United States Department of Agriculture
Agricultural Research Service
Pierce's Disease
Research Summaries

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Component I: *Xylella fastidiosa* (Xf) Systematics, Genomics, Biology, Ecology, Epidemiology

Goal 1: Clarify the taxonomy/nomenclature of Xf

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

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

Project Name: *Xylella fastidiosa* Genome Sequences

Associated CRIS Project Number(s) and Title(s):
5302-22000-007-00D, Epidemiology and Management of *Xylella fastidiosa* (Xf) and Other Exotic and Invasive Diseases and Pests

Start and Termination Dates:
5302-22000-005-00D: Start Date: 5/16/01– Termination Date: 9:30/05
5302-22000-007-00D: Start Date: 10/1/05 – Termination Date: 3/14/07
5302-22000-008-00D: Start Date: 3/15/07 – Termination Date: 3/14/12

Scientific Personnel:
Edwin L. Civerolo, Supervisory/Research Plant Pathologist, Parlier, California, (0.9 FTE)
Marie-Anne Van Sluys, University of Sao Paula, Sao Paulo, S.P., Brazil (Cooperator)

Research Goal(s):
Component I. Xf Systematics, Genomics, Biology, Ecology, Epidemiology

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

Research Objective(s):
1. Sequence, assemble and annotate the genome of a Xf strain associated with Pierce's disease in California.

2. Comparatively analyze the genome sequences and annotations of Xf strains associated with Pierce's disease, almond leaf scorch disease and oleander leaf scorch disease in California and citrus variegated chlorosis disease in Brazil.

Research Approach:
Under a Specific Cooperative Agreement between ARS and FAPESP (Brazil), *Xf* strains associated with Pierce's disease (strain Temecula-1), almond leaf scorch disease (strain Dixon) and oleander leaf scorch disease (strain Ann-1) in California were provided to cooperators at the University of Sao Paulo, Sao Paulo, S.P., Brazil. DNA was isolated by standard techniques. Complete genome sequences were generated by using a combination of ordered cosmid and shotgun strategies. Various shotgun libraries with different insert sizes (0.8-2.0 kb and 2.0-4.5 kb) were cloned. Cosmid libraries with inserts ranging from 30-45 kb) were also constructed. Cosmid ends with a Phred quality above 20 (approximately 26-fold genome coverage, were used in the scaffolds. Selected cosmids were fully sequenced. Sequence gaps were identified by linking information from forward and reverse reads and were closed by primer walking, PCR sequencing, and insert subcloning. Sequences from both ends of most cosmid clones were used to confirm the orientation and integrity of the contigs. The sequences were assembled using the Phred+Phrap+Consed package. All consensus bases had a Phred quality of at least 20. Most of the sequencing was performed with BigDye terminators and ABI Prism 3700 DNA sequencers. Annotation was based primarily ORF identification using GLIMMER, GeneMark and alignment against the National Center Biotechnology Information protein database. BLASTX searches were used to find additional putative protein-coding genes. All ORFs were inspected manually by an annotation team. For each ORF, links to Cluster of Orthologous Groups of Proteins, Protein Family Database and Kyoto Encyclopedia of Genes and Genomes were used. RNA species were identified by using BLASTN, secondary structure analysis, and tRNAscan-SE. Gene maps, lists, comparative Xf-CVC and Xf-PIERCE'S DISEASE data, and GenBank submissions. Whole genomes were compared at the nucleotide level using UUMmer with default values. Genomes were compared

at the amino acid level. Genes were considered orthologs where *e*-values were 10^{-5} or less. Genes with no hits or the e-value was 10^{-5} or more in other genomes were considered to be strain specific.

Actual or Potential Deliverables:

- The sequence and annotation of the genome of *Xf*(Xf) strain Temecula-1 (associated with Pierce's disease in California) was completed. (2003)
- The sequence and annotation of the genome of Xf strain Ann-1 (associated with oleander leaf scorch in California) were completed. (2006).
- The sequence of the genome of Xf strain Dixon (associated with almond leaf scorch disease) was completed (2006).
- The comparative analyses of the structure and organization of the genomes of Xf strains Temecula-1, Ann-1 and 9a5c (associated with citrus variegated chlorosis in Brazil) were completed. (2006)

Achieved and/or Potential Benefits:

- Identification of Xf-specific DNA sequences for molecular (e.g., PCR)-based methods to diagnose Xf-caused diseases, and detect and identify Xf strains.
- Identification of genes that encode proteins potentially involved in specific ecological adaptations, pathogenicity and host-pathogen interactions and that are potential targets for mitigating Xf infection and/or disease development.

Project Name: Assessing the Potential of Forage Alfalfa to Serve as a Source of *Xylella fastidiosa* for Spread to Almond and Grape

Associated CRIS Project Number(s) and Title(s):
5302-22000-007-00D, Epidemiology and Management of *Xylella fastidiosa* (Xf) and other Exotic and Invasive Diseases and Insect Pests

Start and Termination Dates:
5302-22000-005-00D: Start Date: 5/16/01– Termination Date: 9:30/05
5302-22000-007-00D: Start Date: 10/1/05 – Termination Date: 3/14/07
5302-22000-008-00D: Start Date: 3/15/07 – Termination Date: 3/14/12

Scientific Personnel:
Mark Sisterson, Research Entomologist, Parlier, California, (1.0 FTE)
Russ Groves, Research Entomologist (no longer with ARS)

Research Goal(s):
Component 1. Xf systematics, genomics, biology, ecology, epidemiology

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

Research Objective(s):
Assess the potential for forage alfalfa to serve as an inoculum source of Pierce's disease and almond leaf scorch strains of Xf.

Research Approach:
Six alfalfa sites in the San Joaquin Valley are being sampled seasonally to determine levels of Xf infection. Sweep samples are collected monthly to determine abundance of potential Xf vectors. Potential vectors are frozen and will be assayed using molecular methods to determine if they carry Xf. Neighboring vineyards and almond orchards are surveyed at the end of each year to gain a representative sample of infected grape and almond. Genetic markers will be used to compare the Xf strains found in alfalfa to those carried by vectors and those found in infected grape and almond.

Actual or Potential Deliverables:
- Knowledge/Information regarding the degree to which forage alfalfa can serve as an inoculum source of Xf.

Achieved and/or Potential Benefits:
- Knowledge/Information regarding the potential role of alfalfa as an epidemiologically significant inoculum source of Xf will aid in developing strategies to manage the spread of Xf.

Peer Reviewed Publications post 2004 NAS Report: None

Project Name: Assessing Secondary Spread of *Xylella fastidiosa* in Almond

Associated CRIS Project Number(s) and Title(s):
5302-22000-007-00D, Epidemiology and Management of *Xylella fastidiosa* (Xf) and other Exotic and Invasive Diseases and Insect Pests

Start and Termination Dates:
5302-22000-005-00D: Start Date: 5/16/01– Termination Date: 9:30/05
5302-22000-007-00D: Start Date: 10/1/05 – Termination Date: 3/14/07
5302-22000-008-00D: Start Date: 3/15/07 – Termination Date: 3/14/12

Scientific Personnel:
Russ Groves, Research Entomologist (no longer with ARS)
Jianchi Chen, Research Molecular Biologist, Parlier, California, (1.0 FTE)

Research Goal(s):
Component 1. Xf systematics, genomics, biology, ecology, epidemiology

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

Research Objective(s):
Determine incidence, spatial pattern, and degree of secondary spread of almond leaf scorch stains of Xf in almond orchards.

Research Approach:
Three almond orchards in the San Joaquin Valley were surveyed seasonally for the incidence of ALS affected trees over three years. Trees were ranked based on the presence of symptoms. Samples were taken from all trees suspected of being infected by Xf. PCR and ELISA tests were used to determine if trees were indeed infected. Xf was also isolated from selected trees. Spatial patterns of disease incidence where compared between years to determine the extent of secondary spread.

Actual or Potential Deliverables:
- Knowledge/Information regarding the degree of secondary spread of Xf in almonds.

Achieved and/or Potential Benefits:
- Epidemiological knowledge/information that secondary spread of Xf in almond orchards in the San Joaquin Valley is rare. This indicates the growers do not necessarily need to remove infected trees to slow pathogen spread. Instead, the decision to remove infected trees should be focused on the degree of lost productivity.

Project Name: Significance of Riparian Hosts in the Spread of Pierce's Disease

Associated CRIS Project Number(s) and Title(s):
5306-21220-003-00D, Sustainable Vineyard Production Systems

Project Start and Termination Dates
5306-22000-012-00D: Start Date: 11/07/99 Termination Date: 7/31/04
5306-22000-013-00D: Start Date: 08/01/04 Termination Date: 4/30/08

Scientific Personnel: Kendra Baumgartner, Davis, California, (0.25 FTE)

Research Goal(s):
Component I. Xf Systematics, Genomics, Biology, Ecology, Epidemiology

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

Research Objective(s):
1. Analyze spatial and temporal patterns of Xf-caused diseases in the presence of indigenous sharpshooter vectors and GWSS; and

2. 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.

Research Approach:
Riparian areas contribute to PIERCE'S DISEASE, as evidenced by a correlation between disease incidence and proximity of vines to riparian areas, but the blue green sharpshooter's (BGSS) generalist feeding habit makes it difficult to predict which riparian species are important inoculum sources (competent reservoirs) for the spread of Xf to grapevines. Xf also has a broad host range that includes all winegrape varieties and some riparian plants, but its limited persistence and low titers in most species means that not all Xf hosts are likely to be competent reservoirs. Riparian hosts have been identified mainly from greenhouse studies. Greenhouse evaluation of the host range of a vector-transmitted, generalist pathogen, however, makes for weak epidemiological inference, based on the fact that disease risk is impacted by not only host genotype, but also by a complex range of environmental factors that may differentially affect the host, the pathogen, and the vector. In the field, such hosts are situated within plant communities (vegetation types), where their relative abundance can vary. Therefore, we used a field-based approach to identify the competent reservoirs of Xf in riparian areas, based on the relationship between PIERCE'S DISEASE incidence, BGSS density, and riparian host diversity. Our paired design includes 20 vineyards with severe PIERCE'S DISEASE and 20 without PIERCE'S DISEASE. Canonical correspondence analysis will be used to determine if there is a significant relationship between PIERCE'S DISEASE (severe vs. absent) and riparian host diversity, and if certain species are found in higher relative abundance in riparian areas adjacent to diseased vineyards. We will use multiple regression analyses to identify the independent variables (vector abundance, relative abundance of riparian species that distinguish diseased sites from healthy sites, abundance of infective vectors) that are significantly associated with disease incidence.

Actual or Potential Deliverables:
- Knowledge/Information regarding the significance of riparian hosts in the epidemiology of Xf.

Achieved and/or Potential Benefits:

- By identifying which riparian hosts are correlated with high PIERCE'S DISEASE incidence in vineyards, we will be able to make reliable recommendations to North Coast growers on which species to target for removal. By examining species composition of adjacent riparian zones the growers will be able to make more informed management decisions concerning vineyard establishment.

Project Name: *Xylella fastidiosa* Phage Biology

Associated CRIS Project Number(s) and Title(s):
5302-22000-007-00D, Epidemiology and Management of *Xylella fastidiosa* (Xf) and other Exotic and Invasive Diseases and Insect Pests

Start and Termination Dates:
5302-22000-005-00D: Start Date: 5/16/01– Termination Date: 9:30/05
5302-22000-007-00D: Start Date: 10/1/05 – Termination Date: 3/14/07
5302-22000-008-00D: Start Date: 3/15/07 – Termination Date: 3/14/12

Scientific Personnel: Drake C. Stenger, Research Plant Pathologist, Parlier, California, (1.0 FTE)

Research Goal(s):
Component I. Xf Systematics, Genomics, Biology, Ecology, Epidemiology

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

Research Objective(s):
1. Characterize prophage sequences integrated in different Xf strains.

2. Induce integrated Xf prophage to enter into a productive, lytic cycle.

3. Characterize infectious phage particles.

Research Approach:
1. Xf strains present in the USDA-ARS Parlier collection will be grown in culture and genomic DNA isolated. The presence and genomic location of integrated prophage sequences will be determined by Southern blotting using known prophage sequences (e.g., those resident in completely sequenced *X. fastidiosa* strains Temecula 1, 9f5c, and Dixon). Alternatively, prophage sequences will be identified by PCR using primers developed to known Xf. prophage. Integrated prophage sequences (and X. fastidiosa border sequences) will be cloned and sequenced. Prophage sequences will be compared for sequence diversity and evolutionary relatedness.

2. Cultures of Xf strains known to harbor prophage sequences will be treated with various physical and chemical stresses to induce prophage to enter into a productive, lytic infection. Phage particles will be harvested from induced cultures. Identification of phage particles will be accomplished by electron microscopy of partially purified and concentrated preparations of cell-free culture supernatants. Phage particles recovered from induced cultures will be tested for infectivity by inoculation of Xf cultures both on solid media (e.g., a plaque assay) and liquid media. Inoculated cultures will be examined for newly integrated prophage and for production of phage particles.

3. Induced phage particles will be concentrated and purified using standard virological techniques. Structural proteins will be extracted from phage particles and characterized by gel electrophoresis. Phage DNA present in phage particles will be characterized for topology (liner or circular). Phage DNA will be cloned, sequenced and compared to integrated prophage sequences.

Actual or Potential Deliverables:
This research has just been initiated (January, 2007).

- Potential deliverables include new genomic information for Xf strains (2008), potential new marker sequences for strain specific diagnostics (2008), infectious phage genomes that may be modified for use as Xf gene vectors (2009), and anti-*X. fastidiosa* viruses and/or gene products (2010).

Achieved and/or Potential Benefits:

- Characterization of phage sequences integrated into the Xf genome may provide improved tools for discrimination of strains that may facilitate pathogen population genetics and disease epidemiological studies. Gene vectors based on Xf phage genomes will provide alternative methods to deliver foreign DNA to Xf. Infectious, intact phage or individual gene products derived from phage may have anti-Xf activity that may be exploited as novel means of Xf-caused disease management.

Peer Reviewed Publications post 2004 NAS Report: None.

Project Name: Multi-locus SSR Markers for Genotyping and Genetic Diversity Assessment of *Xylella fastidiosa* in California

Associated CRIS Project Number(s) and Title(s):
5302-22000-007-00D, Epidemiology and Management of *Xylella fastidiosa* (Xf) and other Exotic and Invasive Diseases and Insect Pests

Start and Termination Dates:
5302-22000-005-00D: Start Date: 5/16/01– Termination Date: 9:30/05
5302-22000-007-00D: Start Date: 10/1/05 – Termination Date: 3/14/07
5302-22000-008-00D: Start Date: 3/15/07 – Termination Date: 3/14/12

Scientific Personnel: Hong Lin, Research Plant Physiologist, Parlier, California, (1.0 FTE)

Research Goal(s):
Component I. Xf Systematics, Genomics, Biology, Ecology, Epidemiology

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

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

Research Approach:
1. Perform genome-wide sequence analysis to identify Simple Sequence Repeat (SSR) loci from four Xf genomic sequencing databases (PD, CVC, ALS and OLS). Design and develop PCR-based multi-locus SSR markers for pathogen genotyping assay and population genetic analyses.

2. Analyze genetic diversity and population structure of Xf populations associated with agricultural crops (e.g., grape and almond, alfalfa) and insect vectors, as well as non-agricultural riparian natural habitats.

3. Construct a large Xf allele frequency database for use as an Xf strain identification system.

4. Use the SSR Marker system to evaluate Xf adaptation, host selection and functional fingerprinting assay for determining avirulent and virulent strains.

Actual or Potential Deliverables:
- Publicly available Xf Simple Sequence Repeat (SSR) primers. Improved Xf pathogen diagnostic and genetic analysis system(s). Improved tool(s) for epidemiological risk assessment based on assessment of Xf population diversity, host-pathogen interaction(s) and adaptation. Development of a high-throughput Xf pathogen diagnostic and genetic analysis system.
- Genetic analyses of California's Xf populations associated with PIERCE'S DISEASE and ALSD in different grapevine- and almond-growing regions. The sensitivity, specificity and ability to detect Xf polymorphism in plant and insect hosts makes this system an ideal tool for studying Xf genetics and epidemiological risk assessment.

Achieved and/or Potential Benefits:
- Strain identification, genetic diversity assessments and population structure analysis are crucial to understanding and controlling disease outbreaks and are also critical to the successful

breeding of PIERCE'S DISEASE resistant grapes. The SSR DNA marker system, combined with a rapid sample preparation protocol provides a high-throughput Xf pathogen diagnostic and genetic analysis system. This system avoids the time-consuming bacterial isolation step and reduces the chance of sample loss due to contamination and/or culture difficulties.

- Genotyping the pathogen, along with information about host-Xf and insect vector-Xf will help understand Xf pathogenesis mechanisms.
- The SSR DNA marker system is useful for differentiating Xf strains, characterizing genetic diversity, and aiding in epidemiological and strain virulence studies.
- Information about the genetic diversity and population structures of Xf pathovars by SSR can be combined with disease index and mathematically modeling to develop an effective disease forecast system to better understand relationship between population dynamics in time and space.

Project Name: *Xylella fastidiosa* Strain Diversity

Associated CRIS Project Number(s) and Title(s):
5302-22000-007-00D, Epidemiology and Management of *Xylella fastidiosa* (Xf) and Other Exotic and Invasive Diseases and Insect Pests

Start and Termination Dates:
5302-22000-005-00D: Start Date: 5/16/01– Termination Date: 9:30/05
5302-22000-007-00D: Start Date: 10/1/05 – Termination Date: 3/14/07
5302-22000-008-00D: Start Date: 3/15/07 – Termination Date: 3/14/12

Scientific Personnel:
Jianchi Chen, Research Molecular Biologist, Parlier, California, (1.0 FTE)

Research Goal(s):
Component I. Xf Systematics, Genomics, Biology, Ecology, Epidemiology

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

Research Objective(s):
1. Evaluation of genetic and phenotypic diversity of Xf strains and its use in the studies of pathogen taxonomy, disease epidemiology, and host-pathogen interactions.

2. Development a comprehensive detection system for rapid, sensitive, and accurate identification of Xf.

Research Approach:
1. Collection of Xf strains from the continental United States began in 2003. Over 400 strains have been collected. Currently, most strains are from almond orchards and vineyards in the Central Valley of California. We have also collected Xf strains from landscape plants in southern California, and Xf strains from oak, plum, and grape from the southeastern United States where Pierce's disease originated. All isolated strains were subjected to phenotypic and genetic characterizations. Phenotypic characters include colony morphology, media selectivity, growth rates, surface motility, etc. Genetic characterizations include both local and global genomic sequence comparison. Global genomic analyses are conducted by random sampling tools such as RAPD analysis. Local genomic sequence studies were achieved by sequence comparison of selecting loci of different evolution rates that include the conserve *rrn* operon and hyper-variable regions.

2. Based on the available bio-informative and phylogenetic information, sets of genes or DNA sequences are identified. Sequence variations (indels, SNPs, etc.) are used to identify and differentiate Xf strains. Candidate genes or sequences such as 16S rDNA, rpmE, gltA, rpfA, rpfB, sucD, dnaN and gyrB) were be evaluated by a comprehensive collection of Xf strains of different genotypes/pathotypes from California, Georgia, Florida and South Carolina. A PCR array detection system is being developed.

Actual or Potential Deliverables:
- A broad-based working collection of Xf strains from different hosts and geographical areas has been established for research.
- New knowledge and understanding of the diversity of Xf strains.
- Improved diagnostic methods and molecular reagents for Xf strain identification.

Achieved and/or Potential Benefits:

- New knowledge and understanding of the diversity of Xf. New knowledge that should be about the microbial ecology of Xf to better understand the epidemiology of Xf-caused diseases developing improved disease management strategies.

Project Name: Identification, Molecular Characterization, and Detection of Foreign and Newly Emerging Domestic Bacteria

Associated CRIS Project Number(s) and Title(s):
1920-22000-029-00D, Identification, Molecular Characterization, and Detection of Foreign and Newly Emerging Domestic Bacteria

Start and Termination Dates:
1920-22000-029-00D: Start Date – 5/01/2003; Termination Date: 4/30/2008

Scientific Personnel:
Norman Schaad 303, Plant Pathology, Fort Dietrich, Maryland. (0.75 FTE)

Research Goal(s):
Component I. Xf Systematics, Genomics, Biology, Ecology, Epidemiology

Goal 1. Clarify the taxonomy/nomenclature of Xf.

Research Objectives:
1. Clarify the classification of the species Xf in order to link all information about host, vector, epidemiology, and control to appropriate names.

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

Research Approach:
Determine the correct taxonomy of Xf so that reliable, rapid molecular-based detection assays and fingerprint databases can be developed.

Actual or Potential Deliverables:
- New knowledge regarding Xf taxonomy
- New or novel Xf diagnostics

Achieved and/or Potential Benefits:
- New information about the phylogenetic relationships of Xf could lead to revision of the taxonomy of this pathogen. For example, Xf may consist of (at least) three subspecies. This will allow for development of improved, reliable specific PCR diagnostic assays. Development of a novel agar-plating PCR assay allows for increased sensitive and accurate detection of the target pathogen.

Project Name: Exotic Pathogens of Citrus

Associated CRIS Project Number(s) and Title(s):
1275-22000-151-00D, Citrus Pathogen Collection

Start and Termination Dates:
1275-22000-151-00D: Start Date: 2/20/1998; Termination Date: 5/1/2002

Scientific Personnel:
John S. Hartung, Research Plant Pathologist, Beltsville, Maryland, (0.25 FTE)

Research Goal(s):
Component I. Xf Systematics, Genomics, Biology, Ecology, Epidemiology

Goal 1. Clarify the taxonomy and nomenclature of Xf.

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

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

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

2. Continue to clarify the classification of the species Xf.

3. Determine potential interactions between different strains of Xf naturally infecting citrus and grapevine in grapevine.

4. Determine interactions between Xf and endophytic bacteria in planta that affect disease expression in infected plants.

Research Approach:
1 and 2: Costa Rica is the only country to report Xf infecting and causing disease in grapevines, citrus and coffee. The subspecies concept, with Xf subsp. pauca occurring in South America only and the other subspecies occurring in North America only is consistent with geography. Costa Rica is midway between North and South America. Are the strains of Xf similar to North or South American strains, or does Costa Rica represent a third center of diversity for Xf Strains isolated from citrus, coffee and grapevine in Costa Rica were analyzed using VNTR to address this question. The strains studied are thus far unique to Costa Rica and are not directly derived from North and South America.

3: Strains of Xf from sweet orange have been cultured together in vitro and coinoculated into periwinkle plants to determine if they interact synergistically or antagonistically. Quantitative PCR based assays have been developed to quantify each strain in plant extracts. Populations of Xf in planta are being monitored.

4: The xylem vessels inhabited by Xf in infected plants also harbor a diverse microflora, including strains of *Methylobacter mesophilicum* and *Curtobacterium flaccumfaciens*. These strains have been shown to affect the expression of symptoms in Xf infected citrus. These bacterium have been isolated and standard and Quantitative PCR based assays have been developed for them, so that their interactions with Xf in planta can be fully characterized.

Actual or Potential Deliverables:

- Objectives 1 and 2. A manuscript describing the new strains of Xf from Costa Rica has been submitted. Publication is expected within the year.

- Objective 3. A manuscript describing the real time and quantitative PCR assays specific for the Pierce's disease and Citrus Variegated Chlorosis strains in coinoculated plants is in preparation. Publication is expected next year.

- Objective 4. A manuscript describing the interaction between *Curtobacterium flaccumfaciens* and Xf in planta has been submitted. Publication is expected within the year.

Achieved and/or Potential Benefits:

- Objectives 1 and 2. A clear understanding of how the strains of Xf in Costa Rica are related to strains from North and South America will inform decision making on control and exclusion programs.

- Knowledge of the interaction of strains of Xf from citrus and grapevine will inform disease management strategies used against Pierce's disease in California.

- Objective 4. A bacterium with potential utility in biological control of diseases caused by Xf, including Pierce's disease, has been discovered.

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

Goal 1: Clarify the taxonomy/nomenclature of GWSS

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

Goal 3: Determine relationships between climatological factors and GWSS overwintering

Goal 4: Determine population dynamics of GWSS with respect to Xf transmission and the occurrence of disease

Goal 5: Determine GWSS feeding behavior and nutritional storage needs for use in developing mass production systems in elucidating pathogen transmission

Project Name: Glassy-winged Sharpshooter and Pierce's Disease

Associated CRIS Project Number(s) and Title(s):
6618-22000-031-00D, Glassy-winged Sharpshooter and Pierce's Disease

Project Start and Termination Dates
6618-22000-025-00D: Start Date: 4/4/02; Termination Date: 6/2/05
6618-22000-031-00D: Start Date: 6/3/05; Termination Date: 6/2/10

Scientific Personnel:
Wayne B. Hunter, Lead Scientist, Research Entomologist, Fort Pierce, Florida, (0.9 FTE)
David G. Hall, Research Entomologist, Fort Pierce, Florida, (0.10 FTE)

Research Goal(s)
Component II. Vector Systematics, Genomics, Biology, Ecology, Epidemiology, Mass Rearing

Goal. Using a genomics approach, produce the most useable data to address all the elements listed under Section II research goals.

Research Approach:
Large-scale expressed sequence tags, ESTs, were produced from three adult sharpshooter species. Within the GWSS the cDNA libraries were made from 5th instar nymphs from salivary-gland, and from midgut tissues. All sequences were released to the scientific community by publishing in the public domain on the NCBI website. Using these sequences, primers to specific gene transcripts were produced for functional genomic studies. Microsatellites were identified for use in separating species and biotypes, and a unigene set was created for the production of microarray analyses of GWSS with respect to biology, pathology, and pathogen interactions.

Actual or Potential Deliverables:

- Insect Genomics: Sharpshooters (Hunter)

Leafhoppers: (Hemiptera: Cicadellidae). Genetic information for three vectors of PD.

Sharpshooter Species	Genetic Sequences	Years.
Glassy-winged Sharpshooter, *Homalodisca vitripennis*,….	25,000	2004-2006
Sharpshooter, *Oncometopia nigricans* ……..	10,000	2005
Blue-green Sharpshooter, *Graphocephala atropunctata*…..…	9,600	2006
Full length protein sequences for GWSS = 30.		2005
GWSS Unigene set…….	~4,300 genes.	2006
GWSS Microarray of ……	2,160 genes.	2006
Proteins characterized GWSS delta-9 desaturase, and GWSS vitellogenin		2006

Three leafhopper viruses were discovered.

- Including a new insect-pathogenic virus, named '*Homalodisca coagulata virus-1.*' 2004-2006
- Evidence of new plant virus transmitted by GWSS (Reoviridae) 2006
- First GWSS virus capsid sequenced, HoCV-1. 2005
- First GWSS virus genome sequenced, HoCV-1. 2006

Achieved and/or Potential Benefits:

- This is the largest genomics dataset yet produced for these sharpshooters. Researchers are now using this genetic information to produce genetic markers, microsatellites, and genetic primers to specific genes of interest, and to conduct functional genomic studies. Identified ~7 enzymes linked to salivation and feeding, and another ~15 gut related proteins and enzymes. These were used to design genetic primers for further studies on functional genomics.
- The microarrays will permit the study of thousands of gene expressions at a time, and will advance our understanding of the genomic basis of disease transmission, pathogen interactions, and sharpshooter/plant interactions.
- The discovery of pathogenic insect viruses which are specific to GWSS and sharpshooters has shown that naturally occurring pathogens against leafhoppers are still being discovered, are reducing GWSS in the field, and may provide a method for long-term GWSS management.
- Produced GWSS gut expression, and salivary gland expression cDNA libraries to identify the digestive enzymes being produced within GWSS guts during feeding, and to identify proteinases and other enzymes being produced by the GWSS salivary glands during feeding, host plant selection, and disease acquisition and transmission. All expressed sequence tags from both projects (~10,000) are published in the public database on the NCBI website.
- Identification of virus sequences from field caught GWSS show an association with GWSS and Reoviruses.

Project Name: Sampling, seasonal abundance, and comparative dispersal of glassy-winged sharpshooters (GWSS) in citrus and grapes.

Associated CRIS Project Number(s) and Title(s):
5347-22620-017-00D, Ecologically-Based Pest Management Strategies for Western Cotton

Start and Termination Dates:
5347-22620-017-00D: Start Date: 3/18/50; Termination Date: 3/17/10

Scientific Personnel:
Jacquelyn Blackmer and James Hagler, Maricopa, Arizona

Research Goal(s):
Component II. Vector Systematics, Genomics, Biology, Ecology, Epidemiology, Mass Rearing

Develop a better understanding of the factors that influence GWSS flight activity in open landscapes (i.e., over barren ground) versus structurally-complex landscapes (i.e., mature citrus grove).

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

Research Approach:
Mark-release recapture (MRR) studies with IgG protein markers and fluorescent dusts were conducted to compare rates of movement of GWSS and STSS in open and structurally-complex landscapes. 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.

Actual or Potential Deliverables:

- Potential deliverables include an identification of critical variables that influence GWSS movement, which will allow us to better determine the rate and extent of their spread. This knowledge will allow us to design more effective area-wide management programs and potentially reduce the spread of Xf and its vector.

Achieved and/or Potential Benefits:

- Environmental variables and host-plant quality influence GWSS 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 PIERCE'S DISEASE, as well as aid area-wide management strategies.

Project Name: Quantifying Landscape-Scale Movement Patterns Of Glassy-Winged Sharpshooter And Its Natural Enemies Using A Novel Mark-Recapture Technique.

Associated CRIS Project Number(s) and Title(s):
5347-22620-017-00D, Ecologically-Based Pest Management Strategies for Western Cotton

Start and Termination Dates:
5347-22620-017-00D: Start Date: 3/18/50; Termination Date: 3/17/10

Scientific Personnel:
James Hagler, Jacquelyn Blackmer and Thomas Henneberry, Maricopa, Arizona

Research Goal(s):
Component II. Vector Systematics, Genomics, Biology, Ecology, Epidemiology, Mass Rearing

Research Objective:
Determine effect of deficit irrigation on xylem flux and GWSS movement in citrus.

Research Approach:
A Latin-square design (3x3) was used to examine whether irrigation scheduling affected stem water potential, xylem flux, and GWSS movement. A drip irrigation system supplied 100, 80 and 60 percent of the normal amounts of water to a mature citrus grove. A GWSS mark-capture technique was used to mark adult GWSSs in each of the three water treatment zones. Specifically, each water treatment area was sprayed with a unique protein using a standard broadcast spray rig. Then, every week for two consecutive growing seasons (2005 and 2006) sticky traps were used to monitor the movement of GWSS relative to the three irrigation regimes. Each captured GWSS was analyzed by protein-specific ELISAs for the presence of each mark to determine its dispersal distance. Additionally, xylem sap was sampled every other week from June through September in 2005 to determine the influence of each water treatment on GWSS host quality.

Actual or Potential Deliverables:
- An enhanced knowledge of how irrigation scheduling might influence xylem flux and in turn GWSS movement. This information could possibly be used to limit or at least predict flight activity of GWSS.

Achieved and/or Potential 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 movement will aid us in predicting the spread of PIERCE'S DISEASE. Knowledge of the effect of irrigation scheduling on GWSS movement could potentially provide a management tool.

Project Name: Development of an Artificial Diet for the Glassy-Winged Sharpshooter

Associated CRIS Project Number(s) and Title(s):
3622-22000-030-00D, Improved Mass Rearing of Insects For Biological Control Programs Through Advanced Nutrition And Quality Control Analyses

Start and Termination Dates:
3622-22000-030-00D: Start Date: 6/16/2005; Termination Date: 6/15/2010

Scientific Personnel:
Thomas Coudron, Chemist, Columbia, Missouri, (.05 FTE)

Research Goal(s):
Component II. Vector Systematics, Genomics, Biology, Ecology, Epidemiology, Mass Rearing

Goal 5. Determine GWSS feeding behavior.

Research Objective(s):
1. Evaluate artificial diet delivery systems for rearing GWSS.

2. Formulate and evaluate artificial diets for the development and reproduction of GWSS.

Research Approach:
The simultaneous testing of over 25 diet delivery systems in combination with over 10 diet formulations was completed. Diet formulations were tested that were based, in part, on previous studies using GWSS (unpublished data), as well as on artificial diets that had been developed for other Hemiptera and also on the xylem chemistry of GWSS host plants. The xylem chemistry of host plants indicated that nitrogen may represent a nutrient limitation for GWSS. Therefore, three potential sources of nitrogen, i.e. proteins, peptides and amino acids, were evaluated via artificial diets. Diets were evaluated based on their effects on life history analyses. The ability of salivary and midgut proteolytic enzymes to digest proteins/peptides was also investigated in order to determine whether less costly nitrogen sources could be substituted for those commonly found in plants.

Actual or Potential Deliverables:
- Effective parafilm-substrate diet delivery systems, devoid of pressurized flow components, were designed that supported immature development and adult feeding which allowed adult survival for more that 30 days. Partial salivary and midgut proteolytic characterization confirmed sufficient activity to demonstrate a significant role for proteases in the digestive tract of GWSS.

Achieved and/or Potential Benefits:
- These studies demonstrated that GWSS manifest nutritional traits common to most insects and in doing so the results provide insights for understanding the nutritional requirements of GWSS. Further, the formulation of functional diets and design of functional delivery systems for immature and adult GWSS demonstrates that the development and optimization of artificial rearing systems for GWSS is fully achievable.

Project Name: Identifying Key Predators of the Various Glassy-winged Sharpshooter Lifestages.

Associated CRIS Project Number(s) and Title(s):
5347-22620-017-00D Ecologically-Based Pest Management Strategies for Western Cotton.

Start and Termination Dates:
5347-22620-017-00D: Start Date: 3/18/50; Termination Date: 3/17/10

Scientific Personnel:
James Hagler and Thomas Henneberry, Maricopa, Arizona, Jesse de León Weslaco, Texas

Research Goal(s):
Component II. Vector Systematics, Genomics, Biology, Ecology, Epidemiology, Mass Rearing

Identify key predators of glassy-winged sharpshooter.

Research Objective(s):
Identify key predators of the various life stages of the glassy-winged sharpshooter (GWSS) using state-of-the-art predator gut content assays.

Research Approach:
Our approach was to use state-of-the-art molecular techniques to analyze the gut contents of field-collected predators for the presence of GWSS remains. To this end, we developed GWSS-egg specific enzyme-linked immunosorbent assays (ELISA) to detect GWSS egg protein and GWSS-specific PCR assays to detect fragments of the GWSS mitochondrial cytochrome oxidase subunit I and II genes (COI and COII) (i.e., GWSS DNA). We conducted laboratory tests to determine how long GWSS egg protein and DNA remains detectable in guts of various predator species. We found that GWSS egg is detectable by ELISA for up to 12 h in lacewings (*Chrysoperla carnea*) and 6 h in lady beetles (*Harmonia axyridis*). Similarly, GWSS DNA is detectable by PCR assay for up to 24 h in lacewings and 18 h in lady beetles. We just completed the analyses of the gut contents of thousands of predators that were collected from various crops in California every year since the summer of 2002. We assayed each predator for the presence of GWSS DNA by PCR. If positive, we knew that the predator consumed at least one GWSS egg, nymph, or adult. Individuals scoring positive in the PCR assay were then assayed by a GWSS egg-specific ELISA to determine if the predator fed on a gravid female or an egg. Combining ELISA and PCR assays revealed which life stage(s) is most vulnerable to predation by any given predator species. To date, ≈17 percent of the predators examined (n=1052 to date) contained GWSS in their guts. We are currently analyzing the data obtained from thousands of other predator samples. Our work represents a first step towards identifying the GWSS predator complex. Ultimately, this research will provide information needed to implement an effective conservation biological control program for GWSS.

Actual or Potential Deliverables:
- Potential deliverables include the precise identification of key predators of each GWSS lifestage. Additionally, the predator gut content assays developed can be used for future studies of predation.

Achieved and/or Potential Benefits:
- Enhanced conservation or augmentation biological control program for GWSS.

Project Name: Development Of Storage Technology For Use In The Mass Rearing Of GWSS And Its Parasitoids

Associated CRIS Project Number and Title:
5442-22000-040-00D, Development of cold storage technology for mass-reared and laboratory–colonized insects

Project Start and Termination Dates
5442-22000-034-00D: Start Date: 6/6/04; Termination Date: 5/24/05
5442-22000-038-00D: Start Date: 6/06/2004; Termination Date: 5/24/2005
5442-22000-040-00D: Start Date: 05/25/05; Termination Date: 5/24/10

Scientific Personnel: Roger Leopold Research Entomologist, Fargo, North Dakota, (0.4 FTE)

Research Goal(s):
Component II. Vector Systematics, Genomics, Biology, Ecology, Epidemiology, Mass Rearing

Component II. Determine GWSS feeding behavior, and nutritional and storage needs for use in developing mass production systems and in elucidating pathogen transmission.

Research Objective(s):
1. Examine the effects of constant and fluctuating temperatures on cold storage of parasitized and unparasitized eggs of the glass-winged sharpshooter.

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

3. Examine the influence of subambient temperature on development and reproduction of the egg parasitoid, *G. ashmeadi.*

4. Investigate use of refrigerated dead glassy-winged sharpshooter eggs for propagation of the parasitoid, *G. ashmeadi.*

Research Approaches:
1. 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 on post-storage parasitism and emergence by egg parasitoid, *G. ashmeadi*. Determine the cold tolerance of the egg parasitoid, *G. ashmeadi*, within GWSS eggs and attempt to extend shelf time of the parasitoid by altering storage temperature regimes.

2. Characterize functional response of the parasitoid to GWSS eggs of different ages. Using various host-parasitoid ratios, determine the effect of host densities and ages on development time of wasps and superparasitism of the hosts. Use these parameters for evaluating hosts and parasitoids following storage.

3. 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.

4. Determine suitability and acceptability of GWSS eggs killed by exposure to a lethal cold temperature as hosts for propagating *G. ashmeadi*. Determine the most acceptable stage for 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.

Actual or Potential Deliverables:

- The functional responses and superparasitism 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. (October 2005)
- 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. (June 2006)
- Dead host eggs stored 20 days remained fully acceptable to the wasps for attack and although the parasitism rate decreased with storage time, > 80 percent parasitoid emergence was realized from eggs stored 30 days. The intrinsic and finite rates of increase, population doubling time, and mean generation time decreased only after storage for 60 days. Our results show that short-term cold storage would be useful for maintaining wasp populations in a mass-rearing program, and that the detrimental effects on host eggs held stored over 30 days do not extend to F_2 generation. (December 2006)

Achieved and/or Potential Benefits:

- The impact of defining the reproductive parameters and life tables for the egg parasitoid, *G. ashmeadi*, allowed us to evaluate the effects of cold storage on the fitness of this wasp and to modify the conditions of a storage protocol to eliminate harmful effects. It also allowed us to define the ultimate limitations of this type of storage protocol. Developing and improving preservation technology provides techniques for efficiently and economically storing insects mass-reared and released for biological control programs.

Project Name: Role of Visual And Olfactory Stimuli In GWSS Host-Finding Behavior

Associated CRIS Project Number(s) and Title(s):
6204-22000-018-00D, Project Title: Biological Control of Invasive and Exotic Pests

Project Start and Termination Dates
6204-22000-015-00D: Start Date: 10/10/1999; Termination Date: 04/08/2003
6204-22000-016-00D: Start Date: 4/9/2003; Termination Date: 6/21/05
6204-22000-018-00D: Start Date: 6/22/2005; Termination Date: 6/21/10

Scientific Personnel: Joseph M. Patt, Research Entomologist, Weslaco, Texas, (80.0 FTE)

Research Goal(s):

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

(*Goal 4 in ARS PIERCE'S DISEASE-GWSS Strategic Research Plan)* Determine population dynamics of GWSS with respect to Xf transmission and the occurrence of the disease. This includes correlating the effects of crowding, sex ratio, reproductive status, and 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.

Research Objective(s):
Determine the relative importance of visual and olfactory cues in host-plant recognition and selection by adult and immature GWSS.

Research 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.

Actual or Potential Deliverables:
New knowledge that:

- GWSS are responsive to host-plant olfactory stimuli.
- Exposure to host-plant olfactory cues enhances the response of GWSS immatures and adults to visual stimuli.
- Immature GWSS can learn to recognize gustatory- and olfactory stimuli produced by host plants.
- Immature GWSS can use information conveyed by novel gustatory compounds to recognize host-plant odor cues.
- Associative learning may have a potential role in host-plant location by adult GWSS.
- GWSS nymphs may be able to distinguish among various proportions and ratios of mixtures of host-plant volatiles.

Achieved and/or Potential Benefits:

- Virtually nothing is known about the 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. In turn, understanding the mechanisms by which nymphs locate their host-plants is fundamental to developing vegetation management programs aimed at suppressing their population growth and dispersal in complex landscapes.

Project Name: Sampling, Seasonal Abundance, And Comparative Dispersal Of Glassy-Winged Sharpshooters In Citrus And Grapes.

Associated CRIS Project Number and Title:
5347-22620-017-00D, Ecologically-based Pest Management Strategies for Western Cotton

Start and Termination Dates:
5347-22620-017-00D: Start Date: 3/18/50; Termination Date: 3/17/10

Scientific Personnel:
Steve Castle, Entomologist, Maricopa, Arizona, (0.10 FTE)
Steve Naranjo, Entomologist, Maricopa, Arizona, (0.10 FTE)

Research Goal(s):
Component II. Vector Systematics, Genomics, Biology, Ecology, Epidemiology, Mass Rearing

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

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

Research Approach:
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.

Actual or Potential Deliverables:
New knowledge/technology about the comparative effectiveness of four different sampling devices to estimate the proportion of the GWSS population that is Xf-inoculative; development of sampling plans in citrus.

Achieved and/or Potential Benefits:

- Estimating the spatial distribution of GWSS within citrus trees has helped to refine the sample unit and further reduce 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.
- The bucket sampler was the most cost efficient technique, providing good reproducibility for estimating both adult and nymphal populations of GWSS.
- Knowledge that yellow sticky trap catches of adult GWSS are highly correlated with all foliage sampling methods, and float the relationships are 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. These findings were used to refine the sample unit for GWSS in citrus.

Project Name: Biotaxonomy of *Homalodisca*, The Genus of The GWSS

Associated CRIS Project Number(s) and Title(s):
1275-22000-225-00D, Systematics of Moths, Leafhoppers, and True Bugs of Importance to Agricultural, Forest, and Ornamental Plants

Start and Termination Dates:
1275-22000-225-00D: Start Date:10/1/04; Termination Date: 3/17/2005

Scientific Personnel: Stuart H. McKamey, Research Entomologist, Beltsville, Maryland, (0.25 FTE)

Research Goal:
Component II. Vector Systematics, Genomics, Biology, Ecology, Epidemiology, Mass Rearing

To circumscribe the genus *Homalodisca*, define all species, investigate the egg-mass coverings (brochosomes) of as many species as possible, and to provide identification aids for all of these.

Research Objective(s):
Establish limits of the genus *Homalodisca* and all species in the genus, determine their valid names, and describe new species as necessary**;** characterize brochosome structure and related behavior to allow identification of egg masses; provide authoritative and accessible identification aids and distribution data for the genus.

Research Approach:
Comparative morphology of *Homalodisca* and related genera, electron-microscopy of brochosomes.

Actual or Potential Deliverables:

- Rakitov, R. 2006. Leafhopper Egg Brochosomes Image Database. (URL http://ctap.inhs.uiuc.edu/takiya/broch.asp).

Achieved and/or Potential Benefits:

- The publications and web-posting listed above provide the tool and consistency needed so that all other information gathered (host plants, ecology, physiology, genomics, etc., which are all priorities in the Xf *a* problem) can be linked to the correct names for meaningful communication about GWSS and any of it relatives, including other known pest species in the United States, Mexico, Central America, and especially Brazil, where the genus transmits citrus variegated chlorosis in oranges. It also enables APHIS and Homeland Security officers in their quarantine efforts against potentially invasive species of *Homalodisca*.

Project Name: Development of A Degree-Day Cooling Model For The GWSS

Associated CRIS Project Number(s) and Title(s):
5302-22000-007-00D, Epidemiology and Management of *Xylella fastidiosa* (Xf) and other Exotic and Invasive Diseases and Insect Pest

Start and Termination Dates:
5302-22000-005-00D: Start Date: 5/16/01– Termination Date: 9:30/05
5302-22000-007-00D: Start Date: 10/1/05 – Termination Date: 3/14/07
5302-22000-008-00D: Start Date: 3/15/07 – Termination Date: 3/14/12

Scientific Personnel:
Russ Groves, Research Entomologist (resigned from ARS). This project has now been transferred to Mark Sisterson, Parlier, California.

Marshall Johnson, University of California-Riverside

Note: This work is being done at the University of California system through an SCA between ARS and University of California-Riverside.

Research Goal(s):
Component II. Vector systematics, genomics, biology, ecology, epidemiology, mass rearing

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

Research Objective(s):
Determine minimum temperature requirements for overwintering GWSS

Research Approach:
Cooling degree days required to kill GWSS were estimated by comparing mortality of GWSS held under 6 different constant temperature regimes with or without a host plant. This information in conjunction with historical temperature data will help establish which portions of California have habitats suitable for overwintering survival of GWSS.

Actual or Potential Deliverables:
- A degree-day cooling model for GWSS.

Achieved and/or Potential Benefits:
- Knowledge of the areas where GWSS can and cannot overwinter will help focus management tactics to the must vulnerable areas.

Peer Reviewed Publications post 2004 NAS Report: None

Project Name: Seasonal Abundance of GWSS In Selected Crop and Non-Crop Habitats.

Associated CRIS Project Number(s) and Title(s):
5302-22000-007-00D, Epidemiology and Management of *Xylella fastidiosa* (Xf) and other Exotic and Invasive Diseases and Insect Pests

Start and Termination Dates:
5302-22000-005-00D: Start Date: 5/16/01– Termination Date: 9:30/05
5302-22000-007-00D: Start Date: 10/1/05 – Termination Date: 3/14/07
5302-22000-008-00D: Start Date: 3/15/07 – Termination Date: 3/14/12

Scientific Personnel:
Russ Groves, Research Entomologist (resigned from ARS). A replacement is being recruited to continue this work.

Research Goal(s):
Component II. Vector systematics, genomics, biology, ecology, epidemiology, mass rearing

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, and infectivity status, host plant quality, feeding ecology, and seasonal and environmental variables with population dynamics and movement of GWSS as an aid to predicting insects and disease spread and applying control strategies.

Objective 1. Determine and characterize the patterns of utilization/preferences of plant hosts among cultivated crops and non-cultivated hosts in agricultural production systems.

Research Objective(s):
Determine seasonal abundance of GWSS in selected crop and non-crop habitat.

Research Approach:
The seasonal abundance of GWSS was compared between lemon, navel orange, pomegranate, olive, avocado, cherry, plum, grape, pistachio, and non-crop weeds. Insect abundance was monitored at three sites for each habitat type. Two types of sampling were employed: sweep/beat sampling and yellow sticky cards. Sweep/beat samples were collected monthly and yellow sticky traps were collected weekly.

Actual or Potential Deliverables:
- Knowledge about the seasonal abundance and preference of GWSS for different crops .

Achieved and/or Potential Benefits:
- Knowledge of the abundance of GWSS in different crop types will help focus control measures to the habitats where they are most common.

Peer Reviewed Publications post 2004 NAS Report: None

Component III: Xf-Vector Interactions

Goal 1: Determine the mechanisms of transmission of Xf by GWSS (and other insect vectors.

Project Name: Xf -Vector Interactions

Associated CRIS Project Number(s) and Title(s):
5302-22000-007-00D, Epidemiology and Management of *Xylella fastidiosa* (Xf) and other Exotic and Invasive Diseases and Insect Pests

Start and Termination Dates:
5302-22000-005-00D: Start Date: 5/16/01– Termination Date: 9:30/05
5302-22000-007-00D: Start Date: 10/1/05 – Termination Date: 3/14/07
5302-22000-008-00D: Start Date: 3/15/07 – Termination Date: 3/14/12

Scientific Personnel:
Elaine A. Backus, Research Entomologist, Parlier, California, (1.0 FTE)

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

Research Objective(s):
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**.**

Research Approach:
The PIPRA Research Priority category for this work is *Understanding transmission of the disease*. The primary Disease Stage category is *E. Transmission of* Xf *to grapevine*. The Focus Area is *Disease Epidemiology*.

1. a) Specific probing behaviors have been identified via electrical penetration graph (EPG) monitoring of stylet penetration, combined with videotaping stylet movements in transparent diet, light and confocal microscopy of salivary sheaths and excised stylets in diets and plants, plus electromyography and X-ray imaging of precibarial valve and cibarial muscles in the precibarium and cibarium (the two parts of the foregut).

b) In the last two years, all-new protocols have been developed for use of green fluorescent protein-transformed Xf (GFP-Xf) to visualize Xf in vector foreguts and in probed grape tissues, including the first-ever sectioning of GFP-containing tissue. For acquisition studies, GWSS are fed on GFP-Xf – infected grape for 14 days. Locations in the foregut are examined via confocal microscopy for GFP-Xf. For inoculation studies, a single-probe inoculation bioassay has been developed to study the success of a standardized, EPG-recorded GWSS probe into grape.

Actual or Potential Deliverables:
- Comprehensive characterization and correlation of nearly 20 feeding waveforms of adult GWSS on susceptible grape. The entire process of GWSS stylet penetration can now be determined and interpreted exclusively by observing EPG waveforms of GWSS.
- Identification of the EPG waveform that represents stylet entry into the xylem, termed the *sharpshooter X wave*. The complex behaviors represented by the X-wave function in: 1) micro-ingestion and egestion of fluid into/out of the precibarium (*waveforms B1w* and *proto-C*), 2) salivation and precibarial valve fluttering (*waveform B1s*), plus 3) mechanically testing the strength of the stylet seal into the xylem (*waveform C*).

- Characterization of the locations and timing of Xf colonization in the GWSS foregut during acquisition. GWSS acquired Xf first into the posterior precibarium, then into the cibarium.
- Knowledge that the availability of binding sites for Xf determines acquisition success. Comparing clean with field-collected insects (whose foreguts are contaminated with a variety of microbes) showed that many more Xf are acquired by clean insects. Thus, competitive microbial binding affects Xf acquisition efficiency, i.e. vector load.
- Knowledge that acquisition success/vector load determines the degree to which inoculation succeeds. Formerly clean GWSS, after acquisition, have a higher rate of inoculation success than do dirty, field-collected GWSS. Differences in inoculation efficiency could lead to certain individuals being "super-spreaders," i.e. responsible for a disproportionately high rate of inoculation than others. (expected in 1-2 years)
- Identification that the *X wave* represents Xf inoculation. Two components of the X wave are *C* and *B1*, which have been shown to be the two waveforms most important for inoculation, when the behaviors they represent are performed in xylem. (expected in 1-2 years)
- Multivariate statistical analysis of EPG data will develop a Stylet Penetration Index (SPI) which, when combined with the single-probe inoculation bioassay, can be used to objectively and rapidly compare among grape genotypes for likelihood of inoculation success. (expected in 1-2 years)

Achieved and/or Potential Benefits:

- Host plant resistance by either genetic engineering or classical breeding:
  - Use of the SPI and single-probe inoculation bioassay will allow comparison among genotypes. This will allow rapid detection of resistance to the vector, via deterrence of inoculation behavior. Resistance to vector inoculation will be an important trait for development of resistant grape varieties.
- Biological control of Xf:
  - Testing of benign, competing microbe species for their ability to block GWSS from acquiring and inoculating Xf. Such a microbe could be sprayed in vineyards.
- Epidemiological, risk assessment simulation models:
  - Data on acquisition and inoculation efficiency, including "super-spreaders," will be input into risk assessment models.
  - Determine whether GWSS/Xf outbreaks or "hot spots" occur as a result of "super-spreaders" interacting with varying climate, temperature, and host plants, as the insect invades new areas of California.

Project Name: Xf -Vector Interactions

Associated CRIS Project Number(s) and Title(s):
5302-22000-007-00D, Epidemiology and Management of *Xylella fastidiosa* (Xf) and other Exotic and Invasive Diseases and Insect Pests

Start and Termination Dates:
5302-22000-005-00D: Start Date: 5/16/01– Termination Date: 9:30/05
5302-22000-007-00D: Start Date: 10/1/05 – Termination Date: 3/14/07
5302-22000-008-00D: Start Date: 3/15/07 – Termination Date: 3/14/12

Scientific Personnel: Elaine A. Backus, Research Entomologist, Parlier, California, (1.0 FTE)

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

Research Objective(s):
1: 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).

Research Approach: The PIPRA Research Priority category for this work is *Understanding transmission of the disease*. The primary Disease Stage category is *E. Transmission of Xf to grapevine*. The Focus Area is *Disease Epidemiology*.

2a) Perform electrical penetration graph (EPG) waveform correlations with salivation, and develop EPG protocols for future work. Develop light and confocal microscopy techniques to visualize GWSS salivary sheaths and GFP-Xf in the xylem cell marked by the salivary sheath, 0, 10, 20 and 40 days following an EPG-identified, standardized probe inoculating GFP-Xf (single-probe bioassay). Dissect salivary glands from field-collected GWSS. Extract, purify, assay and raise antibodies to beta 1, 4-glucanase (EGase), a major component of the watery saliva and known to be a cell wall-degrading enzyme, in collaboration with J. Labavitch (UC Davis). Use immunofluorescent staining of EGase to co-localize saliva and GFP-Xf in plant tissues probed during a single-probe GFP-Xf inoculation bioassay.

2b) Further extract and separate the salivary constituents from salivary glands vs. directly secreted saliva via 1-D and 2-D gel electrophoresis, in collaboration with H. Lin (ARS Parlier) and F. Schreiber (CSU Fresno). Use colorimetric analysis to determine broad categories of enzymatic function for separated proteins. Time permitting, perform amino acid and DNA sequencing of selected proteins from saliva.

Actual or Potential Deliverables:
- Characterization that both sheath and watery saliva are injected into grape xylem cells during the *B1 waveform*, especially its sub-component, *B1w* (citations).
- Knowledge that grape cells into which GWSS has injected saliva show signs of cell wall degradation.
- Knowledge regarding whether salivary enzymes degrade pit membranes of xylem cells adjoining the Xf inoculation cell. (expected in 2-3 years)
- Identification of the degree to which *Xf* distribution in xylem immediately after inoculation

is spatially correlated with distribution of labeled EGase in watery saliva, especially in adjoining xylem cells with damaged pit membranes. If so, additional knowledge that:
    - Xf is carried into the plant via saliva, thus inoculation occurs in part via salivation. (expected in 2-3 years), and
    - GWSS saliva promotes the initial spread and growth of Xf via pit cell membrane destruction. (expected in 2-3 years)
- Identification of other proteins in GWSS saliva and their chemical functions, for further investigation of Xf –saliva interactions. (expected in 2-3 years)

Achieved and/or Potential Benefits:

- Host plant resistance:

  Protein inhibitors of GWSS salivary enzymes (known to exist for EGase) could be genetically engineered into grape, to provide resistance to Xf after initial inoculation that would prevent spread and further multiplication of the pathogen.

Component IV: Xf-Host Plant Interactions

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

Project Name: Virulence Analysis of the Pierce's Disease Agent, *Xylella fastidiosa*: Roles of *Xylella fastidiosa* proteins in virulence.

Associated CRIS Project Number(s) and Title(s):
5302-22000-007-00D; Epidemiology and management of *Xylella fastidiosa* (Xf) and other Exotic and invasive Diseases and Pests.

Start and Termination Dates:
5302-22000-005-00D: Start Date: 5/16/01– Termination Date: 9:30/05
5302-22000-007-00D: Start Date: 10/1/05 – Termination Date: 3/14/07
5302-22000-008-00D: Start Date: 3/15/07 – Termination Date: 3/14/12

Scientific Personnel:
Edwin L. Civerolo, Supervisory/Research Plant Pathologist, Parlier, California, (0.9 FTE)

Research Goal(s):
Component IV. *Xylella fastidiosa*-Host Plant interactions

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

Component VI. Disease and Vector Management – Goal 1: Prevent Xf infection

Research Objective(s):
Identify gene and gene products of Xf that contribute to its virulence.

Research Approach:
Identify, isolate and characterize components of the outer membrane of Xf that are associated with infection and/or disease development. Determine the nature of the Xf component(s) responsible for inducing chlorosis in *Chenopodium quinoa*. Analyze the protein composition of extracts of aqueous Xf cell suspensions by sensitivity to various proteases, heat, pH and organic solvent treatments, as well as physical properties (e.g., mass spectroscopy of tryptic digests, molecular weight, solubility) and biological activity (e.g., chlorosis-inducing activity in *C. quinoa*).

Actual or Potential Deliverables:
- Identification and characterization of mopB as the major outer membrane protein of Xf. (2002)
- Identification of most of the chlorosis-inducing activity of Xf in C. quinoa as mopB. (2002)
- Identification of the likely start of translation for the mopB gene (corresponding to a 40.7K translation product) recognizes a candidate 22 amino acid residue signal peptide that maps to the mopB gene sequence in Xf. (2003).
- Demonstration that mopB is a, or possibly the, major outer membrane protein of Xf. (2003-2004).
- Demonstration that mopB is accessible on the Xf cell exterior and is a member of the ompA family of outer membrane proteins of Gram-negative bacteria. (2000-2004).

Achieved and/or Potential Benefits:
Since mopB probably accounts for at least 10 percent of the Xf cell exterior, mopB is a highly suitable target for inactivation of Xf cells *in planta*.

Peer Reviewed Publications post 2004 NAS Report: None

Component V: Grape Genomics, Genetics, Physiology, and Crop Resistance

Goal 1: Identify grape rootstock germplasm and/or varieties that reduce or mitigate PIERCE'S DISEASE development in susceptible wine grape scions in PIERCE'S DISEASE-prone production areas

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

Goal 3: Identify and characterize physiological PIERCE'S DISEASE resistance mechanisms in Vitis species

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

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

Project Name: Grape Rootstock Variety Influence on Pierce's Disease Symptoms in Chardonnay

Associated CRIS Project Number and Title:
1910-21220-002-00D, Title: Genetics and Genomics of Grape Rootstock and Scion Interactions with Pathogens

Start and Termination Dates:
1910-21220-002-00D: Start Date – 4/24/2003; Termination Date: 4/23/2008

Scientific Personnel:
Peter Cousins, Geneticist (Plants), Geneva, New York, (0.5 FTE)

Research Goal(s):
Component V. Grape Genomics, Genetics, Physiology, and Resistance to Xf in Grapes, Almonds, and Other Commercially Important Species

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

Research Objectives:
To evaluate the impact of rootstock variety on expression of Pierce's disease symptoms in naturally infected vines of the PIERCE'S DISEASE susceptible *Vitis vinifera* scion variety Chardonnay.

Research Approach:
If grape rootstocks could contribute Pierce's disease resistance or tolerance to their scions, this would be a major benefit to viticulture in Pierce's disease prone areas. To evaluate this potential, grafted vines of Chardonnay on five rootstocks (Freedom, Tampa, Dog Ridge, Florilush, and Lenoir) were planted at the Kika de la Garza Subtropical Agricultural Research Center in Weslaco, Texas in July, 2006. Natural inoculation of the vines by insects will be permitted. Evaluation of Pierce's disease response of the vines will begin in 2007.

The Rio Grande Valley is an excellent location for the field evaluation of Pierce's disease resistant plant germplasm and Pierce's disease management techniques. Many insect vectors of Xf are native to the region, including the glassy-winged sharpshooter. Susceptible grapevine varieties are infected naturally with Xf in the vineyard and demonstrate characteristic Pierce's disease symptoms and decline. The Rio Grande Valley is similar to many viticultural regions in California; the region is flat, irrigated, and supports multiple types of crops (citrus, grains, vegetables) in close proximity. The Rio Grande Valley is an ideal test environment due to heavy Pierce's disease pressure, with abundant vectors and inoculum, in contrast to many other locations, especially California, which demonstrate substantial cycling of Pierce's disease incidence. The USDA Agricultural Research Service Kika de la Garza Subtropical Agricultural Research Center in Weslaco, Texas is located in the heart of the Rio Grande Valley and provides an ideal experimental location for the evaluation of Pierce's disease management practices, including rootstock evaluation.

Five rootstocks were chosen for evaluation in this project. Freedom is a complex interspecific hybrid developed as a root-knot nematode resistant rootstock by the USDA, ARS, at Fresno, California; its parentage includes *Vitis vinifera, V. labrusca, V. x champinii, V. solonis,* and *V. riparia.* Freedom is widely used in California viticulture. Dog Ridge is a *V. x champinii* selection recognized for its nematode resistance and resistance to Pierce's disease, but it is rarely used as a rootstock. Lenoir, a *V.*

aestivalis/*V. vinifera* hybrid, was used historically as a rootstock and presently is cultivated as a wine grape in Pierce's disease prone regions (including some parts of Texas). Tampa includes a *V. aestivalis* selection and the juice grape Niagara (a *V. labrusca* hybrid) in its parentage. Florilush is a selection from the cross Dog Ridge x Tampa. Both Florilush and Tampa were selected by the University of Florida as Pierce's disease resistant rootstocks for bunch grapes. Pierce's disease resistance is necessary for rootstock mothervines to thrive in Florida, so the Pierce's disease resistance of Florilush and Tampa should not be construed necessarily as contributing to the Pierce's disease response of the scions.

Actual or Potential Deliverables:

- The anticipated product is a description of the effect of these five rootstock varieties on Pierce's disease expression in Chardonnay. Grape growers could use this description to aid in rootstock selection, particularly if there is an ameliorative effect of rootstock variety on Pierce's disease symptom expression.
- The project was planted in 2006. At least three years of vineyard observations will be needed to evaluate the effect of these rootstock varieties on Pierce's disease symptom expression in Chardonnay.

Achieved and/or Potential Benefits:

- Since the trial was planted in 2006, no benefits have yet been realized from this project. Potential benefits could be the identification of a rootstock that reduces Pierce's disease symptoms in susceptible scion varieties. Deployment of this rootstock alone or in combination with other Pierce's disease management strategies could be a sustainable tool for Pierce's disease management.

Peer Reviewed Publications post 2004 NAS Report: None

Project Name: Effects Of *Xylella* On Diverse Almond And Peach Germplasm

Associated CRIS Project Number(s) and Title(s):
5302-22000-007-00D, Epidemiology and Management of *Xylella fastidiosa* (Xf)-caused Diseases and Xf Insect Vectors

Start and Termination Dates:
5302-22000-005-00D: Start Date: 5/16/01– Termination Date: 9:30/05
5302-22000-007-00D: Start Date: 10/1/05 – Termination Date: 3/14/07
5302-22000-008-00D: Start Date: 3/15/07 – Termination Date: 3/14/12

Scientific Personnel:
Craig A. Ledbetter, Research Geneticist (0.25 FTE) (retired); recruitment for replacement in Parlier, California, is underway.

Research Goal(s):
Component V. Grape Genomics, Genetics, Physiology, and Resistance to Xf in Grapes, Almonds, and Other Commercially Important Species

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

Research Objective(s):
Determine the mechanisms of resistance to Xf infection and subsequent disease development in *Vitis* and selected *Prunus* species.

Research Approach:
Small almond trees are inoculated with known strains of Xf during the early part of the growing season. Almond varieties that are reportedly different in their resistance to ALS will be needle inoculated and then sampled throughout the growing season for the presence of Xf. The ability of the bacteria to infect these plants is followed by real-time PCR and by ELISA. In other experiments different rootstocks will be used with similar scion varieties to examine rootstock effects on Xf establishment and symptom development. Diverse peach rootstocks will be screened for their ability to host Xf strains responsible for Pierce's disease. and ALS.

Actual or Potential Deliverables:
- Technology for standardized methods to inoculate trees and assay for the presence of Xf
- Knowledge about Xf growth *in planta* .
- Knowledge about the timing of ALS symptoms relative to Xf inoculation in various *Prunus* germplasm will be determined.

Achieved and/or Potential Benefits:
- Identification of Xf resistant almonds and peach rootstock germplasm.
- Knowledge of varietal differences in susceptibility to ALS among commercial varieties will allow growers to reduce the impact of this disease if it becomes established and widespread. If resistance to ALS exists in other *Prunus* species, this knowledge might be exploited through breeding with almonds. Such future populations could be screened for Xf susceptible and Xf resistant tree types.

Project Name: Breeding Pierce's Disease Resistant Table and Raisin Grapes and the development of markers for additional sources of resistance.

Associated CRIS Project Number(s) and Title(s):
5302-2200-007-00D, Epidemiology and Management of *Xylella fastidiosa* (Xf) and other Exotic and Invasive Diseases

Start and Termination Dates:
5302-22000-005-00D: Start Date: 5/16/01– Termination Date: 9:30/05
5302-22000-007-00D: Start Date: 10/1/05 – Termination Date: 3/14/07
5302-22000-008-00D: Start Date: 3/15/07 – Termination Date: 3/14/12

Scientific Personnel:
David W. Ramming, Research Horticulturist, Parlier, California, (0.25 FTE)

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

Identify table and raisin grape germplasm and varieties with enhanced resistance or tolerance to Pierce's disease that have commercial fruit quality.

Research Objective(s):
Develop table and raisin grape germplasm selections with enhanced resistance/tolerance to Xf infection and Pierce's disease development and that have commercial fruit quality.

Research Approach:
Hybridize high quality seedless table and raisin breeding lines as female parents with previously identified sources having Pierce's disease resistance/tolerance to combine fruit quality and seedlessness with Pierce's disease 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 Pierce's disease 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 Pierce's disease -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. Use molecular markers developed for *V. arizonica* source of resistance to select resistant genotypes in breeding population. Develop additional genetic markers for Pierce's disease -resistance in other genetic backgrounds and for fruit characteristics.

Actual or Potential Deliverables:
- Table and raisin grape germplasm that is resistant to Pierce's disease with improved fruit quality has been developed. Third generation *V. rupestris* x *V. arizonica/candicans* background families have been developed with Pierce's disease resistance and are being used as parents for table and raisin grape cultivar development. First and second generation southeast United States background families have been developed for increasing the diversity of resistant table and raisin grape germplasm.
- Molecular markers for resistance to Pierce's disease in the *V. rupestris* x *V. arizonica/candicans* background to identify resistant individuals in each progressive generation as made.

- Additional molecular markers for resistance to Pierce's disease in other diverse genetic backgrounds. (expected in 3-4 years).
- Table and raisin grape selections resistant to Pierce's disease with fruit quality good enough to start advanced production/cultural trials. (expected in 3-4 years).

Achieved and/or Potential Benefits:

Many families and seedlings have been created by hybridizing various Pierce's disease sources of resistance with seedless table and raisin grapes. This gives a high chance of finding resistant genotypes with high fruit quality and seedlessness for table and raisin cultivar development.

- *V. rupestris* x *V. arizonica/candicans* resistance source hybridized with seedless table and raisin grape selections produced four F1 families of 188 individuals and 26 resistant individuals were selected after greenhouse tests. Eleven BC1 families with 101 individuals were produced and 9 resistant individuals were identified. Sixteen BC2 families and 427 plants were produced. Fifty-six have been screened for resistance with molecular markers and 21 were resistant. Increase in fruit quality was achieved as evidenced by increase in berry size from 1.82 g largest berries in F1 to 4.85 g in the BC1. Increase in fruit quality also shown by the development of Pierce's disease resistant individuals with aborted seeds smaller than those found in Thompson Seedless in the BC1. Resistance to Pierce's disease has been maintained through three generations. This shows that progress can be achieved rapidly by greenhouse screening and molecular marker selection.
- Southeast United States sources of resistance hybridized with seedless table grapes produced 184 individuals in 3 F1 families and 39 fruiting individuals were identified as resistant. One family has been increased to search for additional molecular markers for resistance in a second genetic background. Selections with fruit quality approaching California standards and having 9.0 g seeded fruit and another with 5.6g seedless fruit have been identified. This shows that fruit quality can be rapidly increased while maintaining Pierce's disease resistance. An additional 15 BC1 families consisting of 267 plants have been produced and are being grown in the field for fruit evaluation. This diversifies the resistance sources to reduce the chances of genetic breakdown of resistance.

Project Name: Physiological Characterization and Proteomic Identification of Pierce's Disease Resistant Mechanisms: Analysis of Xylem Anatomic Structures, Natural Products and Protein Expressions in Stem and Xylem Sap of Pierce's Disease Resistant *Vitis*

Associated CRIS Project Number(s) and Title(s):
5302-22000-007-00D, Epidemiology and Management of *Xylella fastidiosa* (Xf) and other Exotic and Invasive Diseases and Insect Pests

Start and Termination Dates:
5302-22000-005-00D: Start Date: 5/16/01– Termination Date: 9:30/05
5302-22000-007-00D: Start Date: 10/1/05 – Termination Date: 3/14/07
5302-22000-008-00D: Start Date: 3/15/07 – Termination Date: 3/14/12

Scientific Personnel:
Hong Lin, Research Plant Physiologist, Parlier, California, (1.0 FTE)

Research Goal(s):
Component V. Grape Genomics, Genetics, Physiology, and Resistance to Xf in Grapes, Almonds, and Other Commercially Important Species

Goal 3. Identify and characterize physiological Pierce's disease resistance mechanisms in Vitis species. In section V. Grape Genomics, Genetics, Physiology, and Crop Resistance.

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

Research Approach:
1. Use SEM to evaluate anatomical structures of Pierce's disease resistant and Pierce's disease susceptible grapes associated with Xf colonization, movement and population development in xylem tubes.

2. Develop a bioassay to determine the antimicrobial effect of Pierce's disease resistant and Pierce's disease susceptible xylem saps on preventing / suppressing biofilm formation, colonization.

3. Proteomic identification of differential protein expression from xylem sap and stem induced by Xf.

Actual or Potential Deliverables:
- New knowledge concerning grapevine-Xf interactions.
- Potential deliverable will be anti-Xf proteins and/or other chemical compounds that may have utility in suppressing disease development in grapevines.

Achieved and/or Potential Benefits:
- Facilitate the development of varieties with improved resistance and /or tolerance to Pierce's disease.
- Identifying antimicrobial compounds presented in grape rootstock and utilizing Pierce's disease resistant rootstocks to improve susceptible scions is a quick, practical and acceptable approach for improving plant performance against Pierce's disease. In viticulture, grafting with resistant rootstocks is commonly used to overcome pest and disease problems. The mutual translocation of nutrients and growth regulators between the scion and rootstock is evident. Therefore, screening, identifying xylem antimicrobial compounds, and using these Pierce's disease

resistant plants as rootstocks may provide a unique opportunity for improving the expression of Pierce's disease resistance without genetically modifying the scion. This last point is critical because it will be very difficult to produce economically competitive wine grape cultivars through classical breeding or genetic engineering, because of the conservative international wine industry. If a rootstock that can confer Pierce's disease resistance is produced, then the integrity of wine grape cultivars will be maintained.

Project Name: Developing Transcriptional Profiles and Microarray Expression Analysis of Grape Plant Response to *Xylella fastidiosa*

Associated CRIS Project Number(s) and Title(s):
5302-22000-007-00D, Epidemiology and Management of *Xylella fastidiosa* (Xf) and other Exotic and Invasive Diseases and Insect Pests

Start and Termination Dates:
5302-22000-005-00D: Start Date: 5/16/01– Termination Date: 9:30/05
5302-22000-007-00D: Start Date: 10/1/05 – Termination Date: 3/14/07
5302-22000-008-00D: Start Date: 3/15/07 – Termination Date: 3/14/12

Scientific Personnel:
Hong Lin, Research Plant Physiologist, Parlier, California, (1.0 FTE)

Research Goal(s):
Component V. Grape Genomics, Genetics, Physiology, and Resistance to Xf in Grapes, Almonds, and Other Commercially Important Species

Goal 1. Identify grape rootstock germplasm and/or varieties that reduce or mitigate Pierce's disease development in susceptible wine grape scions in Pierce's disease -prone production areas. In section

Component V. Grape Genomics, Genetics, Physiology, and Crop Resistance

Research Objective(s):
1: Identification of grape rootstock varieties that reduce Pierce's disease symptom expression or disease development in susceptible scions.

Research Approach:
1. Construct Pierce's disease expression profiles from Pierce's disease resistant and susceptible genotypes
2. Sequence and annotate expressed genes. Categorize genes according to their putative and construct a Pierce's disease expression database.
3. Design and developed high density microarrary *Vitis* gene chip for gene expression analyses.

Actual or Potential Deliverables:
- We have characterized transcriptomes (5421 ESTs) from 12 tissue specific (stem, leaf and shoot) cDNA libraries. These annotated genes were submitted to the NCBI's ESTdb under the accession numbers DN942225 to DN947645. These publicly available ESTs and all the other EST sequences were analyzed to deduce a non-redundant set of 20,020 ESTs derived from numerous *Vitis* species including Pierce's disease resistant grapes such as *V. shuttleworthii*, and *V. arizonica* x *V. rupestris* hybrids. A custom high density grape microarray gene chip was developed, which represents 191,450 with two replicates of gene probes per slide. Data generated from microarray global gene expression analyses were constructed into an online relational database, VitisExpDB which is open to the public (http://cropdisease.ars.usda.gov/~fruit_tree/).

Achieved and/or Potential Benefits:

- This Pierce's disease expression database provides informative molecular events in response to xylella infection and will help to identify resistant mechanisms in Pierce's disease -resistant grapes.
- Understanding molecular basis of host response to Xf infection is a key step in revealing the mechanisms of Xf resistance and pathogenicity. However, information regarding molecular based Pierce's disease resistance and resistant mechanisms is limited. Using SSH and Microarray based functional genomics, we have identified 9 such candidate genes involved in host resistance from the native *Vitis* sps. germplasm. The identified candidate genes will be evaluated for their function and introduced into the elite Pierce's disease susceptible vinifera varieties for developing resistant germplasm. Along with the above list, we have also identified a group of ESTs with putative role in resistance mechanisms that will be used to facilitate molecular-assisted breeding for Pierce's disease genetic breeding program.

Component VI. Disease and Vector Management

Goal 1: Prevent Xf infection

Goal 2: Determine GWSS suppression factors, particularly natural enemies

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

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.

Project Name: Alternatives to Conventional Chemical Insecticides for Control of Glassy-winged Sharpshooter

Associated CRIS Project Number and Title:
1931-21220-011-00D, Integrated Orchard Management for Deciduous Tree Fruit Crops

Start and Termination Dates:
1931-21220-011-00D: Start Date:12/15/98; Termination Date: 12/14/03

Scientific Personnel:
Gary J. Puterka, Research Entomologist, Kearneysville, West Virginia, (0.2 FTE)

Research Goal(s):
Component VI. Disease and Vector Management

Goal 3. Develop kaolin-based particle film technology to suppress Glassy-winged Sharp Shooter (GWSS) populations to levels that reduce or minimize Peirce's Disease in Grape.

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

Research Approaches:
1. Evaluate effectiveness of season-long applications of Surround WP at three locations in Temecula, California to prevention of GWSS infestation.
2. Evaluate effectiveness of early season applications of Surround as a barrier to prevent GWSS movement from citrus into grape in Bakersfield, California.

Actual or Potential Deliverables:
- Technology based on season-long applications of Surround WP for high level of control both GWSS and the indigenous sharpshooter complex that transmits Xf, the causal agent of Pierce's disease. Achieved (2000/2001) Technology based on early season applications of Surround WP as a barrier to prevent GWSS movement from citrus into grape determined that: a) Three Surround treatments greatly reduced GWSS infestations and prevented egg-lay in grape while six insecticide applications did not, b) an 800 ft. Surround treatment barrier reduced GWSS movement beyond the barrier into grape while insecticides did not, and; c) Surround WP treatments prevented egg lay both within treated grape and in grape beyond the Surround barrier while insecticide applications did not. Achieved (2001/2002).

Achieved and/or Potential Benefits:
- Research results regarding the effectiveness of season-long applications of Surround WP were transferred to Engelhard Corporation (Iselin, New Jersey), the makers of Surround WP. The product was registered by the U.S. Environmental Protection Agency in 2002 for use against GWSS and other sharpshooters that transmit Xf in grape and citrus. Achieved (2002).
- Surround WP as a barrier to GWSS. Research results were transferred to APHIS and California Department of Food and Agriculture. Surround WP was utilized as a barrier in 2002 to contain the spread of GWSS in grape in their Area-Wide GWSS Management Program.

Project Name: Exploration for Biological Control Agents in the Native Range of Glassy-Winged Sharpshooter

Associated CRIS Project Number and Title:
6204-22000-018-00D, Biological Control of Invasive and Exotic Pests

Project Start and Termination Dates
6204-22000-015-00D: Start Date: 10/10/1999; Termination Date: 04/08/2003
6204-22000-016-00D: Start Date: 4/9/2003; Termination Date: 6/21/05
6204-22000-018-00D: Start Date: 6/22/2005; Termination Date: 6/21/10

Scientific Personnel:
John A. Goolsby, Research Entomologist, Weslaco, Texas, (.40 FTE)

Research Goal(s):
Component VI. Disease and Vector Management

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

Research Objective:
Explore for nymphal parasitoids of GWSS in Texas

Research Approach:
The glassy-winged sharpshooter is native to Northeastern Mexico and the Southeastern United States, and little 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). 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.

Actual or Potential Deliverables:

- The 'South East Texas Survey' for nymphal parasitoids of GWSS started in June 2005 and has continued monthly at 15 sites from Weslaco to College Station, Texas with wild mustang grape, *Vitis mustangensis*. The objectives of the survey are to: a) discover new natural enemies of GWSS; b) determine the phenology of GWSS and related proconiine sharpshooters in their native habitat; and c) assay wild collected sharpshooters to determine when and if they are infective with Pierce's disease, Xf. Exploration for new natural enemies is focused on discovery and culture of the species of big-headed flies (Pipunculidae) attacking GWSS. Larval pipunculids have been dissected from hand collected *Oncometopia orbona* sharpshooters feeding on mustang grapes. Numerous adult pipunculids have been collected in sticky and Malaise traps. The species attacking GWSS is in the genus *Eurydorylas* and is most active in the late winter and early spring. We have developed sentinel tethered nymphs and customized our Malaise traps to collect live individuals for culture. Several live individuals have been field collected and transferred to GWSS colonies to attempt to rear them. Progeny of these wild collected pipunculids will be immediately transferred to CDFA rearing facilities in Riverside, California (2007). The phenology and distribution of GWSS and two other closely related sharpshooter species has been documented for 18 months. This will be the first description of the field ecology and phenology of GWSS in its native range.

Achieved and/or Potential Benefits:

- Nymphal parasitoids of the proconiine sharpshooter, *Oncometopia orbona* have been collected and identified as Pipunculidae, big-headed flies. Many adult pipunculids have also been collected from traps adjacent to GWSS populations on native mustang grape. We are attempting to collect these parasitoids from GWSS and transfer them to CDFA for mass rearing and release. The potential impact of these biological control agents could be a lower density of GWSS in the agroecosystem, thus lowering the risk of Xf transmission to grapes and other susceptible crops.

Project Name: Microbial Control of the Glassy-Winged Sharpshooter, *Homalodisca Coagulata* With Entomopathogenic Fungi.

Associated CRIS Project Number(s) and Title(s):
5303-21220-003-00D, Integrated Management of Pests Affecting Cotton: Plant Genetics, Biocontrol, and Novel Methods of Pest Estimation

Start and Termination Dates:
5303-21220-003-00D: Start Date: 5/25/05 – Termination Date: 5/24/10

Scientific Personnel: McGuire, M. R., Shafter, California, (0.1 FTE)

Research Goal:
Component VI. Disease and Vector Management

Evaluate the microbial control potential of the entomopathogenic fungus, *Beauveria bassiana* against the glassy-winged sharpshooter (GWSS).

Research Objective(s):
Evaluate the performance of selected isolates of *B. bassiana* against adult GWSS in caged studies.

Research Approach:
Three isolates of *B. bassiana* demonstrated their potential for GWSS control in preliminary assays. These isolates included two from California and one from Texas. The Texas isolate was recovered from GWSS while one of the California isolates was recovered from soil from a GWSS habitat and the other from the three-cornered alfalfa hopper, *Spissistilus festinus*. These isolates were further evaluated in caged studies where adult GWSS were allowed to feed on potted cowpea (*Vigna ungiculata*) plants sprayed with conidial suspensions. Each cage contained a single cowpea plant sprayed with $1X10^{10}$ conidia in 40 ml of 0.01 percent Silwet L-77 solution. Fifty adult GWSS were released into each cage and their mortality was monitored daily for up to 14 days. All three isolates caused mortality and infection in GWSS. Although, the differences were not statistically significant, the California isolates appeared to cause higher levels of infection than the Texas isolate.

Actual or Potential Deliverables:
- Information on the feasibility of utilizing microbial control agents was generated and published that could be used by others to develop microbial control strategies for GWSS. These strategies will most likely be developed in areas where chemical pesticides may not be used due to homeowner concerns, environmental sensitivities, or in organic production.

Achieved and/or Potential Benefits:
- Biological control of the GWSS with entomopathogenic fungi.

Project Name: Management of Pierce's Disease

Associated CRIS Project Number(s) and Title(s):
5302-22000-007-00D, Epidemiology and management of *Xylella fastidiosa* (Xf) and other Exotic and invasive Diseases and Pests.

Start and Termination Dates:
5302-22000-005-00D: Start Date: 5/16/01– Termination Date: 9/30/05
5302-22000-007-00D: Start Date: 10/1/05 – Termination Date: 3/14/07
5302-22000-008-00D: Start Date: 3/15/07 – Termination Date: 3/14/12

Scientific Personnel:
Edwin L. Civerolo, Supervisory/Research Plant Pathologist, Parlier, California, (0.9 FTE)

Research Goal(s):
Section VI. Disease and Vector Management

Goal 1. Prevent Xf Infection

Research Objective(s):
Determine if systemic acquired resistance can be induced in grapevines to Pierce's disease.

Research Approach:
Evaluate the effect(s) of commercially available materials in the greenhouse and in the field on the development of Pierce's disease of grapevines. Grapevines were treated with foliar applications of Actigard (Acibenzolar-*S*-methyl) and Messenger (harpin proteins). In field trials, Flame Seedless grapevines were sprayed four times at 2-month intervals when new shoot growth was 15-25 cm long until fruit set in each of two years (2001 and 2002) with 0, 160, 320, and 480 g harpin ai per ha. In the greenhouse, harpin-treated and untreated control plants were inoculated with Xf via insect transmission. Disease incidence in the field and greenhouse plants were assessed at 30-day intervals throughout the growing season in the field and for 4 months following inoculation in the greenhouse. Xf was isolated from treated and untreated control plants. The identity of presumptive Xf colonies was confirmed by pathogenicity, ELISA and PCR.

Actual or Potential Deliverables:
- New information about inducing resistance in grapevines with commercially available materials to Pierce's disease as a potential strategy for disease management. (Deliverable by 2004)

Achieved and/or Potential Benefits:
- At least one commercially available product, Messenger (harpin), alone can potentially significantly reduce the incidence of Pierce's disease in the field and development of symptoms in greenhouse plants artificially-inoculated with Xf via insect transmission.

Project Name: Genetic Characterization of The Glassy-Winged Sharpshooter and Its Natural Enemies To Support and Enhance The Biological Control Program

Associated CRIS Project Number(s) and Title(s):
6204-22000-018-00D, Biological Control of Invasive and Exotic Pests

Project Start and Termination Dates
6204-22000-015-00D: Start Date: 10/10/1999; Termination Date: 04/08/2003
6204-22000-016-00D: Start Date: 4/9/2003; Termination Date: 6/21/05
6204-22000-018-00D: Start Date: 6/22/2005; Termination Date: 6/21/10

Scientific Personnel:
Jesse H. de León, Research Molecular Biologist, Weslaco, Texas, (1.0 FTE),
Walker Jones, Research, Entomologist, Montpelier, France

Research Goal:
Section VI: Disease and Vector Management

Develop biologically-based pest management strategies to mitigate Xf-caused diseases by controlling or suppressing populations of Xf insect vectors (including, but not necessarily limited to, the GWSS).

Research Objectives:
1. Determine the origin of the glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar) [(=*H. coagulata* (Say)] (Hemiptera: Cicadellidae) that invaded California and determine the population/geographic structure. Determine the origin of the GWSS to determine the source of invasion in order to collect pre-adapted or co-evolved natural enemies.

Research Approach:
Collect GWSS from across its geographic range from Florida to Texas and California. Screen and characterize various PCR-based DNA fingerprinting methods to develop molecular genetic markers for the GWSS to genetically characterize this invasive pest.

Actual or Potential Deliverables:
- DNA polymorphisms in GWSS.
- Molecular genetic markers for GWSS.
- Significant GWSS gene diversity among regions, among populations within regions, and among populations
- Estimated population genetic structure among GWSS.
- Demonstrated that the GWSS that invaded California is of Texas origin.
- GWSS populations in the United States genetically distinct, clustering into two main groups or clades, a 'southeastern' and a 'southwestern and western' clade.
- GWSS that recently invaded the Pacific Island of French Polynesia clustered genetically with the Texas and California or the 'western and southwestern' clade,

Achieved and/or Potential Benefits:
- Methods incorporating simple sequence repeats (SSR) are the most sensitive and efficient methods to distinguish different sharpshooter species (*H. vitripennis*, *H. liturata*, and *H. insolita*) and detect geographic variation in GWSS.

- GWSS molecular markers are useful for determining genetic variation within and among populations, gene flow mechanisms, population /geographic structure, and the origin of the GWSS that invaded California.
- Knowledge that more than one 'founding event' of GWSS introductions occurred in California.
- Knowledge that strongly suggests that GWSS invaded French Polynesia via California.
- Since the origin of the California GWSS infestation is Texas, the most effective pre-adapted natural enemies for release in California should be found in Texas. Natural enemies have usually co-evolved with the pest of interest in the area of origin and therefore have good host-finding abilities.

Project Name: Genetic Characterization of the Glassy-Winged Sharpshooter and Its Natural Enemies To Support and Enhance The Biological Control Program

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Project Start and Termination Dates
6204-22000-015-00D: Start Date: 10/10/1999; Termination Date: 04/08/2003
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6204-22000-018-00D: Start Date: 6/22/2005; Termination Date: 6/21/10

Scientific Personnel:
Jesse H. de León, Research Molecular Biologist, Weslaco, Texas, (1.0 FTE)

Research Goal:
Section VI: Disease and Vector Management

Develop biologically-based pest management strategies to mitigate Xf-caused diseases by controlling or suppressing populations of Xf insect vectors (including, but not necessarily limited to, the GWSS).

Research Objective:
Objective 2. In collaboration with James Hagler (ARS Maricopa), identify key predators of the different life stages of GWSS and smoke-tree sharpshooter (STSS), *H. liturata*.

Research Approach:
Develop diagnostic SCAR (sequence characterized amplified region) markers and markers designed toward multi-copy mitochondrial COI and COII genes. Use these markers, along with monoclonal antibody, to identify GWSS and/or STSS remains in the guts of field-collected predators.

Actual or Potential Deliverables:
- GWSS and STSS-specific molecular diagnostic markers for identifying key predators (2005).

Achieved and/or Potential Benefits:
- The diagnostic markers (SCAR and COI and COII) were highly specific toward the GWSS [*H. liturata* and (STSS)] and were able to identify GWSS remains at all life stages (eggs, nymphs, and adults) in predator gut contents.
- Field studies of predators in natural environments using both molecular markers (e. g., COI) and a monoclonal antibody (ELISAs) have shown excellent success. (V. Fournier et al., in preparation).
- The most frequent predators to test positive included the assassin bug, spiders from certain families, lacewings, and praying mantis.
- Development of diagnostic markers for GWSS will aid in understanding the ecology of GWSS-predator interactions in natural environments. This information will be included in area-wide pest management programs of this invasive pest.
- The specific diagnostic markers will also be useful 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 morphological identification.

Project Name): Genetic Characterization of the Glassy-Winged Sharpshooter and Its Natural Enemies To Support and Enhance The Biological Control Program

Associated CRIS Project Number(s) and Title(s):
6204-22000-018-00D, Biological Control of Invasive and Exotic Pests

Project Start and Termination Dates
6204-22000-015-00D: Start Date: 10/10/1999; Termination Date: 04/08/2003
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6204-22000-018-00D: Start Date: 6/22/2005; Termination Date: 6/21/10

Scientific Personnel:
Jesse H. de León , Research Molecular Biologist, Weslaco, Texas, (1.0 FTE), Walker Jones, Research Entomologist, Montperlier, France, Mamoudou Sétamou (Citrus Center-Texas A&M), David J. W. Morgan (CDFA)

Research Goal:
Develop biologically-based pest management strategies to mitigate Xf-caused diseases by controlling or suppressing populations of Xf insect vectors (including, but not necessarily limited to, the GWSS).

Research Objectives:
Genetically characterize GWSS natural enemies or egg parasitoids belonging to the genus *Gonatocerus* Nees.

Objectives 3 and 4: Determine whether *Gonatoceus ashmeadi* (Girault) (Hymenoptera: Mymaridae), and *G. morrilli* (Howard), primary egg parasitoids of GWSS, exist in nature as cryptic species complexes or whether new species exists.

Research Approach:
Collect geographic populations (California, Louisiana Texas, Florida) of *G. ashmeadi* and *G. morrilli*. Survey molecular methods [ISSR-PCR DNA fingerprinting, amplification and sequencing of internal transcribed spacer region fragments (ITS1 and ITS2) and mitochondrial cytochrome oxidase subunits COI and COII genes]. Perform phylogeographic analyses inferred by ITS2, COI, and COII fragments. For *G. morrilli*, develop molecular diagnostic markers to detect and discriminate it from the very closely related California native species, *G. walkerjonesi*.

Actual or Potential Deliverables:
- Knowledge that geographic populations of *G. ashmeadi* are genetically differentiated inferred by ISSR-PCR DNA fingerprinting.
- Discovery that one of the primary egg parasitoids of GWSS from California is actually a new species, *G. walkerjonesi* (Triapitsyn) (Hymenoptera: Mymaridae) (S. Triapitsyn, University of California-Riverside).
- Several diagnostic markers [inter-simple sequence repeat-polymerase chain reaction (ISSR-PCR) DNA fingerprinting, size of the internal transcribed spacer region 2 (ITS2), 'one-step' *G. morrilli* and *G. walkerjonesi* specific markers targeting the ITS2 fragments] were developed that distinguished the two very closely related species from California (*G. walkerjonesi*) (native species) and Texas (*G. morrilli*) (imported species).
- *G. annulicornis*, an egg parasitoid from South America, and *G. walkerjonesi* were shown to be phylogenetically more similar to each other than *G. walkerjonesi* was to *G. morrilli*. Utilizing a generalized molecular clock estimate for insect mitochondrial DNA of 2.3% per million years to

roughly indicate the length of time since divergence, based on COI sequence data, *G. walkerjonesi* and *G. morrilli* were estimated to have diverged 2.83 million years ago.

Achieved and/or Potential Benefits:

- Texas is the origin of GWSS that invaded California. Since natural enemies have usually co-evolved with the invasive pest, this gives us a source of where to collect pre-adaptive natural enemies (e g., *G. morrilli*).
- It may be important for the biological control program to be aware that geographic populations of *G. ashmeadi* are highly differentiated and that there may be a geographic population that may be better suited to the California environment.
- J. de León (ARS-Weslaco) exported *G. morrilli* from Texas (origin of GWSS) to California in the spring of 2005 to restart the *G. morrilli* biological control program.
- Several molecular diagnostic markers are available to monitor the success of establishment and to evaluate dispersal and efficacy of *G. morrilli* in California.
- The discovery of the new species (*G. walkerjonesi*) has now allowed CDFA and California researchers to correctly identify the specific areas of California where the native species predominates, giving researchers a critical understanding of the natural fauna of California's native parasitoids and guiding them to make more precise decisions. Before this, it was incorrectly determined that *G. morrilli* was native to California.
- As a result of the discovery of *G. walkerjonesi*, University of California-Riverside scientists have determined that *G. walkerjonesi* is recovered more frequently in the coastal region of California, whereas the common impression was that *G. ashmeadi* predominated in California.

Project Name: Genetic Characterization of the Glassy-Winged Sharpshooter and Its Natural Enemies To Support and Enhance The Biological Control Program

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6204-22000-018-00D: Start Date: 6/22/2005; Termination Date: 6/21/10

Scientific Personnel:
Jesse H. de León , Research Molecular Biologist, Weslaco, Texas, (1.0 FTE), Guillermo A. Logarzo (ARS-Argentina), Serguei V. Triapitsyn (UC-Riverside), David J. W. Morgan (CDFA).

Research Goal:
Section VI: Disease and Vector Management

Develop biologically-based pest management strategies to mitigate Xf-caused diseases by controlling or suppressing populations of Xf insect vectors (including, but not necessarily limited to, the GWSS).

Research Objectives:
Objectives 5, 6, and 7. Genetically characterize *Gonatocerus tuberculifemur* (Ogloblin) (Hymenoptera: Mymaridae), *G. metanotalis* (Ogloblin) and *Gonatocerus* species 3 (*G*. sp. 3), prospective egg parasitoid candidate agents from South America. Determine if these species exist in nature as cryptic species complexes or whether new species exist.

Research Approach:
Collect *G. tuberculifemur, G. metanotalis* and *Gonatocerus* species 3 (*G*. sp. 3) from regions in South America (Argentina and Chile) (G. Logarzo, ARS-Argentina and S. Triapitysn, University of California-Riverside). Conduct genetic (J. de León, ARS-Weslaco), morphological (S. Triapitsyn, University of California-Riverside), and cross-mating studies (G. Logarzo, ARS-Argentina). Genetic studies include ISSR-PCR DNA fingerprinting and phylogeographic analysis [e.g., sequencing of mitochondrial COI partial gene and other genes (ITS2, COII, and D2 loop of 28S DNA)].

Actual or Potential Deliverables:
- Genetic characterization of prospective South American egg parasitoids that are potential candidates as biocontrol agents for GWSS, *Gonatocerus tuberculifemur*, *G. metanotalis* and *G*. sp. 3.
- Identification of geographic-specific ISSR-PCR markers in a population of *G. tuberculifemur* (clade 2) from Argentina (San Rafael, Mendoza Province).
- A phylogeographic analysis of *G. tuberculifemur* inferred by the COI gene clustered the populations into two well-supported clades with very strong bootstrap values (96-100%).
- Molecular diagnostic markers (ISSR-PCR and PCR-RFLP) were developed for *G. tuberculifemur* to genotype isofemale lines in order to perform cross-mating studies.
- Phylogenetic analysis of the COI gene of two morphotypes (red or black gasters) of *G. tuberculifemur* identified in Argentina showing that the color of the gasters could not be used to distinguish *G. tuberculifemur*. Rather, the two morphotypes clustered into two clades, in agreement with the results of the geographic populations.

- Preliminary hybridization studies with isofemale lines created from *G. tuberculifemur* individuals belonging to the two diverged clades were reproductively incompatible.
- Based on preliminary morphological studies the individuals from the two diverged clades of *G. tuberculifemur* are actually different species. Will be described as such by S. Triapitsyn (University of California-Riverside).
- *G. tuberculifemur* clade 2 (San Rafael, Mendoza Province) or the new species will be sent to CDFA and University of California-Riverside researchers in early 2007 for further studies.
- ISSR-PCR DNA fingerprinting uncovered several different banding patterns in geographic population of *G. metanotalis* from Argentina, South America, indicating that these populations are genetically distinct.
- A phylogeographic analysis of *G. metanotalis* inferred by COI sequence data, clustered the populations in three distinct clades supported by very strong bootstrap values (100 percent), uncovering geographic or haplotype structure.
- Genetic distinction of the morphologically similar *G*. sp. 3 and *G*. *tuberculifemur* (clades 1 and 2) inferred by ISSR-PCR DNA fingerprinting. COI sequence data distinguished *G*. sp. 3 from *G. tuberculifemur* clade 1, but not *G. tuberculifemur* clade 2 (new species), making ISSR-PCR, in this case, more sensitive than COI sequence data. Hybridization studies, which are being planned, will resolve whether *G*. sp. 3 and *G. tuberculifemur* clade 2 are actually different species.

Achieved and/or Potential Benefits:

- Knowledge that *G. tuberculifemur* is a complex of more than one species is critical to the biological control program in California.
- Of utmost importance, is the fact that *G. tuberculifemur* clade 2 is from a location (San Rafael, Mendoza Province) in South America that 'climate matches' with California, but not within any region of the southeastern United States. This is critical, because based on climate matching theory, if this species is released in California, it is not expected to migrate to the southeastern region of the United States and attach non-target leafhoppers in that region. This greatly reduces the risk factors of releasing *G. tuberculifemur* clade 2.
- Knowing that *G. metanotalis* may be a complex of several species is also critical to the biological control program.
- Correctly identifying or distinguishing *G. tuberculifemur* from *G*. sp. 3 is also critical for the biological control program.

Project Name : Genetic Characterization of the Glassy-Winged Sharpshooter and Its Natural Enemies To Support and Enhance The Biological Control Program.

Associated CRIS Project Number(s) and Title(s):
6204-22000-018-00D, Biological Control of Invasive and Exotic Pests

Project Start and Termination Dates
6204-22000-015-00D: Start Date: 10/10/1999; Termination Date: 04/08/2003
6204-22000-016-00D: Start Date: 4/9/2003; Termination Date: 6/21/05
6204-22000-018-00D: Start Date: 6/22/2005; Termination Date: 6/21/10

Scientific Personnel:
Jesse H. de León, Research Molecular Biologist, Weslaco, Texas, (1.0 FTE) and David J. W. Morgan (CDFA).

Research Goal:
Section VI: Disease and Vector Management

Develop biologically-based pest management strategies to mitigate Xf-caused diseases by controlling or suppressing populations of Xf insect vectors (including, but not necessarily limited to, the GWSS).

Research Objectives
Objective 8: Post-release evaluation of the *G. morrilli* biological control program in California against GWSS based on molecular diagnostic markers. Test the utility of several molecular diagnostic markers to aid in detecting and discriminating *G. morrilli* from the very closely related *G. walkerjonesi* in the field.

Research Approach:
Randomly analyze post-released specimens of *G. morrilli* stored in ethanol from several counties in southern California (D. Morgan, CDFA) from years 2002 to 2006 and test specimens with the developed molecular diagnostic markers (ISSR-PCR DNA fingerprinting, amplification of the ITS2 rDNA fragment, and 'one-step' species-specific ITS2 markers for *G. morrilli* and *G. walkerjonesi*, respectively) (J. de León, ARS-Weslaco).

Actual or Potential Deliverables
- Knowledge of several molecular diagnostic markers for *G. morrilli* and their utility was available to the CDFA and University of California cooperators (2005-2006).

Achieved and/or Potential Benefits:
- The developed molecular diagnostic markers were highly successful in detecting and discriminating *G. morrilli* (imported from Texas) from *G. walkerjonesi* (native) in California.
- The markers were used successfully to monitor egg parasitoid colonies against contamination with unwanted species.
- Amplification of the ITS2 fragments of post-released *G. morrilli* specimens (2002 to summer of 2005) detected only the native *G. walkerjonesi* ITS2 genotype from California, suggesting that *G. morrilli* imported from Texas was not establishing in California and raising concern as to whether the *G. morrilli* biological control program was successful. Analysis of the original *G. morrilli* 'release' colony by ISSR-PCR DNA fingerprinting, uncovered that the colony was contaminated with California's own native species (*G. walkerjonesi*). The results demonstrated that what was

being released in California 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, in the spring of 2005, *G. morrilli* from Texas (the origin of GWSS) (J. de León, ARS-Weslaco) was sent to CDFA and University of California-Riverside to restart the *G. morrilli* biological control program and for further studies by University of California-Riverside scientists. Sending this species from the area of origin can increase the probably of establishment, and therefore success, based on the co-evolution of the parasitoid with its host (GWSS). In the fall of 2005 and in the winter and fall of 2006, the *G. morrilli* (Texas) ITS2 genotype was detected in three locations where the new *G. morrilli* release colony was previously released in southern California, demonstrating the utility of the developed diagnostic markers. Detection of *G. morrilli* for two years in a row is very encouraging.
- Molecular technology to monitor *G. morrilli* from start to finish to determine the success of the biological control program in California by evaluating establishment, dispersal, and efficacy of the natural enemy, and improve mass rearing by save guarding for contamination of unwanted species.
- Application of molecular markers as diagnostic tools for enhancing the precision of classical biological control programs is critical. These same methods 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.
- A consequence of these molecular genetic studies by J. de León (ARS-Weslaco), in conjunction with CDFA and University of California-Riverside, a greater investment by CDFA is being made toward the production, release, and monitoring of *G. morrilli* (imported natural enemy). *G. morrilli* in now currently the second most produced biological control agent by CDFA in both of its rearing facilities and it is now being released over a range of environments that include organic, urban, coastal, and inland locations.

Project Name: Genetic Characterization of the Glassy-Winged Sharpshooter and Its Natural Enemies To Support and Enhance The Biological Control Program

Associated CRIS Project Number(s) and Title(s):
6204-22000-018-00D, Biological Control of Invasive and Exotic Pests

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6204-22000-018-00D: Start Date: 6/22/2005; Termination Date: 6/21/10

Scientific Personnel:
Jesse H. de León, Research Molecular Biologist, Weslaco, Texas, Guillermo A. Logarzo (ARS-Argentina), Serguei V. Triapitsyn (UC-Riverside), and David J. W. Morgan (CDFA).

Research Goal(s):
Section VI. Disease and Vector Management

Develop biologically-based pest management strategies to mitigate Xf-caused diseases by controlling or suppressing populations of Xf insect vectors (including, but not necessarily limited to, GWSS).

Research Objective:
Objective 9. Determine the phylogenetic relationships of both North and South American *Gonatocerus* Nees species, egg parasitoids of the glassy-winged sharpshooter.

Research Approach:
Collect (G. Logarzo ARS-Argentina; S. Triapitsyn, UC-Riverside; and David Morgan, CDFA) various species belonging to the genus *Gonatocerus* Nees from regions in both North and South America and send to J. de León (ARS-Weslaco) for genetic analysis. Resolution of phylogenetic relationships requires information about variability not only at the level of populations within a species but also between species. Therefore, a molecular systematic approach was implemented based on sequencing both the mitochondrial COI gene and the internal transcribed spacer region 2 (ITS2).

Actual or Potential Deliverables:
- Knowledge of the phylogenetic relationships of North and South American *Gonatocerus* Nees species, egg parasitoids of GWSS, inferred by both COI and ITS2 sequence data.
- COI sequence data was included for the following species: *G. triguttatus*, *G. morrilli*, *G. walkerjonesi*, *G. ashmeadi*, *G. tuberculifemur* clades 1 and 2, *G. metanotalis* clades 1, 2, and 3, *G. fasciatus*, *G. annulicornis*, *G. novifasciatus*, *G. incomptus*, *G. atriclavus*, *G. nigrithorax*, *G.* sp. 3, *G.* sp. 6, *G.* sp. 15, *G. uat*, and *G.* nr *metanotalis*. Outgroups included: *Anagrus atomus* and *A. erythroneurae* (mymarid genus).
- ITS2 rDNA fragment sequences from 11 species of *Gonatocerus* egg parasitoids.
- Phylogenetic trees inferred by both COI and ITS2 sequence data, confirming species boundaries.

Achieved and/or Potential Benefits:
- The phylogenetic relationships generated by the ITS2 fragment were in excellent agreement with those delineated by the taxonomic data of S. Triapitsyn (University of California-Riverside).
- The ITS2 fragment appears to be phylogenetically more informative or valuable than that inferred by COI sequence data.

- Combining molecular phylogenetic analyses with classical taxonomic data in defining the relationships of GWSS egg parasitoids belonging to the genus *Gonatocerus*, leads to the most accurate phylogenetic relationships available.
- Extended phylogenetic knowledge that *G. tuberculifemur* clusters with and therefore belongs to the *morrilli* subgroup of the *ater* species group of *Gonatocerus*.
- Twenty-seven mitochondrial cytochrome oxidase subunit I (COI) gene sequences were submitted to GenBank in the phylogeographic studies of *Gonatocerus tuberculifemur*.
- Thirty-two COI sequences were submitted to GenBank for the phylogenetic studies of GWSS egg parasitoids belonging to the genus *Gonatocerus* Nees.

Project Name: Modeling Spread of *X. fastidiosa*

Associated CRIS Project Number(s) and Title(s):
5302-22000-007-00D, Epidemiology and Management of *Xylella fastidiosa* (Xf) and Other Exotic and Invasive Diseases and Insect Pests

Start and Termination Dates:
5302-22000-005-00D: Start Date: 5/16/01– Termination Date: 9:30/05
5302-22000-007-00D: Start Date: 10/1/05 – Termination Date: 3/14/07
5302-22000-008-00D: Start Date: 3/15/07 – Termination Date: 3/14/12

Scientific Personnel:
Mark Sisterson, Research Entomologist, Parlier, California, (1.0 FTE)

Research Goal(s):
Component VI. Disease and vector management

Goal 1. Prevent Xf infection

Research Objective(s):
Develop models to forecast and assess risk of disease incidence

Research Approach:
Currently available information on Xf and its vectors will be synthesized to develop two simulation models regarding the complex interactions between Xf and its vectors and host plants. One model will focus on Xf as spread by GWSS and the other model will focus on Xf as spread by native sharpshooters. The models will be written in C++ and will be spatially explicit. The GWSS model will have three patch types: 1) grape, 2) citrus, and 3) native vegetation. The native sharpshooter model will have two patch types: grape and riparian habitat. The models will include movement between patch types, seasonal population dynamics of the vector, management practices in crop fields, and seasonal dynamics of Xf infections (e.g., overwinter curing). Simulations will focus on using sensitivity analysis to determine which features of the system drive pathogen spread. In addition, the models will be used to simulate proposed disease management plans to determine their potential for success.

Actual or Potential Deliverables:
- Models of the GWSS and native sharpshooter pathosystems will be developed (2008).

Achieved and/or Potential Benefits:
- The models will identify which attributes of this complex pathosystem are most important for pathogen spread and where disease management strategies should be focused.

Peer Reviewed Publications post 2004 NAS Report: None

Project Name: Induced Resistance To *Xylella Fastidiosa* Infection and Development of Pierce's Disease.

Associated CRIS Project Number(s) and Title(s):
5302-22000-007-00D, Epidemiology and Management of *Xylella fastidiosa* (Xf) and Other Exotic and Invasive Diseases and Pests.

Start and Termination Dates:
5302-22000-005-00D: Start Date: 5/16/01– Termination Date: 9:30/05
5302-22000-007-00D: Start Date: 10/1/05 – Termination Date: 3/14/07
5302-22000-008-00D: Start Date: 3/15/07 – Termination Date: 3/14/12

Scientific Personnel:
Edwin L. Civerolo, Supervisory/Research Plant Pathologist, Parlier, California, (0.9 FTE)
Kayimbi M. Tubajika (Research Associate)

Research Goal(s):
Component VI. Disease and Vector Management

Goal 1. Prevent Xf Infection

Research Objective(s):
Objective 1. Develop effective management of Xf diseases based on host resistance.

Research Approach:
Greenhouse and field trials in commercial vineyards to evaluate the effects of available chemical and biologically-based systemic resistance inducers on Xf infection and disease development were established. Materials (e.g., Actigard, Messenger) were applied to potted plants in the greenhouse or in the ground in a grape vineyard in Kern County following manufacturers' guidelines. In the field experiments, plants were treated with Messenger (0, 160, 320 and 480 g harpin ha^{-1}) at 2-month intervals beginning when shoot growth was 15-25 cm long and continued until fruit set. PIERCE'S DISEASE symptoms were assessed at 30-day intervals beginning in mid-July.

For the greenhouse tests, by using potted grapevine plants artificially infected with Xf by GWSS inoculation. GWSS adults were collected from citrus orchards in Ventura County and confined on bean, sorghum and pea seedlings. Randomly selected GWSS adults were assayed by ELISA and IC-PCR using primers RST31 and RST33. No Xf were detected by these tests. The remaining insects were caged for 48 hrs on grapevines known to be infected with Xf. After acquisition, groups of GWSS were caged for 24 and 48 hrs on harpin-treated or untreated healthy (i.e., not infected with Xf). After removal of the sleeve cages, plants were sprayed with acetamiprid to kill surviving GWSS.

Actual or Potential Deliverables:
- Knowledge about, and technology to, induce resistance in grapes to Pierce's disease.

Achieved and/or Potential Benefits:
- Potential technology to induce resistance in grapes to Pierce's disease based on harpin proteins to activate plant defense mechanisms would potentially mitigate disease development.

Project Name: Epidemiology of Pierce's disease in the lower San Joaquin Valley.

Associated CRIS Project Number(s) and Title(s): 5302-22000-007-00D, Epidemiology and Management of *Xylella fastidiosa* (Xf) and other Exotic and Invasive Diseases and Pests

Start and Termination Dates:
5302-22000-005-00D: Start Date: 5/16/01– Termination Date: 9:30/05
5302-22000-007-00D: Start Date: 10/1/05 – Termination Date: 3/14/07
5302-22000-008-00D: Start Date: 3/15/07 – Termination Date: 3/14/12

Scientific Personnel:
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Kayimbi Tubajika, USDA-ARS, Bakersfield, California (Cooperator)

Research Goals:
Component VI: Disease and Vector Management
Goal 1: Prevent Xf infection

Research Objectives:
Objective 1: Determine/Identify factors that affect the distribution and management of PD in the lower San Joaquin Valley

Research Approach:
Twelve vineyards were surveyed during two consecutive growing/production seasons in the GWSS area-wide management pilot project in Kern County in the lower San Joaquin Valley. The vineyards were selected based on their proximity to overwintering hosts of the GWSS, age, grape cultivar and previous GWSS infestation. Each vineyard was assessed visually for PD symptoms at various times (30-day interval) each year and geo-referenced using GPS technology. PD incidence was confirmed by DAS-ELISA and IC-PCR. Xf was isolated from the petioles of randomly collected leaf samples. The identity of presumptive isolated Xf colonies was determined by DAS-ELISA and IC-PCR. The GWSS vector was monitored using yellow sticky cards in each vineyard; and on yellow sticky cards placed in citrus, almond, stone fruits and berries; on on 12m-high poles placed adjacent to eucalyptus trees bordering citrus trees. The spatial patterns of PD were analyzed by ordinary runs, indices of dispersion, two-dimensional distance class, and geostatistical analyses. Commercially-available materials were evaluated in the field [kaolin (Surround®), harpin (Messenger®) and imidacloprid (Admire®)]and greenhouse [Messenger®] for their effect on PD development/incidence

Actual or Potential Deliverables:

- New knowledge about the nature of the spatial distribution of PD in the presence of the glassy-winged sharpshooter.
    - New knowledge that the GWSS was the most likely Xf insect vector in the area-wide management pilot project area accounting for rapid increase in PD incidence via vine-to-vine of Xf within and across rowsNew knowledge that there were no disease gradients relative to GWSS source (e.g., citrus)
    - New or confirming knowledge regarding potential differential field PD-resistance/-tolerance among grape cultivars (genotypes)

- New knowledge regarding the role and importance of primary Xf inoculum sources and mechanisms of pathogen dispersal
- New knowledge about potential induction of resistance to Xf infection/PD development

Achieved and/or Potential Benefits:

- The results of this project suggest that effective PD management is likely to be based on practices that significantly reduce insect vector populations, removal of infected vines (i.e., Xf inoculum sources); and resistant/tolerant varieties.

Potential new approach (i.e., induced resistance) for mitigating PD