ARS *Xylella fastidiosa* Diseases – Glassy-winged Sharpshooter

STRATEGIC RESEARCH PLAN

K. Hackett, E. Civerolo, R. Bennett, D. Stenger

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Coordination: County Agricultural Commissioners, CDFA, National Invasive Species Council, UC-Cooperative Extension, California Pierce's Disease (PD)-GWSS Board, UC-Department of Agriculture and Natural Resources (DANR) PD-GWSS Grant Program, USDA-APHIS

Laboratory Changes: The ARS Phoenix Laboratory has been relocated to Maricopa; however, the term Phoenix has been retained for this document.

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EXECUTIVE SUMMARY

The bacterium *Xylella fastidiosa* (Xf) causes serious diseases in many agronomic, horticultural, and landscape ornamental plants – including Pierce's disease (PD) of grape, and leaf scorch diseases of almond, peach, plum, pear, and oleander in the United States, and, citrus and coffee in South America. PD, alone, threatens a California wine, table, and raisin grape-dependent industry valued at $45 billion, and almond leaf scorch threatens the $2.5 million almond industry; oleander leaf scorch attacks the main roadside planting, oleander, in California. The Xf strain that causes citrus variegated chlorosis, if it were to be introduced into the United States, would be particularly devastating to citrus production in California and Florida. The recent association of Xf with scorch disease-like symptoms in olive in southern California may also represent a potential risk to olive production.

While Xf strains that cause PD (and almond leaf scorch disease) have been vectored in California by several native sharpshooter (leafhopper) species, the introduction of the glassy-winged sharpshooter (GWSS) into southern California in the 1990's has resulted in epidemics of disease in grape there (Temecula, in Riverside County) and in the lower San Joaquin Valley (Kern County). This is due to the GWSS's large numbers, behavior of feeding at the base of canes (thus spreading the pathogen beyond prunable wood), and tendency to migrate deep into vineyards from areas where the insect overwinters in citrus and other plants.

ARS responded to the PD epidemic by organizing an Emergency PD/GWSS Research Response Team (PD/GWSS Team) that made site visits to areas of the PD epidemic in southern California in 2000, and developed a Strategic Plan of action. Since that time, the ARS effort on xylella diseases and GWSS has expanded greatly, through redirection of resources and personnel (there are currently 30 senior scientists engaged in full- or part-time PD/GWSS research at 12 locations), receipt of additional base-funding, and due to a greatly expanding network of Federal, state, and university collaborations. To coordinate the current research effort, ARS conducted a strategic planning process in 2003 that resulted in the document "ARS *Xylella fastidiosa* Diseases – Glassy-winged Sharpshooter, Strategic Plan." The 2006 updated document is submitted herein.

ARS has taken the approach of responding in the short-term to solutions that will suppress GWSS populations, as a means of interfering with transmission of Xf and reducing the incidence of xylella diseases. There has been considerable success in this effort (as acknowledged by a Secretary's Honor Award to the PD/GWSS Team in 2003), brought about through a collaborative effort with our partners and achieved, in part, through our development of kaolin-clay based repellents, and evaluation and demonstration of the effectiveness of foliar and systemic insecticides. Having slowed the epidemic, ARS is now focusing on mid- and long-term research directed toward sustained control of xylella diseases and the sharpshooter. These research efforts include: i) comprehensive studies of the systematics, biology, ecology, epidemiology and genomics of Xf strains and sharpshooter vectors on a variety of crops (particularly, grape and almond) and reservoir hosts; ii) exploration of Xf-vector-plant interactions; and, using this baseline information, iii) evaluation of integrated pest management approaches for mitigating the impact of the Xf-caused diseases. An important component in this

effort has been ARS' support for sequencing, with Brazil, of Xf strains that cause scorch diseases in grape, almond, and oleander.

ARS has also supported grape genomics at the University of California, Davis, with the long-term goal of developing resistant varieties of grape, and has developed technology for transforming grape that can be used in elucidating gene function, identifying plant genes responsible for resistance and the disease process, and, primarily, facilitating breeding programs.

INTRODUCTION

Background of Problem

Recent invasions of exotic insect pests into California (e.g., pink hibiscus mealybug, vine mealybug, olive fruit fly, imported fire ant, and Africanized honey bee) highlight the threat of invasive species to agricultural production in the State.

One recent introduction, the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say), is an effective vector of *Xylella fastidiosa* (Xf), a bacterial pathogen that causes devastating diseases in a wide variety of agronomic and horticultural crops, as well as landscape ornamentals and shade trees (Hopkins & Purcell, 2002; Purcell 1979; Purcell & Hopkins, 1996; Purcell et al., 1979, Purcell et al., 1999). Some of these diseases are emerging as serious, destructive diseases of important crops (e.g., almond, citrus, coffee, grapevines, peaches, plum) in North and/or South America. In addition, a number of other non-crop plant species are hosts for Xf (Purcell et al., 1999). The threat to wine, table, and raisin grapes (>750,000 acres; total economic value >$45B) and almonds (>7,550,000 acres; value >$2.5M), alone, if realized, would be devastating to the California economy.

Currently, there are no effective treatments for xylella diseases, including Pierce's disease (PD) of grapevines, almond leaf scorch disease (ALSD), plum leaf scald disease (PLSD) and phony peach disease (PPD), each of which can result in serious economic losses during crop production. Control of xylella diseases is currently directed toward reduction of vector numbers.

Although Xf strains can multiply to some degree within a large number of plants that are inoculated with the bacterium, relatively few plants support moderate to high bacterial populations and fewer still allow systemic movement of Xf (Purcell et al., 1999). Further, high bacterial titer, in itself, is not always closely correlated with disease (D. Cook, pers. comm.). Considerable genetic and pathogenic variability occurs among Xf strains (Chen et al., 1995, 2000; da Costa et al., 2000; Hendson et al., 2001; Hopkins & Purcell, 2002; Mehta & Rosato, 2001; Pooler & Hartung, 1995; Purcell & Hopkins, 1996; Qin et al., 2001). Additionally, phylogenetic studies divide *X. fastidiosa* into three subspecies: *X. fastidiosa* subsp. *fastidiosa* (grape, alfalfa, almond and maple), *X. fastidiosa* subsp. *multiplex* (peach, plum, sycamore, elm, and almond), and *X. fastidiosa* subsp. *pauca* (citrus) (Schaad et al., 2004).

PD occurs in some California vineyards every year. Serious losses have traditionally occurred in the Napa Valley, but, with the introduction of GWSS into southern California, losses are now extensive in parts of the San Joaquin Valley (e.g., Kern County) and the Temecula Viticulture Area. This has resulted in the need for extensive grape replanting. In Florida and other southeastern states (usually with native GWSS populations present), PD has precluded commercial production of European varieties. Some muscadine grapes and American wild grape *x* European grape hybrids (*Vitis vinifera*) are tolerant or resistant to PD (Hopkins & Purcell, 1996; Purcell & Hopkins, 2002; Varela et al., 2001).

Xf is transmitted by xylem-feeding insects. The primary insect vectors of Xf are leafhoppers (family Cicdellidae), particularly sharpshooters (subfamily Cicdellinae); however, spittlebugs

(family Cercopidae) are also Xf vectors (Hopkins & Purcell, 2002; Purcell & Hopkins, 1996). Transmission of Xf, especially early in the season, by native sharpshooter species in California occurs primarily by adult insects that migrate into vineyards and other crop plantings from outside hosts (Varela et al., 2001). In California, at least 20 species of xylem-feeding insects are capable of transmitting Xf (Hill & Purcell, 1997); however, only four species are considered epidemiologically significant for PD (Varela et al., 2001).

GWSS was first detected in California in 1989 (Sorenson and Gill, 1996), following its introduction from the southeastern U.S. Since then, it has become invasive, spreading and becoming established throughout southern California and into the southern San Joaquin Valley (Blua et al., 1999, 2001). The GWSS feeds on a wide variety of horticultural crop and ornamental plants, and transmits Xf prolifically by virtue of its large populations, and tendencies to migrate far into vineyards, and to feed at the base of the cane, beyond the prunable wood; consequently, the PD epidemic has exploded in areas in which this vector is present. The epidemiologies of other xylella diseases [e.g., ALSD, oleander leaf scorch disease (OLSD), and scorch diseases of other landscape plants] are also likely to change in areas in which the GWSS is present. The presence of the GWSS also poses a potential threat to other landscape and ornamental plants, as well as cultivated horticultural crops. This threat will be amplified if other exotic plant pathogens are introduced into California [e.g., Xf strains that cause citrus variegated chlorosis (CVC), PPD, PLSD, and pear leaf scorch disease (PeLSD)], and if other Xf strains or pathotypes emerge (e.g., olive leaf scorch).

Thus, xylella diseases are caused by three or more subspecies of Xf (*fastidiosa, multiplex*, and *pauca*) and a wide range of insect vectors, with extensive host ranges for two of the pathogens and the insect vectors. These diseases, especially PD and CVC, are potential threats to the production of major crops in California. A foundation for management of this pest and diseases caused by Xf and its vectors will require a better understanding of the factors that influence disease epidemiology, as well as vector and pathogen biology and ecology.

ARS Emergency Research Response

On January 21, 2000, then Deputy Secretary Richard Rominger and ARS Administrator Floyd P. Horn charged the ARS Pacific West Area and the ARS National Program Staff with assessing the Agency's research capability and implementation needs for mitigating the impact of the GWSS and PD in California. On February 17-18, 2000, ARS assembled an Emergency Research Response Team (ARS Team) of senior level program managers and technical experts to provide this assessment. Given the magnitude of the invasive species threat to agricultural crop production, and the lack of any effective pre-harvest ARS disease and insect pest management technical capacity in California, response to the complex PD-GWSS epidemic had to be National and complement ongoing research conducted by scientists in the UC system and CDFA. Although some plant pathology research capability and germplasm resources were available in California, key elements necessary for a full response from ARS were lacking. In particular, the Team concluded that the following critical research areas were not being fully addressed: Xf biology, ecology and pathology; GWSS biology and ecology, including Xf transmission (acquisition and inoculation); biological control of invasive insect species, including vectors of plant pathogens such as GWSS; chemical control of insect vectors,

including the effects of insecticides on non-target organisms; molecular biology of exotic plant pathogens, including Xf strains; plant physiological and biochemical responses to Xf infection and disease development; and pathogen and insect vector modeling. The Team also concluded that ARS capacity building should focus on pest (i.e., insect, pathogen) invasion biology.

A Congressional field hearing on the PD problem was held at the St. Supery Winery and Vineyards in Rutherford, Napa Valley, California, on February 22, 2000. The Under Secretary for Marketing and Regulatory Programs represented the USDA at this hearing. Representatives of the ARS Team were present to provide information to the Deputy Under Secretary that was obtained, primarily, during the Team's February 17-18, 2000 site visit to the Temecula Viticulture Area and through subsequent research planning sessions.

The ARS Team then developed an Action Plan (ARS Emergency PD/GWSS Research Response Plan, 2000) for addressing the PD-GWSS problem in California. The plan included immediate actions and potential, longer-term, research activities based upon available resources. Through increased base-funding and program redirections, ARS now has more than 30 senior level scientists engaged full or part-time on GWSS and PD (and other xylella diseases).

Through a Specific Cooperative Agreement between USDA-ARS and Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (FAPESP) in Brazil, the genome of a grape strain of Xf associated with PD in California was sequence and annotated. This was followed by the sequencing and annotation of the genomes of ALSD and OLSD strains of Xf. The genome sequence of the California Xf-PD strain provides information for elucidating the molecular basis of pathogenicity, as well as for determining phylogenetic relationships among Xf strains. Genes identified that potentially function in Xf-host interactions include those that code for hemolysins, hemagglutin, adhesions, and cell wall degrading enzymes. All of these, and likely others, are potential targets for disrupting Xf-host interactions. In addition, genome sequences which have been made available through this research have been used to develop new Xf-PD, -ALSD and –OLSD specific primers for the pathogen-based, clinical diagnosis of Xf infection by real-time PCR.

Also, through a contract with Doug Cook (UC-Davis), an inventory of grape genes for transcriptional profiling was initiated. Among more than 60,000 EST's identified in this work, 6,550 were from Xf-infected, resistant *Vitis* hybrids. Several genes that appear to be up-regulated in response to Xf infection were identified. Promoters for these genes could be used to drive Xf-induced and/or tissue specific expression of transgenes. In addition, real-time reverse transcription-PCR for gene expression was developed. These upregulated host transcripts can serve as markers of Xf infection and may be more sensitive than pathogen-based PCR primers for disease diagnosis. Moreover, transcriptional response pathways may be correlated with disease resistance, tolerance or susceptibility. This work has led to testable hypotheses on the importance of water stress (via xylem blockage and cavitations), pathogen population level, and host vs. pathogen vs. vector contributions to the diseases process.

A transformation system for somatic embryos of grape was developed by Ralph Scorza (ARS-Kearneysville) in collaboration with Dave Ramming (ARS-Parlier) and Dennis Gray (UF-Apopka). Using this methodology, leaf-derived somatic embryos of the grape cultivar

Thompson Seedless were transformed through the use of a combined treatment of *Agrobacterium tumefaciens* infection and microprojectile bombardment. Thompson Seedless plants were produced that expressed the Shiva-1 lytic peptide gene. Plants expressing the lytic peptide gene were evaluated for resistance to PD and showed a potentially useful level of resistance (Scorza, R. and D. J. Gray. United States Patent 6,232,528 B1, issued May 15, 2001).

VISION

Economic, effective protection of agronomic, horticultural and landscape crops from Xf-caused diseases and Xf-vectors, using scientifically-based, environmentally-sound, and cost-effective methods that are worker and consumer safe.

MISSION AND GOAL

Develop effective management strategies to reduce or mitigate losses due to Xf-caused diseases and Xf-vectors during crop production that are safe, environmentally-sound, socially-acceptable, and economical. The overall goal of this research is to find treatments to mitigate or cure diseases caused by Xf, control or reduce the spread of Xf, and control or suppress populations of Xf insect vectors (including, but not necessarily limited to, the GWSS). Included in the goal is the development of PD resistant germplasm with high fruit quality and identification of genes and molecular markers for PD resistance.

COORDINATION AND COMMUNICATION WITH CUSTOMERS, STAKEHOLDERS AND PARTNERS

ARS program managers and scientists work actively with partners in the scientific community (state and Federal), regulatory agencies/organizations (state and Federal), and industry to develop, review, and coordinate its research on a National level, to effectively meet the needs of its customers, beneficiaries, and stakeholders.

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RESEARCH COMPONENTS/RESEARCH AREAS

I. Xf Systematics, Genomics, Biology, Ecology, Epidemiology

ARS Research Context:

ARS laboratories in Beltsville, Frederick and Parlier have focused on Xf strain relationships, particularly genetic differentiation of strains and rapid diagnosis, through use of real-time PCR formats and microarray-PCR based systems, and systematics. The Beltsville and Parlier have also studied the interactions of Xf with host plants.

Scientists at Texas A&M University, University of Houston, UC-Berkeley, UC-Davis and UC-Riverside, as well as at ARS-Parlier, are conducting research on various aspects of the biology of Xf strains, including, crop and non-crop plants, natural reservoirs of diverse Xf strains, Xf diversity Xf-host plant interactions and Xf-insect vector interactions. Lisa Morano, at University of Houston, is identifying strains in Texas (and is also involved in the effort to determine whether potato scorch is caused by a xyella). Research on the genetics of Xf virulence and identification of genes related to resistance to Pierce's disease is being conducted at UC-Davis, UC-Riverside and University of Florida. Genes for resistance to Pierce's disease are being identified at UC Davis. Following FAPESP Brazil's successful sequencing of an Xf-CVC strain from citrus, ARS sponsored the sequencing of an Xf-PD strain from grape, an Xf-ALSD strain from almond, and an Xf-OLSD strain from oleander, thus providing the first opportunity for a comparative genomics approach for understanding the bacterium's taxonomy and systematics (at ARS-Parlier and ARS-Beltsville), and its interaction with vectors and plant hosts. Rapidly evolving genes in the bacterium are being investigated (UC-Riverside) because they will most likely be the ones useful for identification of strains, and for control. Progress has also been made by ARS scientists at Beltsville and others at UC-Davis in developing transposon mutants for studying Xf genetics. DNA microarrays and mutational analysis are being used to identify Xf virulence genes at UC-Riverside and ARS-Parlier, ARS-Beltsville, and ARS Frederick.

The ARS-Parlier laboratory is investigating the epidemiology of Xf diseases in California, particularly, determining seasonal fluctuations of genotyped strains of Xf in cultivated crops and reservoir hosts as a function of abiotic factors; while UC-Riverside is focusing on strains in landscape plants. Qi Huang, of the FNPRU (US Arboretum) at BARC also has a program on Xf and ornamental/landscape plants (oak, sycamore, oleander, beech; eastern hardwoods/ecosystem). The ARS-Parlier laboratory is determining spatial and temporal patterns of Xf-caused diseases as a function of GWSS and other sharpshooter species. The fundamental mechanism of Xf-PD transmission by GWSS is being studied at UC-Berkeley and ARS-Parlier.

Naturally-occurring Xf-inoculum sources are being determined at UC-Riverside (for southern California), and by ARS scientists at Parlier (for California's Central Valley), and UC-Davis (for north coastal California). Xf transmission pathways are being identified at ARS-Parlier and UC-Riverside. Epidemiological assessments are being done cooperatively by ARS, CDFA, UC-Cooperative Extension, UC-Riverside and UC-Berkeley (Kearney Agricultural Center) in major grape growing regions in southern California, the Central Valley of California, and north coastal California.

The Almond Board of California is funding research on the epidemiology and control of ALSD, as well as on transmission of ALSD strains of Xf, at UC-Davis and UC-Berkeley. This research includes collaborations with ARS scientists at ARS-Parlier and UC-Davis. [JC Chen (with UC collaborators) also receives funds from the C Almond Board for ALSD epidemiology research.]

Goal 1: Clarify the taxonomy/nomenclature of Xf.

Current Situation: The taxonomy of the organism has been revised. Revision of the species should help in development of improved diagnostics and in understanding host/vector relationships.

Objective 1: Continue to clarify the classification of the species Xf to link all information about host, vector, epidemiology, and control to appropriate names.

Approach: ARS will establish the taxonomy of species/subspecies of *Xylella,* determine appropriate names, and describe new species/subspecies, as appropriate.

Cooperators: This research was and is being done by Norman W. Schaad (ARS-Ft. Detrick) in collaboration with George Lacy (VPI, Blacksburg, VI), M'Barek Fatmi (Hassan II University, Agadir, Morocco), and C.-J. Chang (University Georgia).

Benchmarks:

Conducted DNA-DNA relatedness assays using high stringency conditions and sequence the 16S-23S intergenic spacer (ITS) region of typical strains from 8-10 different hosts, including grape and almond.

Accomplishments:

The taxonomy of 26 strains from 10 hosts, including grape, almond, alfalfa, peach plum, and citrus has been completed. Results show that the 26 strains can be divided into three unique subspecies: *piercei*, *multiplex*, and *pauca*.

1) Schaad, N.W., Postnikova, E., Lacy, G., Fatmi, M., and Chang, C.J. 2004. *Xylella fastidiosa* subspecies: *X. fastidiosa* subsp. *piercei,* subsp. nov., *X. fastidiosa* subsp. *multiplex* subsp. nov., and *X fastidiosa* subsp. *pauca* subsp. nov. *Systematic and Applied Microbiology* 27:290-300.

Expected Benefits: The taxonomic revision of Xf will be an authoritative source for identification of the causal agents of distinct diseases and be useful to researchers, public and private diagnostic laboratories, and quarantine efforts by state and federal officers. Having specific names for each of the causal disease agents will assist in communication.

Goal 2: Improve diagnostic procedures for detection, identification, and differentiation of Xf.

Current Situation: Methods for detecting Xf infections and diagnosing Xf-caused diseases include symptomatology, pathogen isolation, serological assays, and nucleic acid based approaches. Each of these approaches has advantages and disadvantages depending upon the specific need and application. Because of the sensitivity, specificity, adaptability, speed, and relative ease of use, PCR-based assays (including real-time PCR formats) are widely used for Xf detection and genotype identification. However, the application of PCR-based assays for large-scale, rapid, high-throughput uses can be limited by the presence of inhibitors, laborious extraction protocols, and uneven distribution of low levels of the pathogen in infected hosts (especially in the early stages of infection).

Objective 1: Develop real-time PCR formats for clinical detection and identification of Xf.

Approach: Develop and improve rapid, sensitive and quantitative real-time PCR-based assays for Xf for use in the LightCycler instrument from Roche (Hartung, Beltsville) and the Smart Cycler from Cepheid (Schaad, Frederick; vice-Civerolo, Parlier).

Cooperators: This research was and is being done by John S. Hartung (ARS-Beltsville), Norm Schaad (ARS-Frederick), and will be done at ARS-Parlier. Evaluation and validation involves grape and wine growers in California, and other ARS and UC scientists.

Benchmarks:

A standard PCR assay specific for Xf was first described in 1995.

Applied Cleaved Amplification Product Polymorphism (CPS) analysis to show that the PCR-amplified product can be easily used to distinguish Xf-CVC strain from Xf-PD strains.

Described a real-time, quantitative PCR assay for a one-hour diagnosis of PD using the portable, rapid cycling Smart Cycler platform.

Compared several extraction methods to select the best method to rapidly isolate Xf DNA prior to real-time PCR.

Described a real-time, quantitative PCR assay for Xf using the LightCycler platform and used it to study host pathogen interactions.

Designed and evaluated additional, new or improved primers for PCR-based differentiation of Xf strains and pathotypes. Designed and made commercially available a dry bead formulation containing all PCR ingredients, including Xf-specific primers and probe for improved, more robust and rapid real-time PCR assay.

Described a simple agar-absorption and direct PCR assay protocol for rapid diagnosis of PD without influence of plant inhibitors.

Accomplishments:

The real-time PCR method was applied to quantify Xf-CVC in sweet orange seed. Methods have been developed that can rapidly detect, quantify, and distinguish strains of Xf.

1) Li, W.-B., W. D. Pria, Jr., P. M. Lacva, J. S. Hartung. 2003. Presence of *Xylella fastidiosa* in Sweet Orange Fruit and Seeds and its Transmission to Seedlings. *Phytopathology* 93 (8):953-958.

2) Qin, X., Miranda, V. S. , Machado, M., Lemos, E. and Hartung, J.S. 2001. An evaluation of the Genetic Diversity of *Xylella fastidiosa* isolated from Diseased Citrus and Coffee. *Phytopathology* 91(6):599-605.

3) Qin, X and Hartung, J.S. 2004. Expression of green fluorescent protein in *Xylella fastidiosa* is affected by passage through host plants. Current Microbiology 49:215-220.

4) Schaad, N. W., D. Opgenorth, and P. Gaush. 2002. Real-time polymerase chain reaction for one-hour on-site diagnosis of Pierce's disease of grape in early season asymptomatic vines. *Phytopathology* 92:721-728.

5) Fatmi, M.., V. D. Damsteegt, and N. W. Schaad. 2005. A combined agar-absorption and BIO-PCR assay for rapid, sensitive detection of *Xylella fastidiosa* in grape and citrus. *Plant Pathology* 54:1-7.

Expected Benefits: Any research project that needs to rapidly quantify Xf, for example, in plant samples or insect vectors, will benefit from this procedure.

Objective 2: Develop DNA microarray-PCR based identification and detection systems for Xf.

Approach: Develop a set of gene or DNA sequences, defined as diagnostic sequences that can be used to identify specific Xf pathotypes. Use complete and annotated Xf genome sequences to select the appropriate DNA sequences. Evaluate these sequences for their potential to differentially identify Xf pathotypes/genotypes. Using these sequences, design and construct DNA microarrays on glass slides, and use these microarrays to analyze genomic variation among Xf variants from broad geographical areas and hosts.

Cooperators: Scientists at ARS-Parlier are collaborating with California grape and almond growers.

Benchmarks:

Identify diagnostic sequences and evaluate their specificity at Xf species and pathotype levels. Establish and characterize a large collection of Xf strains.

Construct, evaluate, and modify Xf-DNA chips using the available Xf strain collection.

Standardize and continue improvement of Xf-DNA chips and evaluate their potential in clinical application.

Accomplishments: Research has just been initiated.

Expected Benefits: Xf-DNA chips will be used for quick and accurate identification of Xf and its pathotype. The dynamic design ensures newly available research information for Xf identification and characterization.

Goal 3: Elucidate biotic and abiotic factors that affect Xf biology and ecology.

Current Situation: Xylella diseases are complex pathosystems. Xf has an extensive and diverse host range, including many cultivated horticultural crops and ornamental landscape plants, as well as native plant species, in California. Many of these species are limited propagative hosts for Xf; only a few are systemic hosts. Also, many non-cultivated native plant species harbor asymptomatic infections. The epidemiological significance of these various Xf hosts as inoculum sources for spread of the pathogen to cultivated crops is not fully known. Since its introduction into California around 1990, the invasive, aggressive GWSS vector has become established in several counties in southern California and spread into other areas of the State (e.g., portions of the San Joaquin Valley). The GWSS is becoming widely distributed, and feeds and oviposits on a wide range of perennial crops (e.g., citrus), ornamental species and non-cultivated native plant species. The presence of this insect vector is expected to affect the occurrence, distribution, and economic importance of xylella diseases. The epidemiology of xylella diseases in California is differentially affected by the presence of native insect vectors and the GWSS. Knowledge of the factors that affect the epidemiology of xylella diseases can provide useful information regarding (but not necessarily limited to) primary and secondary inoculum sources, mechanisms of pathogen dispersal, and the dynamics of spatial and temporal disease incidence. This information is critical for developing effective disease management strategies.

Objective 1: Determine the genetic diversity and relatedness of Xf strains in crops and reservoir hosts.

Approach: Symptomatic leaves will be collected from selected vineyards and orchards, and patterns of disease spread will be recorded. Pathogens will be isolated and subjected to genomic variation analysis, such as RAPD analysis. Genomic DNA will be isolated from the various Xf isolates using the CTAB minipreparation. Computer-image analysis of the DNA banding patterns obtained during RAPD and rep (repetitive extragenic palindromic)-PCR analyses will be performed.

Cooperators: This research is conducted by Jianchi Chen with collaborators at ARS-Parlier in cooperation with California grape and almond growers, and Farm Advisors.

Benchmarks:

Established experimental designs, initiated sample collections, initiated isolation of Xf strains from diverse sources in different geographical areas, and initiated diversity analyses.

Continue and adjust, if necessary, sample collection and diversity analyses, and complete this phase of characterization of Xf population genetic diversity.

Accomplishments: Report in progress.

Expected Benefits: Clarification of whether single, multiple, or mixed populations of Xf strains are associated with PD and ALSD in California. This knowledge is potentially useful for the assessment of the risk of xylella pathogens to horticultural crops other than grapes and almonds (e.g., peaches, plums, citrus, olives). Determination of inoculum sources. This knowledge will be useful in developing improved, effective strategies to manage Xf-caused diseases, not only in California, but elsewhere as well.

Objective 2: Analyze spatial and temporal patterns of Xf-caused diseases in the presence of indigenous sharpshooter vectors and GWSS.

Approach: Disease incidence is determined by surveys based on visual symptoms. Selected samples are assayed for Xf by isolation, ELISA, and/or PCR to confirm the association of the pathogen with disease symptoms. Two-dimensional maps of the spatial distribution of disease incidence are generated for experimental field units per commodity. Xf vector populations are also monitored (e.g., using yellow sticky traps, sweep nets) and identified. The spatial patterns of disease incidence are interpreted by statistical means (e.g., by ordinary runs analysis, two-dimensional distance class analyses and geostatistics).

Cooperators: Kayimbi Tubajika (ARS-Parlier) collaborates with Jennifer Hashim and Don Luvisi (UC-Cooperative Extension); Dave Bartels (APHIS), and grape growers in Kern and Santa Barbara Counties in California. Parlier scientists will collaborate with UC-Riverside and UC-Berkeley scientists to analyze PD and ALSD incidence data collected in the San Joaquin Valley.

Benchmarks:

Established field plots in 11 vineyards in Kern County (where the GWSS is present); initiated PD incidence surveys; established initial parameters of PD incidence (e.g., percent infected plants, percent vineyards with disease gradients, percent vineyards with apparent ‘edge effects’).

Continued PD incidence data collection in Kern County. Initiated PD incidence data collection in Santa Maria (Santa Barbara County), vineyards where the GWSS is not present. Began analysis of collected data.

Completed analysis of PD incidence in selected vineyards in Kern County. Continued analysis of PD incidence data collected in Santa Maria.

Complete comparative epidemiological analyses of PD incidence data collected in areas with and without the GWSS (e.g., Kern County, Santa Maria, and Coachella Valley).

Accomplishments:

PD incidence data collected in Kern County from 2001 through 2003 were analyzed by ordinary runs and two-dimensional class analyses, and illustrated using multispectral images. Based on these analyses: 1) PD incidence in Kern County ranged from <1 percent to 8 percent in different vineyards; 2) no disease gradient or edge effect was detected in any vineyard; and, 3) spatial disease gradient analyses consistently described the non-randomness of the patterns of PD-affected vines, and an increase in the degree of clustering of PD-affected vines, as disease incidence increased.

1) Tubajika, K.M., Civerolo, E. L., Ciomperlik, M. A., Luvisi, D. A. and Hashim, J. M. 2004. Analysis of the spatial patterns of Pierce's disease incidence in the lower San Joaquin Valley in California. Phytopathology 94: 1136-1144.

2) Groves, R. L. , Chen, J., Civerolo, E. L., Freeman, M. W., and Viveros, M. A. 2005. Spatial analysis of almond leaf scorch disease in the San Joaquin Valley of California: factors affecting pathogen distribution and spread. Plant Dis. 89:581-589.

Expected Benefits: Improved xylella disease management strategies based on knowledge of disease epidemiology whether or not the GWSS is present.

Objective 3: Determine seasonal fluctuation(s) of Xf in cultivated crops (including, but not necessarily limited to, grapevines and almonds) and reservoir hosts, and relate these fluctuations to abiotic factors.

Approach 1: Evaluate the significance of riparian hosts in the epidemiology of PD in the North Coast grape-growing region of California. Among systemically infected riparian hosts, seasonal differences in Xf population levels likely affect their importance as Xf reservoirs. The efficiency of Xf acquisition and inoculation by *Graphocephala atropunctata* (blue-green sharpshooter, BGSS) is influenced by the Xf levels in host plants; the higher the concentration, the higher the probability of BGSS acquiring Xf while feeding. Therefore, in riparian hosts, seasonal fluctuations of Xf levels may influence spread of the pathogen and incidence of PD by affecting the proportion of BGSS that acquire Xf when feeding on these hosts. Xf acquisition is also influenced by vector host preference; a systemic riparian host that is fed upon more frequently by BGSS will likely serve as a more significant source of Xf. By measuring seasonal concentrations of Xf in riparian plants, it will be determined if, and when, concentrations are high enough for acquisition of the pathogen by BGSS. This research will focus on five systemic riparian hosts: *Rubus discolor* (Himalayan blackberry), *R. ursinus* (California blackberry), *Sambucus mexicana* (blue elderberry), *Vinca major* (periwinkle), and *Vitis California* (California grapevine). Future research will focus on BGSS feeding preference for these riparian hosts.

Cooperators:

Kendra Baumgartner (ARS-Davis) is collaborating with Alexander H. Purcell (UC-Berkeley).

Benchmarks:

Propagated California grape, California blackberry, Himalayan blackberry, blue elderberry, and periwinkle (100 plants/species) in the greenhouse.

Mechanically-inoculated all plants with the STL strain of Xf (a strain isolated from PD-symptomatic vines in Yountville, California) and, 4 months later, used PCR to confirm infection. Transferred infected plants from the greenhouse to screenhouses at two sites: Oakville (Napa County, California) and Hopland (Mendocino County, California). Began seasonal Xf quantitation using dilution plating and real-time PCR (October, 2003).

Continue seasonal Xf quantitation. Determine the effects of plant species, season, and location on mean Xf concentration using an analysis of variance. Compare Xf quantitation techniques. Begin research on BGSS feeding preference for the five riparian hosts.

Accomplishments:

Determined that populations reached detectable levels in all five riparian host species during October, 2003. Every replicate plant of periwinkle and California grapevine showed leaf scorch symptoms characteristic of PD, and high concentrations of Xf. Since none of the Himalayan blackberry showed symptoms, despite high Xf population levels, Himalayan blackberry may be tolerant of Xf infection.

Determined that Xf concentrations in California grapevine, Himalayan blackberry, and periwinkle are sufficient for acquisition by BGSS in early autumn. Xf isolations coincided with increased flight activity of young adult BGSS, which peaks in mid summer and remains high through early autumn. Assuming BGSS feeds on California grapevine, Himalayan blackberry, and periwinkle in early autumn, 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 dormant season. However, young adult BGSSs that acquire Xf in mid summer to early autumn and survive the winter are still capable of transmitting Xf the following spring after budbreak.

Expected Benefits: Riparian revegetation management is a method of PD control that focuses on removal of hosts of BGSS and Xf (host plants other than grapevines), followed by revegetation with native, non-hosts. This method has some positive aspects. With lower BGSS populations, fewer insecticide applications are used. Also, some of the plants targeted for removal (Himalayan blackberry and periwinkle) are invasive weeds. However, removal of riparian vegetation is very disruptive to wildlife, and increases the probability of stream bank erosion. Also, some of the riparian hosts are extremely difficult to eradicate. The fewer the riparian plants removed before revegetation, the less disruption there would be to wildlife habitat.

The success of revegetation management depends on a thorough understanding of how riparian hosts contribute to the spread of Xf and the incidence of PD. Although removal of riparian hosts can reduce local populations of BGSS, impact on the riparian area as a reservoir of Xf has not been quantified. Although Xf hosts are known, it is not known if, or when, these hosts attain high enough populations of Xf for acquisition by BGSS in the field. If the results of this research reveal that only a few of the riparian hosts recommended for removal serve as major sources of Xf, grape-growers can concentrate on removing fewer riparian plants, thereby reducing the total amount of riparian habitat disruption.

Approach 2: Small-plot, caged field experiments will be conducted in southern California to investigate the ability of a PD strain of *Xf* to infect and overwinter within selected annual and perennial non-crop wild plant species and, further, to examine the relationships among time of plant infection and the efficiency of systemic infections. A total of ten plant species have been pre-selected for these experiments based upon information regarding their extensive distribution and abundance in urban and agricultural environments, their ability to serve as ovipositional and feeding hosts for vectors, and their ability to support infections of *Xf*.

Cooperators: Russ Groves (ARS-Parlier) has collaborated (and his replacement will collaborate) with Jianchi Chen (ARS-Parlier), California grape and almond growers, and Farm Advisors.

Benchmarks:

Newly-germinated plants of experimental summer annual and perennial species will be needle-inoculated at each of three stages of plant growth – representing vegetative, flowering, and post-flowering developmental stages. Plants will be assayed monthly for infection and GWSS acquisition studies will be conducted.

Continued examination of infection retention in perennial non-crop species and evaluation of winter annual species as potential systemic sources of *Xf* inoculum.

Accomplishments: Report in progress.

Expected Benefits: Determination of the extent to which different non-crop species support only propagative versus fully systemic infections, and, further, assessment of the retention of *Xf* infection over the course of the season. This information will supplement our understanding of important reservoir plant hosts by characterizing the extent to which selected non-crop weed species can serve a dual role as overwintering hosts for *Xf* and as acquisition sources for pathogen spread.

II. Vector Systematics, Genomics, Biology, Ecology, Epidemiology, Mass Rearing

ARS Research Context:

ARS, uniquely, at the Systematics Entomology Laboratory (SEL) in Beltsville, is conducting research on the systematics of leafhoppers, including GWSS. Identification of tiny wasps that parasitize leafhoppers is also being done by scientists at SEL, in cooperation with scientists at ARS-Weslaco and UC-Riverside. Nymphal parasitoids of GWSS and related sharpshooters in the family Pipunculidae are being identified by J. Skevington (Ag Canada) in collaboration with J. Goolsby (ARS-Weslaco).

Artificial diet development was the responsibility of Allen Cohen (ARS-Starkville), until his recent retirement; the ARS laboratory at Columbia will now carry on this work. This work complements studies by APHIS-Mission, North Dakota State University at Fargo, and CDFA (Riverside and Bakersfield), which has resulted in identification of plant hosts for mass rearing GWSS.

Because the biology of leafhoppers vary according to biotic and abiotic factors, studies of GWSS biology are being done at several laboratories, working with different populations of GWSS, and under different climatic (e.g., temperature, humidity) and geographic (e.g., day length) conditions, cropping systems, and management practices, and, with the intention of using the acquired data for varied research purposes. Major research collaborations in this area are between ARS-Phoenix and UC-Riverside (southern California), ARS-Parlier and the Kearney Agricultural Center in Parlier (California's Central Valley), ARS-Davis with UC-Berkeley (north coast of California), and with ARS-Weslaco and UC-Riverside. The ARS program at Parlier is comprehensive, as regards consideration of the majority of these biotic and abiotic factors (e.g., addressing all plant hosts – crops, ornamentals, and native plants), while focused on determining host preference and GWSS population density and movement/dispersal [in citrus and grape]. ARS-Phoenix is developing monitoring methods for GWSS, and testing seasonal abundance and comparative dispersal of GWSS and smoke-tree sharpshooter (STSS) in citrus and grape. UC-Riverside is relating GWSS population size to counts obtained from different sampling methods**.** Traps for GWSS adults and nymphs being developed at UC-Riverside will facilitate design of monitoring schemes. ARS-Weslaco has developed novel behavioral assays for determining the relative attractiveness of olfactory and visual cues in host plant recognition and selection by GWSS and is conducting comprehensive studies on GWSS cognitive ecology. Such information will be combined with investigations of hosts (tree quality), vectors (crowding, sex ratio, reproductive status), seasonal and environmental conditions, and pathogen interactions, with the goal of predicting disease spread, and finding life cycle targets for interrupting transmission of the causative bacteria. This work complements in-house (ARS-Parlier) and collaborative research between ARS-Fargo and North Dakota State University to determine the physiological, behavioral, anatomical, and ultrastructural mechanism(s) of feeding (e.g., stylet penetration of canes), as well as studies at UC-Davis to determine the insect's reproductive biology as related to its physiology.

In addition to ARS-Parlier, UC laboratories (Riverside, Berkeley, Davis) are also conducting multifactor studies of GWSS biology and ecology in the San Joaquin Valley. These studies

focus on GWSS survival in relation to xylem flux and chemistry in grape, and in relation to the physiology of host selection in citrus. At UF-Quincy, work focuses on GWSS behavior, physiology (especially as related to host selection, nutrients, and malnutrition), and natural enemies as limits to GWSS populations. Researchers there, as well as in California at ARS-Parlier and UC-Riverside, are also looking, comparatively, at California crop phenology as a means of predicting the relative course of disease expression in these different climates. Also, other laboratories, e.g., those at UC-Riverside, focus on the effects of GWSS feeding on grape fruit quality and yield.

ARS-Weslaco is determining what factors account for the natural low population levels of GWSS in its native habitat; molecular markers are being used to characterize the genetic variation among geographic populations of GWSS to determine if there may be a species complex. Related research on identification of key predators and pathogens of various GWSS life stages is being conducted at UC-Berkeley, UC-Riverside and University of Florida, and ARS-Shafter.

Goal 1: Clarify the taxonomy/nomenclature of GWSS.

Current Situation: Three species of the genus *Homalodisca* are already known to transmit the Xf strain that causes PD. It is expected that all species in the genus have the capacity to be, or become, important Xf vectors. Of the 19 species in the genus, 13 are exotic for the United States and 17 are exotic for California. Most species of *Homalodisca,* were they to be introduced into, and become established in, California, are serious potential economic threats to several important agricultural crops. Current correct names are in flux and the genus is in need of to be clear about which species are present and disseminating Xf strains.

Objective 1: Stabilize classification of the genus Homalodisca to link all other information about host plants, ecology, physiology, and genomics to correct names.

Approach: ARS will establish taxonomic limits of the genus *Homalodisca* and the limits of all species in the genus, determine their valid names, and describe new species as necessary and/or appropriate. The brochosome structure will be characterized to allow identification of egg masses. Authoritative and accessible identification aids and distribution data will be provided for the genus, as well as specimen support for the study of other sharpshooter leafhoppers as regards their relationship to *Homalodisca* and their potential to transmit Xf.

Cooperators: Stuart McKamey (ARS-Beltsville) is collaborating with Roman Rakitov and Daniela Takiya (INHS), Andrew Hicks (University of Colorado), Carolina Godoy (Instituto Nacional de Biodiversidad, Costa Rica), Gustavo Moya Raygoza (Universidad de Guadalajara, Mexico), and Marco Gaiani (Facultad de Agricola, Universidad Central de Venezuela, Marcy).

Benchmarks:

Examine and analyze *Homoladisca* species and complete preliminary character matrix; request additional material from institutional collections, extract label data, and determine latitude/longitude coordinates. Complete illustrations for three species of *Homalodisca*. Conduct expedition to Costa Rica for exotic species of *Homalodisca*.

Conduct expeditions to Mexico and Venezuela (pending restoration of political stability in the latter country), complete character matrix and phylogenetic analysis of *Homalodisca* species. Postdoctoral fellow (supported by ARS-Beltsville) will complete characterization of brochosomes and submit results for publication. Complete illustrations for remaining species of *Homalodisca*. Compile identification key for image-driven, web-based access. Complete revision of *Homalodisca* and submit for publication.

Accomplishments:

The taxonomic revision of *Homalodisca* is in progress. In addition to the specimens held by the National Museum of Natural History, ARS-Beltsville has borrowed over 1,000 specimens from over a dozen institutions, has extracted locality data, and converted the data to decimal degree geographic coordinates for over 1,500 specimens, has begun characterization of species variation, and has generated a preliminary data matrix. Dr. Rakitov has characterized egg brochosomes and related behavior of 8 species of *Homalodisca*. The Costa Rica fieldwork (June-July 2003) by ARS-Beltsville and cooperators Rakitov, Hicks, and Godoy yielded behavioral data, host data, and samples of egg masses, parasites, and fresh material for molecular and morphological analyses of *Homalodisca* and many close relatives, including several rare genera. The taxonomic revision of *Homalodisca* will be the authoritative source for identification of all species in the genus and will therefore be useful to many GWSS/PD/xylella researchers, state and Federal agricultural staff, as well as for commodity risk assessments conducted by APHIS and the quarantine efforts of the Department of Homeland Security. The Costa Rica specimens provided more complete geographical data for *Homalodisca* and are facilitating development of a more stable and predictive classification of all sharpshooters, including leafhopper vectors of *Xylella* worldwide.

Expected Benefits: Words are the tools of communication and taxonomy is the vocabulary of species. The objective of this aspect of GWSS research is to stabilize the classification of the genus *Homalodisca* so that all other information gathered (host plants, ecology, physiology, and genomics, which are priorities in solving the *Xylella* problem) can be linked to correct names for meaningful communication.

Goal 2: Develop sampling procedures to reliably estimate GWSS population densities and movement.

Current Situation: Since its introduction around 1990, the GWSS has become established in several agricultural production areas, including southern California, and Kern County in the San Joaquin Valley. The sharpshooter has also been detected in other important areas, such as Tulare County in the San Joaquin Valley. The sharpshooter has exacerbated the incidence of PD, and

may affect the epidemiology of other Xf-caused diseases such as (but not necessarily limited to) ALSD. Sampling procedures to reliably estimate GWSS population densities are needed to develop effective pest management strategies based on understanding factors that affect the vector's behavior and dispersal.

An important behavior of the GWSS that contributes to PD epidemics is long distance dispersal flights of the vector into vineyards (Blua et al., 1999; Blua et al., 2000; Sorenson & Gill, 1996). Most current knowledge of these dispersal differences among GWSS and other Xf vectors have been obtained using sampling methodologies in vineyards and citrus orchards [e.g., a UC-Riverside project in Temecula, California (Blua et al., 2000)], and these studies are well served by the use of qualitative (relative) measurements. Now, however, sampling methods are being used to also determine timing of biorational and convention pesticide treatments and to judge their efficacy [e.g., Temecula, California, (Hix et al. 2002), and Bakersfield, California (Wendel et al., 2000)]. This application implies that the sampling method(s) accurately quantify populations. For example, if a given treatment against GWSS results in "zero counts" by beat sampling, does that mean there are no GWSS in the treated area, that they were all killed by the treatment, or could some be left alive – too few to be detected? In the latter case, are those remaining alive enough to vector PD within or out of the treated area? To answer these questions, it is vitally important that sampling methods be validated by total population counts.

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

Approach 1: Compare four sampling tools for estimating GWSS density in terms of precision and cost. Develop and validate sampling procedures and plans for citrus and grapes for research and decision-making applications. Extend sampling plans to estimate the proportion of the GWSS population that is inoculative with Xf.

Cooperators: Steve Castle and Steve Naranjo (ARS-Phoenix) are collaborating with Nick Toscano (UC-Riverside) in Riverside, Temecula, and Ventura Counties.

Benchmarks:

Comparative quantitative analyses were used to identify the bucket and beat net as the most efficient sampling methods for GWSS in citrus. The bucket method has the advantage of being useful for sampling all heights within the tree.

Catches on yellow sticky traps, a common monitoring tool, were correlated to on-plant populations of GWSS in citrus; however, the relationship was variable across years.

Spatial distribution studies revealed that more GWSS are found in the upper half of the citrus tree canopy, and more are found on the south sides of trees. This information was used to refine the sample unit designation.

A preliminary sequential sampling plan was developed for fixed-precision sampling of GWSS on citrus.

A progressive increase in the proportion of adults positive for Xf occurred from the time of adult emergence in late June, 2002, through April, 2003.

Test and refine the sequential sampling plan for GWSS on citrus, develop and test and binomial sampling plan for management application.

Accomplishments:

Four sampling methods were evaluated (bucket, beat net, D-Vac, and A-Vac) to determine which technique is the most reliable and cost efficient. The bucket sampler was the most cost efficient technique and provided good reproducibility for estimating both adult and nymphal populations of GWSS.

Yellow-sticky trap catches were compared with foliage sampler catches to determine the degree of correlation between these techniques. Yellow sticky trap catches of adult GWSS are highly correlated with all foliage sampling methods, but the relationships were variable between years. The spatial distribution of nymphal and adult GWSS was studied in citrus orchards in Riverside, California, using a bucket sampling method. On average, about 2.4 times as many GWSS were collected in the upper half of the tree canopy compared with the lower half, and about 1.6 times as many were collected on the south side of trees compared with the north side. The coefficient of variation (CV=SD/mean) was nearly 2 times lower in samples taken from the upper half of the canopy compared with the lower half, but there were no differences in the CVs among different compass directions. These findings were used to refine the sample unit GWSS in citrus.

Based on the bucket sampling method, density-dependent sample size and sample cost estimates have now been determined, and a preliminary sequential sampling plan for estimating relative population density of GWSS in citrus has been developed. Further work will be needed to independently test the validity of this sampling plan. For pest management application, additional research will be needed to define treatment thresholds.

This sampling program has been applied towards estimating incidence of Xf in GWSS adults. ELISA, PCR, and culturing techniques for the detection of Xf in GWSS are being carried out to obtain an accurate estimate of the proportion of individuals within the population that are capable of transmitting Xf. ELISA has provided the most consistent results. A progressive increase in the proportion of adults positive for Xf occurred from the time of adult emergence in late June, 2002, through April, 2003. The mean titer of Xf in heads and thoraxes also increased progressively through this period, suggesting that the potential for transmission of Xf may rise as the spring generation of adults age.

Expected Benefits: Sampling is a fundamental component for the study of population dynamics and central to development of robust strategies for pest management. ARS research has focused on the development of an efficient method for estimating densities of GWSS in citrus. Based on considerations of precision and cost, a bucket sampler has been identified as an efficient sampling method. Further study of the spatial distribution of GWSS within citrus trees has helped to refine the sample unit and further reduced sampling costs. A preliminary sequential

sampling plan that will enable researchers and pest managers to precisely estimate the relative density of GWSS at a minimal cost has been completed.

Approach 2: Methods and materials to fumigate GWSS populations in single orange trees were developed and tested, and, using these methods, procedures were developed to compare sampling methods with absolute counts of all GWSS in citrus trees. Obtain data on how many GWSS are actually present in a given block of citrus, obtained total population counts of GWSS from individual trees, and began correlating sample numbers of beat counts to total populations. Provide information for selection of sampling methods and interpretation of sampling data.

Cooperators: Tom Henneberry and James Hagler (ARS-Phoenix) are collaborating with Matthew Blua and Carlos Coviella **(**UC-Riverside) on sampling research associated with insecticide treatments, primarily at Riverside.

Benchmarks:

Developed and tested methods and materials to fumigate and count GWSS population in single orange trees; developed procedures to compare sampling techniques with absolute counts of all GWSS in citrus trees.

Obtained data to answer how many GWSS are present in a given block of citrus, obtained total population counts of GWSS from individual trees, and began correlating sample numbers of beat counts to total populations.

Continued with 2002 objectives, obtained data biweekly, analyzed data, and correlated sampling methodologies to total population counts.

Will provide information for the selection of sampling methods and interpretation of sampling data.

Accomplishments:

Methods and materials were developed and tested to elucidate the actual size of a GWSS population in single orange trees, and total population counts of GWSS were obtained from individual trees.

Procedures were developed for comparing sampling techniques with absolute counts of all GWSS in small citrus trees.

Data are beginning to answer how many GWSS are actually present in a given block of citrus.

Efforts are underway to correlate sample numbers from beat counts to total GWSS populations.

Expected Benefits: These studies have already demonstrated that: 1) exceedingly large numbers of GWSS may be present in individual citrus trees (400-600 in 6-7 ft trees and 4000 in conventional sized trees); 2) beat or visual sampling may not detect GWSS at actual population

levels of 20 to 80 GWSS or even higher; and, 3)appreciation of these numbers has led to investigations of whether GWSS populations actually do impact citrus harvest. Also, this work will be used to understand how sampling methods in current use relate to actual population numbers, i.e., to interpret qualitative sampling measurement data with respect to quantitative population numbers.

Goal 3: Determine relationships between climatological factors and GWSS overwintering.

Current Situation: The arrival of GWSS in southern California has dramatically changed the epidemiology of PD. The insect is now present in the San Joaquin Valley and was first detected in Kern County in 1998. However, the insect's rapid population expansion first observed in southern California appears to be constrained to discrete regions within the Central Valley in or adjacent to citrus production areas where overwintering populations are greatest and winter temperatures are relatively mild when compared to expansion at lower elevations in the valley. Presently, there is limited information on the overwintering biology and ecology of GWSS in this large and important agricultural producing region. This lack of knowledge of the basic field ecology of GWSS limits our understanding of the spatial and temporal distribution of GWSS populations and the risk this pest poses for crop (e.g., grape and almond) production.

Objective 1: Determine GWSS population dynamics and overwintering.

Approach: The seasonal population dynamics and overwintering survival of GWSS populations are comparatively examined in agricultural areas of the Central Valley. Adult GWSS feeding and survival in climate-controlled growth chambers are determined to establish the threshold for feeding activity under different combinations of host type and temperature regimes. Using this information, describe differential GWSS mortality and fecundity in different regions and among combinations of host species.

Cooperators: ARS-Parlier (J. C. Chen, H. Lin, E. Backus) is collaborating with Marshall Johnson (UC-Riverside).

Benchmarks:

Experimental GWSS colonies are being established and maintained in a reproductive diapause condition comparable to overwintering populations.

Experimental bioassays will be conducted in environmental chambers to determine the GWSS temperature-dependent feeding activity threshold.

Results will be coupled with high resolution, 1-km scale, and climatological data to spatially define overwintering refugia for GWSS.

Accomplishments: Report in progress.

Expected Benefits: The results of these experiments will aid our ability to define specific environmental constraints that influence population dynamics and overwintering success of

GWSS. Moreover, the results from these experiments will be coupled with high resolution, 1-km scale, climatological data to spatially define locations where GWSS populations may be unable to successfully overwinter, or, conversely, where populations may find overwintering refuge from extended periods of critical temperatures.

Objective 2: Determine the phenology and demography of Homalodisca coagulata in southern California citrus.

Approach: Effective pest management is often realized through timely control actions initiated at a critical point in the growth and development of pest populations. Knowledge of the phenology of a pest organism can increase awareness of infestation patterns and help identify periods of pest vulnerability. Development of a better understanding of the ecology of a pest species towards more effective pest control includes learning seasonal patterns of abundance and scarceness. Further refinement of a management strategy can be obtained by characterizing the demographic structure of a pest population to enable more expert targeting of particular developmental stages. Because of the fundamental importance of citrus to the population dynamics of GWSS in California, populations were studied over two consecutive years in southern California citrus groves. GWSS populations were sampled weekly over the two year period to determine relative abundance and demographic structure of these populations throughout the annual cycle.

Cooperators: ARS-Phoenix (S. J. Castle, S. E. Naranjo) in collaboration with UC Riverside (Nick Toscano)

Benchmarks:

Established study sites at UC Riverside's Agricultural Operations and began weekly sampling in April.

Expanded study area and continued weekly sampling in experimental citrus orchards. Applied systemic insecticide timed to coincide with the emergence of first instar nymphs in April.

GWSS samples processed and identified to nymphal instar; sex ratios of adults also evaluated.

Data analyses and a phenological model developed to aid with decision making for GWSS management.

Accomplishment:

Castle, S. J., Naranjo, S. E., Bi J. L., Byrne, F. J., and Toscano, N. C. 2005. Phenology and demography of *Homalodisca coagulata* (Hemiptera: Cicdellidae) in southern California citrus and implications for management. Bulletin of Entomological Research **95**, in press.

Expected Benefits: A better understanding of the seasonal occurrence of GWSS in southern California citrus has been attained with this study. The important question of how many generations of GWSS are produced in citrus was determined to be one effective generation with

the second generation diminished overwhelmingly due to parasitism. The demographics of GWSS populations were incorporated into a phenology model that will help pest managers predict when specific stages are present and enable them to use selective treatments.

Goal 4: Determine population dynamics of GWSS with respect to Xf transmission and the occurrence of disease. This includes correlating the effects of crowding, sex ratio, reproductive status, infectivity status, host-plant quality, feeding ecology, and seasonal and environmental variables with population dynamics and movement of GWSS as an aid to predicting insect and disease spread, and applying control strategies.

Current Situation: GWSS feeds on a wide variety of cultivated crops and landscape ornamental plants, as well as on native plant species, and vectors Xf, which causes PD and many other plant diseases. Effective management of Xf-caused diseases will depend on understanding the biology and ecology of this and other vectors of the causative agent(s), as well as knowing what proportion of the population is actually vectoring Xf. Of particular interest is understanding the behaviors of these vectors as related to the spread of Xf.

Objective 1: Determine and characterize the pattern(s) of utilization/ preferences of plant hosts among cultivated crops and non-cultivated hosts in agricultural production systems.

Approach: The seasonal host utilization patterns of sharpshooter species within and among a variety of cultivated, perennial crop plant species and non-crop, wild plant species will be examined. Crop utilization patterns are being monitored within perennial crop species including grape, citrus (navel and lemon), stonefruit (peach and plum), olive, cherry, pistachio, and avocado at each of three locations for each crop type through weekly sampling for the presence of sharpshooter adults using yellow sticky, beat-net, and sweep-net counts of all life stages, and visual inspections. At each location, sharpshooter and spittlebug adults associated with orchard ground cover and surrounding non-crop vegetation are sampled using a standard sweep net. The presence of *Xf* in a subsample of vectors captured on yellow cards and on perennial and non-crop species are determined using PCR and strain specific primers are used to investigate the pathotype profile.

Cooperators: Jianchi Chen (ARS-Parlier) is collaborating with Kent Daane UC-Berkeley) and Marshall Johnson (UC-Riverside).

Benchmarks:

Experimental field plots have been established in GWSS-infested areas to monitor seasonal population dynamics and timing of dispersal. Currently, the overwintering population dynamics of vector species on crop and non-crop species is being evaluated.

Continue to evaluate vector population dynamics through a second cropping season and overwintering period. Identify the pathotype profile of *Xf* from infectious vectors collected in 2003.

Further evaluate the infection status of vectors collected in the 2004 growing season and the 2004-05 winter seasons.

Accomplishments: Report in progress.

Expected Benefits: The results of these studies will provide further insight into the relative importance of different crop types as predominant overwintering habitats, ovipositional substrates, preferred feeding hosts, and sources for *Xf* acquisition and transmission to susceptible crops.

Objective 2: Determine the relative importance of visual and olfactory cues in host recognition and selection by adult and immature GWSS.

Approach: Although *H. coagulata* is strongly attracted to bright yellow objects, the relative importance of plant chemical cues in its host plant detection has not been demonstrated with any certainty. To observe and quantify behavioral responses to combinations of olfactory and visual stimuli, novel olfactometry and behavioral assays were developed which were tailored to several key behaviors displayed by GWSS; e.g., a strong tendency to feed and remain on stems and display a distinctive scanning behavior prior to departing a host plant. Responses were measured with no-choice tests in which a single, binary color-odor combination was presented to individuals perched on a release stick. Analysis of data generated with these assays provided an assessment of the relative effects of visual, olfactory, and visual x olfactory stimuli on close-range host plant detection in adult and immature GWSS.

Cooperators: Joe Patt (ARS-Weslaco) is collaborating with Mamoudou Sétamou (Texas A&M University Citrus Center).

Benchmarks:
Develop novel olfactometery and accompanying behavioral assays and conduct initial experiments on response to visual, olfactory, and visual x olfactory stimuli.

Develop methodology to measure response to specific volatile profiles emitted by host plants.

Accomplishments:

The relative effects of spectral (color) and chemical stimuli on host plant detection in immature and adult GWSS was successfully studied using two novel bioassays and factorial experimental designs. Both main effects and interactive effects of the stimuli were observed. The residence and orientation times of nymphs on perch sticks decreased in the presence of host plant chemicals. In adults foraging behaviors were enhanced and phototaxic behaviors curtailed in the presence of host plant chemicals. Although the sharpshooters responded to color stimuli presented alone, host plant chemicals (e.g., foliar volatiles) enhanced their response to color stimuli. The results also indicate that information from visual and chemical cues is integrated, with labial dabbling behavior more strongly influenced by visual cues and probing behavior more strongly influenced by chemical cues.

Patt, J. M. and M. Sétamou. 2006. Chemical and visual stimuli affecting host plant recognition in *Homalodisca coagulata*(Hemiptera: Cicdellidae). *In Review. Environmental Entomology.*

Expected Benefits: Virtually nothing is known about the underlying behavioral and chemical ecology underlying GWSS recognition and selection of host plants. The results from this study will provide basic information that can help determine how GWSS selects host plants within complex landscapes.

Objective 3: Determine how biotic and abiotic factors influence the relative movement of GWSS and the native smoke-tree sharpshooter (STSS) to help understand the dynamics of the spread of Xf.

Approach: Mark-release recapture (MRR) studies with IgG protein markers were used to compare rates of movement of GWSS and smoke tree sharpshooters (STSS) in simple and complex host-plant assemblages. The associations between sharpshooter movement and environmental parameters, as well as host-plant characteristics were determined. The spatial scale of movement was analyzed by regression analysis and a diffusion model. Mark-capture (MC) studies using inexpensive protein markers are underway to compare rates of movement of GWSS in citrus exposed to different water management strategies.

Cooperators: James Hagler and Jackie Blackmer (ARS-Phoenix) are collaborating with ARS-Parlier, Kent Daane (UC, Berkeley), and Marshall Johnson (UC, Riverside) on the MRR and MC research.

Benchmarks:

Established validity of using an IgG protein marker for GWSS dispersal studies. It had no effect on longevity and remained effective for at least 19 days under field conditions.

Established that STSS dispersed further than GWSS in a MRR regime; however, the dispersal of both species was similarly affected by temperature and wind speed.

GWSS dispersed more slowly in a complex host assemblage when compared to a simple assemblage, and the timing of dispersal was correlated most strongly with time of day and fluctuations in xylem pressure.

Further determine how fluctuations in host-plant quality influence sharpshooter movement. Developed three inexpensive protein marking ELISAs that can be used for marking GWSS and it's natural enemies directly in the field using conventional spray rig equipment for large-scale MR studies.

Applied the MC protein marking system to a large-scale study deducted to quantify GWSS and natural enemy dispersal in citrus exposed to various water management strategies.

Accomplishments:

Immunoglobulin (IgG) proteins were tested as potential markers for dispersal studies with GWSS and STSS. Both chicken and rabbit IgG proteins were effective in marking sharpshooters. The marker remained effective under field conditions for at least 19 d and had no effect on survivorship of GWSS.

MRR studies with GWSS and STSS were conducted in 2001 in Moreno Valley in a simple landscape (abandoned alfalfa), and in 2002 in a complex landscape (citrus orchard). Both species readily dispersed horizontally to 90 m and vertically to 7 m; the most distant traps contained a larger percentage of the STSS. For these releases, temperatures above 17º C were correlated with increases in take-offs, and wind speeds over 3 m s^{-1} resulted in a significant reduction in take-off activity. In MRR studies, recapture rates were 12 percent in the simple landscape and 1.6 percent in the complex landscape. Linear regressions of recapture data with the diffusion model provided significant fits to the data for all releases except two. Calculations of dispersal distances using the diffusion model showed that 95 percent of GWSS had moved 90 m in 6 h or less, while 95 percent of STSS had moved 155 m in the same period. In the complex landscape, GWSS movement was much slower; 95 percent of GWSS were recaptured within 99 m of the release site, during a 72 h recapture interval. In 2004, we developed and tested three inexpensive protein marking ELISAs. Each ELISA is specific to either chicken egg whites, soy milk, or nonfat dry milk. The retention of the proteins on insects in the field is as good or better than those reported above for the extremely expensive rabbit IgG and chicken IgG proteins. In 2005, we sprayed these proteins with conventional spray equipment separately to large blocks of citrus that were exposed to three different irrigation regimes (e.g., 100, 80 or 60 percent of the normal amount of water used to irrigate citrus). Currently, we are assessing the dispersal patterns of GWSS and its natural enemies between these large blocks of citrus. The ELISA analyses will allow us to precisely determine the origin of every GWSS (and other insects) in this experiment.

In 2002, we investigated whether plant factors (i.e., amino acids, osmolality, xylem pressure) and environmental parameters (i.e., wind speed, temperature, relative humidity, barometric pressure) influenced sharpshooter population dynamics and movement in a citrus grove setting. Number of egg masses and adults were counted on branches that were sampled for xylem sap. Collection date, tree, and cardinal direction were noted, and xylem pressure, and amino acids (total, essential and amides) were measured. In conjunction with xylem sap collections, movement of sharpshooters was monitored with yellow and clear sticky traps at 4-h intervals during the day and throughout the night. During replicated sampling periods, 40 times more sharpshooters were trapped on yellow sticky traps in comparison to clear sticky traps and the majority, regardless of sex, were trapped between 1000 and 1400 h. Higher trap catches were associated with increasing temperatures above 18ºC, but were not significantly associated with changes in wind speed, relative humidity or barometric pressure. Trap catches varied significantly over the trapping season, but did not differ due to trap location, indicting that there was no strong edge effect for GWSS. Relative to xylem sap collections, xylem pressure and amides varied due to collection date and time of day, and xylem pressure was positively correlated with trap catches. Osmolality, total amino acids, essential amino acids, and percent amides had no apparent relationship with trap catch. GWSS egg counts varied significantly due to collection date and

cardinal direction, with the majority of eggs were observed on the east and south sides of the trees.

Blackmer, J.L., J.R. Hagler, G.S. Simmons & L.A. Cñas. 2004. Comparative dispersal of *Homalodisca coagulata* and *Homalodisca liturata* (Homoptera: Cicdellidae). Environ. Entomol. 33: 88-99.

Expected Benefits: Environmental variables and host-plant quality influence insect population dynamics and the timing and extent of their dispersal. An understanding of how these factors influence GWSS development and movement will aid us in predicting the spread of PD, as well as aid area-wide management strategies. The new serological method for MRR and MC studies of sharpshooter movement will facilitate efforts to delineate the insect's dispersal and spread of Xf.

Objective 4: Determine adult GWSS feeding and oviposition preference for, and nymphal development rates and survivorship on, healthy and Xf-infected grape representing different stages of plant infection.

Approach: Plant physiological status and resulting behavioral responses by phytophagous insects can impact vector ecology and patterns of pathogen spread. The proposed study is designed to determine the effects of Xf infection and resulting plant physiology on the distribution, performance, and behavior of GWSS and its associated natural enemies in controlled greenhouse experimental bioassays and in a small-plot field experiments.

Cooperators: ARS-Parlier is collaborating with Matthew Blua (UC-Riverside).

Benchmarks:

Field plots established in Riverside, California.

Experiments are planned.

Accomplishments: This research is just being initiated.

Expected Benefits: Taken together, these analyses will provide a comprehensive evaluation of how plant infection can influence the population dynamics of the vector, and, in return, PD epidemiology. This knowledge will be critical to evaluate the importance of early pathogen detection and development of crop management practices aimed at minimizing the extent of within-field, secondary spread. Elucidation of the preference for, and performance upon, Xf-infected versus healthy *Vitis vinifera* plants will aid our understanding of the mechanism of spread of PD and the speed with which in-field secondary pathogen spread can occur.

Objective 5: Determine proportion of the GWSS adult population that is carrying Xf, and what proportion of these that actually transmit Xf to experimental grapevine and oleander plants.

Approach: The rate of Xf transmission in the natural environment is a fundamental component of the epidemiology of Xf, but one that is thus far poorly defined. By investigating the proportion of GWSS infected with Xf (i.e. positive for presence of Xf) and determining the proportion of these that are inoculative (i.e. positive for transmission of Xf), greater understanding of the relationship between GWSS densities and Xf incidence in vineyards or other plant stands will be obtained. Measurement of GWSS infectivity and inoculativity may address the issue of whether or not there is an upper threshold of GWSS numbers that can be tolerated in a given region. An approach that balances findings from the laboratory with monitoring information from the field will improve our understanding of how epidemics of Xf occur in vineyards and elsewhere.

Cooperators: ARS-Phoenix (S. J. Castle) in collaboration with UC Riverside (Nick Toscano).

Benchmarks:

Preliminary surveys of GWSS adults conducted to determine the proportion positive for Xf using ELISA and PCR detection procedures.

Systematic sampling of GWSS adults began in August to evaluate potential changes in proportion positive for Xf relative to the proportion that transmit Xf to experimental plants.

First transmission results using field-collected GWSS adults show that a high proportion (>50 percent) of GWSS and STSS adults collected in February transmit Xf to grapevine.

Accomplishments: This research is still in progress, but already there have been notable findings concerning dynamics of the Xf reservoir within the GWSS population.

Field collections of GWSS made between August 2004 and February 2005 showed an increasing proportion of the population positive for Xf. The mean titer of Xf in the field samples also increased through the fall months, but then diminished from peak levels during 3 collections made in the winter months of December and February. Differences among collection locations were observed in the proportion of the respective populations positive for Xf.

Results from a single transmission experiment conducted 6 February 2005 demonstrated that field-collected adults not only test positive for Xf by ELISA, but also transmit Xf to grapevine test plants (var. Chardonnay). An initial evaluation of xylem fluid collected from multiple branches per test plant revealed as many as 11 plants out of 15 exposed to individual field-collected STSS adults become infected with Xf. For test grapevines exposed to individual field-collected GWSS adults, as many as 5 plants out of 8 tested positive for Xf following 3 day inoculation access periods for individual insects. Additional transmission tests could not be completed due to an absence of GWSS adults in the field at UC Riverside's Ag Ops.

Expected Benefits: The proportion of the GWSS population actually transmitting Xf in the natural environment is an essential element towards a clearer understanding of the risks to Xf-vulnerable crops associated with variable densities of GWSS. Results from this study will provide an estimate of vector infectivity as well as vector inoculativity, an important distinction

in terms of those insects that are positive for Xf (by ELISA or PCR) compared to those actually transmitting Xf to plants. Information obtained on the seasonal incidence of Xf in GWSS populations will also provide insight into times of the year in which GWSS populations are more or less threatening to a grape vineyard or almond orchard.

Goal 5: Determine GWSS feeding behavior, and nutritional and storage needs for use in developing mass production systems and in elucidating pathogen transmission.
Current Situation: Control of PD will largely be dependent upon areawide suppression of GWSS until resistant plant varieties are developed. Unfortunately, more than 130 plants can serve as hosts for GWSS and as reservoirs of the disease, often necessitating insecticidal applications in urban and wilderness areas adjacent to commercial fields. Biological control of GWSS in these areas would be more economically and environmentally desirable, and an important IPM strategy for this pest. Mass production of GWSS is needed for use in biological control programs. Production of one of the most effective biological control agents, *Gonatocerus* spp., parasites of GWSS eggs, would be critical to the biological control approach, but has been hampered by the difficulty and cost associated with producing sufficient numbers of GWSS eggs on which to rear the parasites. Development and formulation of artificial diets are needed for designing improved, reliable GWSS mass rearing protocols. Better knowledge of GWSS nutritional needs and feeding behavior would facilitate mass rearing, improve understanding of GWSS feeding ecology, and also would help elucidate pathogen transmission mechanisms. (Also, see Research Area III, Goal I, for studies of GWSS feeding in relation to Xf transmission.)

Objective 1: Determine feeding mechanism of GWSS and study stylet penetration into host plants by documenting the path of mouthparts from the epidermal layer to the vascular tissue and determine if feeding includes parenchyma or phloem tissue en route to xylem tissue. Determine ultrastructural characteristics of the salivary sheath, its chemistry, and interaction with all plant tissues along the stylet path from plant surface to xylem tissue.

Approach: Roger Leopold (ARS-Fargo) rears GWSS at the Bioscience Laboratory, on the North Dakota State University campus. This provides the ARS-Fargo team (including James Buckner) with a continual supply of immature and adult insects feeding on a variety of host plants. For morphology and ultrastructure of sharpshooter mouthparts, immature and adult GWSSs are examined using confocal scanning light microscopy (CLSM) of intact, cleared, and dissected specimens.

Cooperators: James Buckner (ARS-Fargo) is collaborating with Thomas Freeman (Electron Microscope Center, North Dakota State University, Fargo, ND) and Roger Leopold (ARS-Fargo).

Benchmarks:

A description of GWSS mouthpart morphology and characterization of plant penetration has been completed.

This project has been terminated and a new research direction has been implemented.

Accomplishments:

The gross morphology and ultrastructure of the labrum, labium, and stylet fascicle was described. Stylet probing in host tissue was found to be largely intercellular, with salivary sheaths frequently showing multiple branches.

Leopold, R. A., T. P. Freeman, J. S. Buckner, D. R. Nelson. 2003. Mouthpart morphology and stylet penetration of host plants by the glassy-winged sharpshooter, *Homalodisca coagulata* (Homoptera: Cicdellidae). Arthropod Structure & Development 32(2-3):189-199.

Expected Benefits: Cellular and ultrastructural data on GWSS mouthpart penetration of host tissues is required if we are to fully understand the interactions of the insect, the bacterium, and the host. Benefits will be a better understanding of nutrient uptake, pathogen transmission, and host suitability.

Objective 2: Advance our understanding of GWSS nutritional requirements and response to chemical components in the food stream.

Approach: Increased knowledge of GWSS nutritional needs, digestive physiology and impact of nutrition on the efficiency of diet utilization would simplify development of artificial rearing methods, including formulation of artificial diets and diet-delivery systems. Development of genomic biomarkers to monitor fitness traits related to nutrition would accelerate and enhance formulation of high performance diets.

Cooperators: Tom Coudron is collaborating with Elaine Backus (ARS-Parlier), Joe Patt (ARS, Weslaco), Wayne Hunter (ARS, Ft. Pierce), and Roger Leopold and George Yocum (ARS, Fargo).

Benchmarks:
Evaluate performance of immature and adult GWSS when reared on several combinations of diet formulations and delivery systems.

Two potential sources for nitrogen, i.e. proteins or peptides, are being studied by determining the fate of dietary proteins/peptides and the ability of salivary and midgut proteolytic enzymes to digest proteins/peptides.

Differentially expressed genes will be selected and evaluated for use as nutritional biomarkers.

Accomplishments: Studies indicate different survival responses by GWSS adults to changes in the carbohydrate and amino acid content of their diet. Collaboration with Jones and Setamou (ARS, Weslaco) has demonstrated continuous feeding by adult GWSS for up to 39 days on artificial diets presented through a specialized feeding tube. In addition, molting was observed with immature GWSS that fed from this system. Best performance of GWSS reared on an artificial diet was accomplished through the simultaneous testing of different formulations and delivery system designs, i.e., testing of over 25 diet delivery systems in combination with over

10 diet formulations Optimal performance parameters for artificial diets were different for adults versus immature GWSS.

Expected benefits: An artificial diet and delivery system will result in increased output and performance or health of mass-reared GWSS at a reduced cost of production. A better understanding of biochemical and physiological linkages between nutrition and developmental performance will assist in optimizing diets. The discovery of nutritional biomarkers that respond to dietary changes will be used to direct diet formulation so as to result in diets that support improved performance and fitness criteria.

Objective 3: Determine xylem sap components and GWSS feeding biology as clues to developing an artificial diet for GWSS rearing.

Approach: Develop an improved understanding of the specifics of the feeding dynamics of GWSS in order to more adequately satisfy nutritional requirements in an artificial feeding system. The assumptions behind this work are that the profile of components in xylem sap used by GWSS will be an optimal or at least suitable diet for this species, and that details of the feeding biology of the insect will help identify its dietary needs. Studies are conducted to detail the plant's sap profile before and after feeding.

Cooperators: Allen C. Cohen (ARS-Mississippi State, retired) collaborated with David Morgan (CDFA-Riverside), Isabelle Lauziere (APHIS-Mission), and Stephanie Rill (CDFA-Oswell Street Biocontrol Laboratory).

Benchmarks: *This work was terminated after the retirement of the project leader, A. Cohen.*

Accomplishments:

Examination of 100 salivary sheaths revealed that they are characteristically straight, leading directly from the plant surface to xylem bundles; this is in contrast with branching seen for 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 ingestion of large amounts of xylem sap (which is under negative pressure in the plant's vascular system). The filter chamber is extremely active in peristaltic movements that evidently increase efficiency of concentration of the sap and transfer of water to the Malpighian tubules, which remove water and carry it directly into the hindgut where 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 final nutrient products are absorbed by microvilli that are on the surface of a highly convoluted series of tubules.

One of the most unexpected and important findings in this work is the discovery of exceptionally great amounts of aminopeptidase activity and general peptidase activity in the salivary glands, filter chamber, anterior midgut, posterior midgut, and Malpighian tubules. GWSS has a much greater amount of amino peptidase activity, indicting that this insect uses nitrogen sources other than free amino acids, most likely peptides or even proteins. As a xylem sap feeder, GWSS was expected to lack ability to digest peptides because xylem sap is not known to contain substantial

amounts of peptides or proteins. These findings led to our studies of interactions between GWSS and host plants which revealed that the profiles of free amino acids in xylem sap in infested and un-infested sweet potato plants show an increase in concentrations of most amino acids in xylem sap of infested plants.

As a result of these studies, further research efforts were made to provide an artificial diet that contained short peptides, including tryptic soy and casein digests, along with sugars, a dilute salt and vitamin mixture, and small amounts of organic acids that characterize xylem sap, the natural food of this species. Also, a flow-through feeding system based on membrane feeding was used successfully to present the diet.

The molecular mechanisms underlying plant defense against sap-feeding insects are slowly being uncovered, with emphasis on xylem-feeders of woody perennials—especially fruit trees. Using cDNA arrays, we analyzed transcriptional changes of 1731 non-redundant citrus transcripts that resulted from xylem-feeding GWSS. In addition, we compared herbivory elicited changes to that of mechanical damage to identify GWSS-specific responses. GWSS feeding led to a significant expression change in 51 transcripts. Of these, 14 were also changed by mechanical damage. Sequence similarity searches to public database GenBank entries indicted that the responsive transcripts broadly function in direct defense, defense signaling, ROS scavenging, transport, cell wall modification, photosynthesis, and abiotic stress response. In particular, GWSS feeding resulted in a transcript profile that resembled wounding, likely through jasmonmic acid-independent pathways as well as an association with dehydration stress. Interestingly, seven of the GWSS-responsive transcripts failed to significantly match any public protein sequence, signifying their potential as novel genes functioning in plant defense, wound response, or abiotic stress.

Transcriptional changes resulting from herbivory by the xylem-feeding leafhopper *Homalodisca coagulata* (Say) (Hemiptera: Cicdellidae) in the vascular transcriptome of *Citrus sinensis* L. Osbeck. Jerry Mozoruk, Michael G. Bausher, Ronald D. Cave, Wayne B. Hunter, and Laura E. Hunnicutt. 2005. Plant Science (submitted).

Expected Benefits: Development of a suitable diet for GWSS would lead to techniques for the mass rearing of promising natural enemies of this important pest species.

The molecular mechanisms underlying plant defense against sap-feeding insects are being identified, with an emphasis on xylem-feeders of grape and fruit trees. This would permit further analysis of induced plant responses to disease and insects that will advance development of improved plant cultivars.

Objective 4: Study feeding ecology of GWSS by determining amino acid and carbohydrate composition of xylem fluid in plants in relation to variable densities of feeding GWSS.

Approach: Establish a study site in a mixed planted citrus orchard at the interface of lemon trees and Valencia orange trees. Monitor GWSS densities weekly on both citrus species and sample xylem fluid from the same set of trees every two weeks for biochemical analyses. A statistical

analysis will then evaluate whether particular nutritional complements of xylem fluid are related to higher or lower densities of GWSS.

Cooperators: ARS-Phoenix (S. J. Castle) in collaboration with UC Riverside (J. L. Bi and Nick Toscano).

Benchmarks:

Established study sites at UC Riverside's Agricultural Operations and began weekly sampling in April.

Expanded study area and continued weekly sampling in experimental citrus orchards. Also began xylem fluid analyses using a Perkin-Elmer amino acid analyzer.

Continued to process xylem fluid samples for determination of amino acids and carbohydrates.

Accomplishment:

Bi, J. L., Castle, S. J., Byrne, F. J., Tuan, S. J., and Toscano, N. C. 2005. Influence of seasonal nitrogen nutrition fluctuations in orange and lemon trees on population dynamics of the glassy-winged sharpshooter (*Homalodisca coagulata*). Journal of Chemical Ecology **31**, 2289-2308.

Expected Benefit: Improved understanding of the feeding ecology of GWSS as it relates to nutritional components in plants. Natural shifts in densities of GWSS feeding on various plants correlated to fluctuations in particular amino acids within xylem fluid. The observation that amino acid profiles in plants are not constant help to explain why movement and resettlement on a wide range of hosts is such an essential part of the ecology of GWSS.

Objective 5: Develop artificial (diet) eggs for oviposition and rearing of GWSS parasites.

Approach: Develop an artificial diet for production of artificial "eggs" that could be used to economically rear *Gonatocerus* parasites of GWSS. Screen and optimize existing artificial diets for ability to sustain and promote development of *Gonatocerus* spp. parasites of GWSS. Develop a suitable artificial ovipositional substrate for *Gonatocerus* spp. Develop bioassays that can be used to screen bactericidal proteins linked to PD, or insecticidal resistance proteins linked to GWSS, resistance for their effects on *Gonatocerus* spp., and, perhaps, other nontarget insects. This would enable plant breeders to assess the risk of these proteins before incorporating them into commercial varieties.

Elucidate genes and proteins associated with GWSS digestion and feeding to develop genetic markers linked to diet suitability. (2005)

Examine GWSS vitellogenin production and development of eggs for potential in artificial protein production, and/or down regulation to reduce GWSS populations (Wayne Hunter, USDA, ARS, USHRL).

Identify and characterize enzymes linked to lipid utilization in GWSS (Wayne Hunter, USDA, ARS, USHRL).

Cooperators:

Artificial diet for GWSS research would be done by Tom Coudron (ARS-Columbia). Collaborator: Wayne Hunter, ARS-Ft. Pierce, FL.

Benchmarks: *This work has not been funded and will not likely be continued unless additional resources are found.*

Accomplishments:

Successfully sequenced GWSS vitellogenin and characterize protein.

Hunter, WB. and LE. Hunnicutt. 2005. Vitellogenin cDNA of the glassy-winged sharpshooter Journal Insect Science (submitted).

Identified and characterized Delta-9 Desaturase from GWSS.

C. S. Katsar, W.B. Hunter, and C. A. Cleland. 2005. *In silico* analyses of Delta 9 Desaturase 1 from the Glassy-winged Sharpshooter, *Homalodisca coagulata* (Hemiptera: Cicdellidae). Insect Molecular Biology (Submission).

Expected Benefits: Development of cost-effective and less labor-intensive methods for mass rearing of the parasitoids to be used for the biological control of GWSS.

Objective 6: Develop storage technology for use in the mass rearing of GWSS and its parasitoids.

Approach: Determine the cold tolerance of the egg parasitoids, *G. ashmeadi* and *G. triguttatus* within host eggs of GWSS under specific environmental conditions and developmental stages. Assess whether chilling during an immature developmental stage has latent damaging effects on quality of the adult parasitoid. Determine efficacy of extending shelf life of GWSS eggs for use by mass-reared parasitoids, by the method of pre-conditioning females environmentally and/or nutritionally.

> *6a. Examine the effect of constant and fluctuating temperatures on cold storage of parasitized and unparasitized eggs of the glass-winged sharpshooter.*

Approach: By using controlled environmental chambers, determine the cold tolerance of GWSS eggs stored at constant and fluctuating low temperature over time and evaluate development of eggs and nymphs and reproduction of adults following cold storage. Determine the effect of cold storage using constant or a daily stepwise-fluctuating temperature on post-storage parasitism and emergence by egg parasitoid, *Gonatocerus ashmeadi*. Determine the cold tolerance of the egg

parasitoid, *G. ashmeadi*, during development within GWSS eggs and attempt to extend shelf time of the parasitoid by altering subambient storage temperatures.

6b. Determine functional responses and incidence of superparasitism by the parasitoid, Gonatocerus ashmeadi, using glassy-winged sharpshooter eggs as the host.

Approach: Characterize functional response of the parasitoid to GWSS eggs of different ages. By using various host-parasitoid ratios, determine the effect of host densities and ages on development time of wasps and superparasitism of the hosts.

6c. Examine the influence of temperature on development and reproduction of the egg parasitoid, Gonatocerus ashmeadi.

Approach: By using controlled environmental chambers, determine the effect of temperature on rate of development from egg to adult stage, the temperature and thermal constants of immature stages, adult emergence pattern, life-table parameters and reproduction of *G. ashmeadi* when using the glassy-winged sharpshooter egg as host.

6d. Investigate use of dead glassy-winged sharpshooter eggs for propagation of the parasitoid, Gonatocerus ashmeadi.

Approach: Determine suitability and acceptability of GWSS eggs killed by 5 or 11 day exposure to a lethal cold temperature as hosts for propagating *G. ashmeadi*. Determine the most acceptable stage of stopping GWSS egg development for propagating *G. ashmeadi.* Assess quality of *G. ashmeadi* progeny reared on dead GWSS eggs stored for various periods of time by evaluating progeny fecundity and lifespan.

Cooperators: Roger Leopold and George Yocum (ARS-Fargo) are collaborating with David Morgan (CDFA-Riverside) and Marion Harris and Wenlong Chen (ND State-Fargo).

Benchmarks:

Established laboratory colonies of GWSS, *G. ashmeadi* and *G. triguttatus.* Determined optimum host plants for rearing and oviposition. Initiated cold tolerance studies on parasitized eggs of GWSS.

Determined low temperature limits for development of egg parasitoids and GWSS. Identified preferred ovipositional host plants of the GWSS that also have extended cold tolerance as cuttings for storage purposes.

Determined that daily cycling of cold storage temperature from below the thermal threshold for development to slightly above enhance survival of GWSS eggs and of *G. ashmeadi* when using GWSS eggs as hosts.

Obtained nearly 60 percent emergence of *G. ashmeadi* from killed GWSS eggs stored for 50 days and their progeny had the same fecundity and lifespan as controls emerging from live hosts.

Accomplishments:

By establishing a laboratory colony of GWSS, an ancillary study on GWSS mouthpart morphology and plant penetration was completed. The study identified previously undescribed accessory structures associated with the labrum, labium, and stylet fascicle provided impetus for electrophysiological and TEM studies for understanding the feeding process and host selection by the GWSS.

The functional responses and super-parasitism by the egg parasitoid, *G. ashmeadi* on *H. coagulata* eggs 1- 9 days old were measured over various host densities. The number of host eggs parasitized varies significantly with host density and age. Also, host age and density, as well as the host age × density interaction, contributes significantly to differences found in length of the development time of *G. ashmeadi* within host eggs It was determined that response of this wasp fits the type II model and frequency of superparasitism was randomly distributed over all experimental host densities.

The development, fecundity and life table parameters of *G. ashmeadi* were studied in the laboratory at six constant temperatures between 12 and 32˚C. It was established that lifetime fecundity was greatest at 24˚C, and lowest at 32˚C, with maximum net reproduction also occurring at 24˚C. Greatest intrinsic and finite rates of increase, shortest population doubling time, and mean generation time occurred when *G. ashmeadi* was held at 28˚C.

Leopold, R. A., T. P. Freeman, J. S. Buckner, D. R. Nelson. 2003. Mouthpart morphology and stylet penetration of host plants by the glassy-winged sharpshooter, *Homalodisca coagulata* (Homoptera: Cicdellidae). Arthropod Structure & Development 32(2-3):189-199.

Chen, W.L., R.A. Leopold, M.O. Harris. 2006. Parasitism of the glassy-winged sharpshooter, Homalodisca coagulata (Homoptera: Cicdellidae): Functional response and superparasitism by Gonatocerus ashmeadi (Hymenoptera: Mymaridae). Biol. Cont. 37: 119-129.

Chen, W.L., R.A. Leopold, D.J.W. Morgan, M.O. Harris. Development and Reproduction of the Egg Parasitoid, *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae) as a Function of Temperature. Envrion. Entomol. (submitted).

Expected Benefits: Effectiveness of any biological control agent used for pest control purposes depends on being released at the proper time. Unforeseeable environmental influences, such as those impacting pest migration, population fluctuations, and crop growth, amplifies the need for precise timing, especially when releases of insects are to be integrated into multi-disciplinary control programs. Development of cold storage technology for mass rearing of insects, such as the GWSS and its parasitoids, will allow insectary managers to gain flexibility and enable them to supply a purely biological product on demand.

Goal 6: Determine origin of the GWSS that invaded California and also determine the population genetic structure.

Current situation: Correct identification of a pest is extremely important to biological control programs. Geographic populations of the same species may differ in relevant biological characteristics of importance to biological control. In addition, knowing the native origin of an exotic pest is crucial for collection of natural enemies in the native range. The native range of the GWSS has been presumed to be the Gulf States and northeastern Mexico. Identification of the geographic source of the California infestation will lead to the identification of the sources of invasion, and also identify geographic areas with which to look for adapted natural enemies. To determine the invasive biology of an exotic pest, it is crucial to determine the geographic or population structure.

Objective 1: a) Develop molecular genetic markers for the GWSS to begin to genetically characterize this invasive pest, and b) determine the origin of the GWSS to determine the source of invasion in order to collect pre-adapted natural enemies.

Approach: Collect GWSS from across its geographic range and screen various PCR-based molecular marking techniques.

Cooperators: Jesse de León (ARS-Weslaco) is collaborating with [Walker Jones (formerly ARS-Weslaco)] and David Morgan (CDFA-Riverside). David Boyd (ARS-Poplarville), Jesusa Legaspi (ARS-Tallahassee), Rolando Lopez (Clemson University-Charleston), Robert Lynch (ARS-Tifton), Greg Simmons (APHIS-Phoenix), Russ Mizell (UF-Quincy) among others who have provided samples for testing are cooperating with Jesse de León .

Benchmarks:

Collect GWSS from its known range for DNA extraction.

Screen various PCR-based DNA fingerprinting methods (ISSR-PCR, RAMP, SAMPL, RAPD), develop various molecular markers, and compare sensitivity and efficiency of methods.

Perform a population study of the GWSS with the inter-simple sequence repeat-polymerase chain reaction (ISSR-PCR) DNA fingerprinting method.

Accomplishments:

DNA polymorphisms, for the first time, were detected in GWSS with four PCR-based DNA fingerprinting methods (ISSR-PCR, RAMP, SAMPL, and RAPD). The methods incorporating simple sequence repeats (SSR) were found to be the most sensitive and efficient methods with GWSS template. These methods were not only able to distinguish different sharpshooter species (*H. coagulata, H. liturata*, and *H. insolita*) but most importantly they were able to detect geographic variation in GWSS. The development of molecular genetic markers for the GWSS is of great significance because it now allows us to genetically characterize the GWSS, for example, it allows us to 1) determine genetic variation within and among populations, 2) determine gene flow mechanisms, 3) determine the population or geographic structure, and 4) determine the origin of the GWSS that invaded California.

de León, J. H., W. A. Jones, and M. González. 2002. Detection of DNA polymorphisms in glassy-winged sharpshooters (*Homalodisca coagulata*) by PCR-based DNA fingerprinting methods, pp. 171-172. *In* Proceeding, Pierce's Disease Research Symposium, 15-18 December 2002, San Diego, California. Complied by M. Athar Tariq, Stacie Oswalt, Peggy Blincoe, and Tom Esser, Sacramento, California

de León, J. H., and W. A. Jones. 2004. Detection of DNA polymorphisms in *Homalodisca coagulata* (Homoptera: Cicdellidae) by Polymerase Chain Reaction-based DNA fingerprinting methods. Annals of the Entomological Society of America 97: 574-585.

In the population study of the GWSS, compound inter-simple sequence repeat (ISSR) primers containing C-repeat motifs in their sequences were utilized to estimate the population genetic structure of GWSS from a total of 19 populations (544 total individuals) throughout the United States. Results showed significant partitioning of gene diversity at three levels, among regions, among populations within regions, and among populations. The results estimated the population genetic structure of the GWSS, but most importantly, the study demonstrated that the GWSS that invaded California is of Texas origin, but more than one 'founding event' occurred in California. The data showed that GWSS populations in the United States were genetically distinct, clustering into two main groups or clades, a 'southeastern' and a 'southwestern and western' clade.

Since the origin of the California GWSS infestation is Texas, the most effective natural enemies for release in California should be found in Texas. A separate study of egg parasitism in south Texas showed that an indigenous species parasitizes about 90 percent of GWSS egg masses throughout the season, perhaps at least partially explaining the uncommon occurrence of GWSS there.

de León, J. H., W. A. Jones, and D. J. W. Morgan. 2003. Population genetic structure of the glassy-winged sharpshooter determined by ISSR-PCR DNA fingerprinting, pp. 211-214. *In* Proceeding, Pierce's Disease Research Symposium, 9-11 December 2003, San Diego, California. Complied by M. Athar Tariq, Stacie Oswalt, Peggy Blincoe, and Tom Esser, Sacramento, California.

de León, J. H., W. A. Jones, and D. J. W. Morgan. 2004. Population genetic structure of *Homalodisca coagulata* (Homoptera: Cicdellidae), the vector of the bacterium *Xylella fastidiosa* causing Pierce's disease in grapevines. Annals of the Entomological Society of America 97: 809-818.

Expected Benefits:

Determination of GWSS genetics provides leads as to the degree to which management techniques need to be defined to a taxonomic unit below the species, and also, clues as to where to search for pre-adapted parasitoids.

III. Xf-Vector Interactions

ARS Research Context:

The efficiency by which GWSS serves as a vector of Xf depends on its interaction with the pathogen and the host. ARS-Parlier is focusing efforts on understanding vector acquisition and inoculation of the pathogen, using information gleaned through studies of sharpshooter feeding behaviors, as well as through an understanding of GWSS genomics. (Aspects of studies to elucidate feeding behavior in relation to GWSS nutrition are described in Research Area I, Goal 5.)

This work complements studies on the role of attachment factors in pathogenicity (UC-Berkeley). Other factors being studied include: i) the plant, as a substrate, and its affects on vector retention and inoculation of the pathogen (UC-Riverside and UC-Berkeley); and, ii) the impact of multiple strain infections on acquisition and inoculation (UC-Riverside). This work is aided by development of methods for detecting Xf in GWSS, primarily by PCR-based methods, at ARS-Parlier and UC-Riverside.

Goal 1: Determine the mechanisms of transmission (i.e., acquisition and inoculation) of Xf by GWSS (and other insect vectors).

Current Situation: GWSS is xylophagous and has a wide range of monocot and dicot hosts. The fine structure and function of the mouthparts of xylem-feeding insects, including the GWSS, are largely unknown. Information about the external location of feeding sites, stylet penetration (probing) behavior, salivary sheath formation within plant tissues and specific cells ingested, especially as related to acquisition and inoculation of Xf, is inadequate. Also, the role of watery, digestive saliva in inoculation and/or facilitating movement of Xf in plants is unknown.

Objective 1: a) Identify and quantify all probing (stylet penetration)behaviors of GWSS on grape, and b) identify the precise role of probing behavior in Xf acquisition and inoculation.

Approach: Specific probing behaviors are identified and quantified via electropenetration graph (EPG) monitoring. Waveforms are defined by correlation with stylet activities via videotaping of stylet movements and salivary sheath production in transparent diets, and through light and CLSM investigations of salivary sheath placement in plant tissues. Several methods for detecting Xf in the plant (i.e., PCR, culturing, ELISA, and immunocytochemistry) and in the vector (i.e., SEM examination of Xf in the foregut) are combined with EPG to determine the inoculation success of identified probes. In addition, electromyography or laser vibrometry are used with EPG to correlate specific stylet activities with movement of valves and muscles in the insect's foregut.

Cooperators: Elaine Backus (ARS-Parlier) is collaborating with Greg Walker (UC-Riverside) and Thomas Miller (UC-Riverside).

Benchmarks:

While at the University of Missouri, E. Backus established sharpshooter colony and plants and acquired equipment.

Performed EPG-histology-diet correlation experiments to identify and define AC EPG waveforms, developed AC-DC monitor and began AC-DC waveform correlations. Performed experiments, via several bacterial detection methods, to determine the phases of probing behavior that are correlated with inoculation by identified probes.

E. Backus assumed a new research position at ARS-Parlier, and moved experiments there.

Complete analysis of experiments by dissecting the heads of test insects to determine appearance of Xf colonies in foregut, to associate that appearance with results of probing-inoculation tests. Correlate electromyography of valve muscle movement with EPG waveforms.

Accomplishments:

Preliminary results show that certain EPG waveforms are correlated with detectable inoculation of Xf, and thus that specific probing behaviors control, in part, the inoculation process. Immunocytochemistry is the most sensitive method, and can successfully detect the presence of Xf cells near 8 of 10 identified probes' salivary sheaths, only 5 days after the probe. ELISA is the next most sensitive technique, followed by PCR. Culturing is highly insensitive; bacteria are recoverable from only 1 out of 10 identified probes.

Expected Benefits: Definitive knowledge of the mechanisms of transmission (i.e., acquisition and inoculation) may lead to clues to interrupt transmission. Use of EPG to produce standardized, reproducible, identified probes will greatly aid research on transmission and Xf-plant interactions. Also, identifying EPG waveforms representing inoculation will allow future development of a Stylet Penetration Index, for screening germplasm for resistance to inoculation behavior. Such an index can be used to greatly further research on artificial diets, feeding preferences, and the development of resistant varieties that will reduce crop production and management costs.

Objective 2: *a) Determine whether GWSS watery saliva plays a role in the mechanism of Xf inoculation or in subsequent movement of bacteria in grape. If so, then b), characterize the chemical composition of this saliva to determine which compounds exert the effect(s).*

Approach: a) Immunocytochemistry/histology will be used to visualize and localize antibody-labeled Xf cells, saliva, salivary sheaths, and possible cellular abnormalities in the vicinity of EPG-monitored, identified, inoculating GWSS probes. A needle-inoculation bioassay developed by J. Labavitch, a cooperator, will be used to establish an infection /disease time-course. A histological time-course study will determine whether the presence and distribution of saliva is associated with lateral movement of Xf cells from the site of inoculation, and/or possible cellular abnormalities or other signs of cell wall loosening that could result from actions of salivary enzymes and other solubilizing substances. If so, then b) biologically active salivary gland

substances will be identified using analytical methodologies, and individual substances and combinations of substances will be bioassayed via injection.

Cooperators: Elaine Backus (ARS-Parlier) is collaborating with Thomas Coudron (ARS-Columbia), Wayne Hunter (ARS-Ft. Pierce), and John Labavitch (UC-Davis).

Benchmarks:

Initiate immunocytochemical/histological time course of saliva.

Analyze data from time course study and begin to characterize saliva composition.

Correlate specific saliva component(s) with increased pathogenic characteristics.

Accomplishments:

Preliminary immunocytochemical/histological results (Objective 1) suggest that Xf cells move more rapidly, laterally, away from the site of insect-inoculation (near salivary sheaths from EPG-identified probes), when compared to movement of the bacteria after needle-inoculation (E. Civerolo, pers. comm.). Studies with other insects have repeatedly shown that oral secretions such as saliva account for the major differences between insect- and needle-inoculation of pathogens. Also, preliminary results from the same experiments suggest that cellular abnormalities occur near GWSS salivary sheaths. Such abnormalities are often initiated by saliva-mediated cell wall loosening. We hypothesize that lateral movement of Xf cells through pit cell membranes in adjoining xylem cells could be facilitated by the interaction of vector saliva with Xf chemistry.

Expected Benefits: Understanding the role of saliva in acquisition and inoculation will better delineate the total role of the insect in Xf etiology. For example, saliva could play an important role in differences in transmission efficiency among different vector species. This knowledge would allow development of a method for rapid, biochemical screening of xylem-feeding insects for vector potential that could lead to more rapid response to introduction of new, invasive Xf vectors, or existing native vectors, as Xf spreads.

IV. Xf–Host Plant Interactions

ARS Research Context:

In the research area of Xf-host plant interactions, ARS is focusing on understanding establishment of infection, and determining pathogenicity and virulence factors, which are crucial to the disease process. This research specifically aims at identifying physiological and biochemical responses of grape and almond to Xf infection, determining the basis for, and regulation of, these responses, and identifying host genes involved in the establishment of infection and disease development.

This work is complementary to that at UC-Davis, where researchers are examining Xf-resistant and susceptible *Vitis* germplasm to better define the process of pathogen establishment and pathogenicity. Another approach taken at UC-Berkeley and UC-Davis is to interfere with Xf cell-to-cell communication in xylem lumina, as a means of inhibiting establishment of colonies in the plant host. This will be greatly facilitated by a better understanding of the Xf cell surface, as being researched at UC-Davis and UC-Berkeley. Exopolysaccharide (xantham) gum genes revealed through the collaborative ARS-FAPESP (Brazil) Xf genome sequencing project are being exploited at UC-Riverside with the goal of degrading or inhibiting the production of gum to prevent clogging of xylem vessels, a purported factor in pathogenicity. Complementing this work, ARS-Ft. Pierce and UF-Quincy are collaborating to study gum gene expression in defined media. The gum gene work is part of a greater effort to determine gene function and regulation as it relates to factors associated with pathogenicity, as researched at ARS-Parlier, UC-Berkeley, UC-Davis, and the University of Florida. Differential induction of plant genes by putative Xf virulence genes is being studied at UC-Berkeley and UC-Davis. Determining the roles of vessel cavitations, cell wall metabolism, vessel occlusion and Xf movement in vessels, as studied at UC-Davis, will also yield clues that can be exploited through interruption of the infection/disease process, or through plant breeding. Xf virulence-related proteins, including outer membrane proteins (e.g., mopB), are being identified through a Specific Cooperative Agreement between ARS-Parlier and UC-Davis. Knowledge of the role(s) of these gene products in Xf virulence or pathogenicity could potentially lead to strategies for mitigating disease development. The effect of Xf strain on host plant specificity is being studied using whole or partial Xf genome DNA microarrays at UC-Berkeley and UC-Riverside, and at ARS-Parlier, based, on data obtained through the collaborative ARS-FAPESP (Brazil) Xf genome sequencing project.

Goal 1: Determine the nature of, and basis for, establishment of infection by Xf in grape.

Current Situation: Xylella diseases are complex pathosystems. The nature of, and basis for, colonization of xylem tissue and establishment of infection by Xf is not well understood.

Objective 1: Identify and characterize Xf pathogenicity and virulence factors.

Approach: Xf virulence-related proteins, including outer membrane proteins (e.g., mopB), will be identified, isolated, and characterized, and their role in establishment of Xf infection will be determined through a Specific Cooperative Agreement between ARS-Parlier and UC-Davis.

Mutants of Xf will be required to elucidate the mechanism underlying the ability of the pathogen to systemically colonize the vascular system and cause disease. The ability of Xf strains to colonize and cause disease in alternative hosts will be explored by experimental inoculation. The extent and speed with which Xf can colonize plant tissues will be studied with a combination of confocal microscopy of green fluorescent protein (GFP)-labeled bacteria and quantitative PCR.

Cooperators: John S. Hartung (ARS-Beltsville) is conducting the ARS research on Xf mutants. Vice-Civerolo will continue to cooperate with George Bruening (UC-Davis) in research on Xf virulence-related proteins.

Benchmarks:

In collaboration with George Bruening (UC-Davis), ARS-Parlier demonstrated the chlorosis-inducing-activity of intact Xf cells and a heat-stable, enzyme resistant fraction of Xf cells in *Chenopodium quinoa* (Cq).

ARS-Beltsville demonstrated transformation of Xf with an Xf/*E. coli* hybrid plasmid.

Demonstrated a greater range of diversity present in citrus strains of Xf in comparison with strains from grapevine and other host plants.

In collaboration with George Bruening (UC-Davis), ARS-Parlier developed a method to purify, isolate, and characterize a Cq chlorosis-inducing factor from intact Xf cells.

ARS-Beltsville created Tn5 insertion mutants in Xf (citrus strain) using a triparental mating approach for the first time in Xf. These mutants are simultaneously labeled with GFP.

In collaboration with George Bruening (UC-Davis), continued characterization of Cq chlorosis-inducing factor and identified it as mopB, an outer membrane protein.

ARS-Beltsville developed method to rapidly locate the Tn5 insertions in the Xf genome, and identified them by comparison to the published Xf genome.

In collaboration with George Bruening (UC-Davis), ARS-Parlier continued characterization of mopB, and establishment of its role as a virulence or pathogenicity factor.

Accomplishments:

The ARS-Beltsville laboratory has demonstrated that the ability of Xf strains to infect plants and incite disease is even greater than previously thought. By doing this, the laboratory has also demonstrated that the symptoms induced by Xf in plant hosts are entirely plant responses to infection, since a single strain (sweet orange) can induce the widely divergent symptoms of CVC, PD, coffee leaf scorch and periwinkle wilt in the appropriate host plant. This is useful, because alternative hosts are needed to expedite study of these diseases. The laboratory has developed an efficient mutagenesis strategy for Xf, as well as methods to localize the mutants so

that gene function can be ascertained from genomic sequence data. The laboratory has developed a novel system to follow such mutants *in planta*, using the GFP marker and confocal microscopy. The results of this work suggests that the expression of Xf genes is globally affected by passage through either sweet orange or tobacco plants.

1) Li, W., Zhou, C., Pria, Jr., W.D., Teixera, D.C., Miranda, V.S., Pereira, A.J., He, C.-X., Costa, P.I. and Hartung, J.S. 2002. Citrus and coffee strains of *Xylella fastidiosa* induce Pierce's disease of grapevine. *Plant Disease* 86:1206-1210.

2) Li, W.B., Pria Jr., W.D., Teixera, D.C., Miranda, V.S., Ayres, A.J., Franco, C.F., Costa, M.G., He, C.X., Costa, P.I. and Hartung, J.S. 2001. Coffee Leaf Scorch caused by a strain of *Xylella fastidiosa* from citrus. *Plant Disease* 85 (5):501-505.

3) Qin, X. and Hartung, J.S. 2001. Construction of a shuttle vector and transformation of *Xylella fastidiosa* with plasmid DNA. *Current Microbiology* 43:158-162

4) Qin, X., Miranda, V.S. , Machado, M., Lemos, E. and Hartung, J.S. 2001. An evaluation of the Genetic Diversity of *Xylella fastidiosa* isolated from Diseased Citrus and Coffee. *Phytopathology* 91(6):599-605.

5) Qin, X and Hartung, J.S. 2004. Expression of green fluorescent protein in *Xylella fastidiosa* is affected by passage through host plants. Current Microbiology 49:215-220.

Expected/Realized Benefits: Exploitation of genomic sequence information already available will be facilitated through the use of defined mutants. Such mutants are being used to study Xf/host interactions. The sweet orange strain of Xf has been demonstrated to cause disease in coffee, grapevine, periwinkle, and tobacco. More than 200 GFP-labeled mutant Xf strains are now available. The GFP label is being used to study colonization of grapevine tissues by confocal microscopy. These ARS-developed methods can be generalized, and will be useful to anyone needing labeled, defined mutants of Xf. Knowledge of the role(s) of gene products in Xf virulence or pathogenicity (e.g., mopB) could potentially lead to strategies for mitigating disease development.

Objective 2: Identification of the role of GUM proteins in disease epidemiology for the development of PD resistant plants.

Approach: Gum gene (GUM) expression in response to specific constituents within a defined medium are monitored with real-time PCR. Medium constituents are correlated with plant xylem components. Expression of GUM proteins in susceptible and resistant plants are evaluated on disease symptom severity and rates of transmission efficiency by GWSS.

Cooperators: Wayne Hunter (ARS-Ft. Pierce) is collaborating with Russell Mizell and Pete Andersen (UF-Quincy), Breno Leite (FAMU, Tallahassee).

Benchmarks:

Developed defined media for growth and study of Xf, particularly for screening gum gene expression (UF-Quincy).

Completed Phase I trial of real-time PCR analysis of GUM gene expression in two defined media (ARS-Ft. Pierce and UF-Quincy).

Continue analysis of GUM gene expression in media and *in planta*, and determine role in sharpshooter transmission.

Accomplishments:

Developed defined media for differential growth of Xf *(ARS-Ft. Pierce)*.

Identified role of sulfide bonds in binding of Xf (ARS-Ft. Pierce).

Determined GUM gene expression in Xf *in vitro,* using real-time PCR (ARS-Ft. Pierce and UF-Quincy).

Expected Benefits: Understanding the factors that trigger the differential production of GUM proteins by Xf, and the presence or absence of these elements in plant xylem will aid efforts to develop PD resistant varieties of grape and other crop plants.

V. Grape Genomics, Genetics, Physiology, and Resistance to Xf in Grapes, Almonds, and Other Commercially Important Species

ARS Research Context:

With APHIS and CDFA, ARS contracted (2001-2003) with Doug Cook (UC-Davis) to produce grape ESTs, a project now completed; future plans at Parlier are to develop and test hypotheses based on this genomic data, e.g., by using microarrays and real-time PCR to test the hypothesis that certain plant varieties have systemic resistance reactions to the pathogen that exacerbate the course of Xf-diseases.

Manipulation of the grape genome was facilitated by development at ARS-Kearneysville (R. Scorza), ARS-Parlier (D. Ramming), and UF-Apopka (D. Gray) of a grape transformation system based on *Agrobacterium tumafaciens*, primarily for the purpose of determining gene function. UC-Davis is developing use of *Agrobacterium rhizogenes*-mediated transformation, with the goal of high-throughput screening for genetic resistance to PD in grape, while maintaining desirable grapevine characteristics and grape quality. This method can also be used to rapidly screen for virulence mechanisms in Xf strains.

ARS has wine, table, and raisin grape breeding programs at Geneva (New York), focused on rootstocks and *V. vinifera x Muscadina* crosses, and at Parlier, focused on resistant *V. vinifera* scion stock of high fruit quality and seedlessness. Promising rootstocks are then used by colleagues at UC-Davis and ARS-Geneva for grafting *V. vinifera* scions (e.g., Chardonnay), and *V. muscadina* and *V. vinifera* stocks are used at UC-Davis and FAMU to identify resistance genes, and to create genetic maps for marker aided selection (MAS) to accelerate breeding. An expanded genetic map of *V. vinifera* x *V. muscadina* for fine scale mapping and characterization of PD resistance is being developed at UC-Davis. Molecular markers, in response to grape infection by Xf, are also being investigated, at ARS-Parlier and UC-Davis.

Goal 1: Identify grape rootstock germplasm and/or varieties that reduce or mitigate PD development in susceptible wine grape scions in PD-prone production areas.

Current Situation: The influence of grape rootstocks on development of PD in grapevines in vineyard settings is unknown. Based on previous research, the longevity and productivity of vines of PD susceptible varieties is enhanced in PD zones by the use of particular rootstock varieties. In addition, rootstock variety reduces the development/incidence of *Xylella* disease in peach in field trials in Florida. Rootstocks might confer protection against PD development in susceptible scions grafted to them.

Objective 1: Identification of grape rootstock varieties that reduce PD symptom expression or disease development in susceptible scions.

Approach: Overall, grafted vines are planted in a PD prone vineyard and symptoms of PD development on scions are recorded. Susceptible *V. vinifera* wine grape varieties Cabernet Sauvignon and Chardonnay were grafted to a select group of rootstocks and planted in a vineyard in Tallahassee, Florida, in 2001. Own-rooted rootstocks and scions were also planted.

Vines are managed to control foliar fungal diseases. PD pressure is very high in the experimental vineyard, which has vegetated row middles and abundant *Xylella* vectors. PD symptom expression is scored twice per growing season, for at least three growing seasons. Cooperators: Peter Cousins (ARS-Geneva) is collaborating with Jiang Lu (FAMU).

Benchmarks:

Established pilot experimental block at Center for Viticulture (FAMU). Conducted initial PD symptom evaluation and scoring on test vines.

Expanded experimental block at FAMU. Continued PD symptom evaluation and scoring on test vines.

Continued PD symptom evaluation and scoring on test vines.

Complete PD symptom evaluation and scoring on test vines. Identify rootstocks that reduce PD development in susceptible *V. vinifera* wine grape scion varieties under vineyard conditions.

Accomplishments: Long-term research is in progress.

Expected Benefits: Rootstocks that mitigate PD development in susceptible *V. vinifera* wine grapes under vineyard conditions. Increased survival and productivity of PD-susceptible scion varieties grafted on rootstock(s) in PD-prone areas. Reduced crop production and management costs.

Goal 2: Identify table and raisin grape germplasm and varieties with enhanced resistance or tolerance to PD that have commercial fruit quality.

Current Situation: PD-resistant/tolerant grape germplasm has been identified in: the collection at the National Clonal Germplasm Repository-Tree Fruit and Nut Crops and Grapes, Davis, California; selections obtained from breeders in the southeastern U.S.; and selections from the UC grape breeding program at Davis. However, none of this germplasm or these selections has commercial table and raisin fruit quality or other commercially-desirable horticultural traits (e.g., seedlessness, large berry size, firm fruit without undesirable flavors).

Objective 1: Develop table and raisin grape germplasm selections with enhanced resistance/tolerance to Xf infection and PD development and that have commercial fruit quality.

Approach: Hybridize high quality seedless table and raisin breeding lines (genotypes) as female parents with previously identified sources having PD resistance/tolerance to combine fruit quality and seedlessness with PD resistance. Use embryo rescue methods to allow seedless genotypes to be used as either male or female parents. Screen each breeding cycle for fruit quality and PD resistance. Susceptibility/resistance to Xf infection under greenhouse conditions following artificial inoculation is evaluated by symptom expression and *in planta* movement of the pathogen is assessed by ELISA. Back cross PD-resistant selections with good fruit quality to table and raisin grape selections for the continued improvement of fruit quality. Evaluate

advanced selections in cultural/field trials. Develop genetic markers for PD-resistance and fruit characteristics.

Cooperators: David Ramming (ARS-Parlier) is collaborating with M. Andrew Walker (Department of Viticulture and Enology, UC-Davis).

Benchmarks:

Identified PD-resistant seedlings in table grape genotypes. Hybridized seedless table and raisin grape selections with southeastern U.S. (SEUS) PD-resistant selections and cultivars.

Backcrossed PD-resistant selections identified in 2001 with seedless table and raisin grape selections. Hybridized seedless table and raisin grape selections with PD-resistant selections from multiple sources of PD-resistance. Evaluated seedlings for fruit quality in the field and PD resistance in the greenhouse.

Backcrossed PD resistant selections from second family with seedless table and raisin grape selections. Hybridized seedless table and raisin grape selections with SEUS PD-resistant selections. Evaluated seedlings for fruit quality in the field and propagated the best selections for determining PD resistance in the greenhouse.

Continue hybridizing seedless table and raisin grape selections from multiple sources of PD-resistant selections, and back crossing to new advanced PD-resistant selections. Evaluate seedlings for fruit quality in the field and PD resistance in the greenhouse. Use information from greenhouse PD evaluation to choose parents giving the highest proportion of resistant seedlings.

Accomplishments:

Hybridized a PD-resistant selection from the UC-Davis grape breeding program with four seedless table grape selections. Additional families were created and grown at UC-Davis from crosses between UC PD-resistant selections and ARS-Parlier seedless selections.

Identified PD-resistant table grape seedlings in populations derived from hybridization of four seedless table grape seedlings with a PD-resistant selection from the UC-Davis grape breeding program. Hybridized seedless raisin and table grape selections with southeastern U.S. grape selections and varieties, a PD-resistant French hybrid, and a seeded *V. vinifera* x *V. rotundifolia* hybrid.

Backcrossed PD-resistant selections from the crosses made in 2000 between seedless table grape selections and the UC-Davis PD-resistant selection back to seedless table and raisin grape selections, and produced plants by embryo rescue. Evaluated fruit quality of the first family of a seeded PD-resistant selection from the UC-Davis grape breeding program hybridized with a large seedless table grape from the ARS Parlier breeding program. Several selections within this first generation family 0023 [8909-15 = (*V. rupestris x V. arizonica*) *x* B90-116)] that had low *in planta* populations of Xf and no cane PD symptoms following artificial inoculation were identified, and have been used as parents.

Identified PD-resistant seedlings in populations derived from hybridization of SEUS selections and cultivars. Selections from family 0023 were backcrossed with seedless table and raisin selections. Additional grape selections from the southeastern U.S. were hybridized with seedless table and raisin selections. Numerous seedlings have fruited and the best selected based on fruit quality and low powdery mildew incidence. Twenty-five wine grape selections were made from families whose parents include SEUS breeders from PD-resistant selections crossed with wine varieties. Cuttings have been taken of 80 advanced selections for greenhouse PD-resistance testing.

Expected Benefits: Availability of PD-resistant scion germplasm and varieties with commercially-desirable fruit quality will allow increased survival and productivity of table and raisin grapes in PD-prone areas. Reduced crop production and management costs.

Goal 3: Identify and characterize physiological PD resistance mechanisms in *Vitis* species.

Current Situation: Host plant resistance is a critical component of integrated crop management. Traditional breeding is underway to develop PD resistant plants. Many of the native *Vitis* species show good PD resistance, but the mechanisms controlling this resistance have not been well studied. Given that PD resistance exists in a wide range of genetic backgrounds with different origins, it is expected that the resistance mechanisms [will] may be different among *Vitis* species.

Objective 1: Identify the anatomical and biochemical mechanisms involved in grape plant resistance to Xf.

Approach: To examine the antimicrobial activity of the xylem sap, the sap is extracted from PD-resistant and PD-susceptible cultivars and used in a variety of microbial bioassays designed to test Xf sensitivity. This includes both *in vitro* and *in vivo* bioassays. Based on these antimicrobial bioassays, the cultivar/species exhibiting the most Xf antagonistic activity in the xylem sap is used in reciprocal and interstock grafting experiments with the very susceptible wine grape *V. vinifera* cv. Chardonnay. In these experiments, the transmissibility of antimicrobial compounds from resistant rootstock plants to susceptible scions are examined. Appropriate Xf inoculation procedures are carried out in order to challenge the susceptible scions. Characterization of the observed phenotype in the scion includes quantitative PCR-based assays to determine Xf populations and vascular movement. In addition, graft unions are examined using both light and electron microscopy to determine if pit membrane connections present a physical barrier to Xf movement.

Cooperators: Daniel Kluepfel (ARS-Davis) is collaborating with M. Andrew Walker (UC-Davis) and Hong Lin (ARS-Parlier).

Benchmarks:

Propagate PD resistant and susceptible *Vitis* species/cultivars. Confirm that the plant material is Xf free. Express xylem sap from all cultivars. Design and execute antimicrobial bioassays using the collected sap. Statistically analyze Xf inhibition data to select the most resistant cultivars.

Graft resistant plants identified in 2003 to the susceptible wine grape *V. vinifera* cv. Chardonnay. Inoculate the susceptible scion. Determine Xf population and movement using PCR based bioassay. Determine vascular movement of antimicrobial components in the xylem sap.

Accomplishments: Report in progress.

Expected Benefits: This project will lead to a clearer understanding of PD resistance mechanisms and the extent to which they vary among grape species. More importantly, if we are able to use these resistant plants as rootstocks and express PD resistance in scion plants, a rapid and very effective means of controlling PD will be available to grape growers.

Goal 4: Identify genes responsible for resistance to Xf and GWSS in grapes, and use these genes in traditional or molecularly-based breeding programs.

Current Situation: Host plant resistance is a critical component of disease and insect pest management to ensure overall plant health. Traditional plant breeding has been the primary strategy for developing PD-resistant table, raisin and wine grapes. However, development of commercially-acceptable, pest (e.g., Xf, GWSS)-resistant varieties by conventional plant breeding procedures is time-consuming. This process can be accelerated by marker-assisted selection (MAS) of pest resistant genotypes. However, availability of known genes linked to resistance to Xf infection and/or GWSS-grape interactions for MAS is limited.

There are no resistance gene markers available that are linked to disease and insect resistance. Grapes native to the southeastern U.S. have resistance to PD and other economic diseases of cultivated grapes. Development of a marker-assisted selection program will accelerate the production of new grape varieties with disease and insect resistance, while reducing grower costs.

Objective 1: Identify genetic markers linked to disease and sharpshooter resistance for rapid selection of new varieties with PD resistance.

Approach: Overall, total RNA isolation and large scale EST gene sequencing of grape mRNA are done using disease resistant varieties. Identified genes are compared to known genes from *Vitis* and putative markers are selected. Progeny from crosses of "susceptible *x* resistant" grape varieties are evaluated using the selected markers. Genetic markers linked to susceptibility or resistance are catalogued. Further sequencing will identify additional markers. In an experimental vineyard that has abundant xylella vectors and high PD pressure, evaluate subsequent progeny from crosses to develop an adequate marker population linked to disease and sharpshooter resistance. Development of marker-assisted selection program. Focus will be on Florida grape varieties.

Cooperators:

Wayne Hunter (ARS-Ft. Pierce) is collaborating with Jiang Lu (Florida A&M University [FAMU]).

Benchmarks:

Produced F1 progeny from *Vitis* susceptible *x Vitis* resistant grape (representing 20-40 different crosses). This represents independent populations and ~5,000-7,000 seedlings planted to the field, at the Center for Viticulture (FAMU). Conducted initial PD symptom evaluation and scoring on F1 population.

First cDNA library extracted, sequenced 2000 ESTs (ARS-Ft. Pierce). Identified 117 putative markers selected for screening progeny populations (FAMU). Collected and processed grape material for further sequencing. Produced progeny (~5,000-7,000 seedlings) from (49 different cross attempts), to expand experimental block at FAMU. Continued PD symptom evaluation and scoring on primary populations from previous crosses.

Completed 15,000 ESTs from a PD resistant grape *V. shuttleworthii* (ARS-Ft. Pierce). Identified ~220 putative markers to screen for disease resistance. Genes were made public via electronic publication through NCBI. Continued PD symptom evaluation and scoring on primary populations. Produced another 5,000-7,000 seedlings from new crosses, planted second crossed population seedlings, collected and prepared tissues from each individual vine for marker analysis (FAMU).

Future: Complete another 15,000 ESTs from PD resistant *V. shuttleworthii*. Validate marker set, and continue to screen new markers identified. Make crosses and produce progeny (5,000-7,000 seedlings) from resistant stock for desired fruit quality traits. Evaluate marker-assisted selection capabilities and determine future industry needs.

Accomplishments:

Completed the first cDNA library, representing 2000 ESTs, from PD resistant grape, *V. shuttleworthii* (ARS-Ft. Pierce).

Identified 117 potential makers, currently being evaluated (ARS-Ft. Pierce).

Completed 15,000 ESTs from PD resistant grape (ARS-Ft. Pierce, UF-Quincy).

Identified 220 potential markers, published in public database (ARS-Ft. Pierce, UF-Quincy, FAMU).

Training sessions provided by ARS-Ft. Pierce to FAMU scientist and staff, “Bioinformatics-Data processing”.

Continue evaluation of markers. This work has led to the identification of genetic markers that are currently being used in the grape marker-assisted-selection program at FAMU in the creation of new grape cultivars with increased disease and insect resistance. Unique genes identified from *V. shuttleworthii* were accepted for inclusion by the company, Affymetrix, in their development of The "Affymetrix Vitis GeneChip," now commercially available. This chip is being used for global gene discovery in grapes by the viticulture industry, and by universities in Nevada and California. The chip has been tested and proven to be of exceptionally high quality. Information at the Affymetrix website: www.affymetrix.com.

Expected Benefits: A set of genetic markers linked to disease and insect resistance will accelerate selection of new, disease-resistant grape varieties. A marker-assisted selection program will also save time and money, while providing a valuable new tool to viticulture industries.

Objective 2: Develop DNA molecular markers for grape genes conferring resistance to Xf infection and PD development, and transform grape rootstock with resistance genes.

Approach: Propagate, and establish groups of, grape genotype selections from siblings derived from segregated crosses (e.g., *V. vinifera* x *V. arizonica*) that are highly resistant and highly susceptible to Xf infection and PD symptom development. Plants are inoculated with pathogenic Xf-PD strains. Total RNA is extracted from pooled samples of stems, leaves, and petioles which reflect different tissues at various stages of disease development. The purified mRNA from different genotypes (including uninfected and infected, as appropriate) is used for cDNA library construction by standard protocols (including suppression subtractive hybridization to remove common housekeeping genes, which will enrich differentially expressed genes of interest). Expressed genes of interest are cloned, sequenced, and annotated, and PD-specific transcriptional profiles are developed. cDNA microarrays are used to identify genes linked to possible metabolic pathways and to elucidate the mechanism(s) of PD resistance and pathogenicity.

Cooperators: H. Lin (ARS-Parlier) is collaborating with Jiang Lu (FAMU) and M. Andrew Walker (UC-Davis).

Benchmarks:

Selected, propagated, and maintained appropriate resistant and susceptible grape genotypes. Inoculated plants with Xf, as needed. Extracted total grape RNA and purified mRNA. Constructed cDNA libraries; prepare and sequence cloned cDNA fragments.

Analyze sequence data, construct expression profile databases, and identify potential markers for resistance to Xf infection, PD development, and GWSS-grape interactions.

Clone and sequence full-length resistance genes. Construct vectors for transforming plants with resistance genes, conduct *in vitro* gene expression analyses, transform resistance genes into somatic grape tissue embryos, isolate and bioassay transgenic plants, and initiate plant performance evaluation.

Accomplishments: Long-term research is in progress.

Expected Benefits: Identification of DNA markers linked to resistance genes in grapes infected with Xf, PD development, and GWSS-feeding. This will yield molecular markers of resistance genes for marker-assisted selection programs to facilitate breeding for, and rapid selection of, PD- and GWSS-resistant genotypes. PD- and GWSS-resistant grape scions [or transgenic PD-resistant rootstocks (Note, however, that genetically modifying the scion would not currently be accepted by the wine industry)] will enable breeders to develop PD-resistant grapes while maintaining the integrity of wine varieties (e.g., high grape quality) through classical breeding approaches.

Goal 5. Determine level of resistance to xylella diseases in commercial almond varieties, and in selected *Prunus* species.

Current Situation: Almonds and other *Prunus* species of commercial importance are high value crops susceptible to Xf-caused leaf scorch diseases. The potential impact of Xf-GWSS on these crops is not known but could be high. Little is known of the relative susceptibility of different commercial varieties to these diseases, nor is it known if there is a source of resistance that could be introduced to mitigate the effects of more widespread disease. The interaction between scion and rootstock is also of interest in influencing the course of infection and disease. For instance, almond trees on peach rootstock infected with Xf decline rapidly. However, a 12-year infected almond/Marianna 2624 tree that annually develops severe scorch symptoms shows an absence of limb dieback and produces edible almonds. Based on PCR assays, the Peerless scion was positive, and the plum rootstock sucker negative, for Xf. Also, Xf was cultured from the scion tissues. It appears that Marianna 2624 is immune to Xf and the tree does not decline because photosynthates flow unimpeded down the phloem into the rootstock, which in turn, develops structural roots and absorptive root hairs to supply water and minerals to the scion.

Objective 1: Determine resistance to Xf infection among inoculated almond varieties and other Prunus species.

Approach: Small almond trees (commercially available bare root) are inoculated with known strains of Xf (some associated with PD and some with ALSD). The ability of the bacteria to infect these plants is followed by real-time PCR and by immunoassays at several times after inoculation. Bacterial populations are localized from several trees of susceptible varieties. A similar approach is used to monitor susceptibility of selected material from the *Prunus* collection at the USDA ARS Germplasm Repository at Davis using rooted cuttings when feasible. The susceptibility of the California native wild species *P. andersonii* and *P. fremontii* to infection by Xf strains will be examined. Native *Prunus* species from the southern U.S. (e.g. *P. mexicana*, *P. munsonia*, *P. serotina*, and *P. umbellata*) may be tested for resistance to *Xylella* as well.

Cooperators: Craig Ledbetter (ARS-Parlier) will be collaborating with Hong Lin, and vice-Civerolo (ARS-Parlier), and Bruce Kirkpatrick (UC-Davis).

Benchmarks:

Methods to inoculate trees and assay the presence of Xf will be standardized, and the time period for bacterial growth determined. Six commercial varieties of almonds and six other *Prunus* species from the USDA Germplasm Repository (Davis) will be tested for their ability to support growth of Xf.

Six more almond accessions, including *P. webbii*, and six other *Prunus* species will be analyzed. The relative susceptibility of the plants to Xf will be determined.

Accomplishments: Work was initiated in October 2003.

Expected benefits: Knowledge of varietal differences in susceptibility to ALSD among commercial varieties will allow growers to reduce the impact of this disease if it becomes established and widespread. If resistance to ALSD exists in *Prunus* species, plant breeders can test and devise methods to incorporate it into developing lines.

Objective 2: Confirm almond varietal susceptibility to Xf caused disease through field observations.

Approach: In collaboration with the work outlined in Research Area I, Goal 2, Objectives 1&2, information will be collected on the variety of almonds affected by xylella diseases. Incidence (number of trees infected and intensity of symptoms) of ALSD will be determined by variety in the growing areas with the highest incidence of ALSD. Leaf samples from selected affected orchards will be collected and dried to corroborate varietal assignments using microsatellite markers or other DNA-based techniques.

Cooperators: Collaboration will be sought between a plant pathologist, Jianchi Chen (ARS-Parlier), Farm Advisors, individual growers, and the California Almond Board.

Benchmarks:

Establish working relationships with cooperators and set up database for reporting.

Complete analysis of reported data on varietal susceptibility to ALSD. Conduct field verification of disease symptoms; verify almond varieties with microsatellite or by other analyses if data is anomalous.

Accomplishments: Work is being initiated.

Expected benefits: These results will verify greenhouse studies on resistance to ALSD, providing reliable information to growers on desirable varieties.

Objective 3: Determine mechanism(s) of resistance to Xf among almond varieties and Prunus species.

Approach: If resistance to Xf is found to exist in almonds or *Prunus* species, it will be studied to determine the mechanism of resistance and its transferability to commercially important *Prunus*. Assays of xylem fluid will be conducted to determine the presence of a substance inhibitory to the growth of Xf. Physiological responses will be compared in resistant and susceptible varieties and physiological responses will be correlated with bacterial load. Gene expression in resistant *Prunus* after Xf inoculation will be determined by analysis of mRNA and these results will be compared to those in grape.

Benchmarks: (This work will not be initiated until resistance to Xf is shown to exist.)

The inhibitory effect of xylem fluid is tested on Xf growing on plates. Concentrations of abscisic acid and selected antioxidants is determined in leaves of almond trees after inoculation with Xf.

mRNA will be isolated from tissue of susceptible and resistant varieties at several times (to be determined) during infection. mRNA will be sequenced for the most abundant messages and compared to sequences from grape tissue.

Accomplishments: This work has not yet been initiated.

Expected Benefits: Identified resistance genes from almond and other *Prunus* species that can facilitate choice of breeding stock.

Objective 4: Evaluate almond leaf scorch disease (ALSD) on almond trees growing on the plum rootstock Marianna 2624.

Approach: Establish an orchard trial comprised of three almond cultivars (Butte and Peerless) on two rootstocks (peach and Marianna 2624 plum) and Nonpareil on peach with two treatments (Xf inoculated and healthy) and 5 replications. Transplant trees in 2004. During summer 2005, graft inoculate trees with symptomatic scion shoots. Observe for symptoms in 2006 and regraft as needed.

Cooperators: Jerry K. Uyemoto (ARS) is collaborating with Douglas Gubler and Bruce Kirkpatrick, Department of Plant Pathology, and Joe Connell and Tom Gradziel, Department of Pomology (UC-Davis). Collaborations with Russ Groves and Jianchi Chen (ARS-Parlier) also developed.

Benchmarks:

Transplant almond trees in random block design with 5 replications; irrigate and fertilize trees; label trees as per treatments.

In spring, shape trees by selective pruning. In August, graft trees with diseased almond shoots, evaluate graft-take 30 days later and regraft as necessary. Measure trunk circumferences and analyze statistically.

Evaluate for ALSD symptoms and re-inoculate as necessary. Measure trunk circumferences and analyze statistically.

Continue evaluations of graft-inoculations, growth effects on trees, and begin nut quality comparisons. Measure trunk circumferences and analyze statistically.

Accomplishments: This project is in early stages of development.

Expected Benefits: Use of Marianna 2624 rootstock to mitigate effects of ALSD on almond nut quality and as potential strategy for continued nut production in endemic ALSD areas. Diseased almond trees grown on peach rootstocks decline rapidly, while a single chronically symptomatic (for past 15 years) Peerless almond tree on Marianna 2624 plum roots exhibits a full canopy and produces edible nutmeats. Tree is located in our research plot at UC Davis.

VI. Disease and Vector Management

ARS Research Context:

A number of approaches will be needed to provide long-term, cost-effective management of PD. In the short-term, ARS has focused on GWSS suppression through use of repellents at Kearneysville [this method has proven successful and the research is complete] and application of foliar and systemic chemicals to grape and citrus (ARS-Phoenix). On citrus and grape, UC-Riverside is also testing neonicotinoids, crabby, and other insecticides and insect growth regulators for control of GWSS and other pests. Research at UC-Riverside is focused on determining baseline toxicity and developing monitoring techniques to detect early resistance to insecticides being employed. Attempts are also being made at UC-Riverside (including the UC-Kearney Agricultural Center in Parlier) to determine the effects of these insecticides on natural biological control agents. In addition, scientists at ARS-Phoenix (Maricopa) and UC-Riverside are testing sub-lethal doses of neonicotinoids on GWSS feeding and transmission of PD. Area-wide GWSS pest management programs are ongoing in Southern California (Temecula Valley and Coachella Valley), Central Valley of California (Kern County) and Coastal California (Ventura County). These programs are coordinated efforts of USDA-APHIS, the California Department of Food and Agriculture, Agricultural Commissioners, UC-Riverside and growers.

A second approach, at ARS-Parlier, is to prevent Xf infection through use of chemicals that induce heightened plant resistance. UC-Davis has completed testing a wide variety of prophylactic and therapeutic chemicals for use against Xf in grape.

Breeding for Pierce's disease resistance, map-based identification and positional cloning of Xf resistance, and optimizing MAS for resistance in grapes is being done at UC-Davis. ARS-Parlier collaborates in the breeding and MAS work. Field evaluation of responses of grape rootstocks to natural infection by Xf and disease development is being done by ARS-Geneva and FAMU. Transcriptional profiling and analysis of gene expression in response of grapes to Xf infection is underway at UC-Davis, as well as at ARS-Parlier.

ARS is developing bio-based approaches for GWSS management, including, at Weslaco, classical biological control through importation of natural enemies from southern U.S. and southern South America; while UC-Riverside is focusing on agents from the central and eastern U.S. Egg parasitoids of GWSS released in southern California are being surveyed. The biological control effort includes several studies at UC-Riverside on the biology and application of agents (e.g., exotic mymarid parasitoids), and their identification (e.g., of the *Gonatocerus ashmeadi* complex). ARS-Weslaco is developing molecular markers that can be used to distinguish between the various geographic sources of released native parasitoids, and also methods to distinguish between native and exotic parasitoid species. Cold storage methods for parasitized (and non-parasitized) GWSS eggs are being developed at ARS-Fargo to facilitate mass production of parasitoids for biological control. At ARS-Phoenix and Weslaco ELISA and PCR methods are being developed to detect GWSS in the guts of predators.

ARS-Shafter, following earlier work at ARS-Weslaco, is developing fungi for control of GWSS, and the University of Florida is studying mycopathogens and the effect of their exotoxins on GWSS.

A large pilot demonstration project in Kern County is being led by APHIS and UC-Cooperative Extension, with participation by CDFA and Agricultural Commissioners in Kern and Tulare Counties, using techniques developed, in part (e.g., repellents, insecticides, contemporaneous with classically released parasitoids)~~,~~ by ARS, and monitoring schemes by UC-Cooperative Extension. This project has resulted in significant suppression of the GWSS. This areawide GWSS management program is being implemented in Tulare and Ventura Counties. An IPM program in the Temecula Viticulture Area was also developed and implemented by UC-Riverside scientists for areawide implementation. This has been funded, in part, by APHIS (operations) and ARS (monitoring). ARS-Davis is collaborating with UC scientists at Davis and Berkeley to develop an IPM program for PD in California north coast winegrape-growing areas using biological, chemical, and cultural approaches.

A unique approach to controlling Xf transmission is to find endosymbionts that might be used as competitors or antagonists of the pathogen, e.g., the use of non-pathogenic strains of Xf, as is being investigated at UC-Riverside. Efforts are also underway at UC-Riverside, Yale University, UC-Berkeley, and California State University (CSU)-Hayward to isolate other symbiotic bacteria from sharpshooters. To prepare for eventual use of this strategy, the environmental fate of a genetically-marked endophyte is being studied at UC-Riverside and CSU-Hayward. At UC-Davis, scientists are looking for grapevine endophytes and evaluating these for potential biological control of Xf. Chimeric antimicrobial proteins are being investigated at UC-Davis, Los Alamos National Laboratories, and at ARS-Parlier for possible use against Xf via transformed grapevines**.**

An alternative to the use of endophytic bacteria is to use viruses that attack the vector or the pathogen. GWSS pathogenic viruses are being isolated and characterized at UC-Davis, and additional searches are underway at CSU-Hayward for GWSS viruses and Xf bacteriophages.

Goal 1: Prevent Xf infection.

Current Situation: Xf strains infect a number of cultivated crops, as well as uncultivated plant hosts. Xf-diseases have been present in California since the mid-1880's. Despite occasional costly local epidemics, however, growers generally learned to cope with these diseases until the introduction, establishment, and spread of the invasive GWSS. The GWSS is a more aggressive vector of Xf than other sharpshooter insect vectors of Xf that are indigenous to California. Unlike indigenous sharpshooter insect vectors of Xf, the GWSS can build up to very high population levels; has a very wide range of hosts on which it feeds, reproduces and oviposits; can feed on woody tissues (and potentially transmit Xf) throughout the year; and, is a strong aggressive flier. Once the GWSS acquires Xf, it can transmit the bacterium for life, unless or until it molts. Other diseases of important cultivated horticultural crops caused by Xf pose a threat to crop production in California. These include CVC, PPD, and PLSD. In 2002-2003, scorch disease-like symptoms on olive trees in urban landscape settings were associated with Xf

infections. Economic, sustainable, and effective strategies to manage Xf diseases (e.g., PD, ALSD) are currently not available, especially in areas where the GWSS occurs.

Objective 1: Develop effective management of Xf diseases based on induced host resistance.

Approach: Establish greenhouse and field trials in commercial vineyards to evaluate the effects of available chemical and biologically-based systemic resistance inducers on infection and disease development. Materials (e.g., Messenger, Actigard) are applied to potted plants (for greenhouse tests) or in-ground plants (e.g., in vineyards, orchards) following manufacturers' guidelines. Plants are generally inoculated via Xf-inoculative GWSS (or other insect vectors) for 24- and/or 48-hours, after which time the insects are killed by spraying with an insecticide. As necessary or appropriate, plants are inoculated with Xf following a standard pin-pricking method. Plants are monitored and rated or scored for disease symptoms. Visual observations are confirmed by pathogen re-isolation, ELISA, and/or PCR.

Cooperators: vice-Civerolo and Kayimbi Tubajika (ARS-Parlier) are cooperating with Bruce Kirkpatrick (UC-Davis), grape and almond growers, and industry (e.g., Eden Bioscience) representatives.

Benchmarks:

Established field trial to evaluate the effects of Messenger and Actigard in commercial vineyards in the Temecula Viticulture Area in Southern California.

Established field trials to evaluate the effect of Messenger on PD development in a commercial vineyard in the San Joaquin Valley (Tulare County).

Continued evaluation of Messenger in a commercial vineyard in Tulare County in California. Established field trial to evaluate effect of Messenger on PD development in Santa Maria in the Central Coast of California winegrape-growing region. Established greenhouse test to a evaluate effect of Messenger on Xf infection of grape plants and PD development.

Completed evaluation of Messenger on Xf infection and PD development in commercial vineyards in Temecula, Tulare County, and Santa Maria. Completed first greenhouse experiment to evaluate effect of Messenger on Xf infections and PD development.

Expand greenhouse experiments to evaluate effect of Messenger on Xf infections and disease development in grape and almond plants. Expand field evaluation of Messenger effect on PD development in commercial vineyard setting in an area in which the GWSS is not present (e.g., in a Napa Valley, California, north coastal winegrape-growing region).

Continue greenhouse experiments to evaluate effect of Messenger and other potential systemic resistance inducers (chemical and biologically-based) on Xf infections and disease development in grape and almond plants.

Accomplishments:

Two seasons after treatment, the mean incidence of PD in grapevines treated with the harpin-containing Messenger (2.2.5-6.5 oz/acre) was significantly lower in the first small field trial than in untreated grapevines.

In the first greenhouse experiment, fewer plants treated with Messenger were infected with Xf and had reduced PD symptoms than untreated control plants following exposure to Xf-inoculative GWSS.

Expected Benefits: An effective treatment for PD based on increased or induced resistance to Xf infection that is safe and environmentally-sound will reduce losses due to the diseases. Potential broad application of induced resistance to other Xf-caused diseases (e.g., ALSD).

Objective 2: Develop increased host resistance to Xf infection based on antimicrobial proteins that target moieties on the Xf cell surface.

Approach: Identify protein(s), carbohydrates, and/or lipids that function in the establishment, growth, colonization, and/or movement of Xf *in planta.* Construct novel antimicrobial chimeric proteins composed of two moieties, an initial Xf-specific cell recognition domain (e.g., outer membrane protein-recognizing) and a defensin Xf-specific carbohydrate killer domain (e.g., lipid-recognizing). Express chimeric proteins in plant cells and test their efficacy in lysing Xf *in vitro*. Deliver anti-Xf chimeric protein(s) to grape plants by injection of purified protein(s) or by engineering plants to deliver the protein(s) into the xylem. Test the susceptibility of anti-Xf chimeric treated plants to Xf infection and PD development by standard methods.

Cooperators: vice-Civerolo will cooperate with George Bruening and Abhaya Dandekar (UC-Davis) and Goutam Gupta (Los Alamos National Laboratories, Los Alamos, New Mexico)

Benchmarks:

Select appropriate Xf-specific domains for chimeric protein construction. Express most promising domains in plants cells and assess the activity of the individual domains.

Construct appropriate chimeric proteins and assess anti-Xf effects *in vitro*.

Deliver chimeric proteins to Xf-susceptible grape plants. Evaluate susceptibility of chimeric-treated plants to Xf infection and PD development.

Accomplishments:

An Xf outer membrane protein, mopB, has been isolated, purified and characterized. mopB is a candidate for the Xf-recognition domain of an anti-Xf chimeric protein.

Expected Benefits: A protein inhibitor that interrupts Xf-host interactions (or otherwise inactivated the pathogen) is a novel alternative approach to the use of chemical therapeutics (e.g.,

antibiotics, metal-containing sprays, insecticides) to manage PD and, possibly, other Xf-caused diseases.

Goal 2: Determine GWSS suppression factors, particularly natural enemies.

Objective 1: Determine what parasitoids maintain GWSS at low numbers in their original geographic range.

Approach: Survey egg masses and nymphs on the most important host plants and determine parasitism and predation rates. Colonize the most important natural enemy species identified and determine key biological characteristics, e.g., development, longevity, fecundity and searching efficiency, at different temperatures and habitats (host plants) throughout the native GWSS geographic range.

Cooperators: Researchers at ARS-Weslaco are collaborating with David Morgan (CDFA), who is making releases of Texas parasitoids and will lead post-release evaluations.

Benchmarks:

Surveyed egg parasitism and predation throughout the reproductive season on two primary host plants. Released Texas parasitoids in California.

Continued second year of sampling. Determined basic biological data for native egg parasitoids. Released Texas egg parasitoids in California.

Completed third year of sampling. Began recording behavioral traits of egg parasitoids. Released Texas parasitoids in California.

Complete all biological and behavioral studies, identifying the most effective parasitoid under California conditions. Determine if the Texas egg parasitoid, *G. triguttatus*, has been successfully established in California on GWSS eggs.

Evaluate impact of released egg parasitoids in California.

Accomplishments:

Although nymphs were collected from a large number of host plants, they were seldom abundant. Spiders were the most often observed predator of both nymphs and adults. The preferred oviposition hosts were Texas mountain laurel and crape myrtle. GWSS were rarely seen in citrus orchards. Sampling revealed that nearly 90 percent of GWSS egg masses were parasitized throughout each season, with most of the eggs in each mass parasitized. The parasitic wasp, *G. triguttatus*, was the dominant species, but *G. morrilli* and *G. ashmeadi* were occasionally recovered. *G. triguttatus* were provided to CDFA and APHIS for release in California, where it did not previously occur.

Demonstrated that a parasitic wasp attacking the egg stage contributes significantly in keeping GWSS populations under control in the sharpshooter's native range.

Expected benefits: If *G. trigutattus* can become well established and as effective in California as it is in Texas, GWSS populations should be significantly and permanently reduced.

Objective 2: Develop molecular genetic markers that can distinguish between geographic populations of native parasitoids.

Approach: Collect native egg parasitoids from across the geographic range of GWSS and screen various PCR-based molecular marking techniques to determine if the parasitoids can be reliably identified using these methods.

Cooperators: Jesús de León (ARS-Weslaco) and Walker Jones (previously ARS-Weslaco) are collaborating with Isabelle Lauziere (APHIS-Mission), Russ Mizell (UF-Quincy), and David Morgan (CDFA-Riverside).

Benchmarks:

Obtained different species of parasitoid specimens preserved in alcohol. Tested different molecular methods (ISSR-PCR, RAPD-PCR, ITS amplification and sequencing) to compare sensitivity.

Determine the genetic structure and identify population/species-specific markers of pre-released native and south American *Gonatocerus* parasitoids.

Use markers to determine the genetic structure and geographic origin of parasitoids recovered in California following several years of releases.

Accomplishments:

By ISSR-PCR DNA fingerprinting we determined, for the first time, that there is geographic variation within *G. ashmeadi* (the most widespread egg parasitoid of GWSS). Results indicted that geographic populations were highly differentiated (G_{ST}) and, importantly, population-specific markers were identified. It is interesting to note that populations with some degree of genetic differentiation (differences in marker/allele frequencies) may be considered as races. More work is needed to confirm this possibility.

Molecular methods (ISSR-PCR and ITS region amplification) were able to distinguish eight *Gonatocerus* species.

Molecular methods suggested that Texas and California *G. morrilli* may be separate species rather than geographic strains.

de León, J. H., and W. A. Jones. The utility of ISSR-PCR in distinguishing and estimating genetic structure in *Gonatocerus* species (Hymenoptera: Mymaridae): Egg parasitoids of *Homalodisca coagulata* (Homoptera: Cicdellidae). Biol. Control.

Expected Benefits: Two egg parasitoids (*G. ashmeadi* and *G. morrilli*) already occurred in California when GWSS was discovered there. Large numbers of these species from other states were cultured and released. The ISSR-PCR marker technique may allow workers to be able to distinguish released stock from the native population, providing a way to determine the success of the release program. It can also detect whether outcrossing is occurring among native and released parasitoids of the same species. The genetic population of these species can be followed over time to record changes in the genetic structure of these parasitoid species. The technique also makes it possible to identify not only species, but also the geographic origin of species within GWSS eggs prior to emergence.

Objective 3: Explore for exotic egg parasitoids from related sharpshooters that are pre-adapted to California sub-climate and habitat types.

Approach: Using climate-matching resources, an array of sites throughout the grape-growing regions of California are matched to identical sub-climate types in South America, where other species of related sharpshooters are known to occur. Sharpshooters are collected and caged on potted citrus trees for egg deposition. Egg-infested trees are then placed at pre-planned sites. Citrus orchards are also searched for naturally-occurring eggs. Additional exploration will take place in cooler, drier parts of Argentina that are also a good climatic match with the San Joaquin Valley of California. Parasitioids from this region may pose lower risk of non-target attack to native sharpshooters from the southeastern U.S. Egg masses are shipped to U.S. quarantine for evaluation on GWSS as well as on non-target leafhoppers prior to petition for possible release for establishment in California.

Cooperators: Foreign explorations are being conducted primarily by Guillermo Logarzo (ARS-Buenos Aires) and Eduardo Virla (PROIMI, Tucuman, Argentina), and taxonomic identifications are provided by Serguei Triapitsyn (UC-Riverside), all in collaboration with Walker Jones (ARS-Weslaco) (ARS-EBCL-France). Quarantine handling in Texas was initially done by Isabelle Lausière (APHIS- Mission), followed by Walker Jones while at ARS-Weslaco, and now is being done by John Goolsby (ARS-Weslaco) and in California by S. Triapitsyn and Mark Hoddle (UC-Riverside). Releases and evaluations will likely be conducted by David Morgan (CDFA-Riverside) and APHIS personnel.

Benchmarks:

Using CLIMEX software (both old and new versions) and Klammdiagrams by Walker Jones (ARS-EBCL-France) identified sites in South America that closely match appropriate sub-climate types in California. Matches were also identified in South America that did not match areas of the southeastern U. S. Surveyed for egg parasitoids from related sharpshooters and sent for identification.

Conducted extensive collecting in target areas in South America to ship live parasitoids to quarantine facilities in Mission, Texas and Riverside, California. Conducted biological studies on GWSS eggs, and host range studies on non-target leafhoppers.

Import parasitoids and evaluate host suitability of several non-target leafhoppers native to California. Provide data to action agencies to determine if release is desirable. Continue explorations in South America for additional parasitoid species.

Release and evaluate effectiveness of selected imported parasitoids. Explore for more germplasm of parasitoid species approved for release.

Climatic tolerance testing in quarantine of geographically mixed populations of *Gonatocerus tuberculifemur* from Argentina showed that its fecundity when reared under warm, summer Gulf Coast conditions was twice that of individuals reared under summer, Mediterranean conditions similar to Shafter, California. Successful reproduction was recorded on two sharpshooters native to the southeastern U.S., *Oncometopia orbona* and *O. ingrains.* Since these experiments were conducted on geographically mixed populations, therefore adding uncertainty to the results, we will need to repeat them on pure geographic populations of *G. tuberculifemur* to confirm that more than one strain was not present in the population.

SABCL conducted surveys in Mendoza province, Argentina for cool-adapted populations of *Gonatocerus tuberculifemur*.

Jesse de León (ARS-Weslaco) and Guillermo Logarzo (ARS-SABCL-Argentina) will conduct genetic and hybridization studies, respectively from several populations in Argentina and Chile to confirm the possibility of different strains or species.

Accomplishments:

Using CLIMEX software and Klammdiagrams, identified sites were identified in Peru, Chile, and Argentina as closely matching appropriate sub-climate types in California. ARS researchers at the South American Biological Control Laboratory in Buenos Aires, Argentina, initially found and preserved 9 species of *Gonatocerus* and several trichogrammatoid species from sharpshooter eggs, most new to science (identified by S. Triapitzyn). Subsequent collecting in Argentina, Peru and Chile (by G. Logarzo and E. Virla) resulted in successful colonization of seven *Gonatocerus* spp. in culture on GWSS eggs in quarantine in Mission, Texas and Riverside, California. Biological studies on GWSS eggs were completed and host range studies were initiated. Studies in Texas quarantine showed that two species of imported parasitoids did not attack two species of non-target California leafhoppers (conducted by W. Jones).

Expected Benefits: Although certain native egg parasitoids are highly efficient in finding GWSS eggs in their native locations, they may not be as efficient when released in California; e.g., *G. triguttatus* occurs only in south Texas and south Florida, both humid, subtropical areas. The imported parasitoids, by contrast, are already pre-adapted to both the climate and habitat (citrus), so the potential for significantly enhanced suppression of GWSS is high. Such natural control would be self-sustaining and benefits would accrue annually, with no additional input.

Objective 4: Identify possible bioactive compounds from the GWSS-natural enemies-host plant tritrophic system that could be used to enhance biological control and monitoring of GWSS.

Approach: Develop behavioral assays designed to accommodate the specific behavioral attributes of GWSS egg parasitoids. Use these methods to determine if volatiles from egg masses or plants upon which GWSS are feeding or ovipositing are attractive to parasitoids, and if GWSS are attracted to other GWSS and/or host plant volatiles. Develop, modify, or adopt techniques to effectively collect, isolate, identify and field test bioactive compounds.

Cooperators: This research is being conducted by Joe Patt (ARS-Weslaco) and Mamoudou Sétamou (Texas A&M University). Chemists and other collaborators are expected to participate as the studies proceed.

Benchmarks:

Develop customized behavioral assays to detect the presence of bioactive compounds in GWSS-natural enemies-host plant tritrophic system. Evaluate orientation behavior of parasitoids and predators to plants with feeding or ovipositing GWSS. Develop techniques to collect, isolate, and analyze bioactive compounds.

Continue performing assays to detect presence of bioactive compounds. Continue sampling and analysis studies of putative bioactive compounds.

Complete bioassays and identification of bioactive compounds. Initiate and evaluate attraction-based trapping technologies.

Accomplishments:

A behavioral assay that permits evaluation of close-range attraction of Gonatocerus spp. to GWSS and host plants has been designed and is being evaluated. An evaluation of headspace-SPME (short-path microextraction) as an efficient technique for collecting volatiles from GWSS and host plants is underway.

Expected Benefits: Behavioral assays customized for evaluating the searching behavior of *Gonatocerus* spp. will prove useful in determining the relative importance and interactive effects of olfactory and visual cues in these insects' searching behavior. This information will, in turn, provide insights into the complex set of cues utilized by *Gonatocerus* spp. during movements between plants and habitats. Information generated by these behavioral assays will provide insight into processes (such as interplant movement by parasitoids) that may be otherwise difficult to detect because of complexities inherent at larger spatial scales.

Objective 5: Quantify predation on GWSS nymphs and adults and qualify predation on GWSS eggs.

Approach: A technique has been developed for marking individual GWSSs, each with a unique protein. In turn, the gut contents of predators can be examined by a multitude of protein-specific ELISAs to determine how many GWSS are consumed and which predator species consumed them. Additionally, predator stomach content analyses have been developed which include both a GWSS monoclonal antibody (MAb) based ELISA (ELISA), which detects a GWSS egg protein and a PCR-based assay, which detects GWSS-specific DNA. The DNA-based approach reveals the prey identity at the species-level, but it is unable to indicate which GWSS life stage is consumed. In contrast, the GWSS egg-specific ELISA targets the egg stage providing a higher level of precision to document predation.

Cooperators: James Hagler (ARS-Phoenix) is collaborating with researchers at ARS-Parlier, Kent Daane (UC-Berkeley), Jesse de Leon (ARS-Weslaco), Marshall Johnson (UC-Riverside), and Valerie Fournier (UC-Berkeley).

Benchmarks:

GWSS MAb development was initiated.

A GWSS MAb-based ELISA was developed and screened for cross reactivity to dozens of insect species.

A GWSS DNA-based PCR assay was developed and screened for cross reactivity to dozens of insect species.

Continued refining both the ELISA and PCR assay.

Began screening field collected predators by ELISA and PCR assay for the presence of GWSS remains in their guts. Initiated field cage studies using the prey (GWSS) marking technique.

Objective 6: Explore for nymphal parasitoids of GWSS in Southeast Texas

Approach: The glassy-winged sharpshooter is native to Northeastern Mexico and the Southeastern U.S., and the origin of the invasive California populations is reported by de León et al. (2004) to be Texas. Most of the information regarding this pest comes from its status as a disease vector in cultivated hosts. Much less is known about the field ecology and phenology of GWSS and its natural enemies in its native habitat in Texas. Based on what we know about other leafhopper species, GWSS should have a suite of nymphal parasitoids including the big-headed flies (Pipunculidae) and chalcid wasps (Dryinidae). Pipunculidae may have good potential as biological control agents because they known to be specialists and exhibit high attack rates. Many species of Pipunculidae are known to over winter as pupae, which may make them pre-adapted to the phenology of GWSS in California. Establishment of nymphal parasitoids would complement the native and introduced *Gonatocerus* spp. egg parasitoids in California.

Monthly surveys in southeastern Texas for nymphal parasitoids of GWSS were initiated in April 2005. The field sites are located in five distinct biogeographic zones are anchored by native

stands of *Vitis* spp. Monthly trapping with yellow sticky cards is used to determine the phenology of sharpshooters and pipunculids and to focus collections. Several methods are used to collect parasitized sharpshooters.including hand collection, nymphal sentinels, sweeping, malaise traps and baits. Sharpshooters are held on cowpeas for emergence of the parasitic pipunculid flies.

Benchmarks:

Initiated surveys in southeast Texas for nymphal parasitoids of GWSS and other Proconiine sharpshooters. An unknown species of Pipunculidae in the genus *Eudorylas* was collected in Texas from a closely related proconiine sharpshooter, *Oncometopia orbona*.

Continued surveys, refined techniques for holding parasitized sharpshooters.

Accomplishments:

Skevington, J. H., Goolsby, J. A. and Setamou, M. New Records of Pipunculidae attacking Proconiine sharpshooters – the implications for parasitism of *Homiletics coagulata* (Auchenorrhyncha: Cicadellidae: Proconiini). *submitted*

Cooperators: John Goolsby (ARS-Weslaco), Jeff Skevington (Ag Canada, Ottawa), David Morgan (CDFA)

Goal 3: Develop kaolin-based particle film technology to suppress GWSS vector populations to levels that reduce or minimize Xf transmission.

Current Situation: GWSS has a broad host range yet citrus harbors this pest throughout the year and is its primary reproductive host. Vineyards that border GWSS infested citrus are especially vulnerable to PD when adult sharpshooters that overwinter in citrus begin migrating into vineyards in the spring. Contact and systemic insecticides are currently used to reduce adult GWSS numbers in grape and citrus; however, they have not been effective in preventing the establishment and spread of PD in grape. GWSS re-infest grape soon after contact insecticides are applied and systemic insecticides risk the spread of PD because GWSS must feed on the plant in order to ingest the insecticide. Investigating novel approaches that repel GWSS adults and prevent their feeding is needed to effectively prevent the spread of PD in grape and other crops.

Objective 1: Investigate the use of kaolin-based protective barriers as an alternative to conventional contact insecticides for the management of GWSS in vineyards.

Approach: A kaolin-based particle film crop protectant (Surround™ WP, Engelhard Corporation, Iselin, New Jersey) that has general insect repelling properties was compared to standard insecticide programs for grape in large replicated field trials. Initial studies were conducted to determine the efficacy of Surround WP (Temecula, California) and those results were used to develop a particle-film based GWSS management strategy for vineyards (Kern County, California).

Cooperators: Gary Puterka and Michael Glenn collaborated with Matt Ciomperlik, David Bartels, and Lloyd Wendell (APHIS-Mission), Don Luvisi (UC-Cooperative Extension Service, Bakersfield), Ed Civerolo (ARS-Parlier), and Kayimbi Tubajika (ARS-Davis).

Benchmarks:

In Temecula, demonstrated that Surround WP was very effective in reducing sharpshooter and leafhopper levels in grape. Transferred this information to makers of Surround WP (Engelhard Corp., Iselin, New Jersey) to expand the labeled uses of Surround.

In Kern County, determined the benefits of using particle film, Surround WP, over conventional insecticides in large plot field trials. Transferred this information to APHIS and CDFA, and assisted these agencies in developing an area-wide pilot study that utilized Surround WP.

In Kern County, documented the effects of the 2001 area-wide pilot study and concluded that this management program reduced GWSS to non-detectable levels in the study area by 2002. This programs success resulted in its adoption and expansion into new GWSS control districts in Kern County.

Research on this Objective was terminated at the end of 2002.

Accomplishments:

Conducted large-acreage replicated field trials at 3 grower location near Temecula and determined that particle film, Surround WP, was very affective in controlling leafhoppers and sharpshooters in grape. Also, determined that season-long applications of Surround did not have negative effects on grape quality and yield and that this material was compatible with other pesticides and spray tank additives typically used in grape. This information was transferred to the makers of Surround WP (Engelhard Corporation, Iselin, New Jersey) which resulted in the material's registration for use against GWSS in grape and citrus.

Conducted a large-acreage field trial in Kern County and determined that three biweekly applications of Surround WP were more effective in controlling GWSS than six weekly applications of conventional insecticides. An 800 ft. barrier treatment of Surround WP in grape bordering citrus prevented GWSS migration from citrus into grape and prevented GWSS movement beyond the barrier. Furthermore, Surround WP prevented GWSS egg deposition in grape so that GWSS cannot establish itself in grape (weekly use of insecticides, by contrast, did not prevent egg deposition). This research established that Surround was effective and that only barrier treatments are necessary to prevent GWSS migration into grape. This information was transferred to CDFA and APHIS where 800-ft. barrier treatments of Surround WP become part of an area-wide GWSS management program.

Continued monitoring of GWSS populations in the area-wide study determined that this program was highly successful and reduced GWSS numbers to undetectable levels by 2002. This

program continues to utilize particle film technology as one of its components, and was expanded into new GWSS control districts in Kern County.

Puterka, G.J., D.M. Glenn, and D. Luvisi. 2001. Particle Film Technology: A new tool for glassy-winged sharpshooter, sunburn, and other ag problems. California Grower Magazine, p. 8-9. 2001.

Puterka, G. J., Reinke, M., Luvisi, D., Ciomperlik, M. A., Bartels, D., Wendel, L., and Glenn, D. M. 2003. Particle film, Surround WP, effects on glassy-winged sharpshooter behavior and its utility as a barrier to sharpshooter infestations in grape. Online. Plant Health Progress doi: 10.1094/PHP-2003-0321-01-RS.

Expected Benefits: This project demonstrated that kaolin-based particle films (Surround WP) are very effective alternatives to conventional chemical insecticides in reducing GWSS infestations in grape. In addition, this research arrived at an economical approach to controlling GWSS in grape by establishing that only an 800-ft. barrier treatment of Surround WP in grape was needed to effectively prevent GWSS migration deep into vineyards, thus resulting in a safe and effective approach to GWSS control in grape.

Goal 4: Suppress GWSS vector populations to levels that reduce or minimize Xf transmission in vineyards/citrus and almond groves, to reduce xylella disease incidence.

Current Situation: Chemical control of GWSS is essential as a short-term solution, and as a component of long-term sound management systems that are economically, ecologically, and socially acceptable. Integrated pest management (IPM) strategies are urgently needed that meld cultural and biological components with efficacious GWSS chemical control, insecticide resistance management (IRM), and integrated crop management (ICM) inputs. Most of the promising agents have been evaluated (as of April 2002, Akey et al., 2002) and are available for use (or to develop data for registration/use) against GWSS. In 2002, one application of the "2nd" generation IGR, Novaluron®, (benzoylphenylurea group), a chitin blocker, had 86 percent efficacy against large GWSS nymphs. These preliminary results for Novaluron® appear promising. ARS-Phoenix is attempting to demonstrate insect vector management of GWSS using repellents, insecticides, biological control, and cultural practices at two sites.

Few pathogens for GWSS have been reported. Fungi and insect viruses can be effective in insect suppression. Sharpshooter viral pathogens are only now being discovered. The role of viruses in the biology and ecology of the GWSS is unknown.

Objective 1: Evaluate agents for GWSS control, generate data suitable for registration support as needed, particularly for biorational insecticides.

Approach: Field trials in small plots are set up to evaluate candidate control agents, using complete block randomized designed field trials in vineyards and citrus. Use conventional, commercial spray equipment at application rates and pressures suitable for production. Use beat and visual count sampling methods. Use proper transformations and statistical analysis to determine efficacy evaluations. Larger blocks (e.g., about 20 & 30 acre total) will be used to

demonstrate the use of the IGR, Applaud, in citrus in two locations, to provide data to state workers for use in recommendations that include compounds suitable for IPM programs.

Cooperators: Tom Henneberry (ARS-Phoenix) is collaborating with Matthew Blua (UC-Riverside), and David Morgan (CDFA-Riverside). Past collaborators have included David Akey (ARS-Phoenix) and Russell Groves (ARS-Parlier), Nick Toscano (UC-Riverside), Gary Puterka (ARS-Kearneysville), Lloyd Wendel (APHIS-Mission), B. Grafton-Cardwell (Parlier), and P. Phillips (UC-Cooperative Extension, Ventura). Cooperators include A. Laird (Deputy Agricultural Commissioner, Ventura County), Ben Drake (Drake Enterprises, Temecula, California), J. Lee (grower Piru, California), and producers.

Benchmarks:

Conducted immediate chemical trials (conventional and biorational insecticides) against GWSS adults in grapes for use in control programs. Foliar tests were conducted by air-blast-sprayer on grape vines with 14 agents (Akey et al., 2001a).

Evaluated insecticides against immatures and adults of GWSS in citrus (this is important because almost all GWSS development occurs on citrus in southern and central California). Systemic tests were conducted (2001 only) with 3 agents at 2 doses by injecting agents in the irrigation system of mini-sprinkler irrigated trees [data unpublished], and foliar tests (2001 and 2002) were conducted by air-blast-sprayer on same-sized orange trees with 16 agents, including 3 rate trials (Akey et al., 2001b, 2002).

Demonstrated insect vector management of GWSS using repellents, insecticides, biological control, and cultural practices in an IPM/ICM approach.

Coordinate this suppression goal with GWSS epidemiology and Xf disease incidence goals to determine what GWSS population level reductions must be achieved to effect PD reduction and what are the defining parameters and circumstances that determine those reductions?

Find biorational insecticides that act on adult GWSS in addition to nymphs and/or eggs. To date, true ovicides have not been discovered or evaluated apart from the IGR, pyriproxyfen, Esteem. Efforts need to be made to1) further evaluate Esteem for efficacy and determine if it can be used without adversely affecting beneficial beetle populations, and 2) discover and evaluate new agents with ovicidal activity.

Confirm positive results for Novaluron® from 2002 work and determine rate.

Accomplishments:

In four field seasons (2000-2003), more than 25 insecticides were evaluated in 12 different chemical action classes.

Efficacy data on conventional chemistries and on environmentally-friendly biorational insecticides have been obtained.

Eighteen trials have been conducted with 12 insecticides against GWSS in the spring/summer of 2002.

Four agents, the pyrethroid, Baythroid®, and three biorationals, Applaud®, Agroneem®, and Neemix®, had excellent to good efficacies in trials in 2001 and were found efficacious again in 2002 second evaluations.

2000 work was done by D. Akey, N. Toscno, and T. Henneberry.

2001-2003 work was done by D. Akey, M. Blua, and T. Henneberry

Expected Benefits: Development of tactics for GWSS control on grapes and citrus as IPM components in grape-citrus-GWSS-PD systems. Provide information needed to develop strategies for insecticide resistance management (IRM), and integrated crop management (ICM) for GWSS.

Objective 2: Determine prevalence of naturally occurring entomopathogens of GWSS and develop microbial control.

Approach 1: Surveys of GWSS populations are made to assess the prevalence of naturally occurring fungal entomopathogens of GWSS in California. These pathogens are cultured, tested for infectivity, host range, and heat tolerance under laboratory conditions and then field tested in citrus plots. Ideally, the fungal entomopathogens would be used to help reduce the overwintering population when populations are most vulnerable to microbial pesticides. In addition, isolates from other areas of the United States are tested as available.

Cooperators: The replacement for Michael McGuire (ARS-Shafter) will be collaborating with Harry Kaya and Surendra Dura (UC-Davis), John Goolsby (ARS-Weslaco), and Russell Mizell and Drion Boucias (UF-Gainesville).

Benchmarks:

Conduct surveys for fungal entomopathogens in areas with high GWSS populations. Isolate and identify the fungi and perform bioassays against adult and nymph GWSS.

Identify commercial entomopathogens that may infect GWSS and conduct bioassays.
2004-2006: Determine pathogenicity, culture conditions, temperature tolerances, and host range of new isolates of entomopathogenic fungi and determine optimal production and formulation methods.

Conduct limited tests with infectious isolates of fungi on citrus trees.

Accomplishments:

GWSS were collected from Kern, Ventura, and Riverside Counties in 2003-2005. Dead insects were placed on water agar and held for fungal emergence. In Weslaco, the fungus tentatively identified as *Pseudogibellula formicarum*, isolated from GWSS in Mississippi, was shown to infect adults and nymphs under laboratory and greenhouse conditions. This fungus has been deposited in the ARSEF (ARS Entomopathogenic Fungus) collection in Ithaca, New York (information provided by Walker Jones). Unfortunately, the fungus is extremely difficult to culture. Additional fungi isolated from a GWSS epizootic that occurred in Florida include a new species of *Hirsutella*, and other fungi in the genera *Psuedogibellulla, Beauveria*, and *Sporothrix*. Work in Florida to characterize and culture the new isolates is progressing.

Assays were done against GWSS with the *Beauveria bassiana* product Mycotrol. Infections occurred with high doses (10^9 spores/ml) in a dip test. However, spray assays with up to 10^8 spores/ml failed to yield any infections. 10^7 spores/ml (in an ARS-Shafter test) is a very high dose and probably not economical under field conditions. *Paecilomyces fumosoroseus* was also tested against GWSS but no mortality or infections occurred.

New isolates of *B. bassiana* were collected in Texas and Florida and tested in California for activity against adult GWSS. These new isolates infected adults at concentrations lower than the commercial isolate and show some promise for use in microbial control. Host range tests and growth rates at different temperatures are currently being conducted.

Expected Benefits: If entomopathogenic fungi can be found and developed as microbial insecticides, another tool in the arsenal for managing GWSS populations in an environmentally sound manner will be available. The fungi would be used in an application to reduce overwintering GWSS populations before their movement into PD sensitive crops.

Objective 1: To identify and characterize viral pathogens of the GWSS.

Approach 2: Genetic sequencing is being used to identify viral pathogens in sharpshooters. Molecular primers are designed for further detection and monitoring by PCR. Insect cell cultures are used for mass propagation of virus for further biological evaluations and genetic characterizations.

Benchmarks:

Identified GWSS Iridovirus.

Characterized GWSS Iridovirus.

Evaluate Iridiovirus for GWSS, and search for additional leafhopper viruses.

Detect and identify GWSS viruses.

Accomplishments:

The first GWSS virus has now been characterized, and movement and infection of the virus through GWSS has been studied, with manuscripts in preparation.

Completed genome of first reported GWSS virus, named "*Homalodisca coagulata* virus-1", HoCV-1. This virus is a contributing factor in reducing GWSS populations in C and in FL.

Discovery of two more GWSS infecting viruses named "HoCV-2 and HoCV-3." These are taxonomically different from HoCV-1 and are currently being prepared for genomic sequencing.

Expected Benefits: Viral pathogens of insects have been used in other insect systems for long-term management strategies. Viral pathogens of sharpshooters provide a unique opportunity for the development of a new management methodology for growers to use against sharpshooters to aid in the reduction of PD spread.

Objective 3: Determine the spatial and temporal profiles of two neonicotinoid insecticides, imidacloprid and thiamethoxam, in citrus and grapevines.

Approach: The systemic insecticide imidacloprid has been the principal treatment used in the areawide GWSS management programs conducted in the Temecula Viticulture Area and in Kern Co. During the first season in Temecula in 2000, many questions arose concerning whether imidacloprid was being taken up by the citrus trees after having been applied through mini-sprinkler irrigation systems. To address these questions, a study was initiated in Riverside in April, 2001 to determine the temporal and spatial distribution of imidacloprid in mature citrus trees. In addition, GWSS populations were monitored weekly to evaluate the impact of the systemic treatments.

Cooperators: S. J. Castle (ARS-Phoenix) with N. C. Toscano (UC Riverside)

Benchmarks:

Establish experimental plots at UC Riverside's Ag Ops and apply first treatments of imidacloprid through the mini-sprinkler irrigation system; monitor GWSS populations

Establish a second study site in citrus with new treatments of imidacloprid and thiamethoxam applied; monitor GWSS populations. Establish a study site in Temecula and begin evaluations of imidacloprid in grapevines.

Process GWSS samples and evaluate xylem fluid samples for imidacloprid concentrations.

Finish data analyses, submit manuscripts to Pest Management Science and JEE.

Accomplishments:

Castle, S. J., Byrne, F. J., Bi, J. L., and Toscno, N. C. 2005. Spatial and temporal distribution of imidacloprid and thiamethoxam in citrus and impact on *Homalodisca coagulata* populations. Pest Management Science **61**, 75-84.

Byrne, F. J., Castle, S. J., Bi, J. L., and Toscno, N. C. 2005. Application of competitive enzyme-linked immunosorbent assay for the quantification of imidacloprid titers in xylem fluid extracted from grapevines. Journal of Economic Entomology **98**, 182-187.

Peak titers of imidacloprid were observed in citrus trees 6-8 week post application with near-peak titers sustained for an additional 6-10 weeks. Within-tree distributions of imidacloprid were relatively uniform with no significant differences between the top and bottom canopies of trees or among any of 4 quadrants sampled within the trees. The sustained and relatively uniform distribution of imidacloprid within the citrus trees significantly reduced GWSS nymphs and adults.

Expected Benefit: The information generated from this study greatly increases our understanding of the dynamics of a key systemic insecticide, imidacloprid, in citrus and grapevines. Growers and pest managers will have much greater awareness of the activity profile of imidacloprid in their orchards and vineyards and be able to make more knowledgeable pest management decisions.

Objective 4: Establish baseline responses of GWSS populations from different regions in California to insecticides.

Approach: Insecticides have played a critical role as a first response to burgeoning populations of GWSS in various regions of California. Although intended as a short-term, immediate action to reduce GWSS numbers in critical viticultural regions, the importance of establishing baseline responses to various insecticides being used against GWSS was nonetheless recognized. Beginning in 2001, populations of GWSS from Riverside, Redlands, and Kern Co. were sampled multiple times to evaluate their responses to various insecticides in laboratory bioassays.

Cooperators: ARS-Phoenix (S. J. Castle) with UC Riverside (N. Prabhaker and N. C. Toscano)

Benchmarks:

Explore various bioassay techniques for testing GWSS nymphs and adults; determine the range of effective doses for various insecticides.

Sample GWSS nymphal and adult populations from three regions in California for testing in laboratory insecticide bioassays.

Complete field sampling and testing; begin data analyses.

Complete analyses and prepare and submit manuscript.

Accomplishments:

Prabhaker, N., Castle, S. J., Byrne, F. J., Toscno, N. C., and Henneberry, T. J. 2005. Establishment of baseline susceptibility to various insecticides for glassy-winged sharpshooter, *Homalodisca coagulata*, by comparative bioassays. Journal of Economic Entomology, in press.

Byers, J. A., and Castle, S. J., 2005. Area-wide Models Comparing Synchronous Versus Asynchronous Treatments for Control of Dispersing Insect Pests. Journal of Economic Entomology, in press.

Expected Benefit: The exhaustive data set on GWSS baseline responses to insecticides will serve as a reference point for many years to come. It forms the basis of an insecticide resistance management program by providing reference data that can be compared to future resistance monitoring results. The development of the simulation model provides support for synchronous insecticide treatments on an area-wide basis and may prove especially pertinent to the GWSS area-wide control programs.

Goal 5: Identify key predators of the GWSS [and the smoke-tree sharpshooter (STSS)].

Current situation: Identifying the key predators of these sharpshooters will help towards establishing a conservation or augmentation biological control program, and will be useful in identifying the impact of natural enemies in field studies. In addition, these markers will be useful as diagnostic markers in identifying any life stage of GWSS and/or STSS, even before they emerge from egg masses, thus saving time and money required to rear these insects to the adult stage for proper morphological identification.

Objective 1: Develop species-specific molecular diagnostic markers that are specific toward the invasive GWSS and the closely related smoke-tree sharpshooter (STSS) (*Homalodisca liturata* Ball). Ultimately, the markers developed will be used to detect GWSS and/or STSS remains in the guts of field-collected predators.

Approach: Develop molecular diagnostic markers for both the GWSS and STSS to identify key predators.

Collaborators: Jesse de León (ARS-Weslaco) is collaborating with James R. Hagler (ARS-Phoenix, AZ), Valerie Fournier (Rutgers University), and Kent Daane (UC-Berkeley).

Benchmarks:

Develop both GWSS- and STSS-specific molecular diagnostic markers. Markers to develop include, sequence characterized amplified region (SCR) markers and markers targeting the multi-copy mitochondrial COI and COII genes.

Test the selectivity of various GWSS- and STSS-specific diagnostic markers (6) with stock genomic DNA.

Test the sensitivity and efficiency of various GWSS- and STSS-specific diagnostic markers on various generalists predators (lacewing, earwig, and ground beetle, among others).

Accomplishments:

Effective control of GWSS requires an area-wide, multi-tactic pest management program. A major component of such an approach is the exploitation of the pest's natural enemies, which, when utilized to their greatest potential, can increase the effectiveness of other control tactics. However, little is known about the predaceous enemies that feed on eggs, nymphs, or adult GWSS. Direct visual field observations of predation are difficult to obtain and the field study of insect predation has often relied on indirect techniques for measurement and analysis. Jesse de León (ARS-Weslaco) in collaboration with an ARS scientist (J. Hagler) in Phoenix, Arizona, two University faculty members: Rutgers University (V. Fournier) and the University of California in Berkeley (K. Daane) developed, for the first time, GWSS-specific molecular diagnostic markers to aid in identifying key predators. The diagnostic markers [sequence characterized amplified region (SCR) and mitochondrial COI and COII] were specific toward the GWSS (and STSS) and were able to identify GWSS remains at all life stages (eggs, nymphs, and adults) in predator gut contents. Preliminary field studies of predators in natural environments have shown good success using the newly developed GWSS-specific diagnostic markers, particularly the COI markers (V. Fournier, unpublished data).

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de León, J. H., V. Fournier, J. R. Hagler, and K. Daane. 2005. Development of molecular diagnostic markers for glassy-winged and smoke-tree sharpshooters for use in predator gut content examinations, pp. 293-297. *In* Proceeding, Pierce's Disease Research Symposium, 5-7 December 2005, San Diego, California. Complied by M. Athar Tariq, Stacie Oswalt, Peggy Blincoe, Amadou Ba, Terrance Lorick, and Tom Esser, Sacramento, California.

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Expected benefits:

The development of diagnostic markers for the GWSS will allow us to begin to understand the ecology of GWSS-predator interactions in natural environments. This information will be included in an area-wide pest management approach to aid in controlling this devastating pest.

Goal 6: Genetically characterize GWSS natural enemies or egg parasitoids in the genus *Gonatocerus*.

Current situation: Accurate identification of natural enemies is critical to the success of classical biological control programs, as it is essential for 1) selecting the most suitable natural enemy, 2) evaluating establishment, dispersal, and efficacy of natural enemies, and 3) improving mass production.

Objective 1: Determine whether *Gonatocerus morrilli*, a primary egg parasitoid of the GWSS, exists in nature as a cryptic species complex.

Approach: Collect geographic populations (California, Texas, Florida) of *G. morrilli*. First, survey molecular methods [ISSR-PCR DNA fingerprinting and amplification of internal transcribed spacer region fragments (ITS1 and ITS2)]. Second, develop molecular diagnostic markers to distinguish geographic populations or cryptic species.

Collaborators: Jesse de León (ARS-Weslaco) is collaborating with D. Morgan (CDFA), and has collaborated with W. Jones and M. Sétamou (ARS-Weslaco). Christopher Tipping and Russell Mizell III (University of Florida, Quincy, FL) are cooperating by providing *G. morrilli* specimens from Florida. Serguei Triapitsyn (UC-Riverside, C) is cooperating by performing taxonomic analyses.

Benchmarks:

Survey molecular methods and develop molecular genetic markers for *G. morrilli* by ISSR-PCR and amplification of ITS2 rDNA fragment.

Start phylogenetic studies, sequencing mitochondrial COI and COII genes and the ITS2 rDNA fragment and start hybridization studies.

Complete phylogenetic studies and hybridization studies.

Accomplishments:

Jesse de León and collaborators discovered that one of the primary egg parasitoids (*Gonatocerus morrilli*) of the GWSS from California is actually a new species (sp. n.). Serguei Triapitsyn (UC-Riverside) described the new species and named it: *Gonatocerus walkerjonesi*; in honor of Dr. Walker Jones. Diagnostic markers [inter-simple sequence repeat-polymerase chain reaction (ISSR-PCR) and internal transcribed spacer region 2 (ITS2)] were developed that distinguished the two very closely related species from California (*G. walkerjonesi*) and Texas (and Florida) (*G morrilli*). Since Jesse de León and collaborators recently determined that the GWSS that invaded California originated from Texas, it is crucial to choose the natural enemy from the origin of the GWSS. Together these are very significant accomplishments. We now have the technology to monitor the success of the biological control program in California using *G. morrilli* as a control agent.

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de León, J. H., W. A. Jones, Mamoudou Sétamou, and D. J. W. Morgan. 2006. Genetic and hybridization evidence confirms that a geographic population of *Gonatocerus morrilli* (Hymenoptera: Mymaridae) from California is a new species: Egg parasitoids of the glassy-winged sharpshooter *Homalodisca coagulata* (Homoptera: Cicadellidae). Biological Control (in press).

Expected benefits:

Accurate identification of natural enemies is critical to the success of classical biological control programs. Lack of proper identification procedures has affected several programs. Populations of *G. morrilli* from Texas and/or Mexico have been released in California since 2001 and it has therefore been difficult to distinguish between native (*G. walkerjonesi*) (California) and imported natural enemies (*G. morrilli*) (Texas) to determine their establishment. The genetic and hybridization results of the current study demonstrated that the native and imported natural enemies were actually different species. In addition, molecular diagnostic markers were developed from these studies that distinguished the two very closely related species.
The USDA, ARS, Beneficial Insects Research Unit (Weslaco) sent *G. morrilli* from Texas (origin of the GWSS) to California in the summer of 2005 to restart the biological control program. The molecular diagnostic markers developed by J. de León will be utilized to monitor the success of establishment and evaluate dispersal and efficacy of *G. morrilli* in California.

Objective 2: Post-release evaluation of the *G. morrilli* biological control program in California against the GWSS. Test the utility of the molecular diagnostic markers to monitor establishment of *G. morrilli* in California. The molecular markers distinguish the native California species (*G. walkerjonesi*) from the imported Texas species (*G. morrilli*).

Approach: Collect post-release populations of *G. morrilli* in several counties in southern California (D. Morgan, CDFA) and test specimens with the developed molecular diagnostic markers (ISSR-PCR DNA fingerprinting and amplification of the ITS2 rDNA fragment) (J. de León). The two closely related species (*G. walkerjonesi* and *G. morrilli*) do not share ISSR-PCR banding patterns and the two species are associated with different sized ITS2 region fragments.

Collaborator: Jesse de León (ARS-Weslaco) is collaborating with David J. W. Morgan (CDFA).

Benchmarks:

Collect post-release *G. morrilli* specimens and send specimens to ARS-Weslaco for genetic analysis.

Start genetic analysis of post-release *G. morrilli* specimens from years 2002-2005.

Continue monitoring establishment of *G. morrilli* and send specimens to ARS-Weslaco.

Accomplishments:

A four-year (2002-2005) survey was conducted on post-release populations collected in several counties in southern California. The results of this study indicted that the developed molecular diagnostic markers were highly successful in detecting and monitoring establishment of *G. morrilli* in California on a small scale. In addition, the markers were used to monitor egg parasitoid colonies against contamination with unwanted species. Amplification of ITS2 fragments of post-released *G. morrilli* populations detected only the native *G. walkerjonesi* ITS2 genotype from California. These results would indicate that *G. morrilli* (Texas species) was not establishing in California. This raised a concern as to whether the *G. morrilli* biological control program was successful. In addition, there was a concern that the original 'release' colony may have been contaminated with an unwanted species. After testing the original *G. morrilli* colony that was used for release by ISSR-PCR DNA fingerprinting, it was determined that the colony was contaminated with California's own native species (*G. walkerjonesi*). The results demonstrated that what was being released was *G. walkerjonesi* and not *G. morrilli*, and therefore that is why only the California *G. walkerjonesi* ITS2 genotype was being detected. After determining that the original release colony was contaminated with California's own native species, the CDFA imported *G. morrilli* from Texas (USDA, ARS Weslaco), the same area where GWSS found in California originated from. Importing *G. morrilli* from Texas for a classical biological control program in California was important because it can increase the probability of success for establishment because the parasitoid has co-evolved with its host (GWSS). In the fall of 2005, the *G. morrilli* (Texas) ITS2 genotype was detected in a location where it had been previously released in southern California, demonstrating utility of the developed diagnostic markers. Intense surveying will continue for the next two years.

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Expected benefits:

We now have the molecular technology to follow *G. morrilli* from start to finish, to determine success of the biological control program in California. We can now evaluate establishment, dispersal, and efficacy of the natural enemy, and improve mass rearing by guarding for contamination of unwanted species. The application of molecular markers as diagnostic tools for enhancing precision of classical biological control programs is revolutionary. Past projects have often failed due to inability to detect cryptic or different species, either prior to release, or afterward. These techniques can be applied to previous biological control projects to determine if failure might have been the result of improper identification of native or released natural enemies.

Objective 3: Determine whether *Gonatocerus ashmeadi*, a primary egg parasitoid of the GWSS, exists in nature as a cryptic species complex or whether geographic variation or genetic differentiation can be detected. *Current situation*: The recognition of intraspecific variation can be as crucial for the success of biological control programs, as is sound species determination. Populations of parasitoid from distinct geographical regions may differ in relevant biological characteristics of importance to biological control.

Approach: Develop ISSR-PCR markers. Collect *G. ashmeadi* from various geographic locations.

Cooperators: Jesse de León (ARS-Weslaco) has collaborated with Walker A. Jones (ARS-Weslaco). David J. W. Morgan (CDFA) and Russ Mizell (University of Florida) are cooperating by sending specimens.

Benchmarks:

Collect *G. ashmeadi* from various locations.

Develop molecular genetic markers for *G. ashmeadi*.

Start genetically characterizing geographic populations of *G. ashmeadi* to determine if cryptic species exist, define geographic variation, or identify geographic-specific markers.

Complete genetic studies of *G. ashmeadi*.

Accomplishments:

Using ISSR-PCR DNA fingerprinting Jesse de León and cooperators identified geographic variation and geographic-specific markers in certain populations of the GWSS egg parasitoid, *G. ashmeadi*. The results showed that all populations (Texas, Florida, Louisiana, California) of *G. ashmeadi* were the same species, a conclusion reached by other researchers. Though, and very importantly, the populations were highly differentiated. This observation is significant because studies have shown that recognition of intraspecific variation can be as crucial for the success of biological control programs as is sound species determination. Populations of parasitoid from distinct geographical regions may differ in relevant biological characteristics of importance to biological control. In other words, there may be a geographic population of *G. ashmeadi* that may be better suited for the Californian environment. In addition, geographic-specific markers were identified in certain populations (Louisiana) and if releases were made from these individuals, it would become possible to monitor success of the biological control program in California.

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Expected benefits:

Biological controls programs can benefit greatly by knowing that geographic populations of an egg parasitoid are highly differentiated. Certain variants may be better suited to a specific environment of interest. In addition, identification of geographic-specific markers can aid greatly in monitoring establishment of the same species in biological control programs.

Objective 4: Genetically characterize the South American egg parasitoid, *Gonatocerus tuberculifemur*; an egg parasitoid candidate of the GWSS. Do cryptic species exist?

Current situation: It is not certain that egg parasitoids native to California will be as effective against the GWSS as they are in their co-evolved native range, thus egg parasitoids of closely related hosts *Tapajosa rubromarginata* (Signoret) were sought for a neo-classical biological control program in California from regions in Argentina and Chile where climate types and habitats were similar to California.

Approach: Collect *G. tuberculifemur* specimens from regions in South America (Argentina and Chile) (G. Logarzo ARS-SABCL-Argentina). Send specimens to Weslaco (J. de León) for

genetic analysis. DNA fingerprinting (ISSR-PCR) and phylogenetic analysis (e.g., sequencing of mitochondrial COI partial gene) will be performed.

Cooperators: Jesse de León (ARS-Weslaco) is collaborating with G. Logarzo (ARS-SABCL-Argentina) and has cooperated cooperating with W. Jones (ARS-EBCL-France) and E. Virla (CONICET-PROIMI-Argentina). W. Jones, G. Logarzo, and E. Virla first initiated this effort in 2000. Serguei Triapitsyn (UC-Riverside, C) is cooperating by performing taxonomic analyses.

Benchmarks:

Initiate and complete collections of *G. tuberculifemur* specimens.

Send specimens to Weslaco (J. de León). Start performing genetic analyses.

Continue performing genetic analyses and perform hybridization studies.

Accomplishments:

Work in progress.

Expected benefits:

The identification of possible *G. tuberculifemur* strains (or perhaps cryptic or different species) in Argentina and Chile could be of significance to a biological control program. If more than one strain or cryptic species is found, biological and genetic characteristics must be performed on both strains to identify a suitable GWSS egg parasitoid candidate.

Objective 5: Genetically characterize the South American egg parasitoid, *Gonatocerus metanotalis*. A prospective egg parasitoid of the GWSS. Do cryptic species exist?

Current situation: It is not certain that egg parasitoids native to California will be as effective against the GWSS as they are in their co-evolved native range, thus egg parasitoids of closely related hosts *Tapajosa rubromarginata* (Signoret) were sought for a neo-classical biological control program in California from regions in Argentina where climate types and habitats were similar to California.

Approach: Collect *G. metanotalis* specimens from regions in South America (Argentina) (G. Logarzo ARS-SABCL-Argentina). Send specimens to Weslaco (J. de León) for genetic analysis. DNA fingerprinting (ISSR-PCR) and phylogenetic analysis (e.g., sequencing of mitochondrial COI partial gene) will be performed.

Cooperators: Jesse de León (ARS-Weslaco) is collaborating with G. Logarzo (ARS-SABCL-Argentina) and has cooperated with W. Jones (ARS-EBCL-France) and E. Virla (CONICET-PROIMI-Argentina). W. Jones, G. Logarzo, and E. Virla first initiated this effort in 2000. Serguei Triapitsyn (UC-Riverside, C) is cooperating by performing taxonomic analyses.

Benchmarks:

Initiate and complete collections of *G. metanotalis* specimens (G. Logarzo).

Send specimens of Weslaco (J. de León). Start performing genetic analyses.

Continue performing genetic analyses and perform hybridization studies.

Accomplishments:

Work in progress

Expected results:

The possible identification of more than one *G. metanotalis* strain (or perhaps cryptic or different species) in Argentina could be of significance to a biological control program. The strains may have different biological characteristics that may affect their ability to function as a proper biocontrol agent. If more than one strains or cryptic species are identified, than both biological and genetic characteristics must be performed on these strains to identify the proper prospective GWSS egg parasitoid candidate.

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ARS Research Team Biographies

David Akey (retired) had 40 years of research experience working with world-recognized authorities on insects of medical and veterinary importance, and agricultural insect pests. In 1974, Dr. Akey joined USDA, ARS, Arthropod-borne Animal Diseases Research Laboratory at Denver, Colorado. and joined the Western Cotton Research Laboratory, Phoenix, Arizona, in 1985. He joined the ARS Emergency Response Team on GWSS in 2000. He is the author or co-author of more than 90 papers, 68 abstracts and a team report, and has given over 90 presentations.

Elaine Backus is a Research Entomologist in the Crop Diseases Pests and Genetics Research Unit in Parlier, California. Formerly a Professor of Entomology at the University of Missouri-Columbia, Missouri. Dr. Backus has nearly 25 years' research experience in feeding mechanisms of hemipterans in relation to plant response, the causation of plant disease, and transmission of plant pathogens. She has studied the relationships among insect anatomy, stylet penetration, salivation, plant physiology, and pathogen transmission. She also has designed and marketed instruments, as well as provided instruction in, electrical penetration graph (EPG) monitoring of insect feeding. Dr. Backus is recognized as a world authority on EPG and hemipteran feeding mechanisms. While at the University of Missouri, she garnered nearly $2 million in extramural research grants. She has edited 2 books, as well as published 5 book chapters, 2 review articles, and nearly 50 scientific articles and abstracts.

Kendra Baumgartner is a Research Plant Pathologist with the Crops Pathology and Genetics Research Unit in Davis, California. Her research program is focused on developing sustainable methods of disease and weed control for winegrapes. Current research projects include examining the role of riparian hosts in the epidemiology of PD, developing effective control practices for *Armillaria* root disease, evaluating the effects of vineyard floor management practices (weed control, cover crops) on soil chemical, physical, and biological properties, and studying the beneficial effects of mycorrhizal fungi on grapevine growth.

Jackie Blackmer is a Research Entomologist with the Western Cotton Research Laboratory in Phoenix, Arizona, where her main focus is on insect behavior, specifically insect-plant interactions and factors that influence dispersal in insects. She currently is investigating plant- or insect-derived stimuli and/or feedback mechanisms that influence feeding, host finding, oviposition, longevity, reproductive development and emigration of cotton insect pests, as well as invasive species (i.e., *Homalodisca coagulata*). She is also interested in how host-plant morphology, physiology, and nutritional components influence biological processes in insects. She has published more than 50 journal articles/ or book chapters, presented numerous invited and scientific talks, won several awards, and been part of several research teams that have brought in more than $1.2 mil.

David Boyd (no longer conducting PD research) is a Research Entomologist at the Small Fruit Research Station, Poplarville, Mississippi. His research areas include integrated pest management of arthropod pests of ornamentals in large production nurseries and biological control of insects. Current research projects include development of pest management tools for the strawberry rootworm in azalea production and the metallic flea beetle in crape myrtle

production. Other projects include development of host plant resistance in ornamentals to key pests.

James S. Buckner is a Supervisory Research Chemist/Biochemist with the Insect Genetics and Biochemistry Unit, Fargo, North Dakota, and has been with ARS for 27 years. His research contributes to the development of biological and biochemical approaches to improve beneficial insects for the biocontrol of pest insect populations. His expertise is in the identification, function, and biosynthesis of insect cuticular and internal lipids. He has characterized surface and internal lipids associated with pest and beneficial insects, and their natural enemies. His current and future research includes characterizing interactions of pest/beneficial insects and their natural enemies, determining the role of lipids on interactions of natural enemies with their hosts, and characterizing feeding mechanisms and vector transmission for homopteran insect (whiteflies, leafhoppers) to determine resistant characteristics in plants. He has published more than 60 refereed journal articles, three book chapters on the chemistry and biochemistry of lipids and has given numerous invited and scientific presentations.

Steve Castle is a Research Entomologist at the Western Cotton Research Laboratory, Phoenix, Arizona. His areas of interest include pest management, population biology, and insect vector/plant pathogen relations. Current research projects include development of pest management tools for combating two invasive insect species in California, improving chemical control methods for silverleaf whiteflies, and establishing baseline susceptibility levels for pink bollworm to transgenic insecticidal cotton cultivars.

Jianchi Chen is a Research Molecular Biologist in the Crop Diseases, Pests and Genetics Research Unit in Parlier, California, and recently joined the Crop Diseases Pests and Genetics Research Unit. Dr. Chen has been working in the area of population characterization of Xf for over ten years. He was among the first to investigate the genetic diversity and discovered the presence of plasmids in Xf. He has a strong interest in applying molecular biology technology to study and resolve disease problems directly related to crop production.

Edwin Civerolo is Director and Research Plant Pathologist, San Joaquin Valley Agricultural Sciences Center, Parlier, California. Past research focused primarily on various aspects of bacterial plant diseases, specifically those affecting stone fruits, citrus and strawberry caused by xanthomonads. Current research is directed on diseases (e.g., PD of grape and ALSD) caused by Xf. This has included pathogen characterization, pathogen detection and identification, disease diagnosis, disease epidemiology and disease management with an emphasis on induced host resistance.

Thomas A. Coudron is a Research Chemist and lead scientist located at the Biological Control of Insects Research Laboratory in Columbia, Missouri, and has 25 years of experience in insect biochemistry and developmental regulation. His primary research focus is to elucidate the effects of nutrition and/or the regulatory role of biological substances on the developmental processes of beneficial insects, with the long-term goal of developing cost-effective mechanisms to *in vitro* rear beneficial organisms for insect and weed control. Currently he is investigating physiological and biochemical links between nutrition and key developmental processes that challenge the production of quality insects via artificial rearing methods. The goal is to optimize

artificial diets used to mass rear beneficial insects and to establish diagnostic techniques to enable the rapid detection and identification of the biochemical and physiological parameters correlated with quality insects reared under *in vitro* methods.

Peter Cousins is a Plant Geneticist in the Plant Genetic Resources Unit, Ithaca, New York. He is charged with breeding, evaluating, selecting, and introducing grape rootstocks, with national responsibility. He trained in grape rootstock breeding and genetics at the University of California, Davis, focusing on describing the genetic control of resistance to the root-knot nematode Meloidogyne incognita in grape rootstocks. Current research projects include screening diverse grape rootstock germplasm for resistance to virulent nematode populations, evaluation of rootstock impact on Pierce's disease expression in the scion, evaluation of rootstocks for wine, table, and raisin grapes, and breeding improved rootstocks with nematode and phylloxera resistance and desirable horticultural attributes.

Jesús (Jesse) H. de León is a Research Molecular Biologist located at the Beneficial Insects Research Unit, Weslaco, Texas. Jesse was recruited from the biomedical field of pharmacogenetics (over 12 years experience) to implement his expertise in molecular genetics and molecular biology toward biological control (Molecular BioControl) of insect pests. Dr. de Leon's main goals are to incorporate modern molecular genetic/molecular biological methods to support classical biological control programs that reduce the use of pesticides, develop molecular markers and DNA fingerprinting methods to genetically identify both pests and natural enemy populations**,** and examine the nature of genetic variation within and among pest and natural enemy populations that could impact the success of biocontrol programs. Current research projects include determining the population genetic structure of both the glassy-winged sharpshooter and its natural enemies (e.g., *Gonatocerus* spp.) by PCR-based DNA fingerprinting and phylogenetic methods and determining the origin of the glassy-winged sharpshooter.

Gary Elzen (retired) was a Research Entomologist within the Beneficial Insects Research Unit of the Kika de la Garza Subtropical Agricultural Research Center, Weslaco, Texas. Dr. Elzen conducted toxicological and behavioral studies to evaluate the suitability of insect natural enemies for use in biologically based pest management. Research emphasis was placed particularly on *Anthonomus grandis grandis, Heliothis/Helicoverpa* spp., *Spodoptera* spp., *Bemisia argentifolii* and its parasitoids, spiders, and the glassy-winged sharpshooter, *Homolodisca coagulata,* and its parasitoids. In addition to conventional toxicological methods, Dr. Elzen utilized a variety of organic analytical techniques (e.g. HPLC, GC), and novel behavioral bioassays.

Michael Glenn (no longer conducting PD research) is a Research Soil Scientist at the Appalachian Fruit Research Station, Kearneysville, West Virginia. His research focused on the response of fruit and shoot growth to chemical and physical changes in the plant environment, particularly in developing management systems that more efficiently utilize the environmental resources, and production systems that minimize pesticide usage. He patented a pest control system based on particle film technology that controls a wide range of disease and insect pests, including the GWSS. The particle film technology also reduces heat and water stress in plants and improves production efficiency. Dr. Glenn has 94 peer-reviewed, scientific publications in the area of soil and water management, plant nutrition, root physiology and particle film

technology, 1 patent related to irrigation scheduling technology, and 8 patents establishing particle film technology.

John Goolsby is a Research Entomologist and recently joined the Beneficial Insects Research Unit, Weslaco, Texas. From 1999-2004, he was director of the Australian Biological Control Laboratory in Brisbane, Australia. Previously to his overseas assignment he was an Entomologist with USDA-APHIS in Mission, Texas and was the co-project leader of the Silverleaf Whitefly Biological Control Program. His research focuses on biological control of pests and weeds. He brings his expertise in foreign exploration and evaluation of natural enemies to aid in the discovery new biological control agents for effective management of GWSS. Dr. Goolsby has authored more 70 publications on biological control and has individually or as a team member been responsible for the discovery, evaluation and release of 12 parasitoid species and 4 weed biological control agents.

Russell Groves (left ARS) was a Research Entomologist in the Crop Diseases Pests and Genetics Research Unit. Dr. Groves has over 10 years experience in applied entomology and a strong background in insects as vectors of plant disease. Previously, his research responsibilities focused on seasonal population dynamics and epidemiology of the thrips/*Tomato spotted wilt Tospovirus* pathosystem emerging as a serious threat in North Carolina plus more recent research responsibilities administered through Cornell University examining aphid-borne, *Potato Y Potyvirus* epidemiology. The focus of his PD/GWSS research aimed at understanding fundamental biology and ecology of the glassy-winged sharpshooter in the Central San Joaquin Valley as it relates to acquisition and transmission of *Xylella fastidiosa* scorch diseases.

James Hagler is a Research Entomologist located at the Western Cotton Research Laboratory, Phoenix, Arizona. His primary research focuses are in the areas of biological control, insect dispersal, and insect behavior. He has senior authored over 50 publications. Dr. Hagler has made significant contributions toward bridging the gap between molecular biology and applied entomology. His molecular probes for detecting prey remains in predator guts are considered state-of-the-art research among his biological control peers. Dr. Hagler is considered a world authority among biological control researchers in the area of evaluating the efficacy of predaceous natural enemies. Over the past 4 years alone, Dr. Hagler has individually or as a team member, secured external funding exceeding $500,000 through highly competitive grant programs. Over the past year alone, Dr. Hagler has secured external funding exceeding $950,000 through highly competitive grant programs.

John Hartung is a Research Plant Pathologist located at the Fruit Laboratory at Beltsville, Maryland. Dr. Hartung conducts research on a broad range of exotic citrus pathogens under quarantine arrangements in Beltsville, and in cooperation with researchers in Japan, France, Brazil and Florida. A major focus of his research has been on strains of *Xylella fastidiosa* that cause citrus variegated chlorosis and coffee leaf scorch diseases in Brazil. Among the accomplishments of Dr. Hartung's group were the demonstration that strains of *Xylella fastidiosa* cause citrus variegated chlorosis and coffee leaf scorch diseases, the characterization of the population of *X. fastidiosa* associated with sweet orange in Brazil as compared with North American strains, the development of widely used PCR-based detection methods for the pathogen, and the demonstration that strains of *X. fastidiosa* from sweet orange can induce

symptoms similar to those of Pierce's disease in artificially inoculated 'Chardonnay' grapevine. His laboratory has also transformed *X. fastidiosa* and created green fluorescent protein tagged, defined mutants of *X. fastidiosa* and observed them using confocal microscopy in inoculated plants. His group has also provided evidence that the sweet orange strain of *X. fastidiosa* can be transmitted through seed.

Thomas Henneberry is Director and Research Entomologist, Western Cotton Research Laboratory. The laboratory is the focal point in the Western United States for basic and applied research on cotton pest management, as well as the physiological relationships of the cotton plant affecting cotton production. He has authored more than 500 publications in scientific journals and other media, including more than 15 book chapters. He serves on numerous state, federal and industry committees to establish multidiscipline research, priorities and approaches. He has been a member of the Entomological Society of America for 45 years, during which time he has served on Program Committees, the Auditing Committee, Secretary-Elect of Section F, and the Executive Committee of the Pacific Branch. Elected as "Fellow" of the Entomological Society of America in 1991, and recipient of Agricultural Research Service "Outstanding Scientist of the Year" award in 1990. He received the Miles Cotton Research Recognition Award, and the USDA Department Award for Superior Service in 1997, was installed in the ARS Hall of Fame in 1998 and received the President's Senior Executive Service Meritorious Award in 1999.

Wayne Hunter is the Lead Scientist on PD and the GWSS at USHRL, Ft. Pierce, Florida. He has focused on using state-of-the-art technologies to rapidly generate information on the biology, development, and pathology of the GWSS to advance the development of environmentally sound methods of GWSS population management aimed to reduce and/or stop the spread of Pierce's Disease. The focus is on the potential use of insect viral pathogens to develop an Area-wide suppression program against GWSS and other vectors of PD. Dr. Wayne Hunter has accomplished meeting several critical objectives within the first year of this CRIS, focused on Pierce's Disease and the GWSS. Dr. Hunter has aggressively pursued the rapid production and release of information to meet CRIS objectives concerning the devastating problems associated with Pierce's Disease and the GWSS. He has produced important biological information on GWSS biology through the annotation of gene expression data from four cDNA libraries (30,000 gene sequences) from adults, nymphs, salivary gland, and midgut tissue of GWSS. Through these focused efforts genes critical to GWSS development, feeding, digestion, and reproduction have been identified along with the discovery of new viral pathogens.

Walker A. Jones was Supervisory Research Entomologist and Research Leader of the Beneficial Insects Research Unit, Weslaco, Texas, prior to his move to France as the new Director of the USDA-ARS European Biological Control Laboratory. He has served with ARS for 22 years, conducting research associated with classical and augmentation biological control using predators, parasitoids, and pathogens. He completed a 3-year survey of the impact of parasitism on the GWSS in one of its areas of origin, finding that egg parasitism is largely responsible for low sharpshooter populations in south Texas. He has led a team that has identified 10 species of sharpshooter egg parasitoids from South America, collected from sub-climate and habitat types identical to that across the grape-growing areas of California, and studied the biology and risk assessment associated with 7 species of parasitoids from Argentina that successfully attack GWSS eggs in quarantine.

Daniel A. Kluepfel is a Research Plant Pathologist in the Crops Pathology and Genetics Research Unit on the campus of the University of California, Davis. Dr. Kluepfel obtained his Ph.D. from the University of Florida, Gainesville, were he worked on Agrobacterium attachment to plant cell surfaces. After post-doctoral positions in Wagenningen, The Netherlands, and the University of Hawaii, he served on the faculty in the Department of Plant Pathology at Clemson University for 16 years. In August of 2003 he accepted the position of Research Leader of the CPGRU and Location Coordinator at University of California, Davis. His research interests involve an examination of the molecular microbial ecology of the rhizosphere as it pertains to the development of biological control agents. Current research projects include efforts to examine global gene expression patterns in *Pseudomonas* sp. during root colonization. Additional projects involved the study of microbial diversity and its impact on disease incidence.

Craig A. Ledbetter is a Research Geneticist at the San Joaquin Valley Agricultural Sciences Center in Parlier, California. He has been with ARS for 16 years and has worked on both *Prunus* and *Vitis* germplasm breeding and evaluation. Present research activities include the development of new apricot and plum X apricot varieties for the fresh and processing markets. He has introduced five new apricots during the last decade. He is also involved in almond breeding with a major emphasis on developing new self-compatible varieties. *Prunus* rootstock breeding is a third emphasis in his research program. With the enormous market for almond nursery stock in the California growing regions, emphasis is placed on well-anchored root systems and nematode resistance. Enhanced vigor in these almond rootstocks is accomplished through the use of a male-sterile facilitated system to produce diverse hybrid peach-almond seed.

Jesusa C. Legaspi is a Research Entomologist with the Insect Behavior and Biocontrol Unit at Gainesville, Florida. Her duty station is at Florida A&M University (FAMU) at Tallahassee, Florida. She also is an Adjunct Associate Professor at the joint USDA-FAMU Center for Biological Control, which was established in 1999. She has over 20 years experience in biological control and integrated pest management of major insect pests in various field crops. For over seven years, she evaluated transgenic sugarcane against an important stalkborer pest and its impact on their natural enemies. Her current research involves ecological and physiological studies of parasites and predators of major pests in vegetables and small fruits such as silverleaf whiteflies and GWSS. She has published over 130 journal articles, book chapters or extension publications, and has presented numerous papers and lectures.

Roger A. Leopold is the Lead Scientist for the Insect Cryobiology Project at the Red River Valley Agricultural Research Center, Fargo, North Dakota. His career in ARS spans > 36 years, most of which were spent in the study of insect reproduction and development. For the past 10 years his specific research interest has been insect cryopreservation and cryobiology. He has given over 70 research presentations at various scientific meetings, seminars, workshops and conferences, 18 of which were invitational at international venues. He has authored or co-authored 60 journal articles, 6 book chapters, 11 proceedings articles, 5 technical reports and misc. articles and 33 published abstracts. He was awarded a CSIRO Senior Scientist Fellowship in 1994 for study in Australia and the CSIRO Sir Frederick McMaster Fellowship for outstanding foreign scientists in 1996. Leopold currently serves on the editorial board of *Cell Cryopreservation Technology*. In the past 4 years he has obtained about $550,000 in extramural

grants. Current research projects include long-term cold storage of tephritid embryos and short-term storage of GWSS eggs and egg parasitoids.

Hong Lin is a Research Plant Physiologist in the Crop Diseases, Pests and Genetics Research Unit in Parlier, California. He recently joined a new team of scientists in the Exotic and Invasive Disease and Pest Unit. Dr. Lin has 20 years research experience in working with biotic/abiotic stress physiology. His research has encompassed plant physiology, plant biochemistry, population genetics, and molecular biology. Currently the focus of his research aims at understanding mechanisms of PD resistance using functional genomic approaches and evaluating the effect of resistant grape rootstock on PD expression in the scion.

Guillermo Logarzo has been a Research Biologist at the South American Biological Control Laboratory since 1987, searching for, evaluating, and shipping promising agents for biological control of insect pests and weeds. He has conducted projects on biological control of the weeds *Xanthium* spp., *Larrea spp.*, and *Sesbania* spp. At present he is searching for natural enemies of the GWSS, tarnished plant bug, and members of the *Heliothis/Helicoverpa* complex. He has traveled extensively throughout Argentina, from Patagonia to the northern tropical rain forest, including semi-arid rangelands, and neighboring countries, and is familiar with pests of tobacco, cotton, and corn.

Michael R. McGuire (no longer conducting PD research) was Supervisory Research Entomologist and Research Leader of the Western Integrated Cropping Systems Research Unit located in Shafter, California, the heart of the new GWSS/PD infestation area. He is now Assistant Area Director, Northern Plains Area, Ft. Collins, Colorado. After receiving his Ph.D. from the University of Illinois in 1985, Dr. McGuire accepted a post-doc position in Bozeman, MT to study early detection of entomopxvirus, a potential microbial control agent for rangeland grasshoppers. In 1988, he accepted a position in Peoria, Illinois, as Research Entomologist and Lead Scientist of a CRIS to invent and develop novel formulations for entomopathogens. The formulations he and his staff developed extended the activity of bacteria and viruses and were licensed by private industry. In 1995, Dr. McGuire became Research Leader of the Bioactive Agents Research Unit in Peoria with a staff of 8 SYs and 17-20 support staff. In 2000, Dr. McGuire became Research Leader and Location Director for the Shafter, California location. He conducted research to develop new, selective biopesticides for the major pests of cotton. Due to his proximity to the newly infested areas, Dr. McGuire was asked to develop a program aimed at finding entomopathogens for control of GWSS. Dr. McGuire is the author or co-author of approximately 85 peer reviewed publications and eight patents.

Stuart H. McKamey received his B.S. at the University of California, Berkeley in 1985, his M.Sc. at North Carolina State University in 1989, his Ph.D. at the University of Connecticut in 1994, and was a Post-doctoral fellow at the National Museum of Denmark, Copenhagen in 1995-1996. Since 1997 he has been a Research Scientist in the ARS Plant Sciences Institute, Systematic Entomology Laboratory, Beltsville, Maryland, and is responsible for the leafhoppers, treehoppers, planthoppers, froghoppers, and cicadas (suborder Auchenorrhyncha, order Hemiptera), which include about 40,000 species. His primary responsibilities include: curation of the U.S. National Insect Collection, Smithsonian Institution; service identifications for domestic and foreign researchers, state extension agents, commodity groups, and the Animal and

Plant Health Inspection Service; and research on the taxonomy and systematics of Auchenorrhyncha with emphasis on leafhoppers. In addition to over a dozen research papers on the taxonomy and identification of treehoppers and leafhoppers, he has produced a world catalog of treehoppers (3,166 species) and a world checklist of leafhoppers (1758-1955, 11,007 species), which is still the starting point for all taxonomic research on leafhoppers. He is nearing completion of an update of the leafhopper checklist through the year 2000 (approx. 21,000 species).

Steven E. Naranjo is a Research Entomologist at the Western Cotton Research Laboratory, Phoenix, Arizona. His areas of emphasis include population ecology, biological control, sampling, integrated pest management, and systems analysis. The major focus of this research is to understand the contribution of natural enemies to pest population regulation and to integrate biological control with current and developing pest management strategies. Research activities include sampling and description of the seasonal population dynamics of key pests and predators in the cotton system, characterization of the predator complex attacking key cotton pests, evaluation of the impact of insecticides and transgenic cotton on abundance and activity of cotton pest predators and parasitoids, development of decision-aids for whitefly and sticky lint in cotton and development of sampling methods and plans for GWSS in citrus and grapes. Dr. Naranjo has authored 118 publications and has presented numerous papers and lectures. In support of efforts at the Western Cotton Research Laboratory he has individually, or as a team member, secured external funding exceeding $1.6 million through competitive and other grant programs.

Joe Patt is a Research Entomologist at the Beneficial Insects Research Unit in Weslaco, Texas where his main focus is the chemical ecology of host plant-herbivore-natural enemy interactions. Primary projects are: 1) determining the relative contributions of visual and olfactory stimuli in the host finding behaviors of GWSS and *Gonatocerus*; and, 2) evaluating the efficacy of SPME (short-path micro extraction) as a means of collecting volatiles of interest from GWSS host plants. Previous studies examined the role of floral volatiles and visual cues on pollinator and parasitoid behavior, the chemical, morphological and behavioral mechanisms governing interactions between flowers and biocontrol insects, the ability of biocontrol insects to recognize and discriminate particular floral cues, the influence of floral nutrients on the development of predaceous insect larvae, the effect of interplanting flowers on beneficial insect activity in vegetable and ornamental crops, and the utilization of sentinel flowers for monitoring abundance and distribution of metapopulations of a threatened skipper.

Gary J. Puterka (no longer conducting PD research) was a Research Entomologist in the Appalachian Fruit Research, Kearneysville, West Virginia, with 23 years of experience in field crop and orchard research with 13 years of service with the USDA-ARS that focused primarily on insect-plant interactions, insect genetics, and the development of alternative technologies for arthropod pest management. Senior or co-authored 78 technical publications (42 senior authored) and two book chapters (one senior authored) and authored ten patents on particle film and sugar ester technologies. Over 90 formal presentations with 40 of these being invitations to present his research or organize and moderate symposiums for professional society meetings. Expertise in developing alternative technologies for arthropod pest management led to special ARS assignments (GWSS in California) and CRADA's that transferred new technologies to industry.

David Ramming is a Research Horticulturist in the Crop Diseases, Pests and Genetics Research Unit in Parlier, California. Dr. Ramming has been responsible for the grape, peach, nectarine, and plum breeding program for over 28 years. He has released 30 varieties from this program. Current research projects include developing table and raisin grapes with resistance to PD and powdery mildew, improving table grape fruit quality, developing grapes with the natural ability to dry on the vine for raisins, and improved fruit quality for peaches, nectarines and plums.

Fred Ryan (retired) was a Research Plant Physiologist in the Crop Diseases, Pests and Genetics Research Unit in Parlier, California. He recently joined this unit after working with the Postharvest Quality and Genetics Research Unit, Parlier, California, on the effects of methyl bromide alternative treatments on fresh commodities and developing and applying molecular markers for quality traits for the grape and *Prunus* breeding programs. He was previously located with the ARS Aquatic Weed Control Research Unit, Davis, California, and worked on the physiology and control of problem aquatic plants. Using DNA based markers, he also has conducted research on genetic variation, origin, and biological control of Russian thistle in cooperation with other researchers in the United States and abroad. He investigated the interaction between *Xylella* and *Prunus* species, with particular regard to mechanisms of resistance in the plant.

Norman W. Schaad is a Research Plant Pathologist (bacteriologist) with the Foreign Disease-Weed Science Research Unit, Ft. Detrick, Maryland. Research interests include molecular characterization, real-time PCR and microarray detection, identification, and systematics of foreign and domestic plant pathogenic bacteria. He has over 20 years experience in working with *Xylella fastidiosa*. While at University of Georgia he co-developed PW medium and the first serological assay for *X. fastidiosa*. Current research projects on *X. fastidiosa* include development of rapid, sensitive real-time PCR assays and the taxonomy of the bacterium. Dr. Schaad received his BS (1964), MS (1966), and Ph.D. (1969) in Plant Pathology from the University of California, Davis.

Ralph Scorza (no longer conducting PD research) is a Research Horticulturist and lead scientist at the ARS Appalachian Fruit Research Station, Kearneysville, WV. He has worked for over 20 years on the genetic improvement of temperate fruit crops including stone fruits (peach, nectarine, plum), grape, and pear, with most of his effort focused on stone fruits. He has introduced two peach cultivars that are currently the standards for their season in the northeastern and mid-Atlantic states ('Sentry' and 'Bounty') and has additionally released a plum and nectarine cultivar. He has developed novel new peach tree growth habits for high-density production systems that will be introduced in 2004. Dr. Scorza has developed a transgenic plum cultivar with high-level resistance to plum pox virus. This plum has been tested in European field tests for the last 7 years where it has shown excellent resistance, productivity and high fruit quality. It is the first transgenic virus resistant temperate tree fruit to be developed and provides a blueprint for the deployment of this technology.

Mark Sisterson is a Research Entomologist in the Crop Diseases, Pests and Genetics Research Unit in Parlier, California. His past research focused on modeling the effects of insecticidal transgenic crops on target and non-target arthropods. His current research is focused on using simulation models to develop a better understanding of the biological and operational factors that influence the spread of Pierce's disease within and between fields. A field and laboratory component will complement the modeling work.

Drake Stenger has recently assumed duties as Research Leader and Research Plant Pathologist, Crop Diseases, Pests, and Genetics Research Unit, San Joaquin Valley Agricultural Sciences Center, Parlier, California. Past research focused primarily on various aspects of viral plant diseases, specifically those affecting small grains, vegetables, sugar beet and strawberry. Current research is focused on Xf-caused diseases and insect vectors of Xf, with emphasis on molecular biology of Xf and viral pathogens of Xf insect vectors.

Jerry K. Uyemoto is a Research Plant Pathologist in the Crops Pathology and Genetics Research Unit on the campus of the University of California, Davis. After obtaining a Ph.D. from the UC-Davis in 1968, work experience includes: Associate Professor, New York State Agricultural Experimental Station, Cornell University, Geneva, New York 14456 (1968-1977) - responsible for virus diseases of apple, grapevine and stone fruit; Professor, Kansas State University, Manhattan DA 66502 (1977-1982) - virus diseases of corn, sorghum, and wheat; Senior Scientist with Advanced Genetic Sciences, Manhattan Kansas (1982-1984) - protoplast and tissue culture of legumes (guar); Visiting Scientist, UC-Davis (1984-1986) pistachio epicarp lesion and cherry x-disease projects; Research Plant Pathologist, USDA-ARS, UC-Davis (1986-present) virus and virus like diseases of stone fruits and grapevines.

George D. Yocum is a Research Physiologist at the Red River Valley Agricultural Research Center, Fargo, ND. Dr. Yocum has 10 years of experience as an insect physiologist including 4 years with ARS, and has a strong background in diapause and stress physiology. He has authored/coauthored 14 peer-reviewed articles and 6 book chapters, and given 25 presentations at scientific meetings. Past research focused on the molecular mechanisms of both pupal diapause and stress response to high and low temperature exposure. Current research involves characterization of the molecular regulation of adult diapause initiation, and investigating molecular responses to suboptimal diet feeding.