCUSSION

A Valencia Experiment (Experiment 1) was established at a site near Newhall (Ventura County). The site has 6 replications of 6 40-tree rows plus a 7th spare replicate with low GWSS populations and high GWSS populations. The low population treatment was established by applying Admire 2F to all 6 rows (4 guard rows + 2 harvest rows, May and August) in each low population replication. Four rows serve as "guard" rows in each replicate with 2 center rows serving as harvest rows. Insects were monitored weekly by trapping, and visually counting adults, nymphs and egg masses. Efforts to establish differential populations were successful. On 8 August 2002, visual searches revealed 6.0 adults/3 min search/tree (\pm 1.0 SEM) in the high population trees versus 0.7/3 min search/tree (\pm 0.4 SEM) in the low population trees. The high and low population trees had 2.7 (\pm 0.6 SEM) and 0.9 (\pm 0.2 SEM) egg masses/25 leaf turns respectively.

One of the harvest rows was harvested in May the other in August. The fruit was sent to Filmore-Piru Packing House for packout and evaluation. Two cartons from 2 sizes (113 and 138) and 2 grades (Choice and Export) from each block and treatment (total of 96 cartons) were selected. Trans-Pacific shipment was simulated by storing the 96 cartons from at the packinghouse for 21 days at 2.8°C (37°F) after which time the fruit was sent to KAC for storage at 20°C (68°F) for 4 days followed by 12.8°C (55°F) for 5 days. The procedure was followed for the May and August harvest rows. For post-harvest evaluation at harvest, initial measurements of general appearance, pitting, puff and crease, peel firmness, thickness, color,

TA, TSS, and % juice were taken from a 20 fruit sub-sample. Fruit was evaluated for general appearance, rind pitting, and decay following simulated shipment. The size distribution for the Valencia Experiment was not statistically significant for the high population and low population trees, which is not surprising since this fruit was harvested within a few weeks of Admire treatment. This demonstrated that the trees were similar at the beginning of the experiment.

At the May Valencia harvest, 10 oranges were taken from 5 trees per replication and evaluated for pitting and signs of potential GWSS ovipositor wounding on the fruit surface. Only 3.1% of the fruit had pitting. There were no signs of attempted oviposition on the remaining 96.9% of the oranges. Also, when the initial fruit evaluation was compared to final evaluation, significantly more fruit had pitting (Figure 1). The pitting is seemingly a postharvest disorder and is not caused by direct damage of the GWSS. The preliminary information suggests a physiological problem possibly a result of GWSS xylem feeding behavior. Research plans for 2002-03 have been modified to address these issues.

A similar experiment was initiated on 21 August 2001 for 'Washington' Navel oranges. A site was established in Mentone with a completely random design with 5 replications with high and low GWSS populations. Each population level has three rows of 43 trees (2 guard rows and 1 central harvest row). The low populations were established by applying 32 oz of Admire 2 via drip irrigation 21 Aug 2001 and 7 May 2002. The central harvest rows of the low population rows were subsequently treated with 3.2 oz of Baythroid (cyfluthrin, a pyrethroid) to minimize encroachment from adult GWSS flying into these rows from heavily infested neighboring groves. However, this encroachment was desired in the high population reps. Baythroid was chosen because it has excellent knockdown of adults and nymphs and kills nymph hatching from eggs up to 21 days following application. Furthermore, pyrethroids tend to repel GWSS.

Preliminary data was collected from the harvest trees on 21 January 2002. This was not enough time to have complete effect on the treated trees, since fruit set had occurred at least 4 months prior to the first Admire treatment. Thirty oranges were randomly collected from each tree in the harvest rows and hand ringed for size, weighed, and total soluble solids (TSS) determined. Size distributions, average orange weight, and TSS for the high and low population trees were not significantly different.

Simulated Trans-Pacific Shipment of Valencia Oranges

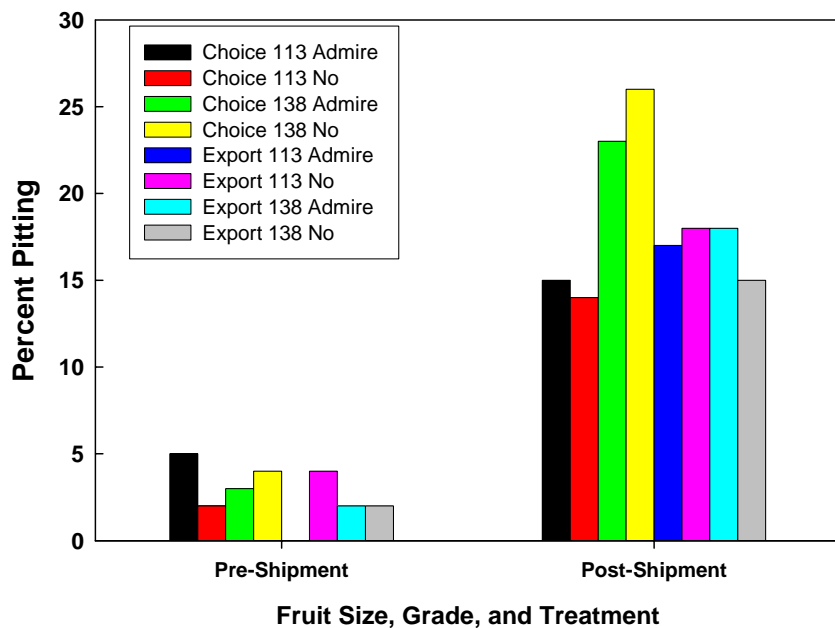


Figure 1. Comparison of initial evaluation versus final evaluation after simulated trans-Pacific shipment of May harvested Valencia oranges. More fruit had Pitting following the storage regime of 21 days at 2.8°C (37°F), the fruit was sent to KAC for storage at 20°C (68°F) for 4 days followed by 12.8°C (55°F) for 5 days.

FUNDING AGENCIES

Funding for this project was provided by the California Citrus Research Board and the University of California Pierce's Disease Grant Program.

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

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Reporting Period: The results reported here are from work conducted from November 2001 to October 2002.

OBJECTIVES

1. Develop a technique to rapidly mark adult GWSS for release-recapture studies.
2. Develop a sampling system for eggs of GWSS and monitor GWSS egg density on citrus.
3. Monitor adult movements of GWSS.

RESULTS AND CONCLUSIONS

We have completed our laboratory and field experiments verifying the stability of fluorescent dust as a method for marking the glassy-winged sharpshooter. We know that the marks will last for at least 30 days and for as long as 80 days under field conditions. Adults captured during the summer months readily survived the capture and marking process. In contrast, the few adult GWSS we captured at the UCR Agricultural Operations citrus groves during the winter months survived poorly after we marked them, probably from age and the stress of being captured and marked. However, Figures 1 and 2 show that the marked and unmarked control GWSS survived equally well. This was true for newly emerged adults collected during summer (Figure 1), and for overwintering adults (Figure 2).

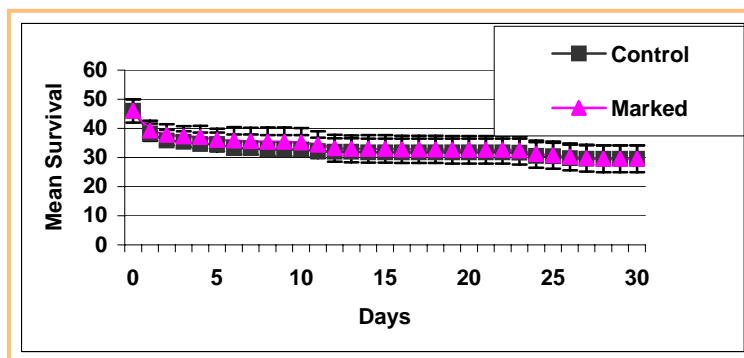


Figure 1. Mean survival of adult GWSS with standard error bars. Insects were collected during July and August.

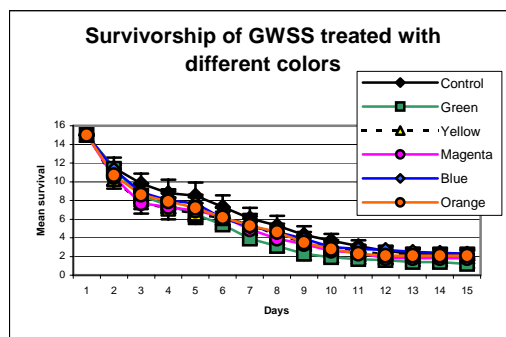


Figure 2. Mean survival of adult GWSS with standard error bars. Insects were collected during January.

Both marked and unmarked control insects released in a barren field were equally able to fly at least 100 meters from the release point within the first five minutes of their release. This field experiment (Figure 3) showed that the drop in recapture

over distance did not differ between marked and unmarked insects. Also, the slope of the line describing the relationship between the numbers of GWSS adults recaptured versus distance did not differ between the marked and unmarked individuals. The barren field experiment also showed a clear-cut affect due to wind speed. At wind speeds above 5 m/s, the efficiency of the yellow sticky cards to capture adult GWSS suddenly dropped (Figure 4) suggesting that the insects cannot steer into the traps or they are simply blown away.

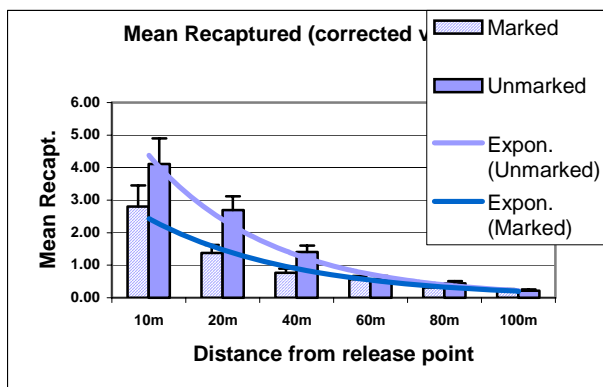


Figure 3. Mean recapture of marked and control insects. Standard error bars and trend lines shown.

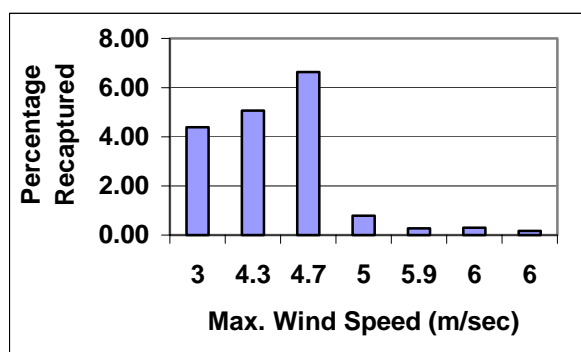


Figure 4. Mean recapture of GWSS with increasing wind speeds.

We now have focused on sampling adult and nymphal GWSS in citrus to estimate changes in their densities over time on a whole tree basis. We began sampling GWSS weekly at the Citrus Experiment Station, Agricultural Operations (UCR) 19 October 2001 and sampled three randomly selected trees per week. We alternated sampling the lemon trees and Valencia trees biweekly using two parachutes to cover each sample tree and confine the Pyronyl Crop Spray® (a natural pyrethrum product). We used the spray to fog the tree canopy beneath the parachute tent. On May 17, 2002 we initiated sampling at a new site in Kern County east of Bakersfield after obtaining authorization to apply pyrethrum in Kern County. The sites we had previously sampled in the Bena Road area beginning in July 2001 were treated October 2001, consequently the GWSS populations at these sites were too sparse to estimate GWSS densities, age structure and survival during the winter spring 2001-2002 period. Adult GWSS densities were too low at both the Agricultural Operations and Kern County sites to track their movements during winter and spring. By fogging Valencia and Lemons trees at Agricultural Operations, UCR, and navel orange trees at a Kern Count grove in 2002, we estimated adult GWSS densities, their sex ratios, the onset of oviposition, and nymphal development. During the last months of this project, we are developing methods of detecting the presence of GWSS in riparian vegetation and, once detected, to estimate their densities, objectives three and four. We propose to test a system based on coated paper that will allow us to detect the ammonia in GWSS excreta and to test it against known GWSS densities. We are currently analyzing the data from these studies statistically and preparing manuscript based on these analyses.

FUNDING AGENCIES

Funding for this project was provided by the California Department of Food and Agriculture.

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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Reporting Period: The results reported here are from work conducted from November 2001 through October 2002.

INTRODUCTION

The glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say) (Homoptera: Cicadellidae), has been identified as the main vector for the xylem restricted bacterium *Xylella fastidiosa*, the causal agent of Pierce's disease. Our project aims to identify those aspects in the GWSS-host plants interaction that may explain variations in GWSS performance and population dynamics of this vector. Following the field population dynamics of a species requires a reliable method for accurately estimating real field densities. Current methods used to estimate GWSS densities in the field, rely mainly on yellow sticky traps or net beatings and insect counts. We have developed a method to take absolute samples of the GWSS to recover all the GWSS stadia except eggs. We will then explore possible correlations between GWSS changes in population densities and performance, with xylem physical and chemical parameters in different host plants.

OBJECTIVES

1. Quantify xylem flux patterns and to 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 and to correlate GWSS demographic statistics with xylem flux and chemistry.

METHODOLOGY

Our sampling system uses military parachutes to cover entire trees and then, by fogging the trees, we recover all the insects. To estimate accurately the recovery rate, we collect adult GWSS in trees surrounding the ones to be sampled and mark the GWSS with a dye. After the randomly selected sample trees are tented, we release 100 marked GWSS in each of them. The amount of marked insects recovered is an estimate of the recovery rate of the population on each of the trees. We then use this number as a correction factor for each tree. The average recovery rate of marked GWSS on these commercially sized citrus trees is 89%. We count all the adult and nymph GWSS in the sample. We also use a specially designed Schölander bomb to measure xylem fluid pressure and to extract xylem fluid for chemical analyses.

RESULTS AND CONCLUSIONS

At this point, the method is being used in orange and lemon trees at three different locations. One is a mixed orange/lemon grove at Agricultural Operations, UC Riverside, and the other two consist of an orange and a lemon grove, in Temecula, California. We also did some limited work at an orange grove in Kern County, and in the Coachella Valley, California. We intend to use this method to follow GWSS population dynamics for two complete years, and to correlate changing GWSS densities over time in different host plants with xylem flux and xylem chemistry over the same time period with the same trees. Our results show that several thousand glassy-winged sharpshooter adults occur per tree too (Figure 1). We also found that adult GWSS densities differ between lemon and Valencia trees at certain times of the year whereas they are similar significantly between Valencia oranges and lemon over the entire year, adult GWSS switch from Valencia to lemon trees starting in mid-January. By the end of February, when the first egg-masses appear, 99.51% of the adult GWSS population can be found on lemon trees.

The results shown here are in agreement with indirect density measurements in the same and other areas. Data from a mark-recapture method used in our other studies, allowed us to use the Lincoln index to estimate GWSS adult population densities. Summer estimations for adult GWSS ranged between 6,000 to more than 8,000 adult GWSS per tree (ca. 600,000 to 800,000/acre) in both Agricultural Operations, UC Riverside and a non-treated orange grove in the Bena Road area of Kern County. We are also counting all nymphal stages and estimating nymph densities. These results for this first year of sampling will also be presented.

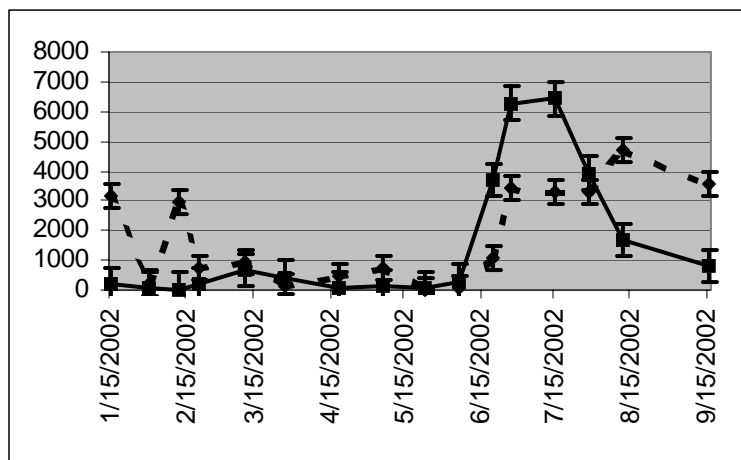


Figure 1. Monthly mean of adult GWSS on lemon trees (dotted line) and orange trees (solid line).

With these data, we have also estimated GWSS sex ratios. Based on the coloration of newly emerged adult GWSS, we estimated the proportion of new adults per sampling date. All these data will be used to calculate recruitment, and mortality for each age and for each generation on both citrus host plants, and therefore, to build a picture of the GWSS population dynamics over time. We are using the Scholander bomb for xylem fluid extraction on the same trees that are sampled for GWSS for subsequent chemical analyses. We are in the process of collecting the samples in both places at UC Riverside and Bakersfield area. We intend to expand this xylem studies to different host plants, namely grapefruit, tangerines, and grapevines during the second year of the project. The chemistry of the xylem samples for the first year is being analyzed, and we will test for possible correlations between fluctuations in xylem chemistry and in GWSS performance that might explain changes in GWSS densities over time.

FUNDING AGENCIES

Funding for this project was provided by the USDA Animal and Plant Health Inspection Service.

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

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Reporting Period: The results reported are from work conducted from 2001-2002.

INTRODUCTION

Leafhopper vectors of *Xylella fastidiosa* (including GWSS) behave very differently than most herbivorous insects. GWSS has evolved many unusual adaptations that enable subsistence on xylem fluid. Adult GWSS may feed on hundreds of different host species, are long lived and exceptionally mobile and fecund. Nutrition affects GWSS female fecundity and longevity, and malnutrition is a primary source of mortality of immatures. We have established that adults prefer to feed on xylem fluid with specific chemical characteristics (high amide concentrations). 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 more efficiently use nitrogen and carbon from high amide concentrations than can young developing nymphs cannot. Given the pivotal status of host plant nutrition on GWSS behavior and survival, we are investigating GWSS behavior and that of its parasitoids in field and laboratory experiments to elucidate how the underlying feeding and oviposition behavioral mechanisms relate to host plant quality. The behaviors involved in host selection can be divided into two extremes. In the first, selection takes place after insects contact the host, the second extreme implies that the insect perceives plant characteristics at a distance and select hosts based on these perceptions. These two extremes can be described as host-plant recognition and host-plant finding. Host plant recognition is less well known in the literature, but our research is addressing behaviors involved in both categories.

OBJECTIVES

1. 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. Determine the relationship of host plant xylem chemistry 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

We examined GWSS host utilization with a series of choice and no-choice tests in cages containing example hosts most relevant to California (Navel orange, Spanish Pink lemon, and *Vitis* sp. - > Chardonnay = grapes). Glabrous soybean as a standard was also included as soy is one of the few hosts on which immature GWSS consistently develop. Cages with adult GWSS (two types run separately - diapausing (late autumn), and reproducing (mid-summer)), and one plant of all 4 species or with 4 plants of each species separately were used to assess host selection, consumption rates, oviposition, nymphal development, growth and survivorship. In choice-tests, Navel oranges were consistently the preferred hosts, 40-65% of GWSS remained on Navel when examined for 10-22 day intervals. Selection of other hosts was dependent on time of year; Spanish Lemon was selected moderately by diapausing insects (25-40%) and *Vitis* and soy were not preferred (<20%). Reproductively-active GWSS also preferred Navel 45-65%, followed by soy (20-30%), with *Vitis* and Lemon lowest (<20%). Despite high selection of Navel (and lemon by diapausing leafhoppers), the two citrus hosts consistently had the lowest consumption rates by adult GWSS. Consumption rates were consistently 1-2 mL/day on *Citrus* while they were 4-7 mL/day on the less preferred *Vitis* and soybean. Consumption was not significantly different between diapausing and reproducing GWSS suggesting that diapausing GWSS are still active in accumulating nutrients, albeit for storage of nutrients for successful overwintering rather than egg production. Navel oranges received the highest rates of oviposition, with *Vitis* also receiving moderate levels of egg deposition. Lemon and soy received very little oviposition. Neonate nymphs (less than 24 hrs) introduced from neutral hosts quickly rejected both *Vitis* and Lemon (<5% remained on hosts after 5 days), and were only able to develop to second instar on Lemon and fourth instar on grape. In contrast, roughly 30% of neonates placed on Navel remained for 5 days; however, these developed slowly and all perished before completion of the second instar. On the soy standard, ca. 35% successfully developed to adults. Preferential selection of Navel thus appears related to reproductive behavior (including oviposition and development of very young nymphs) rather than consumption rates. Navel selection occurred by diapausing as well as reproducing GWSS, indicating that generalized cues in Navel may be operative even when GWSS is not seeking ovipositional sites. We noted a decrease in preference (60 to 40%) for Navel over time by diapausing

GWSS, suggesting that leafhoppers were very gradually shifting to better feeding hosts; for reproductively active GWSS, choice of Navel actually increased with time.

Field evaluation of *Vitis* selection by GWSS:

We examined seasonal trends in GWSS abundance on 12 cultivars of *Vitis*. These included the economically important *Vitis vinifera* (cv. Chardonnay, Chenin Blanc and Exotic), and rootstock species including *V. champinii* (cvs. Dog Ridge and Ramsey), *V. rupestris* (cvs. St. George and Constancia), and *V. simpsoni* (cv. Pixialla). We also examined 4 Southeastern muscadine genotypes from *V. rotundifolia* (cvs. Carlos, Noble, Early Fry, Regale). Vines (4-8 per genotype) were counted at least once a week from late May until the present (late October). GWSS populations on *Vitis* peaked in June with abundances being greater on *V. champinii*, *V. rupestris*, and *V. simpsoni* (13-18 adult GWSS per vine). Abundances were significantly lower on *V. vinifera* (7.5 per vine) and *V. rotundifolia* was significantly lower than all other species (2.2 per vine). Populations on *Vitis* declined in mid-summer but these trends in selection continued until August, at which time GWSS counts on *V. vinifera* rose to approximate those found on *V. champinii*, *V. rupestris*, and *V. simpsoni*. Increased selection of *V. vinifera* persisted until late season, when GWSS populations in Florida decline. Selection of muscadine grapes *V. rotundifolia* was always significantly less than all other *Vitis* species. High suitability of *V. champinii*, *V. rupestris*, and *V. simpsoni* for GWSS may be of interest as these species are often used as rootstocks. We have previously established that rootstocks largely determine xylem composition, and have shown in Florida that selection of rootstock can be used to alter the preference of GWSS for cultivars of *Prunus*. Rootstocks may be a tool for establishing grapes less suitable for GWSS.

One of our primary objectives is to identify plant nutritional variables that may be operational in determining host acceptance, consumption rates, ovipositional preference, etc. Choice experiments were repeated using important host plants from the leafhoppers home range including yaupon holly, burford holly, crape myrtle and soy. Preliminary results of chemical analyses from both sets of choice experiments indicate general trends we have found on Florida hosts previously. Specifically, Navel has proportionally very low amide concentrations. This is consistent with what we have found for other hosts that are poor feeding hosts, but good ovipositional hosts. In a field experiment we evaluated GWSS oviposition rate in response to plant nutritional quality. There were some significant differences in host acceptance and oviposition rate. We also examined oviposition in no-choice experiments (cages with 4 plants of one host species rather a mix of four host species). There were some significant differences in host acceptance and consumption rates indicating that feeding history should be considered in future experiments and can impact GWSS host selection. Completion of our analyses and further experimentation should delineate chemical cues related to ovipositional selection.

Understanding the determinant mechanisms involved in parasitoid plant and host egg finding and recognition (selection and acceptance) is also an objective. We conducted experiments in the field and laboratory to elucidate the cues produced by the plant, the vector or its eggs that parasitoids may be exploiting. We have ostensibly eliminated many possibilities including components of GWSS excreta, but to date we have not positively identified any specific cues directing parasitoid behavior. More candidate cues both chemical and visual need to be evaluated while this work continues.

Understanding the mechanisms involved in adult GWSS host plant finding and recognition is also the subject of several experiments. We have determined that the host plant appears to provide cues that GWSS may recognize in the air prior to landing. Other results suggest that host acceptance is determined by taste at the xylem tissue level. We are presently validating experimental methodologies to further test hypotheses generated from current field results.

FUNDING AGENCIES

Funding for this project was provided by the American Vineyard Foundation and the University of California Pierce's Disease Grant Program.

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

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Reporting period: The results reported here are from work conducted from April 2002 to November 2002.

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 indicates 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 virtually 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 CONCLUSIONS

Laboratory observations and experiments are being conducted in an arena that provides a uniform background and both reduced airborne noise and observer interference. The arena is a 1.1 m x 0.7 m x 0.9 m box that is positioned on a vibration isolation table. The laser and vibrator (see below) are located inside the box.

We recorded vibrational signals using a laser Doppler vibrometer (LDV) (Polytec: model OFV 353 sensor; model OFV 2602 controller, 1.0 mm/s/volt setting) connected to a Macintosh computer equipped with a 16-bit Audiomedia III (Digidesign) sound card. The card was controlled using Peak 3.0 (BIAS) software. Signals were digitized at a sample rate of 44,100 s⁻¹ and stored on the hard drive of the computer. Temporal and frequency features were measured using Canary 1.2.4 (Cornell Laboratory of Ornithology) software. The filter bandwidth for all frequency measurements was set at 43.71 Hz. Calls emitted by males have been recorded and characterized (Figure 1 and Table 1). The male call consists of a long duration whine that rises in frequency followed by a series of pulses. Current efforts on male calling behavior focus on determining daily patterns of calling activity.

At present I am investigating male/female interactions and male search behavior. Females respond to calls emitted by males or to recordings of these signals by emitting a whine-like call that is similar in structure to the male call. Males search in response to female calls in a manner typical of other leafhoppers. Results of this ongoing work will be used to plan specific studies under objective 2.

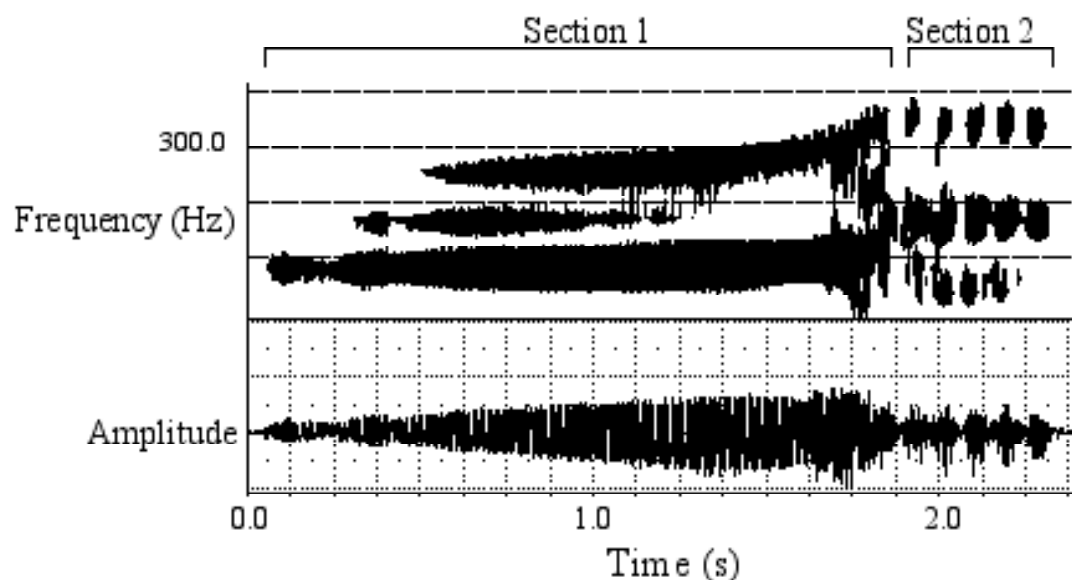


Figure 1. Sonogram (upper panel) and oscillogram (lower panel) of a typical call emitted by a male *Homalodisca coagulata*. Male calls have two distinct sections. Section one consists of a whine that increases in frequency. Section 2 consists of a series of pulses. Refer to Table 1.

Table 1. Analysis of calls emitted by first generation males (N = 15) reared from adults collected on the UC Riverside campus.

Call Features	Mean	\pm SD
Section 1 duration (s)	1.71	\pm 0.167
Section 2 duration (s)	0.68	\pm 0.164
Section 1 initial frequency	75.20	\pm 9.871
Section 1 end frequency	115.60	\pm 19.036
Section 2 initial frequency	65.60	\pm 14.136
Section 2 end frequency	66.20	\pm 8.446
Number of pulses in section 2	6.4	\pm 1.789

All frequency (Hz) measurements are based on the fundamental frequency (i.e. the lowest high energy band). Refer to Figure 1.

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FUNDING AGENCIES

Funding for this project was provided by the USDA Animal and Plant Health Inspection Service.

REPRODUCTIVE BIOLOGY AND PHYSIOLOGY OF GLASSY-WINGED SHARPSHOOTER

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Reporting Period: The results reported here are from work conducted from June 2001 to October 2002.

INTRODUCTION

The glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say), is a serious pest of many tree and vine crops (Turner and Pollard 1959, Nielson 1968). The main concern of the presence of the GWSS in California is that this insect is an efficient vector of the bacterium, *Xylella fastidiosa*, which causes vascular disease in multiple crops, including grapes, citrus, and almonds, along with horticultural plants, including oleander and mulberries (Meadows 2001, Hopkins 1989, Purcell and Hopkins 1996). An adult GWSS need only encounter the Pierce's disease causing bacterium, *X. fastidiosa*, once while feeding on an infected plant and it will then be a vector of *X. fastidiosa* for the rest of its life (Frazier 1965, Purcell 1979, and Severin 1949).

Little is known about the reproductive biology of the GWSS. 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. Our research is focused on the reproductive morphology and physiology of the GWSS. We are examining the seasonal differences in female and male GWSS reproduction between summer and overwintering populations by studying oögenesis and spermatogenesis cycles. This knowledge is important in determining how GWSS might choose plant hosts in the landscape, which and why these host plants are particularly suitable for GWSS ovarian development, and finally how control measures might best be implemented based upon season and stage of reproductive development. Better knowledge of reproductive biology might also lead to better decision support including improved choices of chemical or non-chemical approaches to GWSS control.

OBJECTIVES

1. Collect and prepare GWSS specimens for studying the morphology and anatomy of female GWSS.
2. Study and describe the musculature associated with the female ovipositor.
3. Characterize the reproductive cycle of female GWSS.
4. Study effects of location on female GWSS reproductive cycle.
5. Study effect of host plant type on female GWSS fecundity.

RESULTS AND CONCLUSIONS

Female and male GWSS were collected from June 2001 to October 2001. Due to some problems with specimen preservation, only specimens collected after September 2001 were useful for dissection purposes. Samples were taken on monthly or bimonthly intervals, and a random subsample of 10 females per month were dissected to determine ovarian development of the specimens. The stages of ovarian development were arbitrarily set from 1 to 8, with 1 being the least developed stage associated with youngest adult females and 8 being the fully developed stage associated with the oldest adult females. Stage 1 has 2 small oöcytes per ovariole and no corpus luteum present. Stage 2 has 3 small oöcytes per ovariole and no corpus luteum. Stage 3 has 2 small oöcytes per ovariole and one large ova per ovariole. Stage 4 has 1 small and 1 medium oöcyte, and 1 large ova per ovariole. Stage 5 has 2 small oöcytes per ovariole with a corpus luteum present. Stage 6 has 1 small oöcyte and 1 large ova per ovariole with, or without, a corpus luteum present. Stage 7 has 1 small oöcyte per ovariole with a corpus luteum present. In stage 8 there are only large ova present. Stage 1 and 2 are previtellogenic. Stages 3 to 8 are reproductively active females who are in the process of oviposition (Stages 3, 4, 6 and 8), or have already oviposited at least once (Stages 5 and 7). The average ovary rank per sampling date from October 2001 to June 2002 is plotted in Figure 1. We are still attempting to determine which generations are present on each sampling date. The presence of two generations of insects at most times of the year has led to variation in the ovarian development among the dissected specimens and we are planning to dissect 10 additional specimens for each sampling date. This will result in a total of 20 specimens dissected per sampling date.

The oögenesis and spermatogenesis cycles will be further studied using histological and cytochemical methods as well as transmission electron microscopy (TEM). This study has recently been expanded to include a site in Ventura County, CA to examine possible location effects in California.

Muscles of Ovipositor:

Musculature of the female ovipositor was determined using gross dissection techniques and drawings (Figure 2). Muscle 1 is a dilator, it originates on the VIII tergite (T) and inserts on the common oviduct. Muscle 2, a retractor, connects the pygofer to the VIII T. Muscle 3, a depressor, originates on the VIII T and inserts on the first valvifers (vlf). Muscle 4, a dilator, originates on the pygofer and inserts on the third valvula (vl). Muscle 5, also a dilator, originates on dorsal-posterior portion of the first vlf and inserts on dorsal portion of second vlf. Muscle 6, a retractor, originates on the apodeme of the pygofer and inserts on the dorsal-most portion of second vlf. This muscle consists of two portions: 6a, which originates on the posterior edge of the pygofer apodeme; and 6b, which inserts on the apodeme ridge. Finally, muscle 7 is a protractor, it originates on the dorsal-posterior portion of the pygofer and inserts on the ventral-most portion of the second vlf. The other muscles illustrated are associated with movement of the abdominal segments and are not directly associated with oviposition.

The hypothesized sequence of muscle action during oviposition is described as follows. 1) The contraction of muscle 2 retracts the pygofer by pulling it toward the body and away from the tip of the ovipositor. 2) By the contraction of muscle 4, the paired third valvulae are dilated ventrally to further expose the ovipositor (to reduce friction with the body). 3) Muscle 3 contracts to pull the first vlf dorsally and subsequently causes the first vl to depress and tilt away from body. 4) a. Muscle 7 contracts causing the second vl to protract away from the body, and b. simultaneous contraction of muscles 3 and 7 result in the ovipositor being pushed away from the body. 5) Muscle 6a contracts pulling the second vl toward the body. 6) Muscle 6b contracts resulting in the rotation of the ovipositor. 7) Muscles 3 and 7 will relax as muscle 6a contracts causing the ovipositor to move toward the body again, thus beginning the sawing motion. Muscles 3 and 7 will contract once again, and the seesawing action continues. When muscles 6 and 7 work oppositely, the second vl. – sawing portion – will move toward and away from the body. The simultaneous contraction of muscles 3 and 7 will accentuate the seesawing action of the ovipositor (second vl. slides on a groove in the first vl.). 8) Once a slit has been made in the leaf, muscles 1 and 5 contract causing the opening up of the paired second valvifers, which expands the genital chamber to allow an egg to pass or for copulation. 9) Once the orifice of the common oviduct into the genital chamber has been dilated, the egg will be deposited into the genital chamber and further slide through the opening now present between the paired second valvifers. The egg will then slide down the middle groove of the ovipositor that is present between the paired first and second valvulae.

Oögenesis study:

The details of reproductive cycle of the female GWSS are still not clear based on our limited data. Our data suggest that from October to February, there is a gradual decrease in mean ovarian development rank, indicating that there is probably a shift toward a higher proportion of younger insects in the population during the period. The ovarian rank begins ascending in March, indicating that the population largely consists of older female GWSS (Figure 1).

Host Plant Study:

The greenhouse study conducted this summer has shed light on differences in female fecundity reared on different host plants. In this study adult female and male GWSS were caged on citrus, grape, or oleander and allowed to mate and oviposit on the plants. We were successful in obtaining oviposition and in rearing GWSS from egg stage to adult stage on all three types of host plants. Data are still being collected, and have yet to be summarized for analysis.

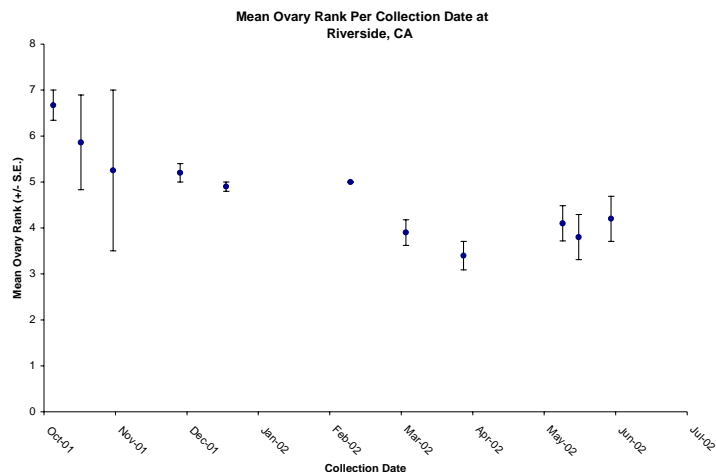


Figure 1. Mean (±S.E.) ovary rank per collection date at Riverside, California.

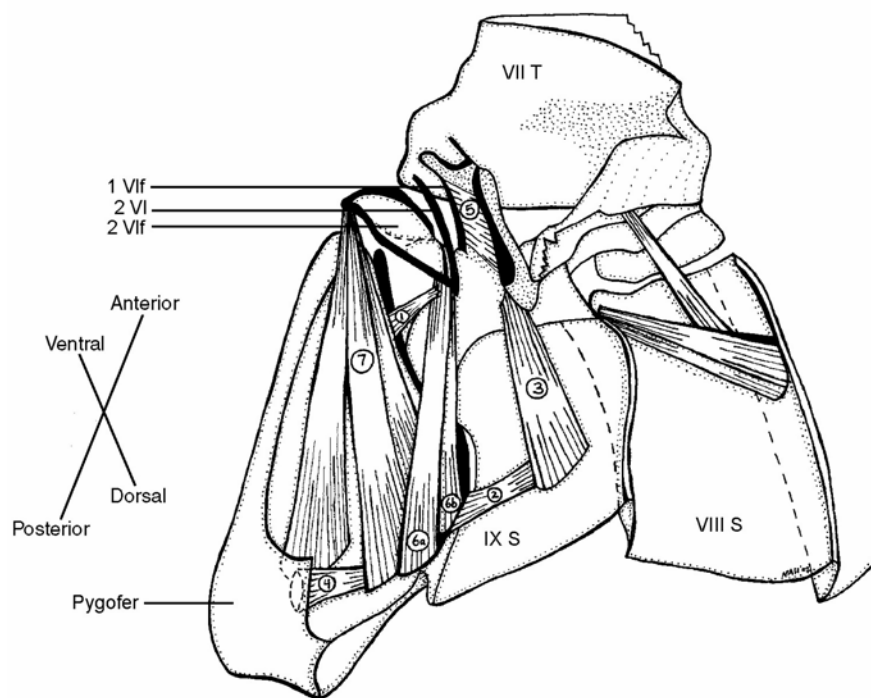


Figure 2. Lateral view of the musculature associated with the female *Homalodisca coagulata* ovipositor.

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FUNDING AGENCIES

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ULTRASTRUCTURAL CONTRIBUTIONS TO THE STUDY OF THE GLASSY-WINGED SHARPSHOOTER AND PIERCE'S DISEASE

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Reporting Period: The results reported are from work conducted from July 1, 2002 to October 31, 2002.

INTRODUCTION

Despite the major research efforts presently underway to minimize the significant economic losses caused by the glassy-winged sharpshooter and Pierce's disease there are major gaps in our understanding of the basic biology of this insect and its interactions with host plants. There is little current literature dealing with the structure of mouth parts of the glassy-winged sharpshooter. Understanding the structure of the mouth parts and their interaction with host plant cells is essential to determining how the insect transfers the bacterium from plant to plant. Details of the mouth parts and feeding behavior may also provide the information necessary to determine why some sharpshooters can feed on infected plants but not transfer the bacteria to healthy plants on subsequent feedings. There is speculation as to why the cavitation does not occur in the xylem tissue despite the large water loss associated with sharpshooter feeding. Preliminary evidence from our laboratory demonstrates that both immature and adult insects probe leaf blades and petioles but never actually penetrate the xylem tissue of the veins. Are these insects actually feeding on cells outside of the xylem tissue? The research outlined in this report will contribute significantly to a greater understanding of glassy-winged sharpshooter feeding and its relationship to Pierce's disease.

OBJECTIVES

1. Describe the morphology and ultrastructure of the glassy-winged sharpshooter mouthparts.
2. Describe the process of stylet penetration and the function of each stylet pair during feeding.
3. Ascertain the path of the mouth parts from the epidermal layer to the vascular tissue of the host plant and to ascertain if the sharpshooter has fed in parenchymatous or phloem tissue en route to xylem tissue.
4. Determine the ultrastructure of the salivary sheath and its association with all plant tissues encountered from the epidermal layer to the xylem tissue.

RESULTS AND CONCLUSIONS

Glassy-winged sharpshooters (GWSS), *Homalodisca coagulata*, were field collected from citrus and eucalyptus in Ventura, California, July 2002. Additional insects and plants were obtained from the Oswald Street Biological Control Station, Bakersfield California. Light and electron micrographic studies were used to describe the sharpshooter mouth parts (Figures 1-5) which consist of a labrum, labium, and stylet fascicle. The three-segmented labium contains the fascicle bundle composed of two external mandibular stylets and two internal maxillary stylets. The stylets are capable of rapidly penetrating leaf tissue or woody stems.

The crescent-shaped mandibular stylets taper to sharp points at their tips (Figure 2) and have elaborate sculpturing along their borders (Figure 3). Each stylet is manipulated by retractor and protractor muscles that allow independent movement of the stylets. On the medial surface of each stylet is a series of cup-shaped flanges that are more prominent near the tip of the stylet. The two mandibular stylets are morphologically distinct.

The maxillary stylets (Figure 4) are longer than the mandibular stylets and are semicircular in cross sectional view (Figure 5). These stylets are interlocked along their entire length with the exception of the very tip. They interlock similar to a mortise and tenon type of joint forming a smooth central tubular food canal and salivary canal (Figure 5). Dendritic canals are evident in both the mandibular and maxillary stylets.

Sharpshooters can relocate from one feeding position to another and be producing exudate within thirty seconds. Many of the salivary sheaths formed are highly branched. Although the sharpshooter is considered to be an exclusive xylem feeder, a high proportion of the salivary sheath branches do not terminate in the xylem tissue (Figure 6).

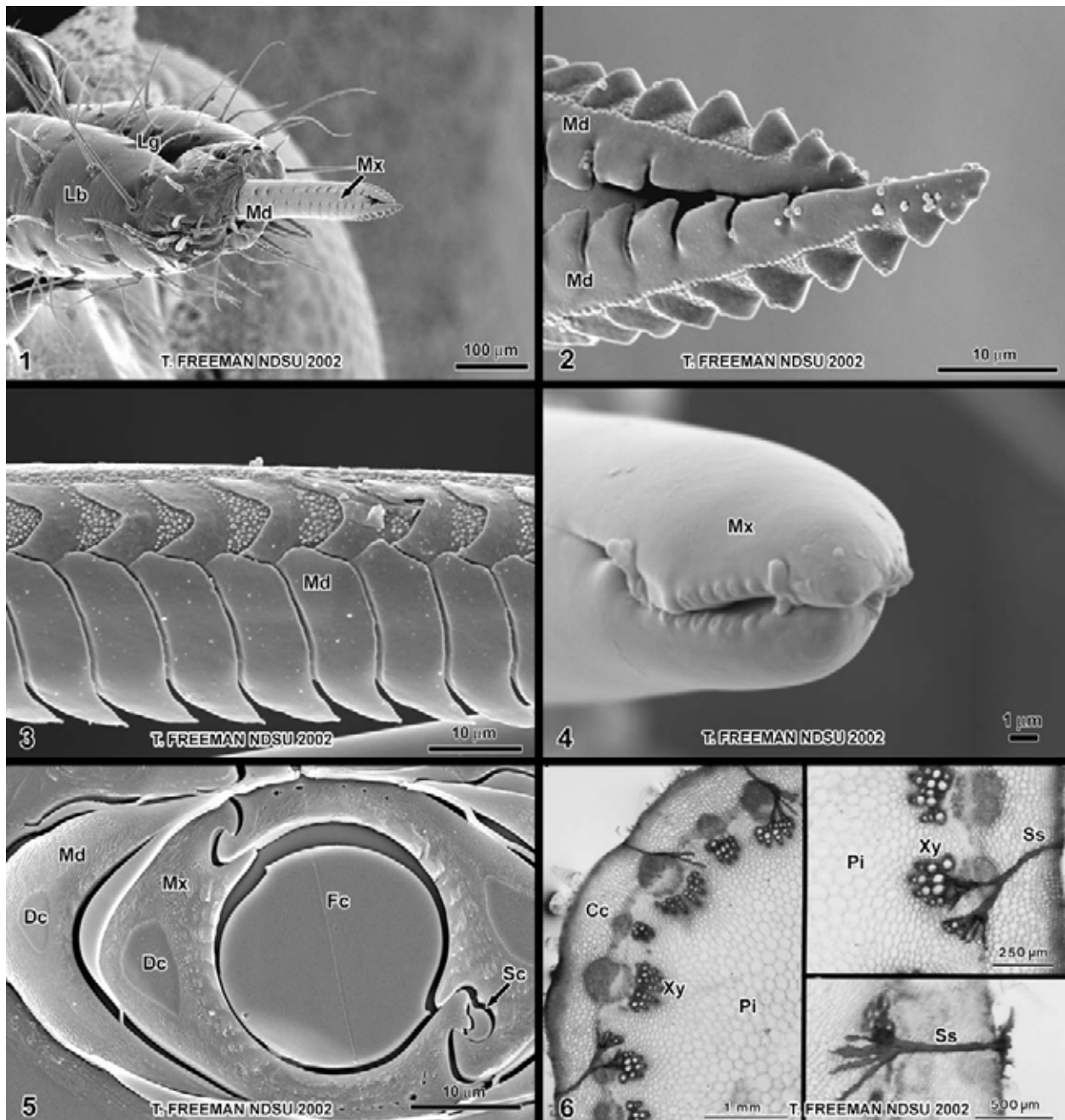


Figure 1. Mandibular and maxillary stylets extended beyond the tip of the labium.

Figure 2. Mandibular stylet tips of a nymph.

Figure 3. Dorsal view of an adult mandibular stylet.

Figure 4. Tips of the maxillary stylets of an adult.

Figure 5. Cross sectional view of the stylet fascicle.

Figure 6. Cross sections of sunflower stems showing salivary sheaths.

Cc - cortex, Dc - dendritic canal, Fc - food canal, Lb - labium, Lg - labial groove, Pi - pith, Md - mandibular stylet, Mx - maxillary stylet, Sc - salivary canal, Ss - salivary sheath, Xy - xylem.

FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

RELATIONSHIP BETWEEN TOTAL POPULATION COUNTS OF GLASSY-WINGED SHARPSHOOTER AND NUMBERS OBTAINED FROM VARIOUS SAMPLING METHODS

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Reporting Period: The results reported here are from work conducted from July 18, 2002 to October 1, 2002.

INTRODUCTION

Most of our knowledge of the dispersion of *Homalodisca coagulata* (glassy-winged sharpshooter, GWSS) has been obtained with relative sampling methods in vineyards and citrus orchards (Blackmer et al. 2001, Blua et al 2001, Puterka 2001). Currently, sampling methods are being used to determine timing of pesticide treatments and to judge their efficacy (Blua and Redak 2001, Henneberry et al. 2001). This use implies that the sampling method used relates in a known way to population density. Unfortunately, this is not the case, and some important questions are raised. If a given treatment against GWSS results in “zero counts” by beat sampling, does that necessarily indicate that there are no GWSS in the area due to the treatments, or could some GWSS be left alive but at density below the detection threshold of the monitoring tool? Could an unknown low density of GWSS be enough to vector PD within or between treated areas? Does the relationship between population sampling precision and accuracy change seasonally? To answer these questions it is imperative to develop a fundamental understanding between actual (=absolute) GWSS density in the field and any relative density estimates derived from various sampling procedures.

OBJECTIVES

The overall goal of our research is to correlate the numbers of GWSS obtained by various sampling methods currently used in population monitoring in citrus with their population density. Part of this goal involves developing and testing sampling methods. Sampling methods chosen for examination were yellow sticky-card monitoring, beat-net sampling, and timed counts. Total sampling involved covering trees with tents, killing all GWSS inside with pyrethrum canisters, and counting dead sharpshooters on cloth under the trees.

RESULTS AND CONCLUSIONS

To determine the efficiency of yellow sticky-card sampling we examined numbers of GWSS caught on the cards as a function of the number of GWSS placed on the card before it was deployed in the field. Mean numbers (and SE) of GWSS caught on cards on which we placed 0, 25, 50, 100, and 200 GWSS was 45 (6.9), 38.4 (4.1), 39.4 (2.0), 30.8 (5.6) and 8.4 (3.3), respectively. We detected a significant ($p < 0.001$) relationship between numbers of GWSS placed on the card and numbers of GWSS caught (Figure 1). This relationship should be considered depending on the use of yellow sticky-cards in GWSS monitoring.

Because this project was initiated in mid July 2002, we have collected and analyzed sampling and total count data for only 7 dates. Thus far, for adult and total GWSS no significant correlations were detected between absolute counts and beat-net samples, timed counts, or sticky-card samples. For adults, GWSS sticky-card samples did not significantly correlate with any other sampling method. For juvenile GWSS, absolute counts correlated significantly ($p = 0.035$) with timed samples. Finally, for juveniles, adults, and total GWSS, beat-net samples correlated significantly ($p < 0.01$) with timed counts.

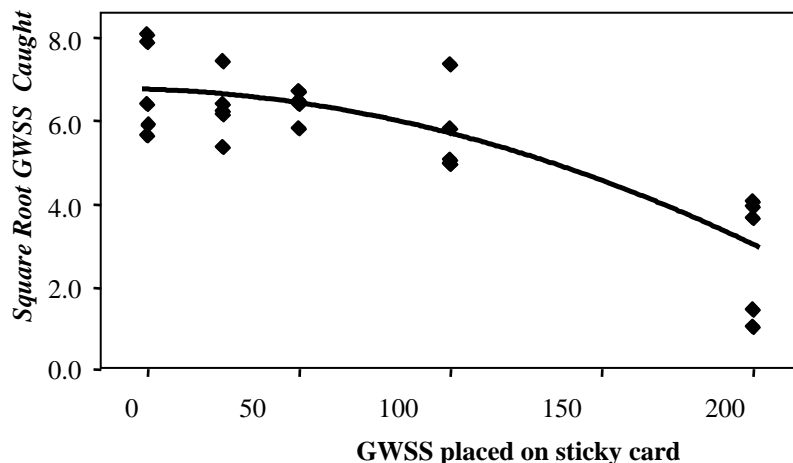


Figure 1: GWSS capture as a function of the number of individuals placed on the yellow sticky card before field deployment. Equation for line is $Y = -8E-05X^2 - 0.0022X + 6.5844$, $R^2 = 0.708$.

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DEVELOPING A METHOD TO DETECT *XYLELLA FASTIDIOSA* IN GLASSY-WINGED SHARPSHOOTER

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Reporting Period: The results reported here are from work conducted from August 30, 2002 to October 31, 2002.

INTRODUCTION

In spite of recent advances to reduce the spread and impact of Pierce's disease spread, few advances have been made in diagnostics of the pathogen, *Xylella fastidiosa*. This is in part due to a perceived lack of need for better diagnostics. Commercial and government laboratories routinely determine the presence of *X. fastidiosa* in plant tissue using serological assays (i.e. ELISA), polymerase chain reaction (PCR), and culturing techniques. Each of these techniques is valuable, the choice depending on the circumstances surrounding their use.

Unfortunately, none of these techniques have been developed to routinely detect *X. fastidiosa* in sharpshooter vectors that are known to spread *X. fastidiosa*. Several laboratories have detected the bacterium in *Homalodisca coagulata*, including laboratories under the supervision of D. Cook, D. Cooksey, H. Costa, T. Miller, A. Purcell, and R. Redak of the University of California. Thus far, the detection limit of *X. fastidiosa* in sharpshooter vectors is not established for any technique. Nor is the relationship between detection in *H. coagulata* and inoculation probability.

Why is it important to detect *X. fastidiosa* in sharpshooter vectors? Precisely so we can define the window of time during which grapevines are most susceptible to an inoculation event leading to chronic/terminal infection. An awareness of this window of time will allow new and promising plant protection tactics to be deployed optimally. Currently, we know that systemic neonicotinoid insecticides not only induce GWSS mortality for several weeks after treatment, but also inhibit feeding for much longer (Blua and Redak 2001, Bethke et al 2001). This later characteristic may be more important to reducing disease spread than the former. If the sharpshooter does not feed, it cannot inoculate the pathogen into a non-infected plant or acquire it from an infected plant. In experiments that optimized *X. fastidiosa* transmission by the GWSS, inoculation efficiency jumped from 2% after 1 hour of inoculation access time to 23% after 6 hours (A. Purcell, personal communication). Substances that limit feeding to seconds or minutes could reduce transmission efficiency to the point of essentially blocking the spread of *X. fastidiosa*. Unfortunately, restraints on the use of neonicotinyl insecticides in grapevines do not allow adequate protection throughout the year. Thus, we need to determine when their use would have the greatest impact on infectious GWSS. These arguments can be applied to other plant-protection tactics including the use of substances that disrupt GWSS behavioral cues, and others that inhibit establishment of *X. fastidiosa* in grapevines.

OBJECTIVES

Our long-term goal is to identify the window of time during which grapevines are most susceptible to inoculation by glassy-winged sharpshooters (GWSS) carrying the Pierce's disease bacterium, *Xylella fastidiosa*. In support of this goal we propose first to generate a method of detecting *X. fastidiosa* in glassy-winged sharpshooter vectors that maximizes sensitivity, and is amenable to large sample sizes.

RESULTS AND CONCLUSIONS

Several commercially available PCR preparation kits are useful in detecting *X. fastidiosa*. These kits use different combinations of cell lysing agents and DNA capture methods. All kits we examined were relatively cheap, easy to manage, and amenable to a large number of samples (Table 1).

All of the kits and procedures detected *X. fastidiosa* from pure culture with the exception of Cell Lytic from Sigma (Table 2). A liquid nitrogen extract of *H. coagulata* heads interfered with Dneasy (Qiagen), FTA genecard (Whatman), and the Single Fly procedure, even though DNA was detected from all extracts. None of the kits detected *X. fastidiosa* from liquid nitrogen-extracted *H. coagulata* heads that were collected from citrus at U.C. Riverside. In a more extensive examination of the gDNA Blood Mini Kit (Eppendorf) our lower detection limit was 9×10^2 CFU with a sharpshooter head background.

Our preliminary attempts to detect *X. fastidiosa* in *H. coagulata* that were allowed to feed on infected grapevines have shown inconsistent results (Table 2). Several issues need to be explored. First, bacterial titer in *H. coagulata* that have acquired it frequently may be lower than our detection limits. Second, bacteria may be "trapped" in areas of *H. coagulata* mouthparts or foregut in ways that inhibit extraction. Third, interfering substances in the insect may inhibit extraction or PCR. Further studies will focus on these possibilities to optimize detection of *X. fastidiosa* in *H. coagulata*.

Table 1: Aspects of PCR kits examined for the detection of *Xylella fastidiosa*.

Kit/Procedure	Company	LYSIS	DNA Capture	Time (24 preps)	Cost /sample
GeneClean	Bio 101	SDS-Detergent	Silica Glassmilk	1.0 hr	\$1.00
Dneasy tissue	Qiagen	Proteinase K	Silica Gel Membrane	1.0 hr	\$2.00
DNAzol	MRC Inc.	guanidine-detergent	Phase Separation	1.5 hr	<\$1.00
FTA genecard	Whatman	Chemical ²	None	<20 min	<\$1.00
gDNA Blood mini	Eppendorf	SDS/Proteinase K	Silica Gel Membrane	1.0 hr	\$1.00
DNA extraction	Fermentus	Chemical ²	Silica glass beads	1.5 hr	\$1.00
Cell Lytic	Sigma	Lysozyme/SDS/Chemical	None	1.5 hr	<\$1.00
Single Fly ¹	N/A	Proteinase K	None	1.0hr	<\$1.00
Phenol Extract	N/A	Phenol	Phase Separation	Varies	<\$1.00

¹Procedure that uses proteinase K for lysis and directly to PCR²Proprietary information**Table 2:** Detection of DNA and *Xylella fastidiosa* (X.f.) by PCR kits examined.

Method	Company	Cultured <i>X. fastidiosa</i>		Cultured X.f. + <i>H. coagulata</i>		Field Collected <i>H. coagulata</i>		X.f. from <i>H. coagulata</i>	
		DNA	X.f.	DNA	X.f.	DNA	X.f.	DNA	X.f.
GeneClean	Bio 101	Yes	+	Yes	+	Yes	-	Yes	-
Dneasy tissue	Qiagen	Yes	+	Yes	-	Yes	-	Yes	-
DNAzol	MRC Inc.	Yes	+	Yes	+	Yes	-	Yes	-
FTA genecard	Whatman	Yes	+	Yes	-	Yes	-	Yes	-
gDNA Blood mini	Eppendorf	Yes	+	Yes	+	Yes	-	Yes	-
DNA extraction	Fermentus	Yes	+	Yes	+	Yes	-	Yes	+
Cell Lytic	Sigma	No	-	No	-	No	-	No	-
Single Fly	N/A	Yes	+	Yes	-	Yes	-	Yes	-
Phenol Extract	N/A	Yes	+	Yes	+	Yes	-	Yes	-

Samples were from cultured *X. fastidiosa* (n=4), cultured *X. fastidiosa* with *Homalodisca coagulata* adult heads extracted with liquid nitrogen (n=8), *H. coagulata* heads from adults collected in citrus and extracted as above (n=4), and heads extracted as above from *H. coagulata* adults allowed to feed for 4 days on grapevines infected with *X. fastidiosa* (n=4). PCR used primers set 31 and 33 (Minsavage et al. 1994).

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FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

DEVELOPING A STABLE CLASSIFICATION OF THE GLASSY-WINGED SHARPSHOOTER GENUS *HOMALODISCA*

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Reporting Period: The results reported here are from work conducted from August 2000 to November 2002.

INTRODUCTION

The glassy-winged sharpshooter (GWSS), the leafhopper principally responsible for the spread of Pierce's disease on grape in California, is the species *Homalodisca coagulata*. This special capacity relates to the tissue upon which all sharpshooters (leafhopper subfamily Cicadellinae) feed: xylem, and the invasive status of the GWSS in California. It is noteworthy that of the 19 species in the genus, only one other species occurs in California and 18 species occur outside the USA (6 of these also occur in the USA). The genus is common in Mexico and also occurs southward through Central America, northern South America, and southeastern Brazil and Paraguay. That is, most species of *Homalodisca*, were they to reach California, have a destructive potential equal to the GWSS regarding the grape industry. The genus *Homalodisca* contains two other species that are already known to vector phytopathogens and it is practically expected that all species in the genus have the capacity to be, or become, serious vectors. Clearly, in a situation like this, we need to be clear about which species we are studying. The genus has never been revised.

Words are the tools of efficient communication and taxonomy is the vocabulary of species. By linking information to genus and species names, a classification of species becomes at once a very efficient system for storage and retrieval of information, and hence for meaningful communication, and a predictive tool, provided that classification is sound. Linking that information to species names that may be based on misidentifications, or belong to entirely different genera, will only add confusion to vector studies. In order to communicate effectively about the GWSS and its congeners, it is essential that everybody use the same names for the same species.

Access to all information on any group of organisms, including *Homalodisca*, is severely impeded by arbitrary generic limits, multiple names for some species and no name for others, or the absence of authoritative identification tools, or all three factors. The status of *Homalodisca* in this regard is below acceptable levels for a group of such economic importance.

OBJECTIVES

Broadly, the objective of the proposed research is to stabilize the classification of the genus *Homalodisca* so that all other information gathered (host plants, ecology, physiology, genomics, etc., which are all identified as priorities in the PD research program) can be linked to the correct names for meaningful communication. This will be accomplished through three major objectives:

1. Establish the limits of the genus *Homalodisca* through comparison to closely related genera, and the limits of all species in the genus, determine their valid names, and describe new species as necessary.
2. Characterization of brochosome structure and related behavior to allow identification of egg masses and females for most species.
3. Provide authoritative and electronically accessible identification aids and distribution data for all species, in addition to a hardcopy publication of the *Homalodisca* revision.

Also important for a revision is determining the relationship of *Homalodisca* to closely related genera. This is presently being addressed by a Ph.D. student and proposal cooperator Daniela Takiya, with outside funding for four years and is consequently not a major objective of this project.

RESULTS AND CONCLUSIONS

This is an incipient, two-year project involving both lab and field work. The revision of *Homalodisca* (Objective 1) has begun. In addition to the specimens held by the National Museum of Natural History, over 1,000 specimens have been borrowed from about one dozen institutions, locality data has been extracted and converted to decimal degree geographic coordinates for 1,500 specimens, and characterization of species and intra- and interspecific variation has begun. The closest genera to *Homalodisca* are *Phera*, *Pseudophera*, and *Oncometopia* (which also contain known phytopathogen vectors), but the distinctions are not satisfactorily established. At present there are 26 names in the genus *Homalodisca*, thought to pertain to 19 species. The most comprehensive study of *Homalodisca* to date was by Young (1968) and was limited to the seven species occurring in the United States. As noted above, however, the genus is more common in Mexico and further south, including Central America, northern South America, southeastern Brazil and Paraguay. The morphology of *Homalodisca* species, incorporating characters of the head, thorax (including wings and legs), abdomen, male and female genitalia, and integumental fine-structure are being analyzed.

To allow identification of egg masses and females (Objective 2), brochosome structure and related behavior is being characterized for as many species as possible, at present for six species. Brochosomes are hydrophobic secretions of malpighian tubules that are found only in leafhoppers. At moulting, nymphs and adults spread the brochosomes over their bodies, presumably to stay dry in wet conditions. In *Homalodisca* and a few related genera, females coat egg masses with these brochosomes, which vary in structure among species. This objective is most important for quarantine purposes, especially with regard to the grape crops.

For Objective 3, an on-line, image-driven key will be produced and placed on the USDA/ARS Systematic Entomology Laboratory server to maximize access and utility. A traditional key to species will accompany the hardcopy generic revision.

Foreign expeditions to support all three objectives are being planned for Mexico, Costa Rica, and Venezuela and will consist of examining existing collections (at the Universidad Nacional Autónoma de México, Instituto Nacional de Biodiversidad, and Museo del Instituto Agrícola, Maracay, respectively), making new collections of *Homalodisca* and associated organisms, such as host plants or natural enemies, and observing oviposition behavior in additional species. All expedition team members are leafhopper specialists with ample fieldwork experience. Collecting in Mexico and Venezuela may reveal males for two of the three *Homalodisca* species presently known only from females (the third species, *H. ignota*, occurs only in Brazil) and will undoubtedly yield new locality records. Obtaining more complete geographic coverage may result in revealing additional variation, which would impact species delimitation, or even new species. Specimens will be shared with collaborating foreign institutions in accordance with host country regulations; all specimens brought to the United States will be deposited in the National Museum of Natural History, Smithsonian Institution.

FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

HOST PLANT RESISTANCE TO THE GLASSY-WINGED SHARPSHOOTER IN GRAPES

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Reporting Period: The results reported here are from work conducted from September 2001 to September 2002.

INTRODUCTION

Although some of the American native *Vitis* species, particularly those originated from the southeastern United States have been known for resistance to the Pierce's disease (Lu 2000; Lu and Ren 2002), their resistant status against the glassy-winged sharpshooter, *Homalodisca coagulata* (Say), GWSS, the vector transmitting Pierce's disease (PD) pathogen (*Xylella fastidiosa*), have not been reported. It would be interesting to know if there is any correlation between resistance to the disease and resistance to the insect vector transmitting the disease. In addition, understanding the mechanism of host resistance to the GWSS, and the insect/plant interactions will add new dimension to control the insect vector in addition to the existed measures. In this connection, a study to survey the GWSS feeding preference on grapevines with different genetic background was conducted at Florida A&M University, Tallahassee, Florida. Our preliminary study indicated that GWSS has feeding preference for certain grapevines. Since one recommendation to manage the GWSS is establishing riparian vegetation surrounding a vineyard, understanding the GWSS feeding habit on different grapes will also enable us to select resistant grape materials against GWSS. The long term goal of this project is to understand the mechanism of feeding preference (or host resistance) among resistant and susceptible grapevines, and the genetic basis of the host resistance to the GWSS.

OBJECTIVES

1. Determine the feeding preferences of GWSS on different grape species and cultivars.
2. Investigate the mechanisms of host plant resistance to GWSS.
3. Understand the interaction between GWSS feeding preferences and physiological responses of the host plant to feeding, and the genetic basis of the host plant resistance to GWSS.

RESULTS AND CONCLUSIONS

The feeding preference of GWSS on different species/cultivars was evaluated in two different ways: 1) count the number of GWSS feeding on grapevines of different species / cultivars in the field; 2) determine the feeding preference by measuring the excretion of the GWSS feeding on difference grape species /cultivars. For the field-count of GWSS on individual grapevines, two separate investigations were conducted during last two seasons. The first survey was conducted on highly susceptible *V. vinifera* cultivars 'Chardonnay,' 'Cabernet Sauvignon', 'Thompson Seedless,' and *V. labrusca* cultivars 'Concord' and 'Niagara', with muscadine grape as a resistant control. One-year old vines grafted on muscadine rootstocks (Ren and Lu, 1999) were used for this investigation. The actual numbers of GWSS feeding on these grape cultivars were counted on a daily basis between 10:00 and 12:00 in the morning from late June to the end of August. As shown in Table 1, the PD resistant grape cultivar *V. rotundifolia* (muscadine grape) had significantly fewer visits by GWSS than did the susceptible grape cultivars 'Chardonnay,' 'Cabernet Sauvignon,' and 'Thompson Seedless.' The frequency of GWSS visits to *V. labrusca* cultivars 'Concord' and 'Niagara', the native American grape susceptible to PD, was intermediate between those found on *V. rotundifolia* and *V. vinifera*.

Table 1: Average number of GWSS on different grape cultivars.

Cultivars	Average numbers of GWSS per vine per observation						
	6/25-30	7/1-10	7/11-20	7/21-31	8/1-10	8/11-20	8/21-31
Chardonnay	1.3	1.0	2.9	0.6	0.0	0.0	0.0
Thompson Seedless	1.1	3.4	5.1	4.1	1.7	1.4	0.3
Cabernet Sauvignon	3.3	6.0	6.6	3.8	1.6	0.8	0.3
Concord	1.3	1.4	1.1	0.3	0.1	0.0	0.0
Niagara	0.4	0.4	0.1	<0.1	0.0	0.0	0.0
Muscadine	0.3	<0.1	0.2	0.0	0.0	0.0	0.0

For the second investigation, adults and nymphs of GWSS were assessed by weekly counts during the period when GWSS were observed in the vineyard in 2002 (from June to October). More than 100 accessions, including pure species and complex hybrids, were included in this investigation. However, only a representative of eight accessions / cultivars (Table 2) was included in this report while the rest of the data are being dissected and analyzed. Similar to the first investigation, the PD resistant muscadine vines received very few visits of GWSS, while PD susceptible grape 'Niagara' received high GWSS counts in the same vineyard. PD tolerant Florida hybrid bunch grapes had an intermediate count of GWSS. As expected, the PD resistant *Vitis* species *shuttleworthii* and *mustangensis* had very low counts of GWSS. Interestingly, the PD tolerant *V. cineria* accession had very high counts of GWSS.

Table 2: GWSS population on selected vines during the 2002 growing season.

Ssp. /cvs.	June	July	August	September	October
<i>Vitis rotundifolia</i>					
Fry	0.1±0.4	0.03±0.6	0.04±0.05	0	0
Carlos	0.1±0.4	0	0	0	0
Florida hybrid bunch grapes					
Blanc du Bois	4.2±3.2	1.2±1.3	3.5±2.6	1.0±1.0	0
Suwannee	1.8±1.7	2.6±0.9	1.5±2.4	0.7±1.6	0
<i>Vitis labrusca</i>					
Niagara	6.8±3.6	0.2±0.5	0.8±0.1	0.7±0.6	0
<i>Vitis shuttleworthii</i> (JL 2001)	0.5±0.6	0.2±0.4	0	0	0
<i>Vitis mustangensis</i> (DVIT 2232)	0.8±0.5	0.6±0.5	0.5±0.6	0	0
<i>Vitis cineria</i> (DVIT 2380)	14.8±7.1	2.8±3.1	1.8±1.3	0	0

The feeding preference was also measured on selected grapes, including resistant, tolerant and susceptible grape cultivars, by collecting the excretion of the GWSS. Two GWSS were introduced and confined in a 50-ml plastic tube in which a shoot was running through for the GWSS feeding. Excretion was collected and used for determining the feeding preference. The experiment was repeated three times (June 24, July 15 and July 22) and two vines were used for each cultivar in each experiment. The data in Table 3 are the average excretion per tube (from two GWSS) collected two days after the GWSS were introduced to the confined tube. In general, more excretion was collected from the bunch grapes than from the muscadine grapes. Among the bunch grape cultivars, more excretion was obtained from the PD susceptible cultivars ('Chardonnay', 'Concord', and 'Niagara') than PD tolerant cultivars ('Blanc du Bois' and 'Blue Lake') and PD resistant cultivar ('Champanelle').

Table 3: Average excretion per tube collected two days after two GWSS were confined in a 50 mL tube with one shoot.

Cultivars	Excretion
Muscadine Grapes	
Carlos	0.34
Fry	1.83
Jumbo	0.49
Bunch Grapes	
Blanc du Bois	0.50
Blue Lake	0.50
Orlando Seedless	1.80
Champanelle	0.45
Chardonnay	3.30
Concord	2.69
Niagara	1.33

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

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INTRODUCTION

Insect dispersal can be influenced by numerous factors, such as increasing population densities, reproductive status, biased sex ratios, host breadth, declining host quality and changing environmental conditions (Denno 1979, 1985; Taylor 1985, Denno et al. 1991, Blackmer and Phelan 1991, Blackmer and Byrne 1993a,b, 1999; Blackmer and Cross 2001). A better understanding of how these various factors influence the movement of the glassy-winged sharpshooter will be crucial in the management of this pest and the spread of Pierce's disease (PD).

OBJECTIVES

1. Compare rates of movement between glassy-winged sharpshooters (GWSS) and native smoke-tree sharpshooters (STSS) to better understand changes in the spread of PD.
2. 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

Mark-release-recapture (MRR) studies with GWSS and STSS were conducted in 2001 in Moreno Valley in an abandoned alfalfa field, and in 2002 additional releases of GWSS were carried out in an 11-ha Valencia orchard in Fillmore, California. Temperature, relative humidity, barometric pressure, wind speed, and wind direction were monitored at the center of each release site with a portable weather station. Physiological parameters, such as egg load, weight and sex ratio were also measured. Recapture data generated from these studies were fit to a diffusion model (Turchin and Thoeny 1993) and model results were used to estimate dispersal distances for each species in each habitat. This model has been shown to accurately describe the movement of numerous insect species (Kareiva 1983, Plant and Cunningham 1991, Turchin and Thoeny 1993, Corbett and Rosenheim 1996, Rudd and McEvoy 1996). At the Fillmore site, sharpshooters were collected and doubly marked with an IgG protein solution (Hagler et al. 1992, Hagler 1997) and a light application of fluorescent pigment. Four colored pigments were used consecutively, which allowed us to separate released sharpshooters from the background population, as well as track sharpshooters for up to 6 wks. Sharpshooters were recaptured on cylindrical yellow sticky traps that were attached at ground, mid-canopy (2-3m) and upper-canopy level (6-7m) to 7-m tall telescoping poles. In a separate study, at the Fillmore location, movement of GWSS was measured relative to time of day, environmental parameters, and xylem flux. Sticky traps were changed and xylem sap was collected at four-hour intervals from 0600 to 2200 hours (N=5).

Linear regressions of recapture data with the diffusion model provided significant fits to the data with high coefficients of determination (R^2) for all of the GWSS and 3 of the 4 STSS releases in 2001, and for 5 of the 6 releases in 2002 (Table 1). In 2001, calculations of dispersal distances using the diffusion model showed that 50 and 95% of the GWSS moved 30 and 90 m in 5-6 hr, respectively, while 50 and 95% of the STSS moved 47 and 155 m, respectively (Table 2). Approximately 7% of the GWSS and 21% of the STSS flew beyond our most distance annuli (90m) in the 2001 releases. In 2002, more than 83,000 GWSS were marked and released between July and early October. Calculations of dispersal distances for these releases showed that 50 and 95% of the GWSS moved 30 and 99 m in 72 hr, respectively (Table 2). Parameters estimated in these trials will be used in further experiments and modeling efforts to determine absolute rates of movement for GWSS.

In separate stepwise regression analyses, trap distance from the release site was the best predictor of trap catch ($R^2 = 0.38$, $P < 0.0001$ for GWSS and $R^2 = 0.31$, $P < 0.0001$ for STSS in 2001; $R^2 = 0.23$, $P < 0.0001$ in 2002). In 2001, the addition of trap height, release date, height and distance interaction accounted for an additional 20-31% of the variability in trap catch. In 2002, the addition of trap height, release date and cardinal position only accounted for an additional 10% of the variability in trap catch. Recapture rates were considerably lower in the citrus orchard as compared to the open field setting (1.6% in 72 hours vs. 12% in 6 hours). Similar to 2001, more sharpshooters were recaptured on the two lower traps (below 3m) than on the upper traps ($P < 0.05$) for all six releases in 2002. Egg loads were fairly even in July and August (3.77 ± 0.38 ; mean \pm SD), but declined in September and October to 0.65 ± 0.33 per female. Weights for male and female sharpshooters fluctuated little throughout the study (0.036 ± 0.003 g for females; 0.027 ± 0.002 g for males), even after egg loads declined. A female biased sex ratio (0.72:1.0; male:female) was evident in the first two releases (in July), but thereafter, a male biased sex ratio (1.2:1.0) was observed.

In a separate study, that measured sharpshooter movement relative to time of day, environmental parameters and xylem flux, we found that sharpshooters were most active, in terms of flight activity, between 1000 and 1400 hours (Figure 1). Of the environmental parameters tested, only temperature explained a significant amount of the variability in trap catch in 2002 ($R^2 = 0.58$, $P < 0.0001$). Sharpshooters were rarely trapped when temperatures fell below 18°C.

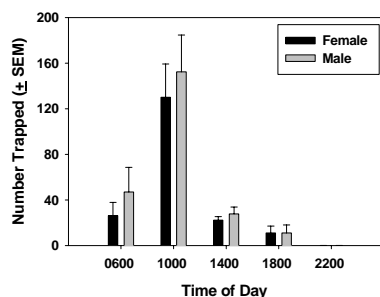


Figure 1. Number of female and male *H. coagulata* trapped relative to time of day.

Table 1. Parameter estimation of diffusion model fit to glassy-winged sharpshooter (GWSS) and smoke tree sharpshooter (STSS) dispersal data for 2001 in Moreno Valley and 2002 in Fillmore, CA.

Trial	GWSS – 2001				STSS – 2001				GWSS - 2002			
	<i>A</i>	<i>B</i>	<i>P</i>	R^2	<i>A</i>	<i>B</i>	<i>P</i>	R^2	<i>A</i>	<i>B</i>	<i>P</i>	R^2
1	134.71	26.98	0.022	0.86	27.45	47.71	0.076	0.70	106.27	37.03	0.011	0.69
2	186.42	26.76	0.017	0.89	27.79	48.29	0.002	0.97	108.01	23.05	0.002	0.88
3	60.60	21.65	0.012	0.91	23.27	30.96	0.009	0.92	136.58	26.33	0.009	0.78
4	107.23	25.26	0.014	0.90	32.63	31.88	0.005	0.95	323.32	17.61	0.0001	0.99
5									179.12	23.36	0.002	0.87
6									8.16	40.18	0.21	0.29

.Data for 2001 are recaptures at 6 hour intervals; data for 2002 are recaptures at 72 hr intervals.

Table 2. Estimates of the radius of a circle enclosing various proportions of dispersal distances for GWSS and STSS after point release in an alfalfa field in 2001, and in a mature orange grove in 2002.

Proportion Enclosed	Estimated Radius (m) for GWSS-2001	Estimated Radius (m) for STSS-2001	Estimated Radius (m) for GWSS-2002
0.50	30	47	30
0.67	43	68	44
0.95	90	155	99
0.99	99	220	145

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*Use of Pesticides and
Alternative Treatments to
Control Glassy-winged
Sharpshooter and Pierce's
Disease*



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

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Reporting Period : The results reported here are from work conducted from July 2002 to October 2002.

INTRODUCTION

The management of Pierce's disease (PD) spread by *Homalodisca coagulata*, the glassy-winged sharpshooter (GWSS) is lacking a fundamental strategy and solid tactics. Among tactics under development is the use of insecticides to minimize numbers of GWSS and inhibit their feeding to reduce their ability to acquire the bacterium from infected vines and to inoculate uninfected vines. Two aspects of insecticides are necessary for this tactic to be successful: (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 examined the impact of insecticides on grapevines against the GWSS (Blua et al 2000, Redak and Blua 2001). We chose to study insecticides of the chemical class known as neonicotinoids because of their reputed inhibition of feeding by sucking-insects, and their long residual activity.

Anti-feedant qualities are one of the important aspects of neonicotinoids. In a 1999 experiment conducted at the University of California, Riverside, GWSS caged on field-grown grapevines treated with Admire (imidacloprid, Bayer Inc) did not feed enough to generate visible amounts of excreta, which they normally produce in copious quantities. In contrast, GWSS on untreated vines generated a substantial volume of excreta. We concluded that Admire inhibits feeding by the GWSS. Our most recent experiments showed this effect for other neonicotinoids, including soil-applied Actara (thiamethoxam) (Syngenta, Inc) and foliar-applied Assail (acetamiprid) (Aventis, Inc) (Bethke et al 2001, Redak and Blua 2001). Most striking is our observation that neonicotinoids applied to grapevines in September of 1999 had a substantial impact on GWSS feeding almost a year later. This may, in fact, be more important to protecting plants from infectious sharpshooters than inducing mortality.

OBJECTIVES

The overall goal of our research is to determine the impact of sub-lethal doses of neonicotinoids on the spread of *Xylella fastidiosa*, the Pierce's disease bacterium, to or from grapevines by the GWSS. In support of this goal, we are examining the impact of sub-lethal doses of Admire on GWSS feeding using electronic monitoring methods under development in the laboratory of E. Backus (2001).

RESULTS AND CONCLUSIONS

Thus far, we have examined the relationship between the amount of Admire applied to potted grapevine seedlings and GWSS mortality after a 24h exposure period. We used these data to select a sub-lethal dose (3.75 mg Admire/pot) for our feeding studies (Figure 1). Ten days after treatment, this amount of Admire induced ca 50% GWSS mortality (Figure 1).

Our investigation of the impact of sub-lethal doses of Admire on GWSS feeding used a factorial experiment with two factors, each with two levels. The first factor was grapevines treatment with Admire or not, and the second factor was grapevines infected with Pierce's disease or not. Feeding behaviors of GWSS on experimental grapevines were recorded with electronic monitoring (Backus 2001). This experiment has been completed and the data are currently being analyzed.

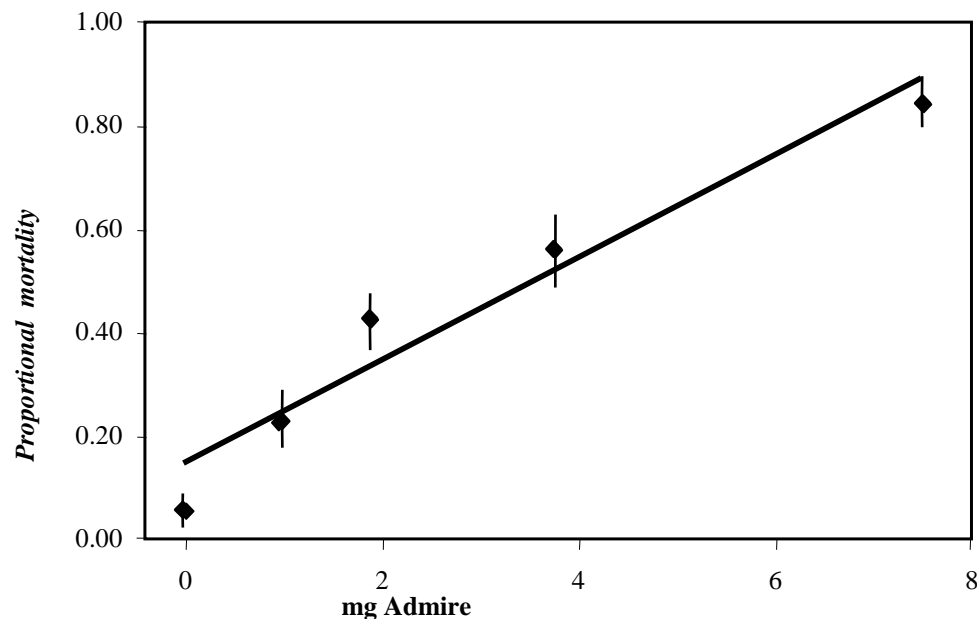


Figure 1. Mortality of *Homalodisca coagulata* as a function of Admire (Bayer, Inc) treatment of potted grapevines. Grapevine seedlings in 700ml pots were treated with 0.00, 0.94, 1.88, 3.75, and 7.50 mg Admire 10 d before *H. coagulata* adults were caged on plants. Mortality was measured 24h after exposure to plants. Points represent means \pm SE (N=17). Regression equation: $y = 0.099x + 0.143$.

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CONTROL OF IMMATURE AND ADULT GLASSY-WINGED SHARPSHOOTERS: EVALUATION OF BIORATIONAL AND CONVENTIONAL INSECTICIDES

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Reporting Period:

INTRODUCTION

Pierce's disease, caused by *Xylella fastidiosa* has become an increasingly important factor in grape production in California since 1996. The glassy-winged sharpshooter (GWSS) is a primary *X. fastidiosa* vector. Serious grape and vine losses have increased as GWSS numbers have increased in southern California (Blua et al. 1999). Purcell et al. (1999) suggested that diseases caused by *X. fastidiosa* are likely to become more prevalent with increased numbers and spread of GWSS. Management methods are urgently needed for GWSS that are economically, ecologically and socially acceptable. Cultural and biological components of developing integrated pest management (IPM) strategies need to be melded with efficacious GWSS chemical control and insecticide resistance management (IRM), and integrated crop management (ICM) inputs. In 2000, we studied GWSS adulticides in grapes (Akey et al., 2001a). Our objectives in the two-year trials (2001-2002) were to identify selective, conventional and biorational insecticides that were efficacious for control of immature and adult GWSS in citrus.

OBJECTIVES

1. Identify selective, conventional and biorational insecticides that were efficacious for control of immature and adult GWSS in citrus.

RESULTS AND CONCLUSIONS

Experiments were conducted with naturally occurring GWSS populations during egg to nymph to adult development during a 4-mo. period (April-July), on 6-7 foot tall orange trees. The experimental designs were two - 3 replicate randomized complete blocks at University of California, Agricultural Operations, Riverside, CA. Plots were 0.114ac in size; 25 by 22ft, 3 trees per plot with guard rows on each side (except for Surround[®] that had plots 3 times larger). GWSS counts were made weekly following applications of treatments (table 1) made with a windmill blast-type sprayer (John Bean Div., FMC) (compliant with Good Lab Practices, GLP). Spray delivery was at 200 psi at 300 gal/ac with 5 swivel-nozzle bodies (Tee Jet) on one side. There were 10 nozzles, each had a core 23, disc 6, and slotted strainer. An adjuvant, Silwet[®] L 77, (Loveland Ind.) was used in all applications (except Surround[®]). Spray penetration was studied previously (Akey et al. 2001a,b). LSD mean separation tests were made if there were significant F values by analyses of variance. Data were transformed by $\sqrt{x + 1/2}$ to adjust zeros in data sets.

Efficacies of materials evaluated for control are shown in table 2. The pyrethroids, Baythroid[®], Capture[®] and Danitol[®] and the neonicotinoids, Provado[®] and Assail[®] were highly effective against GWSS. Also, the insect growth regulator (IGR), Applaud[®] (1 application 0.9333 lb AI/ac), was highly effective against GWSS nymphs. That Applaud[®] rate was ca. half-label and unlikely to affect beneficial insects (study needed). Neem products (5 applications) were efficacious against development of GWSS to large nymphs (neem products had no efficacy or repellency on GWSS adults on grapes; unpublished data, summer/fall 2000). GWSS nymphal control in 2002 (Table 2) with Applaud[®], Agroneem[®] and Neemix[®], and Baythroid[®] confirmed our first-year results (Table 2, Akey et al. 2001b). Novaluron[®] (benzoylphenylurea group), a chitin blocker, was more effective against nymphs than Micromite[®] (also a chitin blocker), diflubenzuron (1 application of each). Sucrose octanoate had minimal efficacy (3 applications). The repellent, Surround[®], significantly decreased numbers of immature and adult GWSS (3 applications).

In summary, Applaud[®] is a prime candidate for IPM programs on GWSS. Pyrethroids are effective conventional agents against GWSS. Neem products may have a place as one tool, combined with others, in organic programs against GWSS. The biorational agents evaluated here will probably be more efficacious in area-wide programs.

Table 1. Trade names, chemistry classes, formulations and rates per acre of foliar insecticides evaluated for immature and adult glassy-wing sharpshooter control in citrus, Riverside, CA, 2001 and 2002.

Immature and adult grassy wing sharpshooter control in citrus, Riverside, CA, 2001 and 2002							
Year	Name		Chemistry		Per Acre		Company
	Trade	Generic	Class	Formulation	Product	lb AI	
Conventional Insecticides							
2001	Capture®	bifenthrin	pyrethroid	2 EC	6.4 fl oz	0.50	FMC
2002	Danitol®	fenpropathrin	pyrethroid	2.4 EC	21.0 fl oz	0.40	Valent USA
2001	Baythroid®	cyfluthrin	pyrethroid	2 E	1.6 fl oz	0.010	Bayer Crop Sc
2001, 02	Baythroid®	cyfluthrin	pyrethroid	2 E	3.2 fl oz	0.020	Bayer Crop Sc
2001	Fujimite®	fenpyroximate	oxime	5 EC	4.0 pt	0.0933	Nichino Amer
2001	Assail®	acetamiprid	neonicotinoids	70 WP	1.2 oz	0.0233	Bayer Crop Sc
2001	Provado®	imidacloprid	neonicotinoids	75 WP	10.0 oz	0.2147	Bayer Crop Sc
Biorational Insecticides and Repellants							
2002	Surround®	Kaolin clay	surface film	100 WP	50.0 lb	50.0	Engelhard
2002	Aza-Direct®	azadirachtin	neem IGR	1.2% wt	1.3 qt	0.0324	Gowan
2001, 02	Agroneem®	neem extract	neem IGR	15.0 %	4.0 qt	1.100	Agro Logistics
		& azadirachtin		0.15%	4.0 qt	0.110	
2002	Neemix®	azadirachtin	neem IGR	4.5 %	1.0 qt	0.46	Certis USA
2001	Trilogy®	neem without azadirachtin	neem IGR	70 %	5 gal	12.74	Certis USA
2002	Applaud®	buprofezin	chitin inhibitor	70 WP	0.2 lb	0.1633	Nichino Amer
2002	Applaud®	buprofezin	chitin inhibitor	70 WP	1.0 lb	0.7000	Nichino Amer
2001, 02	Applaud®	buprofezin	chitin inhibitor	70 WP	1.3 lb	0.9333	Nichino Amer
2001	Esteem®	pyriproxyfen	JH analog	0.86 EC	0.5 qt	0.03	
2002	AVACHem	sucrose	bio-soap	40 %	0.8% v/v	7.9 lb	Ava Chemical Ventures
	Sucrose	octanoate					
	Octanoate						
2002	AVACHem	sucrose	bio-soap	4 %	1.2% v/v	11.9 lb	Ava Chemical Ventures
	Sucrose	octanoate					
	Octanoate						
2002	Micromite®	diflubenzuron	chitin inhibitor	80 WG	6.3 fl oz	0.3125	Uniroyal Chem
2002	Novaluron®	benzoylphenyl urea group	chitin inhibitor	2.4 EC	4.2 lb	0.3125	Uniroyal Chem

Table 2. Mean numbers (\pm SE) and insecticidal efficacy percentages following applications of selected chemicals for glassy-wing sharpshooter control in citrus at Riverside, CA , 2001 and 2002.

2001 Treatments ^{1,2}	Small nymphs		Large nymphs		Adults	
	\bar{x} ³	% ⁴	\bar{x}	%	\bar{x}	%
Baythroid 1	0.1 \pm 0.1	99 d ⁵	0.3 \pm 0.1	95 e	4.3 \pm 1.2	67 efg
Baythroid 2	0.1 \pm 0.1	99 d	0.0 \pm 0.0	100 e	2.7 \pm 0.9	79 g
Capture	0.0 \pm 0.0	100 d	0.0 \pm 0.0	100 e	2.5 \pm 0.9	81 g
Provado	0.2 \pm 0.1	96 d	0.2 \pm 0.1	97 e	3.4 \pm 1.1	74 fg
Assail	0.1 \pm 0.1	99 d	0.1 \pm 0.1	99 e	2.8 \pm 0.8	79 g
Fujimite	3.6 \pm 1.0	45 bc	2.3 \pm 0.6	68 d	4.7 \pm 1.3	64 def
Applaud ⁶	0.7 \pm 0.5	90 d	0.0 \pm 0.0	100 e	5.9 \pm 2.3	55 cde
Esteem	6.7 \pm 2.0	a	4.9 \pm 1.3	31 bc	5.4 \pm 1.4	59 cde
Agroneem	6.8 \pm 1.8	a	5.3 \pm 1.9	26 b	7.1 \pm 1.9	39 bc
Neemix	5.6 \pm 1.8	15 ab	3.0 \pm 0.8	58 d	6.3 \pm 1.4	52 bcd
Trilogy	2.3 \pm 0.7	65 cd	3.4 \pm 1.2	52 cd	7.9 \pm 1.9	39 b
Control	6.6 \pm 1.9	-- a	7.1 \pm 1.7	-- a	13.1 \pm 3.4	-- a

2002 Treatments	Small nymphs			Large nymphs			Adults		
	n ²	\bar{x}	%	n	\bar{x}	%	n	\bar{x}	%
Baythroid	12	0.1 ± 0.1	99 b ⁵	18	0.1 ± 0.1	99 b	21	1.8 ± 0.7	71 b
Danitol	12	0.0 ± 0.0	100 b	18	0.0 ± 0.0	100 b	21	1.0 ± 0.4	85 b
Control	12	7.3 ± 3.8	-- a	18	15.2 ± 4.2	-- a	21	6.1 ± 1.6	-- a
Novaluron	12	6.8 ± 2.3	56 b	18	2.1 ± 0.6	86 c	18	5.4 ± 1.7	22 a
Micromite	12	12.3 ± 1.8	20 a	18	5.6 ± 0.8	63 b	18	7.5 ± 1.6	-- a
Control	12	15.3 ± 3.2	-- a	18	15.2 ± 4.2	-- a	18	7.0 ± 1.8	-- a
Applaud ⁷	21	6.3 ± 1.2	60 b	18	3.7 ± 1.0	78 b	18	2.1 ± 0.7	49 b
Applaud	21	5.4 ± 1.6	66 bc	18	2.4 ± 0.8	85 bc	18	0.8 ± 0.3	81 c
Applaud	21	2.3 ± 1.2	86 c	18	0.0 ± 0.0	100 c	18	1.2 ± 0.4	71 bc
Control	21	15.8 ± 2.6	-- a	18	16.8 ± 4.8	-- a	18	4.2 ± 1.3	-- a
Sucrose ⁸	21	20.7 ± 3.6	-- a	15	10.0 ± 2.1	44 a	12	4.5 ± 1.7	-- a
Sucrose	21	8.2 ± 1.3	26 a	15	3.5 ± 0.7	80 b	12	2.1 ± 0.7	48 a
Control	21	11.2 ± 2.5	-- a	15	18.0 ± 4.7	-- a	12	4.0 ± 1.6	-- a
Surround	9	5.3 ± 1.4	65 b	9	2.1 ± 0.6	89 b	9	19.4 ± 5.9	-- a
Control	9	15.0 ± 4.0	-- a	9	19.4 ± 5.9	-- a	9	2.0 ± 0.9	-- a
Agroneem	12	12.0 ± 2.8	33 a	12	8.8 ± 2.8	68 b	12	20.3 ± 3.9	16 ab
Aza-Direct	12	9.3 ± 2.0	48 a	12	7.6 ± 2.1	72 b	12	15.3 ± 2.8	37 bc
Neemix	12	17.2 ± 5.7	41 a	12	10.3 ± 3.2	62 a	12	12.2 ± 2.4	50 c
Control	12	18.0 ± 4.7	-- a	12	27.2 ± 6.8	-- a	12	24.2 ± 5.4	-- a

¹ Number of applications applied were: one for Baythroid, Capture, Danitol, Assail, Provado, Applaud, Novaluron, and Micromite; two for Fujimite, three for AVA Chem, Sucrose Octanoate, Surround; Agroneem, Neemix, and Trilogy, 2001, five for Agroneem, Aza-Direct and Neemix, 2002.

² n = 3 replicates of each treatment times number of analyzed dates in which the life stage was present post application(s); n 2001 was 21.

³ Means of 3 replicates of each treatment.

⁴ % efficacy = percent reduction from control.

⁵ Means in columns by group(s) with different letters, are significantly different by ANOVA and LSD at $P \leq 0.05$, analyses were based on transformed data, $\sqrt{(x + \frac{1}{2})}$ to adjust zeros in data sets.

⁶ 2001, 0.93 lb AI/ac

⁷ 2002, 0.16, 0.70 and 0.93 lb AI/ac, respectively.

⁸ 0.8 and 1.2%, respectively.

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FUNDING AGENCIES

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

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Reporting Period: The results reported here are from work conducted from March 2001 through August 2002.

INTRODUCTION

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, Engelhard Corp, Iselin, NJ) is based on the inert mineral, kaolin, that forms a film that protects plants from insects and some diseases. Surround WP is exempt from tolerance, can be applied up to the day of harvest, has a 4 hour re-entry period, has 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 received EPA registration in 2002, is made of food grade materials, is exempt from tolerance, and has also shown levels of control of GWSS that is as good as other conventional insecticides. The objectives of this research were to determine how effective these materials were in controlling GWSS in lab and field experiments.

OBJECTIVES

1. Evaluation of particle film, Surround WP, effects on GWSS biology.
2. Efficacy of sugar esters a quick knock-down agents for GWSS control.
3. Prevention of GWSS infestations with season-long and timed spray applications of Surround WP.

RESULTS AND CONCLUSIONS

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 released 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, 20 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.

GWSS nymphs and adults refused to settle on Surround treated foliage when given a choice (Figure 1). When given a no choice, all adults and most nymphs refused to stay on Surround treated plants and cling to the cages until they died. These studies show that Surround is highly repellent to both GWSS nymphs and adults.

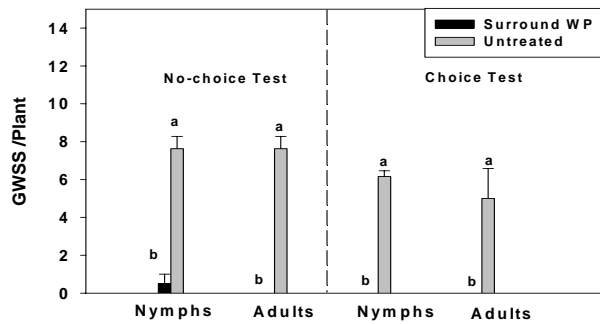


Figure 1. Number of glassy-winged sharpshooter nymphs (n=20) and adults (n=50) after being released in cages containing either a lemon tree treated with Surround WP or left untreated in a no-choice test (right) or given a choice between one limb treated with Surround WP and the other left untreated (left).

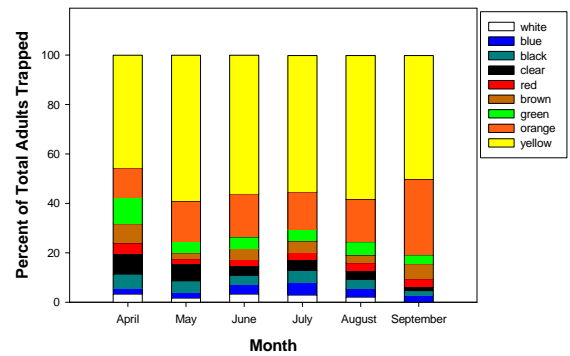


Figure 2. Mean response of glassy-winged sharpshooter adults to 25.4 cm plastic discs painted a spectrum of colors, coated with Tangle Foot™ (Grand Rapids, MI), and placed at a height of 2.0 m in two citrus groves bordering grape, Kern Co., CA.

Surround WP or left untreated in a no-choice test (right) or given a choice between one limb treated with Surround WP and the other left untreated (left).

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 attract GWSS adults 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.

Results during the 2001 season showed that yellow was the most preferred color followed by orange and that white was among the least preferred colors we examined (Figure 2). There was also evidence that GWSS adults responded more to brown in the spring and orange in September while their response to yellow was consistent over the sample period.

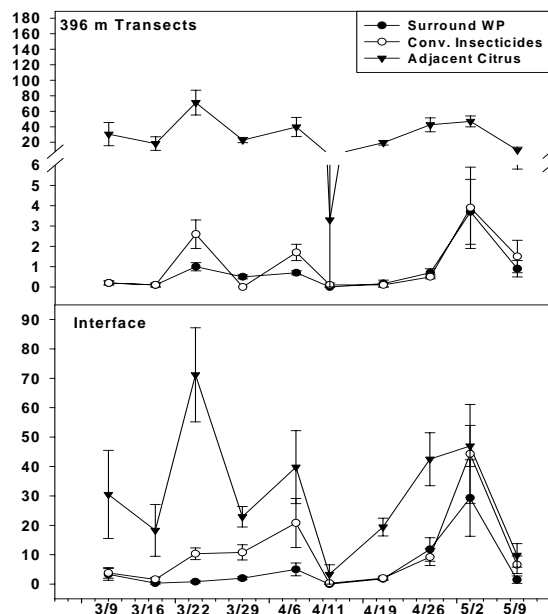


Figure 3. Mean number of glassy-winged sharpshooter adults caught per yellow sticky trap (Trece, Salinas, CA) placed 1.0 m high in the first grape vines in a vineyard bordering citrus (Interface) and placed every 30.5 m in two 365.7 m transects extending into each treatment block (Transect) in Site 1, Kern Co., CA.

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, CA, but only Site 1 produced enough GWSS numbers for study. In this study, we examined the effect of a 247.5 m Surround WP barrier treatment on grape to prevent GWSS adult movement from citrus into grape. Site 1 had treatment blocks 164.6 m wide by 365.7 m long (6.5 ha) replicated 3 times in a mixed block of table and wine grape. Surround treatments only extended 247.5 m into each block with the remaining 152.4 m left untreated while conventional chemical treatments extended the entire 365.7 m distance of the block in order to determine the effect of a 247.5 m treatment barrier. Yellow sticky traps were placed in 2 transects per block and spaced every 100 feet that began where grape interfaced citrus and extended 396 m 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. Effects of the treatments on oviposition was examined on May 4 by visually sampling 25 leaves/vine every 30.5 m along the trap transects. Three bi-weekly treatments of Surround WP were compared to six conventional chemical control program that applied various contact insecticides weekly. Surround treatments of 50 lb Surround WP/100 gal was applied at 50 to 70 gpa on March 13, March 30 and April 14.

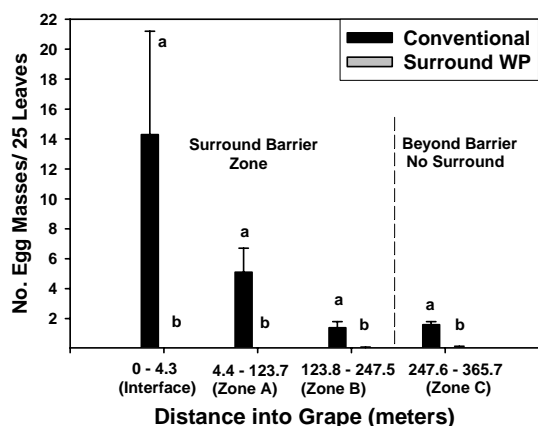


Figure 4. Mean number of glassy-winged sharpshooter eggs on 25 leaves/vine in the first grape vines in a vineyard bordering citrus (Interface) and from samples taken every 30.5 m into each treatment zone in Site 1, Kern Co., CA.

Sugar esters for control of GWSS adults:

Two sugar ester materials that are produced by AVA Chemical Ventures (Portsmouth, MA) were evaluated for efficacy against GWSS adults in a field trial in Ventura Co., CA. A range of concentrations were examined in comparison to another soft insecticide, M-Pede insecticidal soap. Applications were made in late-July, 2002 to 2.0 m citrus trees heavily infested with GWSS. Trees were caged in early morning and treatments were applied using a hand-gun sprayer. Sucrose octanoate was more effective than sorbitol octanoate and M-Pede at lower doses (Table 1). Sorbitol octanoate and M-Pede performed similarly. There was recovery of GWSS adults over time in the sorbitol octanoate treatment which was not as evident for the sucrose octanoate and M-Pede materials making sorbitol octanoate less than desirable for GWSS control. However, further evaluations of sucrose octanoate would be worthwhile. Sucrose octanoate recently became registered as an insecticide with the U.S. EPA as Sucrose Octanoate Esters and which is a new class of insecticide that is safe to humans and the environment.

Table 1. Comparison of glassywinged sharpshooter adult mortalities after treatment with different of sugar esters and insecticidal soap applied to orange trees, Ventura County, CA, July, 2002.

Treatment	Conc.	Mortality over Time (\pm std err.)		
		5 min.	30 min.	60 min.
Sucrose Octanoate	0.8%	93.0 \pm 4.0a	79.0 \pm 6.4bcd	69.0 \pm 7.4cd
Sucrose Octanoate	1.0%	89.0 \pm 4.0a	86.0 \pm 4.8abc	77.0 \pm 6.6bc
Sucrose Octanoate	1.2%	98.0 \pm 1.2a	96.0 \pm 1.8a	97.0 \pm 1.2a
Sucrose Octanoate	1.5%	98.0 \pm 1.2a	98.0 \pm 1.2a	97.0 \pm 1.2a
Sorbitol Octanoate	0.8%	38.0 \pm 7.8c	25.0 \pm 7.5g	17.0 \pm 4.6g
Sorbitol Octanoate	1.0%	76.0 \pm 2.9b	55.0 \pm 7.9ef	37.0 \pm 5.1f
Sorbitol Octanoate	1.2%	95.0 \pm 1.5a	74.0 \pm 7.6cd	59.0 \pm 9.2de
Sorbitol Octanoate	1.5%	91.0 \pm 4.5a	91.0 \pm 4.5ab	88.0 \pm 5.8ab
M-Pede	0.6%	42.0 \pm 9.7c	50.0 \pm 10.3f	49.0 \pm 9.9ef
M-Pede	0.8%	64.0 \pm 5.1b	69.0 \pm 2.9de	65.0 \pm 5.4cd
M-Pede	1.0%	74.0 \pm 4.0b	76.0 \pm 7.6bcd	77.0 \pm 6.0bc
M-Pede	1.2%	99.0 \pm 1.0a	97.0 \pm 97.0a	96.0 \pm 1.8a
Water	100%	0.0 \pm 0d	0.0 \pm 0.0h	0.0 \pm 0.0h

Means followed by the same letter within a column are not significant, REGWQ, $P = 0.05$

FUNDING AGENCIES

Funding for this project was provided by the USDA Animal and Plant Health Inspection Service.

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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Reporting Period: The results reported are from work conducted from December 2001 through November 2002.

INTRODUCTION

The possibility of resistance development when insecticides are used necessitates the development of an effective resistance-monitoring program that enables early detection of even low-frequency resistance alleles in natural populations. The initial step for monitoring of resistance is through development of appropriate bioassay techniques that can establish baseline susceptibility data among populations. Our goal for the first year was to study the effectiveness of selected insecticides that represent various chemistries against GWSS and determine regional comparisons of GWSS responses to these insecticides. Simple and suitable bioassay techniques were developed to detect toxicological responses and to establish baseline susceptibility data of GWSS to various insecticides. Three techniques, petri-dish, leaf-dip and systemic bioassays were described in the previous report (Toscano et al. 2001). Evaluation continued during the second season to assess any changes in responses of GWSS to a wide range of chemistry. The present report compares the toxicological responses of GWSS for a period of two years.

Resistance does not evolve at the same rate for all pests that come under selection pressure. Many factors influence the rate at which resistance develops in a pest. In the case of GWSS, we have no information on the potential for resistance development in this species. One method to estimate the potential for resistance risk is to artificially select resistant strains under greenhouse conditions.

In addition to conventional bioassay methods, we have completed our development of a biochemical assay that measures the levels of sensitivity of sharpshooter acetylcholinesterases (AChEs) to inhibition by organophosphate (OP) insecticides. Insensitivity of the AChE target-site can seriously impair the effectiveness of the OPs in control programs. The assay can be used on all nymphal instars and adults, and is an excellent tool for monitoring the frequencies of AChE variants in populations because it provides inhibition data for individual insects. Monitoring populations of GWSS and smoke-tree sharpshooter that have been exposed, either directly or indirectly, to OPs such as chlorpyrifos will enable us to detect resistant AChE alleles should they arise.

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 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 organophosphate (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

Results showing a two-year comparison of toxicity data to various insecticides using the three techniques are presented in Table 1. In general, GWSS populations are quite susceptible to most insecticides tested. The LC_{50} values indicate considerable variation in susceptibility to insecticides by both techniques. Mortality increased in the treatments over time. Monitoring data for chlorpyrifos and dimethoate indicated a difference of 10- and 15-fold between the two techniques. No significant changes in responses of GWSS to chlorpyrifos were observed from year to year. A slight shift is observed to esfenvalerate towards lower sensitivity. Insects from Redlands appear to be more sensitive than other populations. Similarly, acetamiprid was also quite toxic to GWSS from Redlands with a lower LC_{50} compared to the Riverside or Ventura populations. Among the neonicotinoids, thiamethoxam appears to be slightly less toxic to GWSS populations in 2002 with insects from Redlands showing more sensitivity to acetamiprid than the previous year. No significant differences in responses of GWSS from various locations to endosulfan are observed. In conclusion, two-year comparison of toxicity studies shows that GWSS are still quite susceptible to all insecticides tested so far with small variations between populations from different regions.

In a comparison of AChEs in the GWSS and the STSS, we found a similar response to a wide range of OP insecticides. The enzyme activity in both species was especially sensitive to chlorpyrifos, and was least affected by omethoate, the active form of dimethoate. Using a diagnostic concentration of 10 μ M paraoxon, we assayed insects from Riverside, Redlands and Ventura citrus orchards. We found that the AChE activity in insects from these areas was sensitive to this concentration, thereby providing encouraging evidence for the absence of OP resistance based on insensitivity of the target site.

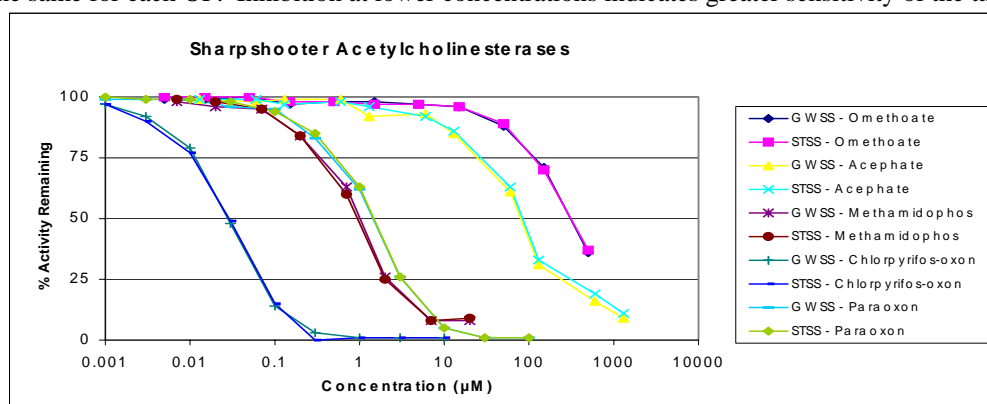
Selected strains of GWSS:

Selection of GWSS strains that are tolerant to an OP, a pyrethroid and a neonicotinoid is underway and will be maintained under selection for a few more generations for further studies.

Table 1. A two year comparison of toxicological responses of GWSS to various insecticides.

Insecticide Class	Insecticides	Sample Location	2001		2002	
			LC ₅₀ Petri dish	LC ₅₀ Leaf dip	LC ₅₀ Petri dish	LC ₅₀ Leaf dip
Organophosphates	Chlorpyrifos	Riverside	0.001	0.013	0.0038	0.0124
		Redlands	0.001	0.015	0.0067	0.0562
		Ventura	0.005	0.032	0.00208	0.045
	Dimethoate	Riverside			0.0091	0.038
		Redlands			0.0176	0.0932
		Ventura			0.0298	0.0699
	Cyfluthrin	Riverside	0.038		0.0023	0.0019
		Redlands	0.004		0.0221	0.252
		Ventura			0.0043	0.338
	Esfenvalerate	Riverside	0.0027	0.022	0.006	0.010
		Redlands	0.00003	0.00004	0.0009	0.0042
		Ventura			0.009	0.034
Cyclodiene	Endosulfan	Riverside	0.042	0.168	0.044	0.551
		Redlands	0.019	0.012	0.0812	0.376
		Ventura			0.0202	0.1431
Neonicotinoids	Acetamiprid	Riverside	0.006		0.00832	0.0723
		Redlands	0.003		0.00349	0.0195
		Ventura			0.00104	0.0089
	Imidacloprid	Riverside	0.01	0.091	0.005	0.072
		Redlands	0.003	0.008	0.0009	0.014
		Ventura	0.04	0.097	0.025	0.074
	Thiamethoxam	Riverside		1.64		0.08
		Redlands		0.61		0.034
		Ventura		1.92		0.121
		Riverside	0.0037	0.0085	0.003	0.004
		Redlands	0.0004	0.0012	0.002	0.008
		Ventura	0.0052	0.0093	0.009	0.020

Figure 1. Inhibition of sharpshooter acetylcholinesterase activity by OP insecticides. The response of both the GWSS and the STSS was the same for each OP. Inhibition at lower concentrations indicates greater sensitivity of the target enzyme.



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

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Reporting Period: The results reported here are from work conducted from April 2002 to October 2002.

INTRODUCTION

Much evidence has accumulated over the past few years pointing to the significant role played by imidacloprid (Admire®) in reducing GWSS populations. In regions of California where imidacloprid has been used in area-wide control programs, populations of GWSS have declined substantially relative to their pre-action levels. For example, remnant GWSS infestations in Temecula appear to be associated primarily with untreated tracts of vegetation such as organic citrus, while their densities in conventional orchards and vineyards are extremely low. Similarly, GWSS population densities have been substantially reduced in southern Kern County as an outcome of the General Beale Road project. In contrast, other areas with high populations of GWSS such as Ventura/Fillmore and Riverside/Redlands that have not yet participated in area-wide control programs still retain high GWSS populations. The significant reduction of GWSS densities in only those regions where concerted action has been mounted is persuasive, even if it is only indirect evidence of the role that imidacloprid treatments have played in curtailing GWSS populations.

By measuring temporal and spatial dynamics of imidacloprid uptake and distribution in mature citrus trees and grapevines, then relating these data to GWSS densities on treated trees and grapevines relative to untreated ones, we have demonstrated the capacity of a single imidacloprid treatment per season to reduce GWSS populations. Questions that initially arose following the first large-scale applications in Temecula in Spring, 2000 concerning the quantity, distribution, and persistence of imidacloprid in citrus trees have now been addressed with the results from our studies. Information that will derive from this project should help optimize future GWSS control efforts.

OBJECTIVES

1. Evaluate the titer 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 titers 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 AND CONCLUSIONS

Imidacloprid was applied to Valencia oranges in Riverside through an irrigation system equipped with microjet emitters at 32 oz. per acre on April 10 and April 4, 2001 and 2002, respectively. Xylem samples were collected every two weeks thereafter with a pressure bomb device and analyzed for imidacloprid concentrations using a commercial ELISA detection kit (Enviroligix, ME). Consistently higher titers were observed in 2002 compared to the previous year (Figure 1). By 16 May 2002, the mean titer had increased to above 20 ppb, then remained above 15 ppb until 25 July before declining. In contrast, mean imidacloprid titers in 2001 never exceeded 15 ppb, but remained between 10-15 ppb from 07 June through 20 July (Figure 1). Within-tree distributions of imidacloprid varied insignificantly among the four quadrants and two elevations from which xylem samples were collected (Figure 2). The near-uniform distribution of imidacloprid to all parts of the mature orange trees had a severe impact on GWSS nymphs and adults (Figure 3). Weekly samples collected from treated and untreated trees revealed a sharp decline in nymphal densities approximately 6 weeks post-treatment that persisted through the end of the nymph developmental season. The emergence of adults in late June and early July coupled with a frenzy of flight activity tended to mask any differences between treated and untreated trees save for the large numbers of dead adults that were observed beneath treated trees. By late July, however, adult densities decreased on treated trees and remained significantly lower than untreated trees through the remainder of the year (Figure 3).

Figure 1. Temporal pattern of imidacloprid titers in Valencia orange trees during 2001 and 2002. Each sampling date gives a scatter of points representing titers of individual trees with the range of imidacloprid titers identified by the vertical lines. The mean titer for each date is defined by the intersection of the traversing line with each vertical line.

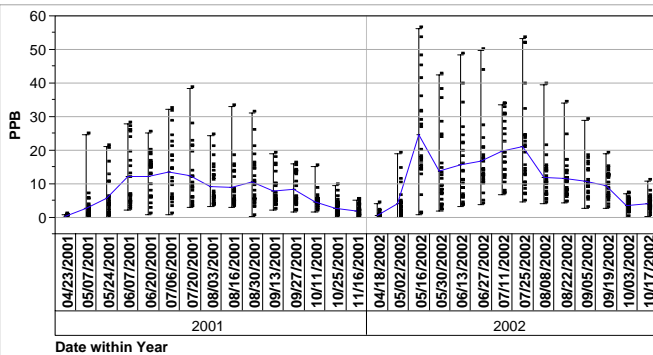


Figure 2. Within-tree distributions of imidacloprid in Valencia oranges according to height (a) or directional location (b). (Explanatory details for this figure are the same as Figure 1).

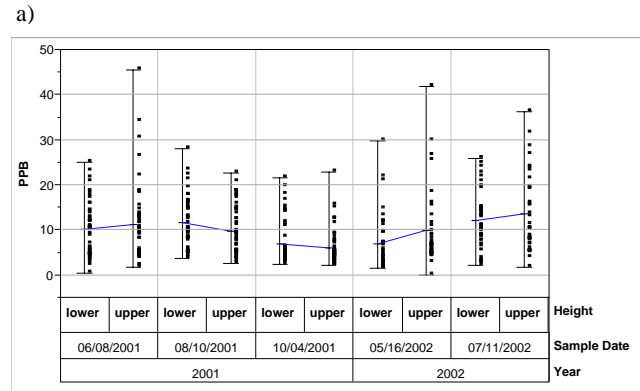


Figure 3. Comparison of GWSS nymphal (top) and adult (bottom) densities on imidacloprid-treated and untreated Valencia oranges.

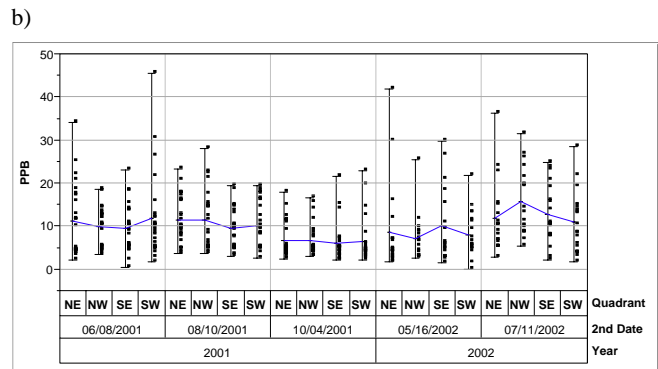
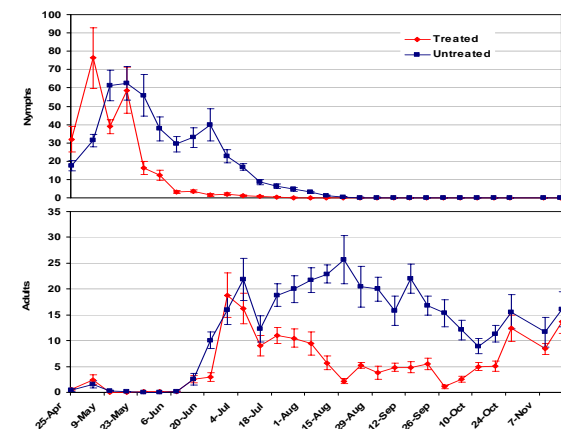


Figure 4. an (\pm SEM) titers of imidacloprid in Cabernet grapevines in Temecula treated with either 16 oz/16 oz (01 May and 26 July) or 32 oz on 01 May 2002. Note the higher titers in grapes compared to oranges as well as the difference between 16 oz and 32 oz rates.

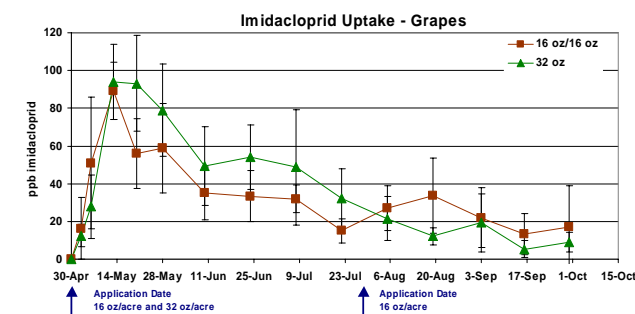
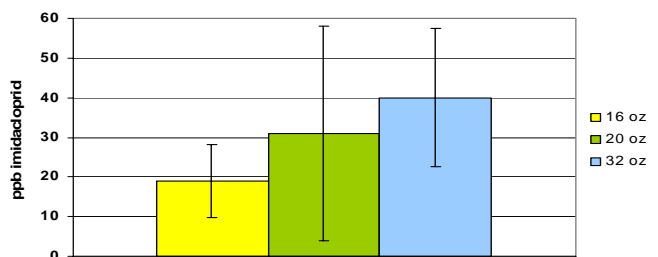


Figure 5. Imidacloprid titers in 1-year-old grapevines treated at 3 rates and measured 8 weeks post-application.



The uptake and distribution of imidacloprid applied to grapevines at 16, 20, and 32 oz/acre were assessed for Cabernet Sauvignon (Figure 4) and Syrah (Figure 5) varieties in Temecula vineyards from 01 May until 31 October 2002. Uptake into vines was rapid, reaching levels above 100 ppb within 10 days. This is in contrast to the uptake dynamics in citrus where maximum levels were only reached at about 6 weeks post-treatment. In particular, the persistence of imidacloprid in trees and vines treated with the 32oz/acre rate was impressive, and gave a clear indication as to why this insecticide has proven to be so successful in area-wide management programs.

FUNDING AGENCIES

Funding for this project was provided by the USDA Animal and Plant Health Inspection Service, the California Department of Food and Agriculture, and the Bayer Corporation.

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

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INTRODUCTION

Citrus nurseries located in glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*, infested areas must ensure that the plants that they ship to uninfested areas of California are free of GWSS. Nurserymen accomplish this by treating the citrus trees with pesticides and by careful visual inspection of leaves for signs of GWSS prior to shipment. All stages of GWSS could potentially be transported. Experiments were conducted to determine the efficacy of various pesticides against adult GWSS and their ability to deposit eggs, and against the nymphs as they attempt to emerge from egg masses.

OBJECTIVES

1. Evaluate the residuality and efficacy of various insecticides against adult GWSS.
2. Evaluate the efficacy of various insecticides against GWSS nymphs hatching from egg masses.

RESULTS AND CONCLUSIONS

Adult GWSS tests:

A combination of 66 lemon and 66 orange citrus trees (15 gallon potted plants) were treated with commercial rates of various insecticides. GWSS adults were collected from an untreated citrus orchard using sweep nets at weekly intervals and caged on the treated trees. The number of live adults after 24 hours and the number of egg masses deposited after 7 days were recorded each week for 11 weeks after treatments were applied.

Residues began to break down as evidenced by survival of adults 2 weeks after treatments were applied for the organophosphate Lorsban (chlorpyrifos), at 3 weeks for the carbamate Sevin (carbaryl), and at 3-4 weeks for the foliar neonicotinoids Assail (acetamiprid) and Marathon (imidacloprid). The systemic neonicotinoids Admire (imidacloprid) caused high mortality for 8 weeks and Platinum (thiomethoxam, unregistered) caused complete mortality of adults for 11 weeks. The pyrethroids Tame (fenpropathrin) and Talstar (bifenthrin) were highly effective, Talstar allowed only one adult to survive 24 h and Tame allowed no adults to survive over the 11 week test period.

In this same experiment, GWSS were able to deposit egg masses one week after application in the Lorsban and Marathon treatments, during week 2 in the Sevin treatment. The foliar neonicotinoids Assail and Marathon prevented egg laying for 4-5 weeks. The Tame treatment allowed 1 egg mass to be deposited during week 4. The other treatments (Admire, Platinum, and Talstar) did not allow a single egg to be deposited during the 8 weeks of the experiment.

Overhead irrigation was applied for 30 minutes 3x per week to half of the trees and significant reduction in residuality of the insecticides was observed for Sevin, Assail, Marathon and Tame.

Emergence of GWSS from egg masses:

In the second experiment, GWSS adults were collected from an unsprayed citrus orchard in Kern County and caged for one week on nursery citrus (15 gallon potted lemons and oranges). The adults were removed, and trees treated with commercial rates of various insecticides to determine if nymphs could successfully emerge from the egg cases. Successful emergence was defined as completely emerged and with fully developed wings.

Provado, Assail, and Sevin did not allow any nymphs to successfully emerge from the egg masses. Actara, Talstar, and Tame allowed 20-40% of the nymphs to successfully emerge, although all died shortly after emerging. The insect growth regulator Applaud (buprofezin) did not have any effect on nymphal emergence.

In summary, these experiments suggest that pyrethroids and the systemically applied neonicotinoids are most effective against adult GWSS and the carbamate Sevin and several of the foliar neonicotinoids are most effective against nymphs attempting to emerge from the egg masses. Based on these experiments, I would recommend that citrus nurserymen apply a systemic neonicotinoid (imidacloprid is the only one registered at this time) 2-8 weeks before shipment is expected. Immediately before shipment, a pyrethroid such as Tame or Talstar should be applied to ensure that the foliage is disinfested of nymphs and adults. In addition, Sevin, Assail, or Provado should be applied just prior to shipment to prevent nymphs from emerging from egg masses. Bethke and Redak (2001, 2002a, 2002b) and Bethke et al. (2001) demonstrated similar efficacy of systemic neonicotinoids and pyrethroids against adult GWSS and efficacy of carbaryl against emerging nymphs for ornamentals.

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FUNDING AGENCIES

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

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Reporting Period: The results reported here are for work conducted from November 1, 2001 to October 31, 2002.

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 (He et al. 2000, 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 (Loomis 1965, 1952, Magoon and Magness 1937). 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. Evaluate the impact of rootstock variety on expression of Pierce's disease symptoms in the scion.
2. 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 August 6 and October 25, 2002 for Chardonnay vines and August 6, September 9, and October 10, 2002 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 for grafted vines and five replicates for ungrafted rootstocks. 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. Symptoms were more severe than in 2001 and increased in average severity from the first to the second screening regardless of the rootstock. Apparent vine death was widespread by October 25. It is likely that *Xylella fastidiosa* established in these vines in 2001, with initial PD symptoms in that year and more severe symptoms in 2002 as PD progressed. The preliminary results indicate that none of the rootstocks evaluated provides amelioration of symptoms sufficient for fruit production under these conditions.

Ungrafted vines of rootstock varieties exhibited a range of symptom levels (Table 1). Ramsey and St. George showed the fewest PD symptoms overall. Although the first screening found other rootstock varieties had less severe symptoms than Ramsey and St. George, those varieties showed a marked increase in symptoms as the season progressed. In 2001 Ramsey and St. George showed relatively more severe PD symptoms; it may be that these varieties are more susceptible when young and increase in resistance with plant age. O39-16 vines showed relatively few PD symptoms in 2001, reflective of the highly PD resistant *Vitis rotundifolia* parentage in this variety. In contrast, in 2002 O39-16 showed severe PD symptoms. The population of bacteria, level of inoculation, or cultural or climatic conditions could be impacting the PD symptom expression in O39-16. 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. It may be that the O39-16 vines will recover in a similar manner. Loomis (1952, 1965) reported that a different rootstock with *V. vinifera* and *V. rotundifolia* parentage extended the life of susceptible scions in Mississippi, but even Chardonnay on O39-16 showed severe PD symptoms in this trial.

Additional rootstocks grafted to Chardonnay and Cabernet Sauvignon were planted in spring 2002 to further investigate the possible influence of rootstock on PD expression. The rootstocks Dog Ridge, 161-49C, and Lenoir are of special interest. Dog Ridge and 161-49C have been reported as increasing vine longevity in areas of high PD pressure (Loomis 1952, 1965). Pierce (1905) suggested Lenoir as a rootstock to manage this disease.

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			
	Aug 6	Oct 25	Sum		Aug 6	Sep 9	Oct 10	Sum
Screening date (2002)								
O39-16	3.4	4.9	8.3	Ramsey	1.6	1.0	1.3	3.9
5BB	3.8	4.6	8.4	St. George	1.2	1.3	1.6	4.1
3309C	3.8	4.8	8.6	101-14	0.7	1.7	2.8	5.2
Ramsey	3.9	4.9	8.8	5C	1.4	1.5	2.9	5.8
5C	3.9	4.9	8.8	110R	1.6	1.5	2.8	5.9
101-14	4.0	5.0	9.0	5BB	1.0	1.8	3.5	6.3
Own-rooted	4.6	5.0	9.6	44-53M	2.0	1.8	3.3	7.1
44-53M	4.6	5.0	9.6	O39-16	1.1	2.7	3.4	7.2
Freedom	4.6	5.0	9.6	Freedom	2.6	2.8	4.3	9.7
110R	4.8	4.9	9.7	3309C	2.6	3.1	4.1	9.8

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*Monitoring
and
Database
Management*



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

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Reporting Period: The results reported here are from work conducted from October 2001 to October 2002.

INTRODUCTION

Decision-making in knowledge-based pest management depends upon sampling methods that provide reliable information on pest densities and distributions. Practical sampling methodology must balance sample precision with simple and cost-effective collection techniques. Four methods are currently being evaluated in citrus orchards as part of our effort to develop a sampling program for glassy-winged sharpshooter (GWSS). These include both hand (bucket and beat net) and gasoline-powered (D-Vac and A-Vac) samplers. In addition, yellow-sticky traps have been used simultaneously to determine the level of correlation between the foliage samplers and commonly used yellow-sticky traps. Data sets for each device will be analyzed for mean-variance relationships according to Taylor's power law and sample-size estimates generated according to fixed levels of precision. Ultimately, sequential and binomial sampling plans will be developed for the precise estimation or classification of population density of GWSS for research and pest management application.

It is well recognized that the major threat of GWSS populations is the potential for vectoring *Xylella fastidiosa* to uninfected plant hosts, in particular grapevines in commercial vineyards. One practical application of a sampling plan would be to precisely estimate densities of GWSS within an orchard or vineyard and then determine what proportion are positive for *X. fastidiosa*. Accurate identification of individuals positive for *X. fastidiosa* is an essential part of an overall appraisal of the risk constituted by a particular population. Work began in April exploring ELISA, PCR, and culturing techniques for the detection of *X. fastidiosa* in GWSS. Sampling and evaluation of the proportion-positive among various southern California populations of GWSS is continuing.

OBJECTIVES

1. Develop, test and deliver statistically-sound sampling plans for estimating density and inoculum potential of GWSS for research and management applications.

RESULTS AND CONCLUSIONS

Evaluation of the four sampling devices continued in citrus orchards in Riverside, CA with the onset of the spring generation of GWSS nymphs in April, 2002. To date, a total of 500 Valencia orange trees have been sampled with each of the four devices to generate 25 data points ($n=20$ per point) that describe the respective mean-variance relationship for each device. The bucket sampler is the most versatile and easiest to use with its extendable pole allowing access to foliage 15-20 ft above ground. Samples obtained with the bucket sampler are also cleaner than those obtained with the beat net, the other hand-operated device, and therefore require less handling during sample processing. The mechanical devices are more expensive to purchase, more cumbersome to use, and do not yield superior results to the hand-operated devices. The range and mean counts of GWSS adults collected with the bucket sampler closely matched the counts obtained with the D-Vac sampler while generally exceeding those obtained with either the A-Vac or beat net samplers (Figure 1). Regressions of log variance upon log mean for each device (Figure 2 - two devices only) yielded the regression parameters a and b (Table 1) that will be incorporated into Taylor's power law ($S^2=am^b$). The results presented here are for adults, but similar processing and data development towards a final sampling plan will be completed for GWSS nymphs as well.

Figure 1. Range (defined by vertical lines spanning each set of points) and mean (intersection of the vertical and traversing lines) counts of GWSS adults collected by each of four sampling devices in Riverside, CA during 2002.

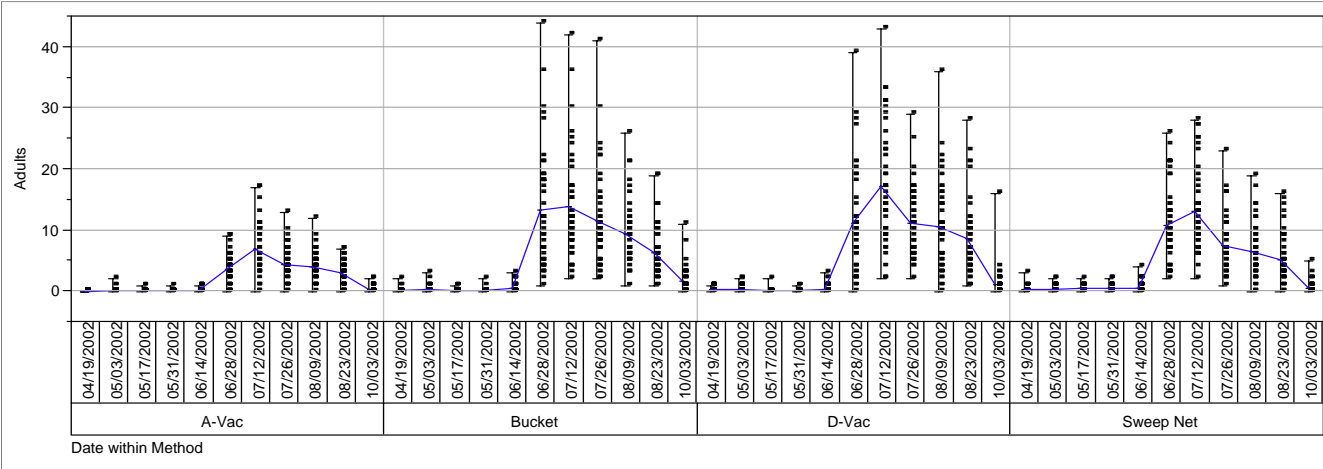


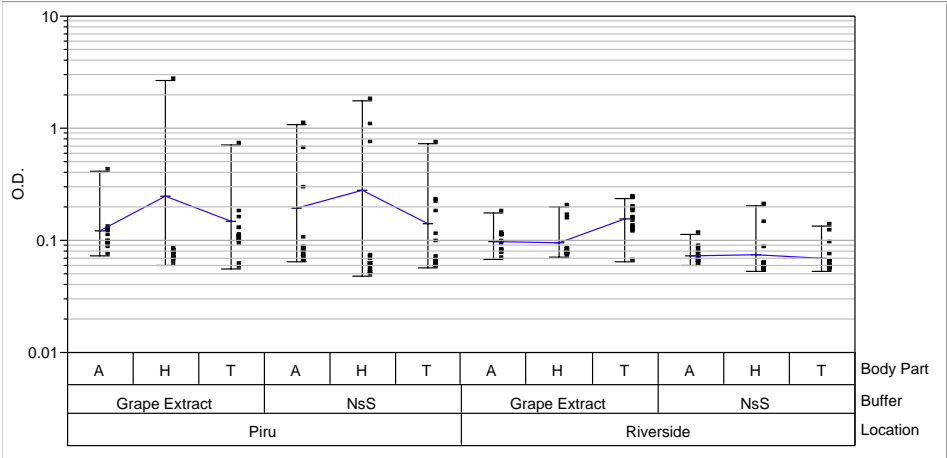
Figure 2: Mean-variance relationships for GWSS adults collected by the bucket (a) and D-Vac (b) samplers.

Table 1: Slope and intercept parameters generated by the regression of log variance on log mean for our sampling devices.

Device	Parameter a	Parameter b	R ²
A-Vac	1.18	1.15	0.98
Bucket	1.35	1.28	0.97
D-Vac	1.28	1.36	0.97
Beat Net	1.17	1.35	0.98

Samples of GWSS adults and nymphs were collected every two weeks from the sampling orchards and frozen for subsequent testing for *X. fastidiosa*. Various methods are being explored to determine the most effective detection system for *X. fastidiosa* in GWSS individuals. For ELISA detection using Agdia, Inc. (Indiana) reagents, different extraction buffers have been examined to determine which one controls nonspecific binding best without suppressing IgG binding to *X. fastidiosa*. When GWSS adult populations from Piru and Riverside were tested by ELISA using either grape extract or NsS buffers, lower optical density readings were obtained for negative controls using NsS buffer. There appeared to be no suppression of positive readings as a similar number of positives were obtained with the NsS buffer and the grape extract buffer (Fig. 3). A higher proportion of the population from Piru tested as strong ELISA positives for *X. fastidiosa* compared to the Riverside population (Fig. 3).

Figure 3. Individual GWSS adults from Piru and Riverside separated into abdomen (A), head (H) and thorax (T) segments and homogenized in grape extract or NsS buffers. ELISA results are presented as optical density (O.D.) units. (See Figure 1 for details)



FUNDING AGENCIES

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

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Reporting Period: The results reported are from work conducted from January 2002 to November 2002.

INTRODUCTION

The glassy-winged sharpshooter (GWSS) *Homalodisca coagulata* is native to the southeastern United States (Young 1958) 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. PD has been a problem in California for more than 100 years, but the GWSS is a more effective vector of *X. fastidiosa* because it can feed on the xylem of seemingly dormant woody stems. Unlike sharpshooters native to California, GWSS can reproduce in grape.

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 2001a). 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 2001a). Many leafhopper species produce brochosomes, but only females are known to produce the rod shaped brochosomes (Rakitov 2000). As reported here in 2001, 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 (Hix 2001b) and demonstrated that even though the AM type trap may have reliability issues, it is clearly not a "blunder trap."

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

Traps were deployed in wine grape vineyards in Temecula with known high populations in addition to vineyards with lower populations. These vineyards were either under organic farming practices or were minimally farmed. Trap types tested included plates, commercially available yellow sticky cards (6), and nymph traps (3 colors). Traps were checked weekly and visual count of egg masses, nymphs, and adults were made. GWSS were sexed, and females with forewing spots of brochosomes or residue were noted.

The data indicated relationships between the number of females trapped and oviposition in associated vegetation. The number of females trapped in July August and September 2002 showed a strong relationship to the number of nymphs found in searches. A search consisted of 3 sets of 25 vines near a designated trap. Analysis of the data from the vineyards provided the expression $y = 3.4X - 2.4$ where y = number of nymphs per search and X is the number of females captured per trap ($R^2 = 0.97$, $F = 378.7$, $P = 0.003$). This expression is only valid in situations where vineyards have not been treated for GWSS.

Yellow plates caught statistically more GWSS than commercially available sticky traps while orange traps usually caught more than the commercial traps. The nymph traps caught first to fifth instar nymphs in moderate 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. Two of the vineyards studied had high populations of GWSS nymphs. GWSS phenology in wine grapes was determined (Figure 1). Moderate GWSS populations can clearly establish and reproduce in vineyards when it is not managed even if nearby GWSS populations are low.

GWSS Phenology in Grape

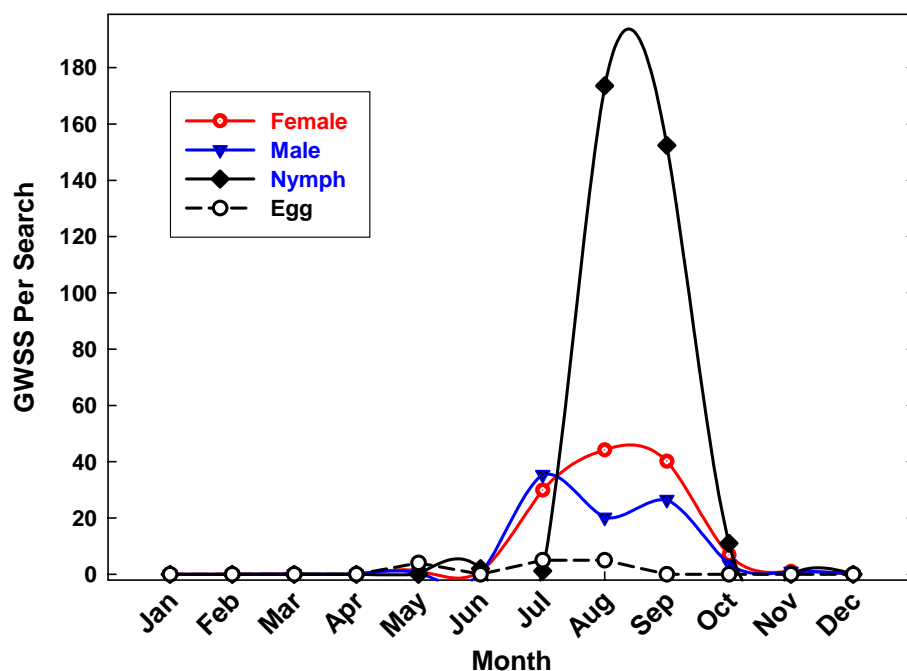


Figure 1. Glassy-winged sharpshooter phenology in wine grapes as determined from vine searches in 5 commercial vineyards in Temecula during the 2001-02 seasons. A search consisted of thorough examination of 3 sets of 25 vines.

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FUNDING AGENCIES

Funding for this project was provided by the American Vineyard Foundation and the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.

SPATIAL DISTRIBUTION OF GLASSY-WINGED SHARPSHOOTERS IN A DIVERSE AGRICULTURAL SYSTEM, AND CORRELATION BETWEEN DIRECT OBSERVATIONS AND STICKY TRAP DATA

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Reporting Period: The results reported here are from work conducted from January 1, 2001 through September 30, 2002.

INTRODUCTION

The glassy-winged sharpshooter (GWSS) is an invasive species that is threatening California agriculture. GWSS live on many different host plants, but the main concern is their potential to vector Pierce's disease in grapes. Pierce's disease can kill susceptible grape vines within 2 years after infection (Varela et al. 2001). An area-wide management program is underway in Kern County to manage outbreak populations of GWSS and slow their northward spread in the San Joaquin valley. To be successful, large scale regional insect management programs require an accurate assessment of pest density and distribution to provide decision support for program managers (Liebhold et al. 1993, Roberts et al. 1993). This report details program activity aimed at understanding the spatial distribution of GWSS populations in Kern County.

OBJECTIVES

1. Determine the spatial distribution and density of GWSS in a diverse agricultural setting.
2. Determine the within field distribution of GWSS and movement between citrus and other perennial host crops.
3. Determine the correlation between yellow sticky trap data and direct observations taken in the field.

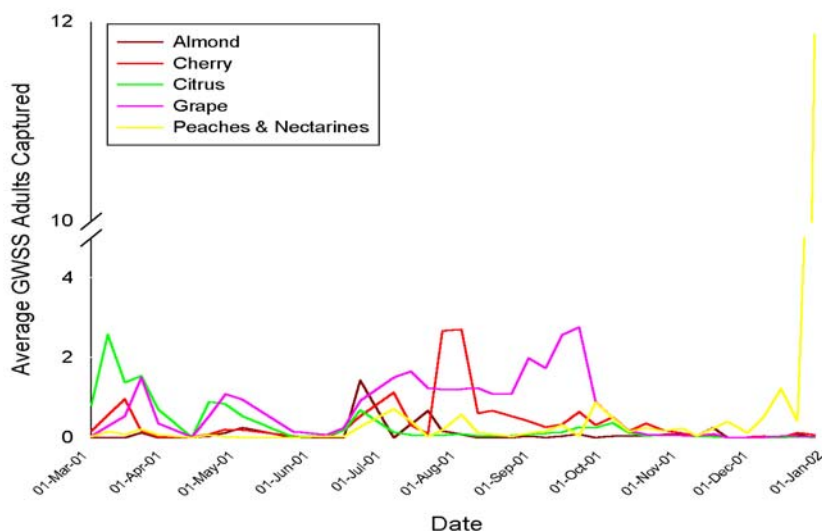
RESULTS AND CONCLUSIONS

A trapping program was initiated to provide pest location and density information. Sticky trap data was collected from a pilot project area consisting of 1457ha (3600ac) of citrus, 1255ha (3100ac) of grapes, and 688ha (1700ac) of other perennial hosts including almond, blueberries, cherries, nectarines, peaches, and pistachios. GWSS adults are highly visually oriented in searching for hosts. Recent work (Hix et al. 2001, Puterka et al. (submitted)) demonstrated that yellow is the most attractive color for trapping. Yellow sticky traps with 36cm² of trapping surface from Seabright Laboratories (Emeryville, CA) were placed throughout the area in a 402m (¼ mile) grid and serviced weekly by CDFA. Traps are placed on 2m bamboo poles to standardize the height of trapping across the entire grid. Within citrus groves and other tree fruits, traps were placed within the row between 2 trees near the corners of the field. Traps in grapes were placed within the trellis 0.1m above the canopy on bamboo poles near the corners of the field. The trapping grid has been expanded from the original pilot project area to include most of the grape/citrus producing areas in Kern County. Over 4000 traps in citrus and grape are serviced on a weekly basis to provide managers detailed information on GWSS locations and density.

In addition to the standard grid, yellow sticky traps were set up in an intensive grid at 11 sites within the pilot project to look at GWSS movement at crop interfaces. Citrus was the common crop at each site. The other crops included 4 grape vineyards, 4 cherry orchards, 2 peach/nectarine orchards, and 1 almond orchard. Trapping grids consisted of 3 transects of traps. Each transect was comprised of 16 traps with 8 traps located in each crop. The traps extended 200m into the crop away from the interface and were approximately 24 m apart. Traps were checked weekly as part of the regular trapping program run by CDFA. Data collection began at the end of February with the first reporting date on March 1, 2001.

When averaged across all sites, the overall trapping pattern for each crop was similar for the entire year, with a few exceptions (see Figure). Early in the season GWSS adult were trapped in the highest numbers in citrus prior to the foliar and systemic treatments in citrus that were applied as the management strategy. Levels in citrus remained low all season due to these treatments. From July to October most GWSS trap captures occurred in grape and cherry. The highest numbers occurred in the site with organic grapes. Late in the year numbers peaked in traps of one peach/nectarine orchard as the grower was pruning the trees and putting on a dormant oil / insecticide treatment. This winter activity points to the need to trap continuously through out the year to detect populations in areas where they may be overwintering.

Crop Interface Trapping Sites Average Across All Sites



Direct observations in the pilot project were made on a monthly cycle with all citrus orchards being sampled at a rate of 10 observations per 40 acres. Grapes and other host crops were sampled at the interface sites where trapping occurred. Two different sampling methods were used in each crop, and these varied based on differences in plant morphology between crops. In citrus, visual counts and beat net sampling methods were used. Two minute visual counts were made of the number of adult GWSS on the terminal growth on approximately ½ of the tree to a height of 2m, and then recorded onto a data sheet. Then, the terminal foliage is beat with a 2.5cm dowel rod to dislodge GWSS into a 62.5cm canvass net. This sample is then transferred to a labeled sealed plastic bag for counting at the laboratory. In the case of grapes, ten equally spaced visual counts are conducted on 4.3 linear meters of canes and recorded. When grape foliage is present, it is swept 20 times with a 37.5cm canvass net over an equivalent area as the visual count. Visual count and beat net sampling methods are used in tree crops, while visual and sweep net sampling methods are used in vine and shrub crops (i.e., blueberries). Finally, each sample unit (tree, vine, etc.) is recorded with a unique GPS coordinate. These results are being mapped and correlations made with sticky trap captures.

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PLANT-GWSS INTERACTIONS: PHYSIOLOGICAL AND BIOCHEMICAL MECHANISMS INVOLVED IN HOST PLANT SELECTION WITH PARTICULAR REFERENCE TO LEMON AND ORANGE TREES

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Reporting Period:

INTRODUCTION

The glassy-winged sharpshooter (GWSS) *Homalodisca coagulata* is an exotic insect in California and is an important vector of *Xylella fastidiosa* that causes Pierce's disease (PD) in grapes. Citrus is a favored host of GWSS throughout the year, and it has been well documented from studies of the Temecula PD epidemic that the proximity of citrus groves to vineyards has influenced the incidence and severity of PD in grapes. It is imperative that effective control strategies be implemented to curb the spread of the vector-vital to this would be establishing the host plant range of the GWSS and determining the physiological and biochemical mechanisms for host selection. One of the key factors contributing to the successful establishment of the GWSS in California has been its ability to utilize more breeding habitats and plant hosts than native PD vectors. Although a comprehensive list of suitable hosts has been identified, comprising 75 plant species in 35 families, little is known about the physiological and biochemical mechanisms involved in host selection of GWSS in California. Elucidation of the physiological and biochemical mechanisms may be usable for developing host plant resistance as a sustainable component of integrated pest management program.

Dietary nitrogen and carbohydrates are important nutritional indices impacting survival, growth and reproduction of phytophagous insects. These nutrients are particularly limited for xylophagous insects, such as GWSS, because xylem fluid consists of over 95% water and is the most dilute food source for herbivores. There are two ways in which the GWSS could compensate for the poor nutrient quality of the xylem fluid. Firstly, they could feed for extended periods of time. This is, in fact, known to be the case, as those of us who have witnessed the sharpshooter rain at first hand will attest. Indeed, it has been estimated that GWSS can process up to 10 ml of xylem fluid per day. Secondly, efficient assimilation of available nutrients during prolonged feeding periods would enhance the nutritional value of xylem. Different host plants may contain different levels of dietary nitrogen and carbohydrate during the year and the differences could play a role in GWSS host selection.

OBJECTIVES

1. Investigate the seasonal population dynamics of GWSS on orange and lemon trees.
2. Study the relationship between densities of GWSS on orange and lemon trees and the nutritional quality of the xylem fluid upon which the insects feed.

RESULTS AND CONCLUSIONS

A lemon and orange mix-planted orchard was used for the experiment. Three blocks of 30 orange and 30 lemon trees were used. Five trees of lemon or orange were randomly selected from each block to monitor the GWSS population dynamics and to extract xylem fluid. A bucket-sampling device was used to sample both immature and adult GWSS. Population dynamics of both adult and immature GWSS was monitored on a weekly basis throughout the season. Xylem fluid in one-year old stems from each of the trees was collected bi-weekly to determine levels of free amino acids, soluble proteins and carbohydrates.

Adult GWSS numbers on oranges were highest from late June to late November 2001 and a smaller peak was observed from early March to early April 2002 (Figure 1). The adult numbers on lemons peaked from mid-July 2001 to late January 2002. The adult numbers were 0.4- to 4.9-fold higher from late June to late July on oranges in comparison with those on lemons, whereas the adult numbers were up to 10.7-fold higher from early August 2001 to late February 2002 on lemons than on oranges. The numbers were 8.7- to 16.4-fold higher from early March to early April 2002 on oranges than on lemons.

Peak immature counts on oranges occurred between late April and early July 2001 and the numbers gradually reached to zero the rest of the year (Figure 1). Peak immature densities on lemons were observed from mid-May to late June 2001. The immature counts were 0.9- to 3.9- fold higher on oranges than on lemons from late April to mid-May 2001. Despite the higher adult counts on lemons between November 2001 and January 2002, nymph counts in the following months were still higher on oranges. The mini-peak of adult numbers that appeared on oranges after January 2002 suggests that the adults had migrated from the lemons to the oranges to reproduce.

Throughout the experimental season, glucose and fructose levels were generally higher in xylem fluid from lemons (Figure 2). Sucrose levels were generally higher in lemon xylem fluid except during the period of early December 2001 to mid-February 2002 when orange xylem fluid had higher levels. During August 2001, levels of xylem asparagine, glutamine, tyrosine, phenylalanine, isoleucine, valine, threonine, histidine, methionine and lysine were generally higher in lemons whereas from February to April 2002, levels of these xylem amino acids were higher in oranges, in correspondence with the higher adult GWSS numbers on these trees (Figure 3).

In summary, from early spring to mid summer adult GWSS numbers were generally higher on oranges and from belatedly summer to late winter the numbers were higher on lemons. Levels of some xylem amino acids were in positive correspondence with the higher GWSS numbers.

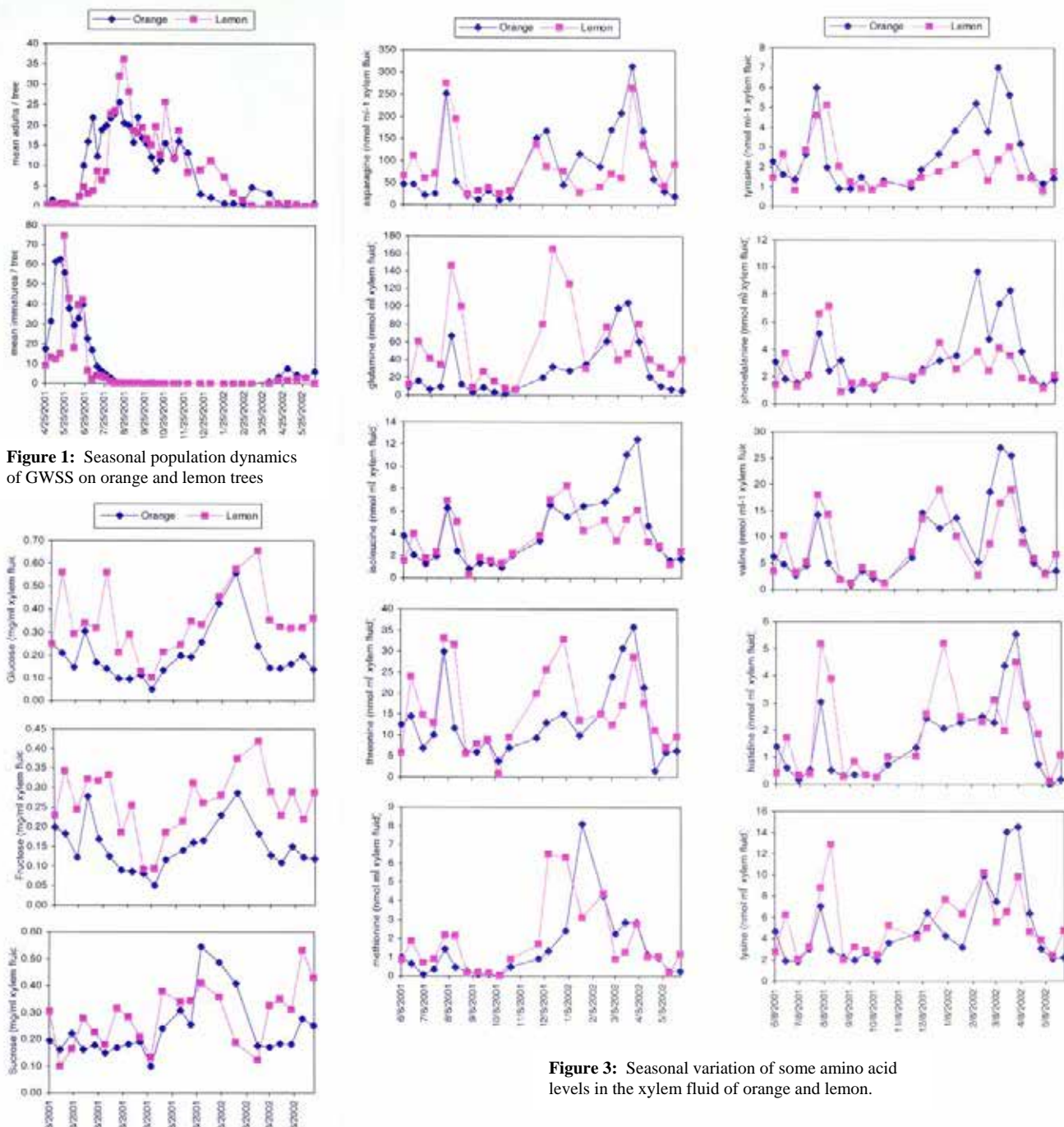


Figure 1: Seasonal population dynamics of GWSS on orange and lemon trees

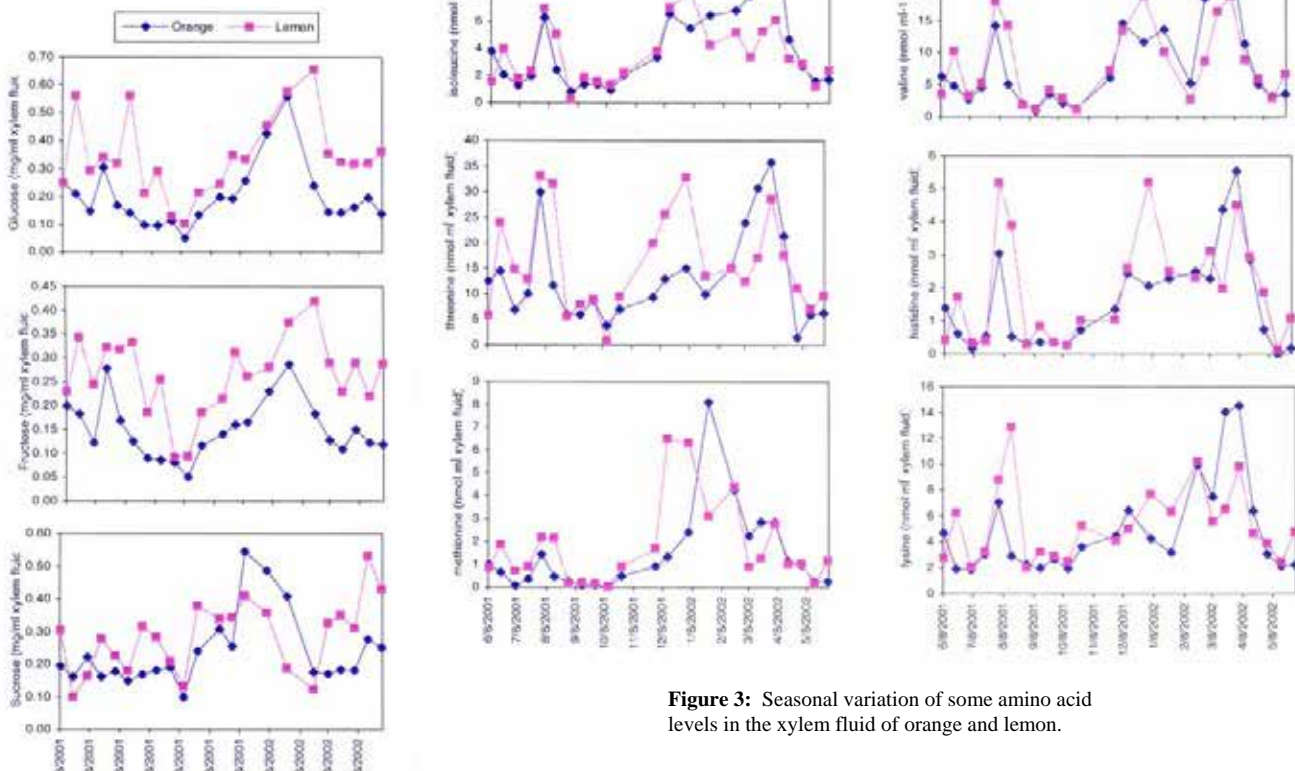


Figure 3: Seasonal variation of some amino acid levels in the xylem fluid of orange and lemon.

FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

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

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Reporting Period: The results reported are from work conducted from March 2002 to November 2002.

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.

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, CA with Admire[®] (imidacloprid) during Apr and May 2000 represented a pivotal shift toward an area-wide management of GWSS. In Mar and Apr 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 and/or made foliar applications of Provado[®], or Danitol[®] in 2002. 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 weekly monitoring in citrus and grapes began in March 2000 and has been continued to December 2002 by trapping (~500 traps), visual counts (adults, nymphs, egg masses), beats in citrus, and A-vacuuming in grapes. This monitoring will continue through at least 30 June 2003. 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. The results from the 2000 project indicated that every tree or acre does not need to be treated. GWSS numbers remained low in citrus treated with Admire in 2001. The populations also remained low in citrus treated with Admire in early 2000, but GWSS were observed at low numbers in some of these groves in July and August 2002.

Troublesome areas or hot spots were identified during early monitoring in January-April 2002. This led to the treatment of 137 acres of citrus with Admire in April 2002. An additional 95 acres of citrus were treated as needed with foliar applications of Assail between July 21 and August 15, 2002. A helicopter was used to make Assail applications to 49 acres with 46 acres treated by speed sprayer. Gavacide C 440 oil was applied to 148 acres of organic citrus in 2002. As in previous years, grape growers were responsible for treatments to grape. Several grape growers treated grapes with Admire or as needed with foliar applications of Danitol, Baythroid, or Provado.

Organic citrus groves and grape vineyards were the problematic areas and populations remained highest in these groves (Figure 1). The results from Gavacide C treatments were promising in 2001, and with the lack of other alternatives in

organic situations, additional treatments were made with 1.25% Gavacide C and water on August 7, 2002 (750 gallons per acre) by speed sprayer to 109 acres of organic citrus. Gavacide C was applied on October 23, 2002 by helicopter to an additional 42 acres that were inaccessible by speed sprayer. The rate applied by helicopter was 15 gallons Gavacide C in 85 gallons water per acre. CCOF and OMRI currently allow the use of most 415 and 440 oils for organic use.

During July and August 2002, it became apparent that some citrus treated with Admire in early 2000 has become re-infested with GWSS. As a result, Admire or Assail treatments are planned for 500-600 acres of citrus in 2003. Based on the success of the program over 2000, 2001, and 2002, it would seem that this level of treatment in citrus every 3 years would keep GWSS populations suppressed in the Temecula viticultural area. This would be coupled with GWSS management within vineyards throughout the valley on a yearly basis.

Some vineyard replanting occurred in 2002 (2% or less) especially in high visibility areas for both aesthetic reasons and to explore the feasibility of reestablishing lost vineyards. Based on a survey of 5 Temecula wineries, 2001 wine grape production ranged from 47% to 77% of what it was in 1995 with production ranging from 3.7 to 7.8 tons per acre in 1995 to 4.3 to 6.0 tons per acre in 2001. However, Temecula is providing few wine grapes to other areas due to economic forces. As a result, the 2000 and 2001 harvests were more than adequate to sustain the Temecula wineries with substantial surplus. In the future, Temecula will likely concentrate on producing quality wine grapes for Temecula's wineries.

Temecula Viticultural Area 14 August 2002

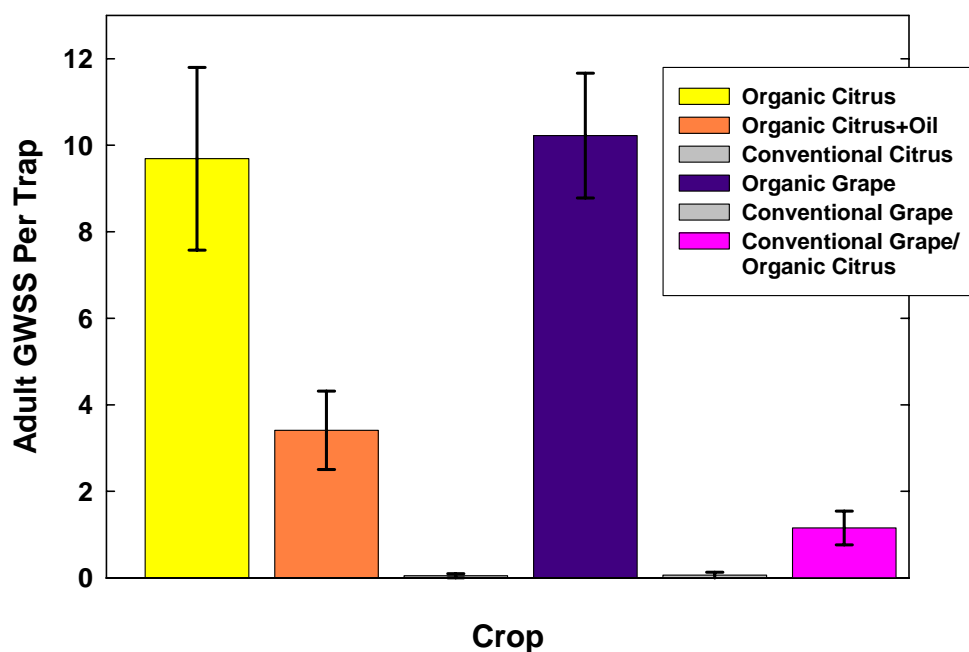


Figure 1. Comparison of GWSS (left to right) trapped in untreated organic citrus, 440 oil treated Organic citrus, Admire treated citrus, organic grape, Admire treated grape, Danitol treated grape Adjacent to oil treated organic citrus. Bars = \pm SEM. N = 15 to 20.

FUNDING AGENCIES

Funding for this project was provided by the USDA Animal and Plant Health Inspection Service, the California Department of Food and Agriculture, Riverside County, and the City of Temecula.

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

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Reporting Period: The results reported here are from work conducted from June 2001 through October 2002.

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, *Xylella 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 coagula*.

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.

RESULTS AND CONCLUSIONS

The research site was established in April of 2001 at the Agricultural Operations facility located on the campus of the University of California, Riverside. 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 and watered with drip irrigation. At total of 10 rows of 100 vines per row was planted. Treatment and treatment combinations evaluated are 1) imidacloprid at full rate, 2) imidacloprid at 1/2 rate, 3) a combination of imidacloprid plus acetamiprid, 4) metalosate, 5) kaolin, 6) imidacloprid-acetamiprid combination plus kaolin, 7) imidacloprid-acetamiprid combination plus metalosate, 8) metalosate plus kaolin, 9) imidacloprid-acetamiprid combination plus kaolin plus metalosate, and 10) control (water only). Treatments involving acetamiprid could not be evaluated until Fall of 2002.

Results indicated that there was a significant difference among treatments with respect to the number of sharpshooters found on experimental plants for both 2001 and 2002 (2001: $F_{8,91}=17.14$, $P<0.0001$, 2002: $F_{9,90}=20.74$, $P<0.001$, Figure 1). As replicates involving acetamiprid are only included in the 2002. As expected plants treated only with metalosate (a potential prophylactic treatment for Pierce's disease) supported similar numbers of sharpshooters as untreated control 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. These patterns have been maintained for the duration of the experiment thus far. No experimental treatment has yet resulted in complete protection from sharpshooters; consequently, all treated plants remain at risk of exposure to *X. fastidiosa*. With the exception of metalosate, all treatments were reasonably effective in reducing sharpshooter numbers throughout the fall season. Differences among treatments were lost as sharpshooter numbers naturally declined at the end of fall. A combination of imidacloprid, acetamiprid and kaolin was most effective at reducing overall sharpshooter numbers; however, it should be noted that a significant number of sharpshooters was found on all treated plants throughout the growing season.

Results of our latest feeding trials demonstrated that imidacloprid, imidacloprid-acetamiprid, and imidacloprid at half-rate applied per twice year significantly reduced sharpshooter feeding (Figure 2). Regardless of treatment type, imidacloprid reduced sharpshooter feeding by approximately 95%. Treating with imidacloprid at half-rate twice a year or combining full rate imidacloprid with acetamiprid did not significantly reduce feeding any further than a single application of imidacloprid at the full label rate.

As of September 2002, several treatments have significantly reduced the incidence of Pierce's disease symptoms in experimental plants. Imidacloprid at full rate, kaolin, kaolin plus metalosate imidacloprid-acetamiprid-kaolin, imidacloprid-acetamiprid-metalosate, imidacloprid plus acetamiprid plus kaolin plus metalosate all significantly reduced the incidence of Pierce's disease relative to untreated controls. Other treatments and treatment combinations (including just metalosate) did not significantly reduce the incidence of PD. Unfortunately, while the above treatments did reduced the incidence of PD relative to controls, they still suffered an approximate average of 30% infections (30% of the treated plants showed symptoms after 1.5 years). Control treated plants displayed an average of 69% infection.

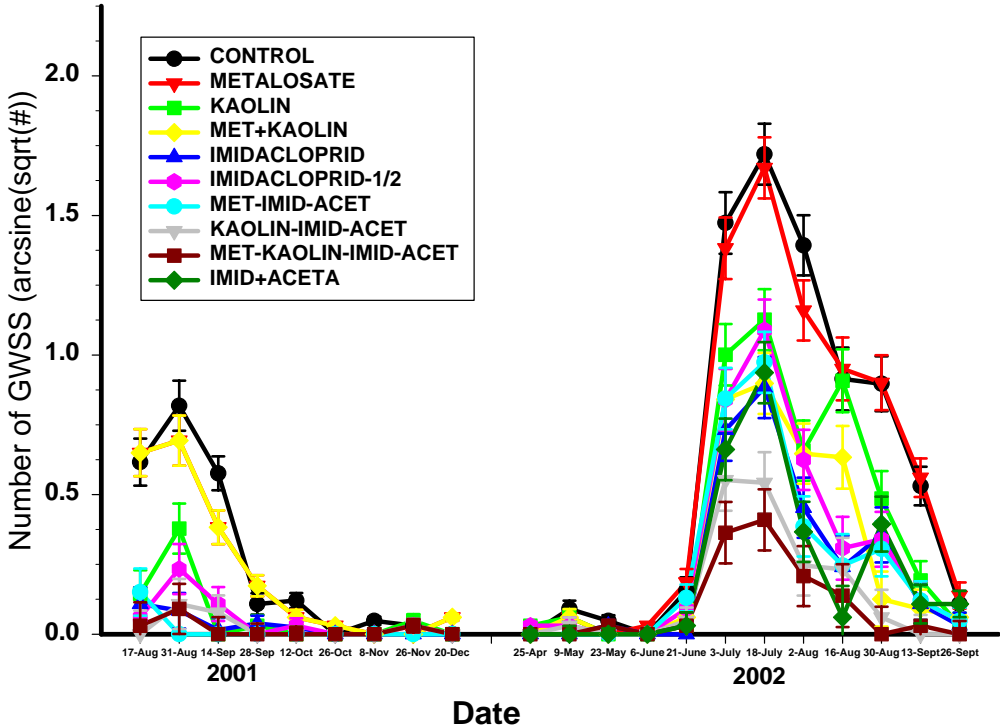


Figure 1: Effect of treatments on numbers of glassy-winged sharpshooters detected in grape plants.

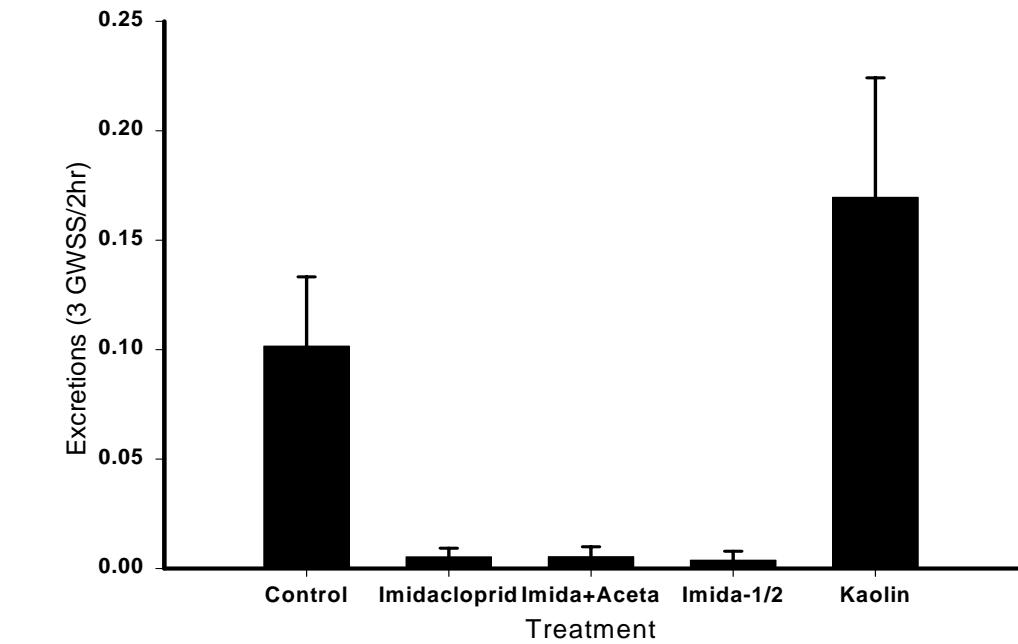


Figure 2: Effect of treatment on feeding rate of glassy-winged sharpshooter on grapes

FUNDING AGENCIES

Funding for this project was provided by the California Department of Food and Agriculture.

MYCOPATHOGENS AND THEIR EXOTOXINS INFECTING GLASSY-WINGED SHARPSHOOTER: SURVEY, EVALUATION, AND STORAGE

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Reporting Period: The results reported here are from work conducted in 2002.

INTRODUCTION

Excluding the observations reported by Turner and Pollard (1959), we know of no studies that have examined the entomopathogens associated with GWSS populations. In general, the lack of pathogens (viral, bacterial, or protozoa) in leafhopper populations may be related to their piercing-sucking feeding behavior. In most cases, these pathogen groups are transmitted orally and would likely need to inhabit the xylem tissue to infect leafhoppers. Pathogens that are transmitted *per os* are typically affiliated with insects with chewing mouthparts. Thus, entomopathogenic fungi, which do not need to be ingested in order to infect insects, are considered to contain the primary pathogens of sucking insects.

Based on a preliminary survey of GWSS (Boucias and Mizell, unpublished 2001) and 20 years of field experience, we expect that the proposed multi-seasonal collections will yield an array of novel mycopathogens that are active against GWSS. This study, will provide a source of mycopathogens with potential for GWSS biological control along with a GWSS microbe collection that will be screened for novel metabolites (exotoxins). In collaboration with an industrial partner, broth filtrates will be screened against an array of eukaryotes and prokaryotes. Of particular interest are the *Hirsutella* spp. isolates affiliated with this insect (Boucias and Mizell, unpublished 2001).

OBJECTIVES

This research will investigate the disease complex associated with glassy-winged sharpshooters in the Southeastern United States. Specific objectives include to:

1. Identify and archive all the major pathogens affiliated with GWSS populations.
2. Estimate the distribution, frequency and seasonality of the major diseases of GWSS.
3. Screen the pathogens for exotoxins with potential toxicity to GWSS and other arthropods.
4. Confirm infectivity of the isolates and the exotoxins and determine which if any pathogens may serve as microbial controls of GWSS and other leafhopper vectors.

RESULTS AND CONCLUSIONS

We have not received final approval of the contract for this grant and cannot proceed full speed until we do. However, we have completed surveys of entomopathogens in field populations of GWSS in north and central Florida with some success. During summer 2002, populations of *Homalodisca coagulata* were sampled at sites in Gainesville and Quincy, Florida, and Cairo, Georgia. Insects were collected and held in sleeve cages on crape myrtle for 2-4 weeks for the detection of pathogens. Sharpshooter cadavers were collected and incubated in a warm, moist environment and observed for fungal and bacterial growth. Populations in Gainesville were observed in low densities on red crape myrtle. There was no indication of fungal infection in these insect populations. Sharpshooter populations in Quincy, Florida were observed in much higher densities on citrus, crape myrtle, and holly, among other hosts plants. Sharpshooters collected in late June experienced a rapid die-off but displayed no signs of fungal infection. The die-off seemed instead to be associated with a bacterial infection, the nature of which has yet to be determined. Cultures of bacteria and fungus associated with the cadavers from this collection are awaiting further identification. In southern Georgia, sharpshooters were sampled on hollies at two different nursery sites. One site yielded a great many mycosed cadavers, whereas the other site yielded only healthy insects. Mycosed cadavers collected from hollies at the infected site were incorporated into a preliminary transmission study. In this experiment, mycosed cadavers were placed on crape myrtle with the live sharpshooters collected from both infected and uninfected areas. Horizontal transmission did not appear to occur within the two week exposure period. Only samples taken from the infected area showed signs of fungal infection. Cultures of the fungi associated with these specimens were examined under SEM and

were identified as a *Hirsutella* spp. Currently selected ribosomal genes are being sequenced to confirm identification of this fastidious fungus.

Some initial work has been done on the composition and function of brochosomes, a unique excretion of GWSS that is used to cover the integument and eggs. Brochosomes may play a role in preventing infection, and are therefore of interest as to how they may interact with fungi or bacteria. Also of interest is the presence of internal parasites in a closely-related sharpshooter, *Oncometopia* spp. Strepsipterans were observed in ca. 20% of *Oncometopia* spp. adults collected in north Florida and south Georgia. No data have shown that the strepsipterans found in the *Oncometopia* spp. can infect *H. coagulata* as well, but more study is needed.

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FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

EXPLORATION FOR FACULTATIVE ENDOSYMBIONTS OF SHARPSHOOTERS

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Reporting period: The results reported are from work conducted from July 1, 2002 to November 1, 2002.

INTRODUCTION

Improved biological control of the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*, in California has been a major objective of research attempts to lower the incidence of this vector of *Xylella fastidiosa* (*Xf*), a bacterial pathogen of grapes, almonds, alfalfa (in California), citrus, coffee (in Brazil) and a variety of other plants (Purcell and Feil 2001). So far, the most promising biological control approaches have been to seek new parasitic wasp species that attack the eggs of GWSS and to discover ways to enhance their effectiveness (Triapitsyn et al. 1998). Pathogens of GWSS have not been employed to date largely because none are known, although recent research is directed towards discovering viruses of GWSS.

Endosymbiotic bacterial associates of leafhoppers are little-understood and unexploited components of their biology that we believe could make a significant contribution to control of these pests. The gravity of the threat posed by GWSS justifies research into this unexplored aspect of its biology. Biological control by itself is unlikely to provide a solution to the GWSS/PD dilemma, but hopefully will serve as a cornerstone to an integrated approach by lowering populations of GWSS to the point where combinations of other control methods such as insecticides, repellents, and habitat management can lower the numbers of infective GWSS in affected crops to manageable levels.

Of particular interest to us are bacterial associates that are facultative (also referred to as “secondary”), i.e., that occur in some individuals or populations but not others, and that could be introduced into, or augmented in pest populations. We use the term symbiont here in the biological sense of “living together” and do not imply mutual benefit (Douglas 1994). Facultative bacterial associates have been described in a variety of Homoptera including leafhoppers (Swezy and Severin 1930, Schwemmler 1974, McCoy et al. 1978, Purcell et al. 1986). The only leafhopper facultative symbiont studied in some depth is BEV, a bacterium that occurs in *Euscelidius variegatus* in France, but apparently not in California (Purcell et al. 1986). Uninfected females of *E. variegatus* inoculated with cultures of BEV transmitted the bacteria transovarially to their offspring. Subsequently, infected leafhoppers had drastically reduced fecundity (by 80%), slowed development (double the normal development time), and increased mortality, relative to uninfected controls (Purcell et al. 1986, Purcell and Suslow 1987). They also transmitted phytoplasma bacteria at drastically reduced rates, but we do not anticipate this would occur with *Xf*.

It is clear from our studies of facultative bacteria in aphids (Chen et al. 2000, Montllor et al. 2002) as well as from the study of BEV, that endosymbiotic associations are complex and have critically important effects, both positive and negative, on the physiology, population biology and vector potential of their hosts. Any component of leafhopper behavior, physiology, or ecology that affects their ability to vector plant pathogens can have major implications for the spread of plant diseases. The most likely impacts that facultative symbionts might have on the control of GWSS involve their potential ability to:

- decrease populations of GWSS to lower equilibrium levels (may be temperature dependent)
- change (+ or -) the rate of successful parasitization by biological control agents
- decrease GWSS fecundity
- facilitate production of GWSS parasitoids (if discovered to be limited by bacterial symbionts)

OBJECTIVES

1. Survey glassy-winged sharpshooter and other sharpshooters in California and the Southeastern U.S. for facultative bacterial endosymbionts.
2. Determine by DNA sequencing the identity of any bacteria discovered.
3. Depending on type of microorganism and relative frequency in surveyed insects, select candidate symbionts to (a) attempt to culture, (b) determine whether they can be transmitted by injection of hemolymph from infected to uninfected GWSS or to other sharpshooter species, (c) determine whether they are transovarially transmitted, (d) determine whether they can be horizontally transmitted through plants and (e) determine whether any are beneficial or pathogenic to GWSS in terms of life history traits (growth, fecundity, longevity, parasitism).

RESULTS AND CONCLUSIONS

Glassy-winged sharpshooters have been collected in Louisiana, Florida and California (two locations) in spring and summer 2002. Four other species of sharpshooters have also been collected in California in summer 2002. DNA has been extracted from hemolymph samples of 170 insects. To date, the 16s ribosomal DNA from 88 of these samples has been amplified with universal eubacterial primers. About 60% of the samples contained enough amplified DNA to detect by electrophoresis, indicating either that some insects do not have detectable bacteria in the hemolymph, or that the hemolymph sample collected was too small. Eubacterial DNA from 25 individual GWSS from Florida, Louisiana and California was digested with the endonuclease Hinf I as an initial screen for differences in the amplified bacterial DNA. These digests have been analyzed by electrophoresis, yielding distinct band patterns. Five different digest patterns have been found to date in these GWSS, and at least two band patterns have been documented from each locality. There appears to be overlap in band patterns among the three localities (i.e., common patterns in insects from the three areas), though this is not yet certain. The presence of a variety of band patterns indicates that several types of bacteria are present in our samples. Although great care has been taken to avoid contamination of hemolymph samples, we do not yet know whether some of the bacterial DNA in our samples might come from an exogenous source. We have also extracted DNA from the primary symbionts of GWSS, which occur in structures called bacteriomes, and which can easily be dissected out of the insect, and from BEV-infected leafhoppers, for comparative purposes.

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FUNDING AGENCIES

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THE AREA-WIDE PEST MANAGEMENT OF GLASSY-WINGED SHARPSHOOTER IN KERN COUNTY

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Reporting Period: The results reported are here are from work conducted from January 2002 through December 2002

INTRODUCTION

The year 2002 marks the second of a proposed three year study to develop and demonstrate the Areawide Management of Glassy-winged Sharpshooter (GWSS) in Kern County, CA. The first year successfully demonstrated the areawide concept as a strategy that will reduce GWSS to very low population densities in a robust multi-crop perennial growing system. Critical to this success is a well organized and coordinated delivery system that growers can adapt to their present pest management programs. The strategy in 2001 was to treat citrus using an action threshold of greater than one GWSS/tree which resulted in 1830 acres out of 3600 receiving a foliar, knockdown, insecticide. All citrus received a single systemic application of Admire ® following the foliar treatments. All acreage, approximately 13,000 acres, is monitored utilizing a combination of direct observations and sticky traps arrayed in a ¼ mile grid.

Grower interest in the results of the pilot study in 2001 culminated in a request to initiate an areawide program in Kern County. An expanded program was implemented in the county. This areawide program utilized the strategies developed in the first year of the pilot study.

OBJECTIVES

1. Adjust management strategies based on current GWSS population levels.
2. Test compatibility of selected insecticides and biological control agents.
3. Develop and implement biological control based strategies. (See Report by Isabelle Lauziere)
4. Implement an area-wide pest management program in Kern County.

RESULTS AND CONCLUSIONS

Results from the monitoring program suggested that in 2002 three of the groves treated last year in the pilot study required treatment with a systemic insecticide. Four groves required only a foliar treatment. Although the GWSS populations in these groves were below the action threshold used in 2001, the decision was made to treat these as hot spots and prevent the possible reinfestation of adjacent groves. Monitoring data indicates that the groves still infested in 2002 were adjacent to eucalyptus windbreaks that border the groves. The windbreaks were initially treated with a foliar insecticide treatment but did not receive a systemic treatment to prevent recurring populations of GWSS. We are currently waiting for an approved label to treat these windbreaks.

GWSS population monitoring in the pilot project study area during 2002 indicates very low levels of infestation. Insecticide inputs during the second year of the study were minimal, suggesting that the area-wide approach to treatment may provide adequate control of the pest over multiple years. The addition of natural enemies in the form of augmentative releases of egg parasitoids may extend this control, potentially building in a long-term sustainable regulating component into the area-wide program.

Limited fiscal resources for an expanded area-wide program in Kern County required the county to be divided into zones that could be effectively managed with available funds. Four zones have been established and are monitored for GWSS populations using a trapping grid of one trap/per 32 acres. The Northern Zone, Edison area, has historically been troubled with large populations of GWSS. This is the first zone to be treated. The management strategy followed the established protocol from the pilot study. The current population of GWSS has been reduced to almost non-detectable levels in citrus. Since Pierce's disease is known to occur in this area of the county, vineyards that continued to have GWSS after harvest were treated to prevent their return into citrus. Hot spot treatments for GWSS in grapes have reduced this population to similar levels as seen in the citrus.

FUNDING AGENCIES

Funding for this project was provided by the USDA Animal and Plant Health Inspection Service.

MONITORING AND CONTROL MEASURES FOR PIERCE'S DISEASE IN KERN COUNTY

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Reporting period: The results reported here are from work conducted from April 2002 through December 2002.

INTRODUCTION

Pierce's disease (PD), caused by the bacterium *Xylella fastidiosa*, is a killer of grapevines. Significant vine loss from PD has occurred in southern California, north coast regions and portions of the southern San Joaquin Valley such as Tulare and Fresno counties over the last 100 years. Native sharpshooters, including the blue-green sharpshooter and the red-headed and green sharpshooters have been largely responsible for PD spread. With the arrival of the glassy-winged sharpshooter (GWSS), a more effective vector, the transmission of the bacterium and subsequent disease threatens Kern County, a major grape production area of the state with more than 87,000 bearing acres and a farm gate value of approximately \$438 million dollars.

Approaches to disease management have changed in Kern County, due largely to an increase in the incidence of disease where PD had not known to exist prior to the introduction of GWSS. To date growers have tried to control the insect and the disease with a combination approach of using both soil-applied and foliar pesticides and roguing out vines expressing PD-like symptoms during the summer. However, there is no data to support whether these strategies will be successful to combat the spread of PD. Many management decisions are currently being made based on anecdotal evidence, testimony and projections. The county provides a unique environment in which to map the incidence of PD, track the spread of the disease over time and investigate the interaction between the insect and the disease, given that both inoculum and the insects are present. Such information would be useful to determine the economic impact of GWSS on California agriculture as well as provide fundamental data on epidemiological factors including but not limited to, host susceptibility to disease, GWSS presence, proximity to preferred hosts of GWSS, proximity to alternative hosts of *Xylella* and individual grower insecticide and disease management programs.

OBJECTIVES

1. Estimate the incidence of PD over time in both GWSS infested regions as defined by the Kern County Agricultural Commissioner's Office and detection efforts by the CDFA and USDA and in areas with no GWSS finds. Data will be collected by mapping case study vineyards for PD in order to determine the quantitative relationships of recorded variables to disease incidence over time.
2. Provide individual support to growers and pest control advisors to ensure that they are aware of the critical nature of PD in the presence of GWSS. The key to this objective is getting grower support to develop their own field monitoring programs and control strategies. This includes individual field meetings if there are questions regarding symptom identification, encouragement of tissue sampling, collecting and shipping samples to the CDFA diagnostic lab, communicating results, providing information on PD management and follow up support.

RESULTS AND CONCLUSIONS

This study focuses on vineyards in Kern County because of its importance as a major grape production area and its short history of GWSS infestation. This situation enables the project to follow the epidemiology and progression of the disease beginning with the arrival of the insect vector, particularly in the northern area of the county (Delano and Highway 65). A number of vineyard blocks throughout the county were selected as case studies for the project (Table 1.). The acreage surveyed within the project represents roughly 5% of the total bearing grape acreage in the county. A profile was created for each vineyard and the variables recorded include: GPS coordinates, cultivar, vine age/plant date, row x vine spacing, pruning and trellising system, weed index, proximity to other host crops of GWSS and *Xylella fastidiosa*, pesticide use information when available and presence and population levels of GWSS. Fifteen cultivars of varying ages were examined during the project and the levels of tolerance to PD are presented in Table 2. All data will be sent to the Center for the Assessment and Monitoring of Forest and Environmental Resources (CAMFER) at University of California at Berkeley under the direction of Barry Hill and Sandy Purcell. The center will compile the data and create a GIS based data set. The resulting data, maps, and information will be shared with collaborating plant pathologists, statistical analysts, agricultural economists, and other legitimate researchers to maximize the opportunity to understand the changed epidemiology of PD, to manage the disease,

and to generate projections for potential economic consequences and risk assessment. Information from this project will be useful in the future in those viticulture areas of the state where the GWSS may become established.

Table 1. Summary of the Pierce's disease survey effort in Kern County.

Areas surveyed for Pierce's disease	Total number of blocks surveyed	Total number of acres surveyed	Number of tissue samples collected	Number of PD + samples
General Beale Pilot Area	42	888.2	1976	908
North: Edison/Bena	7	234	145	109
South A: Arvin	22	314.4	39	6
South B: Arvin	28	259	84	9
Central: Arvin	2	32	0	0
West: Hwy 166	32	801	56	6
Hwy 65 and Delano	83	1586.8	In progress	In progress
Total	216	4115.4		

Table 2. Cultivars included in the study and their respective tolerances to Pierce's disease.

Cultivar	Vine susceptibility <i>Most susceptible=3</i> <i>Less Susceptible=2</i> <i>Most tolerant=1</i> <i>Resistant=0</i>
Green Calmeria	3
French Columbard	2
Jade Seedless	3
Muscat	N/A
Perlette	N/A
Thompson Seedless	1
Superior Seedless	N/A
Red Christmas Rose	N/A
Crimson Seedless	2
Flame Seedless	2
Redglobe	3
Ruby Seedless	2
Purple Autumn Royal	N/A
Black Emerald	N/A
Fantasy Seedless	N/A

All *V. vinifera* cultivars are susceptible to Pierce's disease. Levels of tolerance were assigned based on the rate of spread of bacteria within the plant. Relative tolerances are adapted from Pierce's disease, UCANR Pub. 21600, Varela et al.

FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.



Additional

Reports



DETECTION OF DNA POLYMORPHISMS IN GLASSY-WINGED SHARPSHOOTERS (*HOMALODISCA COAGULATA*) BY PCR-BASED DNA FINGERPRINTING METHODS

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Reporting Period: The results presented here are from work conducted from FY2001 to FY2002.

INTRODUCTION

The glassy-winged sharpshooter *Homalodisca coagulata* (Say) (Homoptera: Cicadellidae), is a xylem feeding leafhopper that is a serious pest because it vectors a strain of *Xylella fastidiosa*, a bacterium that causes Pierce's disease of grapevines (Turner and Pollard 1959; Nielsen 1968). DNA markers have proved to be valuable tools for population genetic studies. DNA fingerprinting methods that do not require prior knowledge of genome sequences include ISSR-PCR (Inter-Simple Sequence Repeat-Polymerase Chain Reaction), RAMP (Randomly Amplified Microsatellite Polymorphisms), SAMPL (Selective Amplification of Microsatellite Polymorphic Loci) and RAPD (Random Amplification of Polymorphic DNA). RAPDs produce dominant markers, whereas ISSR-PCR, RAMP, and SAMPL incorporate Simple Sequence Repeats (SSR) and are capable of identifying co-dominant markers if utilizing 5'-anchored or compound ISSR primers (reviewed in Karp and Edwards 1997), but without known family relationships (segregation/backcrosses) these markers are scored as dominant.

OBJECTIVES

Develop molecular genetic markers for the glassy-winged sharpshooter by the following methods ISSR-PCR, RAMP, SAMPL, and RAPD to estimate the most sensitive and efficient procedure. Screening of the methods was initiated with a small number of insects (3). Identification of DNA polymorphisms (POPGENE software) in natural populations was determined with 10-30 insects with the various DNA fingerprinting methods.

RESULTS AND CONCLUSIONS

Initially, one insect was utilized to screen with the four DNA fingerprinting methods, than three insects (Weslaco, TX) per primer or primer pair (pp) (46 total) were used to estimate the sensitivity and efficiency of each method. The results of this small scale screening procedure are presented in Table 1. A total of 205 polymorphic markers were generated with the four methods, with ISSR-PCR, pp-ISSR-PCR, RAMP, SAMPL, and pp-RAPD producing 34, 41, 58, 32, and 40 polymorphic markers, respectively. The Efficiency Ratio (number of polymorphic markers/number of primers amplified) of each method was as follows: 6.83 (pp-ISSR-PCR), 6.80 (ISSR-PCR), 4.83 (RAMP), 3.33 (pp-RAPD), and 2.91 (SAMPL). The Screening Efficiency (number of polymorphic markers/number of primers screened) indicated that both pp-ISSR-PCR (2.41) and ISSR-PCR (2.27) were the most efficient methods. To test the utility of some of these DNA fingerprinting methods on identifying DNA polymorphisms in a natural population of glassy-winged sharpshooters (Weslaco, TX), 10-30 insects were employed (Table 2). Depending on the sample size, the number of polymorphic loci ranged from 5 (pp-RAPD, reaction #6) to 32 [ISSR compound primer 13, A(CA)₇(TA)₂T] and percentage polymorphic loci was 100% for most primers or primer pairs. Gene diversity ranged from 0.095 to 0.263 for ISSR compound primer 10, G(TG)₄(AG)₄A and pp-RAPD reaction #6, respectively. A small-scale geographic or multi-populations analysis was conducted with ten insects each from Weslaco, TX and Bakersfield and Riverside, California and RAMP (reaction #54). A dendrogram based on Nei's genetic distance by the method of UPGMA and the multi-populations genetic variation statistics are demonstrated on Figure 1. The two California cities, Bakersfield and Riverside formed a cluster that was separated from Weslaco, Texas. The Weslaco population demonstrated the greatest genetic diversity (0.20). Geographic specific markers may also be an indication of subdivided populations. The present results confirmed the utility of the DNA fingerprinting screening procedure and demonstrated extensive genetic variation in natural populations of glassy-winged sharpshooters by the four PCR-based DNA fingerprinting methods.

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FUNDING AGENCIES

Funding for this project was provided by the USDA Agricultural Research Service.

Table 1. Summary of the DNA fingerprinting methods screening procedure. pp, methods incorporating primer pairs.

Method	No. Primers Screened	No. Primers Amplified	No. Polym. Markers	Efficiency Ratio	Screening Efficiency
ISSR-PCR	15	5	34	6.80	2.27
pp-ISSR-PCR	17	6	41	6.83	2.41
RAMP	93	12	58	4.83	0.62
SAMPL	40	11	32	2.91	0.80
pp-RAPD	45	12	40	3.33	0.88
Total	210	46	205		

Table 2. Summary of selected results from the various DNA fingerprinting methods. P, polymorphic loci; %P, percentage polymorphic loci; G. D., gene diversity.

Method	Reaction (#) or primer (p)	Primer(s)	Sample Tm	Size	Loci	P	%P	G. D. (SD)
ISSR-PCR	p-9	CCAG(GT) ₇	52°	30	28	28	100	0.147 (0.124)
ISSR-PCR	p-10	G(TG) ₄ (AG) ₄ A	41°	30	25	25	100	0.095 (0.097)
ISSR-PCR	p-13	A(CA) ₇ (TA) ₂ T	54°	30	32	32	100	0.121 (0.091)
pp-ISSR-PCR	#7	KKVRVRV(TG) ₆	47°	10	15	14	93.3	0.171 (0.116)
		C(CT) ₄ (GT) ₄ G						
RAMP	#54	G(TG) ₄ (AG) ₄ A	43°	10	15	15	100	0.231 (0.117)
		OPM-02						
RAMP	#75	C(AC) ₄ (AG) ₄ A	41°	30	21	21	100	0.197 (0.153)
		OPV-14						
SAMPL	#34	E + AGC	58°	30	14	14	100	0.102 (0.074)
		C(AC) ₄ (AG) ₄ A						
pp-RAPD	#1	OPA-03/A-10	36°	10	11	10	90.9	0.194 (0.165)
pp-RAPD	#6	OPA-03/M-02	36°	30	5	5	100	0.263 (0.155)
pp-RAPD	#17	OPA-10/V-14	36°	30	15	15	100	0.165 (0.158)

Figure 1. Small-scale geographic populations genetic analysis. High molecular weight genomic DNA from ten insects from each location was amplified by RAMP with reaction #54. GSM, number of geographic specific markers.

0.26	0.52	0.78	P	%P	G. D.	GSM
	+-----	Bakersfield, CA	10	55.6	0.16 (0.19)	0
+-----	1					
--2	+-----	Riverside, CA	10	44.4	0.11 (0.15)	1
!						
+-----		Weslaco, TX	15	83.3	0.20 (0.14)	6
Multi-populations:			18	100.0	0.17 (0.12)	



Poster

Abstracts



AC AND DC EPG WAVEFORMS OF GLASSY-WINGED SHARPSHOOTERS ON *CHRYSANTHEMUM* AND GRAPE

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Glassy-winged sharpshooter, *Homalodisca coagulata* (Homoptera: Cicadellidae), is a new vector of Pierce's disease bacterium, *Xylella fastidiosa*, threatening production of grape and other fruits in California. Long-term management of this disease will rely on host plant resistance, which will be (could be approached) aided by a complete understanding of the vector's feeding behaviors associated with bacterial transmission. EPG (electrical penetration graph) monitoring is a powerful tool to investigate feeding behaviors of sucking insects on plants. Both AC and DC EPG were used for the first time to study recorded feeding behaviors of sharpshooters on *Chrysanthemum* and grape. The waveforms were categorized into pathway, xylem ingestion and interruption phases, and were correlated and verified with feeding sites on the plant, insect body postures, watery excretory droplets, and histological observation of salivary sheaths within plant tissues.

USE OF A TRIPARENTAL MATING SYSTEM TO INTRODUCE GREEN FLUORESCENT PROTEIN MARKED TRANSPOSON INSERTIONS IN THE PLANT PATHOGEN *XYLELLA FASTIDIOSA*

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Xylella fastidiosa causes many serious diseases of fruit trees in North America, particularly Pierce's Disease of grapevine and 'Phony' disease of peach. In South America the pathogen causes the recently described Citrus Variegated Chlorosis and Coffee Leaf Scorch diseases, both of which are widespread in Brazil. The magnitude of the disease problems caused by this bacterium led to the organization of a consortium in Brazil, which has determined the complete nucleotide sequence of the genome of a citrus strain of the pathogen. Teams in the United States and Brazil have subsequently sequenced the genomes of grapevine, oleander and almond strains of the pathogen. However in order to exploit the genomic sequence data to enable effective disease control, systems for genetic manipulation of the pathogen are necessary, but have thus far been completely lacking. We report the introduction of foreign DNA into a citrus strain of *Xylella fastidiosa* by use of a triparental mating system. With this system we have introduced a mini-Tn5 transposon that encodes a Green Fluorescent Protein (GFP) gene optimized for expression in bacteria. The mini-Tn5 derivative was inserted into different sites of the genome in independent transconjugants as determined by Southern blotting. The position of the insertions was determined by reference to the genomic sequence data. The GFP gene was also expressed well in *Xylella fastidiosa*, and to different levels in different transconjugants. Four independent transconjugants were separately used to inoculate sweet orange and tobacco seedlings. The transconjugants were able to colonize the plants and were subsequently re-isolated from points distal to the inoculation sites. When the relative fluorescence of the transconjugants that had been passed through either tobacco or sweet orange was compared to that of the same transconjugant maintained continuously in vitro, we observed that passage through either plant host significantly increased the level of expression of the GFP. The increased level of expression of GFP was transient, and was lost upon further culture in vitro. We have developed a system for the introduction of marked mutations which will be useful for both in vitro and in planta analysis of gene expression of *Xylella fastidiosa*.

CITRUS AND COFFEE STRAINS OF *XYLELLA FASTIDIOSA* INDUCE PIERCE'S DISEASE IN GRAPEVINE

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Xylella fastidiosa causes citrus variegated chlorosis disease in Brazil and Pierce's disease of grapevines in the United States. Both of these diseases cause significant production problems in the respective industries. The recent establishment of the glassy-winged sharpshooter in California has radically increased the threat posed by Pierce's disease to California viticulture. Populations of this insect reach very high levels in citrus groves in California, and move from the orchards into the vineyards, where they acquire inoculum and spread Pierce's disease in the vineyards. We now show that strains of *Xylella fastidiosa* isolated from diseased citrus and coffee in Brazil can incite symptoms of Pierce's disease after mechanical inoculation into seven commercial *Vitis vinifera* L varieties grown in Brazil and California. Thus any future introduction of the CVC strains of *X. fastidiosa* into the United States would pose a threat to both the sweet orange and grapevine industries. Previous work has clearly shown that the strains of *X. fastidiosa* isolated from Pierce's disease and citrus variegated chlorosis affected plants are the most distantly related of all strains in the diverse taxon *X. fastidiosa*. The ability of citrus strains of *X. fastidiosa* to incite disease in grapevine is therefore surprising, and creates an experimental system with which to dissect mechanisms used by *X. fastidiosa* in plant colonization and disease development using the full genome sequence data that has recently become available for both the citrus and grapevine strains of this pathogen.

ULTRASTRUCTURE OF THE BACTERIOME-ASSOCIATED ENDOSYMBIONTS OF THE GLASSY-WINGED SHARPSHOOTER, *HOMALODISCA COAGULATA* (HOMOPTERA: CICADELLIDAE)

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Light and transmission electron microscopy were used to examine the intracellular endosymbionts of the glassy-winged sharpshooter, *Homalodisca coagulata*. The organisms described are contained within bacteriocytes, forming organs referred to as bacteriomes. In glassy-winged sharpshooter, bacteriomes consisted of paired reddish organs associated with yellow glandular-like structures located in the ventro-lateral anterior portion of the abdomen. Three morphologically distinct bacteria-like organisms were observed. One spherical in shape, one rod shaped, and one larger, highly pleomorphic organism that often appeared elongated. The red portion of the bacteriome appeared to contain only the spherical organisms, while sections of the yellow portion contained all morphological forms. In some cases individual cells in the yellow portion contained more than one type of organism.

EPIDEMIOLOGY OF *XYLELLA FASTIDIOSA* DISEASES IN CALIFORNIA

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Incidence of Pierce's disease (PD), caused by *Xylella fastidiosa*, continues to increase in many grape varieties in California due to the establishment and spread of the vector, the glassy winged sharpshooter, *Homalodisca coagulata*. Eleven vineyards were surveyed during the 2001 and 2002 production seasons. Each vineyard was assessed visually for PD symptoms and geo-referenced using GPS technology. Commercially available materials [e.g., Particle film barrier (Surround WP) containing 95% kaolin and systemic acquired resistance inducer (Messenger) containing 3% harpin] were also evaluated for their effects on reducing *X. fastidiosa* transmission, and preventing *X. fastidiosa* infections and PD development. The spatial patterns of Pierce's disease epidemics in grower-managed vineyards naturally affected by PD were analyzed by ordinary runs, indices of dispersion, and two-dimensional distance class analyses. Disease incidence ranged from < 1% to 80%. The spatial disease gradient analyses consistently described the non-randomness of the patterns of diseased vines, and an increase in the degree of clustering of diseased vines as disease incidence increased. Three grapevine varieties (Flames Seedless, Chenin Blanc, and Thompson Seedless) in four blocks were treated on March 13, March 30, and April 14, 2001, with Surround WP. During the 2-year study, PD development was lower (6%) in Surround treated plots than in conventional insecticide treated plots (14%). No Surround by grapevine variety interaction was observed in either year. PD incidence among grapevines in a commercial field in Tulare County was 13%, 7%, and 6% with 2.25, 4.50 and 6.50 oz Messenger, respectively. PD incidence in untreated control vines was 18%. Based on these results, effective PD management is likely to be based on practices that reduce initial inoculum and use of resistant varieties. Also, *X. fastidiosa*-infected vine removal and monitoring of surrounding vines for new *X. fastidiosa* infections should be practiced. A greenhouse study to determine the effects of Surround, Admire and Messenger on *X. fastidiosa* transmission and PD development is currently in progress.

