United States Department of Agriculture
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Pierce's Disease Research
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Almeida, R., and E. A. Backus. 2004. Stylet penetration behaviors of *Graphocephala atropunctata* (Signoret) (Hemiptera, cicadellidae): EPG waveform characterization and quantification. Annals of the Entomological Society of America. 97: 838-851.

Abstract: We analyzed the probing (a.k.a. stylet penetration) behaviors of the sharpshooter leafhopper *Graphocephala atropunctata* (Signoret) on grape with an alternating current (AC) electrical penetration graph (EPG) monitor. We characterized waveforms likely to represent stylet penetration pathway phase, and xylem ingestion. The total probing duration of the cohort represented 68 % of all 20 hour monitoring periods for all insects, yet only a small proportion of that probing time was spent in high amplitude/ pathway activities. Few changes in behavior occurred once a probe had started. This was shown by the low number of waveforms events (i.e. uninterrupted occurrences of a behavior) per probe for each waveform type, which varied from a mean of 1 to 2.43. Conditional probability analysis supported that hypothesis, since insects usually terminated a probe and began a new one after ingestion-related events, rather than repeating in the same probe the previously performed waveforms. The size of grape leaves used for the assays directly influenced the amount of time insects ingested from xylem or performed other low-amplitude waveforms. Information from this work establishes benchmarks for future research addressing the mechanisms of *Xylella fastidiosa* Wells et al. transmission and sharpshooter ecology.

Reference: See pages 34-37 in Pierce's Disease Research Summaries.

Backus, E. A., J. Habibi, F. Yan, and M. R. Ellersieck. 2005. Stylet penetration by adult *Homalodisca coagulata* on grape: Electrical Penetration Graph waveform characterization, tissure correlation, and possible implications for transmission of *Xylella fastidiosa*. Annals of the Entomological Society of America. 98: 787-813.

Abstract: The glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say), is an exotic vector of the Pierce's disease (PD) bacterium, *Xylella fastidiosa* (Wells et al), that was first observed in California in 1989. GWSS has since greatly increased the threat of PD to the grape industry, as well as stone fruit, nursery and ornamental industries in California. This is the first in a series of papers that, taken together, will describe how sharpshooter stylet penetration behaviors (especially intricate stylet activities, salivation and ingestion) control transmission (i.e. acquisition and inoculation) of *X. fastidiosa*. Herein, we categorized and characterized AC electrical penetration graph (EPG) waveforms from GWSS stylet penetration on petiole of susceptible grape (cv. 'Cabernet Sauvignon'), paying special attention to waveform fine structures that are likely to be the key to detecting the instant of inoculation. We also correlated waveforms with salivary sheath termini in grape tissues. For the first time in any EPG study of leafhopper or planthopper feeding, we demonstrate through case studies of individual probes how to follow the process of stylet penetration step-by-step as it is occurring, including salivary sheath branching and when the stylets first puncture a xylem cell. Finally, we discuss the implications of our findings for understanding the transmission mechanism of *X. fastidiosa*, in comparison with hypothesized mechanisms in the literature.

Reference: See pages 34-37 in Pierce's Disease Research Summaries.

Backus, E. A., J. Habibi, F. Yan, and M. R. Ellersieck. 2005. Stylet penetration by adult *Homalodisca coagulata* on grape: Electrical Penetration Graph waveform characterization, tissure correlation, and possible implications for transmission of *Xylella fastidiosa*. Annals of the Entomological Society of America. 98: 787-813.

Abstract: The glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say), is an exotic vector of the Pierce's disease (PD) bacterium, *Xylella fastidiosa* (Wells et al), that was first observed in California in 1989. GWSS has since greatly increased the threat of PD to the grape industry, as well as stone fruit, nursery and ornamental industries in California. This is the first in a series of papers that, taken together, will describe how sharpshooter stylet penetration behaviors (especially intricate stylet activities, salivation and ingestion) control transmission (i.e. acquisition and inoculation) of *X. fastidiosa*. Herein, we categorized and characterized AC electrical penetration graph (EPG) waveforms from GWSS stylet penetration on petiole of susceptible grape (cv. 'Cabernet Sauvignon'), paying special attention to waveform fine structures that are likely to be the key to detecting the instant of inoculation. We also correlated waveforms with salivary sheath termini in grape tissues. For the first time in any EPG study of leafhopper or planthopper feeding, we demonstrate through case studies of individual probes how to follow the process of stylet penetration step-by-step as it is occurring, including salivary sheath branching and when the stylets first puncture a xylem cell. Finally, we discuss the implications of our findings for understanding the transmission mechanism of *X. fastidiosa*, in comparison with hypothesized mechanisms in the literature.

Reference: See page 34-37 in Pierce's Disease Research Summaries.

Baumgartner, K., and Warren, J.G. 2005. Persistence of *Xylella fastidiosa* in riparian hosts near northern California vineyards. Plant Disease 89:1097-1102.

Abstract: The spread of Pierce's disease (PD) from riparian hosts to grapevines in California's North-coastal grape-growing region is a function of the proportion of *Graphocephala atropunctata* (blue-green sharpshooters [BGSSs]) that acquire *Xylella fastidiosa* from infected plant tissue. Riparian hosts that do not maintain sufficient *X. fastidiosa* populations for acquisition may not be significant inoculum reservoirs. We examined *X. fastidiosa* populations in systemically infected riparian hosts (California blackberry, California grapevine, elderberry, Himalayan blackberry, periwinkle) at two coastal locations (Mendocino, Napa) with two methods of quantitation (culturing and real-time polymerase chain reaction) from 2003 to 2004. In summer and autumn, *X. fastidiosa* populations were above the threshold for BGSS acquisition in periwinkle, Himalayan blackberry, and California grapevine, at both locations. The only *X. fastidiosa*-positive plants detected in spring at both locations were periwinkle and Himalayan blackberry, suggesting that these species may contribute to long-term survival of *X. fastidiosa*. California blackberry and elderberry may not be important reservoirs of *X. fastidiosa*, given that very few plants of either species maintained infections. Higher *X. fastidiosa* populations in California grapevine, Himalayan blackberry, and periwinkle in Napa, relative to plants in Mendocino, may partially explain the higher PD incidence in Napa vineyards.

Reference: See page 7 in Pierce's Disease Research Summaries.

Bausher M., Shatters R., Chaparro J., Dang P., Hunter W., and Niedz, R. 2003. An

expressed sequence tag (EST) set from *Citrus sinensis* L. Osbeck whole seedlings and the implications of further perennial source investigations. Plant Science 165 (2): 415

Abstract: There are a number of large-scale single past cDNA sequencing entries from annual plants in the NCBI database. However, NCBI has very little information on perennial species. As of November 2002 of the 1,100,000 entries less than 5 percent of the publicly available sequence information represent these plants. Because perennials are unique in their ability to survive pathological and environmental onslaughts without the ability of short cycle reproduction as an escape mechanism, these plants represent a reservoirs of EST information most likely not found in annual species. Tree genomes, especially Citrus, contain genes that encode proteins involved in important traits including- essential oil production, fruit production, dormancy, apomixis, and evergreen and deciduous types. ESTs are an excellent tool to identify and catalog many of the genes responsible for these important metabolic pathways in perennial trees species like Citrus. We describe here a set of ESTs produced from mRNA isolated from 180-day-old whole immature sweet orange Citrus seedlings. From this library, 6758 were sequenced and using several bioinformatics programs producing 922 unique contig composite assemblies with 2561 unique singlets. Based on BLAST comparisons, the data contains genes involved in a number of biochemical pathways, but approximately 30 percent have no significant homology to current dbEST entries. The high level of novel sequences in this database set is evidence that perennial trees are an important source of genetic diversity not duplicated in typical annual model plant species.

Reference: See pages 19-20 in Pierce's Disease Research Summaries.

Blackmer, J.L., J.R. Hagler, G.S. Simmons & T.J. Henneberry. 2006. Dispersal of *Homalodisca vitripennis* (Homoptera: Cicadellidae) from a point release site in citrus. Environmental Entomology 35: 1617-1625.

Abstract: GWSS is an important vector of *Xf*, a bacterium that has caused substantial losses in the viticulture and ornamental industries in California. Area-wide management programs have been implemented to reduce vector populations and limit the spread of this disease. However, there is still a lack of information on the factors that influence this insect's movement within and between host crops. In this study, we used mark-release-recapture (MRR) to examine the dispersal of GWSS in a mature orange grove, *Citrus sinensis* Osbeck. Insects were doubly marked with chicken or rabbit immunoglobulin G (IgG) proteins and fluorescent dusts to enable monitoring over several weeks. Our objectives were to examine the reliability of IgG protein markers relative to fluorescent dusts, determine how sharpshooter movement differed in this landscape relative to a previous study in an open setting, and develop a better understanding of the biotic and abiotic factors that could influence sharpshooter dispersal. Linear regressions of recapture data with a diffusion model provided significant fits to the data in five out of six releases. Recapture data were fitted to a diffusion model and based on parameters generated with the model, estimated dispersal distances for GWSS at 72 h showed 50 and 99% remained within annuli of 31 and 150 m from the release site, respectively. Flight activity was greatest between 1000 - 1400 h, and no flights occurred between 2200 - 0600 h. Only temperature explained a significant amount of the variability in recapture of GWSS with sharpshooters rarely trapped below 18°C.

Reference: See pages 21-22 in Pierce's Disease Research Summaries.

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

Abstract : California's viticulture and ornamental industries have suffered significant losses since the introduction of GWSS, an important vector of the Pierce's disease bacterium. A better understanding of the factors that influence the dispersal of GWSS, as well as other native sharpshooters could enhance our ability to institute area-wide management programs. Studies were conducted to establish the validity of an immunoglobulin G (IgG) protein marker for sharpshooter dispersal studies, to compare the dispersal of GWSS with that of a native sharpshooter, STSS, and to develop a better understanding of the factors that influence their dispersal. Field trials showed that the marker was detectable for at least 19 days and did not affect sharpshooter survival. Four concentrations (0.04, 0.2, 1 and 5 mg/ml) and two different IgG markers (chicken and rabbit) were effective for marking sharpshooters. In mass-mark-recapture studies, approximately 95% of the marked insects flew during the releases and the timing of flight initiation was similar for GWSS and STSS. Mean wind speeds greater than 3 m s^{-1} were associated with a decline in flight initiation for both species. Most sharpshooters were trapped at heights below 4.2 m, and based on sex-ratio comparisons, traps were equally attractive to males and females. Regression analyses of recapture data and a diffusion model were used to assess and compare sharpshooter dispersal. The majority (95%) of GWSS and STSS were recaptured within 90 and 155 m of the release site, respectively.

Reference: See pages 21-22 in Pierce's Disease Research Summaries.

Chen, F., Cheek, L., Lin, H., Kirakosyam, A. Yuan, J. S. and Kaufman. P. 2006. The Study of Plant Natural Product Biosynthesis in the Pre-genomics and Genomics Eras. In: Natural Products from Plants, Second Edition. L. Cseke, A. Kirakosyan, P. Kaufman, S. Warber, J.A. Duke, and H. Brielmann. CRC Press. p203-220.

Abstract: Plants elaborate a vast array of natural products, many of which have evolved to confer selective advantages to the host plant in ecological interactions. These natural products are synthesized through a diverse array of biochemical pathways, most of which belong to secondary metabolism. The elucidation of these biochemical pathways has been a challenging undertaking. It requires the use of advanced tools from the disciplines of analytical chemistry, biochemistry, molecular biology and plant physiology. Before the advent of genomics, biochemical and genetic approaches were the two approaches employed in the study of plant natural product biosynthesis. In the genomics era, various genomic approaches, including transcriptomics, proteomics and metabolomics, have become available. As in general plant biology, the study of plant natural products in the genomics age is undergoing a paradigm shift from reductionist analysis to global analysis. The biosynthesis of plant natural products in several plant species, such as *A. thaliana*, rice, and *Medicago truncatula*, which is a close relative of alfalfa, is currently being investigated at the global level in a high-throughput mode. In addition to facilitating the investigation of natural products biosynthesis, the employment of various genomic approaches to the study will lead to the generation of large data sets, which can serve as a basis for using systems biology to understand the biological function of plant natural products. The elucidation of plant natural product biosynthesis will provide novel information for understanding the biology, ecology and evolution of plants, and also, provide

tools for predictive metabolic engineering to improve plant traits that are determined by natural products.

Reference: See pages11-12 in Pierce's Disease Research Summaries.

Chen, J., Groves, R., Civerolo, E., and Livingston, S. 2006. Surface motility of *Xylella fastdiosa* visualized by oblique illumination. Canadian Journal of Microbiology (Accepted in November 24, 2006).

Abstract: Stereomicroscopic observations using oblique illuminations revealed the presence of two types of movement trails by Xylella fastidiosa strains (A- and G-genotypes) isolated from almond leaf scorch samples on the surface of PW and PD3 culture media. The A-genotype strains showed curved motility trails and the G-genotype strains showed straight motility trails. Haloes were found around some G-genotype colonies due to the excretion of unknown factors/compounds which may be related to the bacterial surface motility.

Reference: See pages 6, 13, and 14 in Pierce's Disease Research Summaries.

Chen, J., Groves, R., Zheng, Y., Civerolo, E. L., Viveros, M., and Freeman, M. 2006. Colony morphology of *Xylella fastidiosa* almond leaf scorch strains and its research applications. Canadian Journal of Plant Pathology (Accepted in November 20, 2006 with pending revisions).

Abstract: Xylella fastidiosa is the causal agent of almond leaf scorch disease (ALSD) which is currently re-emerging in California as a potential threat to almond production. We previously reported the presence of different colony morphotypes of X. fastidiosa ALSD strains on PW (periwinkle wilt) medium solidified with Gel-Rite and their association with genotypes/pathotypes after a low number of serial transfers. In this study, we evaluated the colony morphology variations up through 14 subculture passages. The smooth colony morphotype was consistently associated with G-genotype strains and the rough colony morphotype was associated with A-genotype strains. Colony morphology was successfully used to discern genotypes of an ALSD survey with >95% accuracy. Variations of colony morphology did, however, occur during sub-culturing at a low frequency (<5%). Of a particular interest is the smooth variant of the rough A-genotype strains, described as phase variation. The phenotypic traits described in this study are also of value for further biological and molecular characterization of X. fastidiosa.

Reference: See pages 6, 13, and 14 in Pierce's Disease Research Summaries.

Chen, J., Civerolo, E.L., Jarret, R.L., Van Sluys, M.-A. and de Oliveira, M.C. 2005. Genetic diversity in *Xylella fastidiosa* through sequence analysis of selected randomly amplified polymorphic DNAs. Current Microbiology 50:78-83.

Abstract: Five randomly amplified polymorphic DNA (RAPD) fragments from a strain of X. fastidiosa causing Pierce's disease (PD) of grapevine in Florida were sequenced. These sequences were used to search for similarities in the genome sequence databases of four X. fastidiosa strains that cause PD (PD-Temecula), almond leaf scorch disease (ALSD-Dixon), and

oleander leaf scorch disease (OLSD-Ann-1) in California and citrus variegated chlorosis (CVC-9a5c) in Brazil. Four of the five nucleotide sequences from the Florida PD strain were most similar to PD-Temecula, indicating the homogeneity of X. fastidiosa PD strains from both Florida and California. CVC-9a5c was the most distantly related to the Florida PD strain. The discrepant RAPD, PDX3-1 (547 bp), shared no homology with regions in the genomes of PD-Temecula and CVC-9a5c, but did show homology with two regions in ALSD-Dixon and one in OLSD-Ann-1. PDX3-1 contained the C-terminal portion of an integrase gene (int), highly similar (>40% identities, or, >60% positives) to those in the phage family of Podoviridae, commonly found in Enterobacteriaceae. A 43-bp sequence at the 3' end of PDX3-1 was almost identical (42/43) to that of the anti-codon and T C arms of the two tRNAlys genes found in all of the four X. fastidiosa genomes. This sequence could be the att site for DNA integration.

Reference: See pages 6, 13, and 14 in Pierce's Disease Research Summaries.

Chen, J., Groves, E.L. Civerolo, M. Viveros, M. Freeman and Y. Zheng. 2005. Two *Xylella fastidiosa* genotypes associated with almond leaf scorch disease on the same location in California. Phytopathology 95:708-714.

Abstract: Almond leaf scorch disease (ALSD) has recently reemerged in the San Joaquin Valley of California threatening almond production. ALSD is caused by Xylella fastidiosa, a nutritionally fastidious bacterium. Single nucleotide polymorphisms (SNPs) in the 16S rRNA gene (16S rDNA) of X. fastidiosa strains were identified to characterize the bacterial population in infected trees. Genotype-specific SNPs were used to design primers for multiplex polymerase chain reaction assays of early passage cultures. Two genotypically distinct types of X. fastidiosa strains, G-type and A-type, coexist simultaneously in the same infected almond orchard. This was substantiated by restriction fragment length polymorphism analysis of a different genetic locus, RST31-RST33, which has previously been used to identify and differentiate X. fastidiosa strains. Furthermore, unique bacterial colony morphology was consistently associated with the A-type X. fastidiosa strains. To our knowledge, this is the first report of a mixed genotype infection of X. fastidiosa disease on the same location under natural environmental conditions. The concept of mixed genotype infection could affect the current epidemiological study based on the assumption that one genotype causes ALSD on one location and, therefore, the disease management strategy.

Reference: See pages 6, 13, and 14 in Pierce's Disease Research Summaries.

Chen, J., **Civerolo EL, Jarret RL, Van Sluys MA, de Oliveira MC. 2004**. Genetic Discovery in *Xylella fastidiosa* through sequence analysis of selected randomly amplified polymorphic DNAs. Curr Microbiol. 49:1-6.

Abstract: Xylella fastidiosa causes many important plant diseases including Pierce□s disease (PD) in grape and almond leaf scorch disease (ALSD). DNA-based methodologies, such as randomly amplified polymorphic DNA (RAPD) analysis, have been playing key roles in genetic information collection of the bacterium. This study further analyzed the nucleotide sequences of selected RAPDs from X. fastidiosa strains in conjunction with the available genome sequence databases and unveiled several previously unknown novel genetic traits. These include a sequence highly similar to those in the phage family of Podoviridae. Genome comparisons among X. fastidiosa strains suggested that the ‘‘phage’’ is currently active. Two

other RAPDs were also related to horizontal gene transfer: one was part of a broadly distributed cryptic plasmid and the other was associated with conjugal transfer. One RAPD inferred a genomic rearrangement event among X. fastidiosa PD strains and another identified a single nucleotide polymorphism of evolutionary value.

Reference: See pages 6, 13, and 14 in Pierce's Disease Research Summaries.

Chen, W.L., and Leopold, R.A. 2007. Progeny quality of *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae) reared on stored eggs of *Homalodisca coagulata* (Say) (Homoptera: Cicadellidae). Journal of Economic Entomology. (In Press).

Abstract: This study assessed the effects of refrigerated storage on the suitability of *Homalodisca coagulata* (Say) eggs as hosts for propagation of the parasitoid *Gonatocerus ashmeadi* Girault. Development of host eggs was terminated by chilling at 2°C for 5 days before storage was initiated at 10°C for up to 70 d. Parasitism, adult emergence rate, development time and sex ratio were used to gauge the suitability of the eggs as hosts after storage. Demographic growth parameters were also used to assess the quality of the wasp progeny through the F_2 generation. Host eggs stored 20 d remained fully acceptable to the wasps for attack. Although the parasitism rate decreased with storage time, >80% adult parasitoid emergence was realized from eggs stored 30 days. After 70 d storage, adult emergence rate was decreased by 48%, fecundity decreased by 53%, female production 19%, development extended 3 d, and female longevity was shortened 5 d, respectively. Emergence patterns of F_1 but not F_2 adults varied with storage time of the parental and grandparental hosts, respectively. The F_1 population had a type I survivorship pattern regardless of the length of storage. The F_2 generation, emergence rate, development and sex ratio did not vary with storage time when the F_1 parents parasitized fresh host eggs. Demographic parameters for the F_1 population showed that net reproductive rate was >20 although it decreased significantly after the parental host eggs were stored for >30 days. Intrinsic and finite rates of increase, population doubling time, and mean generation time decreased only after storage for 60 d. Our results show that short-term cold storage could be used for maintaining wasp populations in a mass-rearing program and that the detrimental effects of chilling host eggs in storage for over 30 d do not extend to F_2 generation.

Reference: See pages 25-26 in Pierce's Disease Research Summaries.

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

Abstract: The functional response by the egg parasitoid, *Gonatocerus ashmeadi*, and superparasitism of *Homalodisca coagulata* eggs were found to be related to host age and density when studied under laboratory conditions. Several aspects relating to parasitism of 1-, 3-, 5-, 7-, 9-d-old *Homalodisca coagulata* eggs were measured under varied densities ranging from 1:1 to 1:60 parasitoid to host ratios. The functional responses for the parasitoid to host eggs of all age groups most closely fit the type II and III models that describe responses to changing densities. The instantaneous attack rate by the parasitoids on 1-d-old hosts, as specified in the type III model, is significantly greater than that of other ages. This rate is also

greater in the type II model but is not statistically significant. The number of host eggs parasitized varies significantly with host density and age, but not when analyzed by a host age × density interaction. However, host age and density, as well as the host age × density interaction, contribute significantly to the differences found in length of the development time of *G. ashmeadi* within host eggs. This parasitoid showed a significantly greater tendency toward superparasitism at parasitoid-to-host ratios≤1:10. The maximum number of parasitoid eggs found in a single host egg was 18. The frequencies of superparasitism for *G. ashmeadi* display an aggregated distribution over all observed host densities. Our results also suggest *G. ashmeadi* eliminates the supernumerary parasitoids through physiological suppression.

Reference: See pages 25-26 in Pierce's Disease Research Summaries.

Chen, W.L., Leopold, R.A., Morgan, D.J.W., and Harris, M.O. 2006. Development and reproduction of the egg parasitoid, *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae) as a function of temperature. Environmental Entomology 35(5):1178-1187.

Abstract: The development, fecundity and life table parameters of *Gonatocerus ashmeadi* Girault, were studied in the laboratory at six constant temperatures between 12 and 32˚C. At 12 ˚C, the parasitoid failed to develop beyond the 3rd instar and durations of the egg stage and the 1st and 2nd instars were prolonged. Development from the egg stage to adult emergence varied from 27.1 d at 16˚C to 9.5 d at 28˚C. Temperature thresholds for development were estimated at 4.0, 3.8, 9.1, 6.2, and 12.8˚C and thermal constants were 29.9, 35.0, 26.7, 32.3 and 75.4 degree days for the egg, 1st through 3rd instars, and pupal stages, respectively. To complete development from egg to adult, 206.7 degree days were required above the lower temperature threshold of 8.5˚C. The optimum temperature for egg to adult development was 29.2˚C. Survival from egg to adult was 67.4% at 16˚C and ranged from 83.4-86.7% between 20-32˚C. At 16-32˚C, the population had a Type I survivorship pattern. At 16˚C, longevity of adult females and males averaged 27.1 and 19.0 d respectively, but declined to 6.4 and 6.9 d at 32˚C. Adding honey to the diet of mated females did not increase their longevity. At 20-32˚C, peak adult emergence occurred on the 1st day of emergence, but at 16°C, it was greatest on the 2nd day. When exposed to temperatures ranging from 16-32˚C, the female: male sex ratio was similar, ranging from 3.4 to 5.6. Lifetime fecundity was greatest at 24˚C, and lowest at 32˚C, with the 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.

Reference: See pages 25-26 in Pierce's Disease Research Summaries.

Dara, S. K., McGuire, M. R. and Kaya, H. K. 2007. Isolation and Evaluation of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) for the Suppression of the Glassy-winged Sharpshooter, *Homalodisca coagulata* (Homoptera: Cicadellidae). Journal of Entomological Science 42: xx-xx.

Abstract: The glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Homoptera: Cicadellidae), is a significant threat to California agriculture as a vector of the bacterium, *Xylella fastidiosa* Wells, causative agent of Pierce's disease. Control strategies target vector populations to prevent the spread of the bacterium. One of the potential means of controlling *H. coagulata* is the use of entomopathogenic fungi. To discover naturally-occurring fungal

pathogens that are adapted to *H. coagulata* and its habitats, soil samples from organic citrus and conventional pomegranate orchards in Tulare and Riverside counties in southern California were screened for the presence of pathogens. Using a modified *Galleria* bait method that required small quantities of soil, 124 isolates of *Beauveria bassiana* (Balsamo) Vuillemin and four isolates of *Metarhizium anisopliae* (Metschnikoff) Sorokin (Deuteromycotina: Hyphomycetes) were recovered. Additionally, 22 isolates of *B. bassiana* were recovered by plating soil suspensions on selective growth media. Natural fungal infections were not detected in *H. coagulata* populations from periodic sampling in Kern, Riverside and Ventura counties in southern California. Some of the *B. bassiana* isolates recovered from soil and other insect hosts in southern California were evaluated against *H. coagulata* along with those isolated from *H. coagulata* in Texas and Mississippi. Growth of the selected isolates also was evaluated at 15, 23, 28 and 32 ^{o}C. The Texas isolate and two California isolates of *B. bassiana* were virulent against *H. coagulata* and warrant further study.

Reference: See page 54 in Pierce's Disease Research Summaries.

de León, J. H., G. A. Logarzo, and S. V. Triapitsyn. 2006. ISSR-PCR DNA fingerprinting uncovers distinct banding patterns in *Gonatocerus* species 3 (*G*. sp. 3) individuals emerging from different host tribes: A prospective egg parasitoid candidate agent for the glassy-winged sharpshooter in California, pp. 48-51. *In* Proceeding, Pierce's Disease Research Symposium, 27-29 November 2006, San Diego, CA. Complied by Tom Esser, M. Athar Tariq, Raygina Medeiros, Melinda Mochel, and Sean Veling, Sacramento, CA.

Abstract: We started work to genetically characterize a prospective glassy-winged sharsphooter (GWSS), *Homalodisca vitripennis* (Germar) [=*H. coagulata* (Say)] egg parasitoid biological control candidate agent from South America known as *Gonatocerus* species 3 (*G*. sp. 3). This species is morphologically very similar to *G. tuberculifemur*, another prospective agent from South America. We asked two questions, 1) are *G*. sp. 3 and *G. tuberculifemur* the same species and 2) are two collections of *G*. sp. 3 individuals emerging from different host tribes (Proconiini and Cicadellini) genetically distinct. Or, in both cases, are we seeing genetic variation of the same species. Two molecular methods were utilized to begin to study these species, the very sensitive inter-simple sequence repeat-polymerase chain reaction (ISSR-PCR) DNA fingerprinting and mitochondrial cytochrome oxidase subunit I gene (COI) variation. ISSR-PCR analysis performed together on both *G*. sp. 3 and *G. tuberculifemur* uncovered the following: 1) as previously shown, *G. tuberculifemur* geographic populations were genetically distinct, 2) *G*. sp. 3 and *G. tuberculifemur* were very clearly distinct, and 3) banding patterns differences (about four bands) distinguished the two collections of *G*. sp. 3. A single most parsimonious tree clustered the current specimens in the following fashion: 1) as previously shown, the geographic populations of *G. tuberculifemur* clustered into two well-supported distinct clades with very strong bootstrap values (90-99%), and 2) the *G*. sp 3 collections clustered along with clade 2 (San Rafael population) of the *G. tuberculifemur* populations, though one *G*. sp. 3 collection (Jan 05; Proconiini host) forms a unique clade with moderate bootstrap support (63%). Even though, the divergence between the two *G*. sp. 3 collections was very small, the two shared no haplotypes. The current results confirm that ISSR-PCR DNA fingerprinting using a 5'-anchored ISSR primer is an excellent molecular diagnostic tool for distinguishing *G*. sp. 3 from both clades of *G. tuberculifemur*. COI sequence variation effectively distinguished *G*. sp. 3 from *G. tuberculifemur* individuals from clade 1, though it did not effectively separate *G*. sp. 3 from *G. tuberculifemur* individuals from clade 2 (San Rafael

population). We conclude that based on ISSR-PCR analysis, *G*. sp. 3 and *G. tuberculifemur* and both collections of *G*. sp. 3 are clearly genetically distinct. The only way to confirm whether these specimens are actually cryptic or different species is by performing hybridization studies. These molecular results are important to the biological control program in California.

Reference: See pages 56-66 in Pierce's Disease Research Summaries.

de León, J. H., G. A. Logarzo, and S. V. Triapitsyn. 2006. Development and utility of a 'one-step' species-specific molecular diagnostic marker for *Gonatocerus morrilli* designed toward the internal transcribed spacer region 2 (ITS2) to monitor establishment in California, pp. 60-63. *In* Proceeding, Pierce's Disease Research Symposium, 27-29 November 2006, San Diego, CA. Complied by Tom Esser, M. Athar Tariq, Raygina Medeiros, Melinda Mochel, and Sean Veling, Sacramento, CA.

Abstract: In addition to the 'one-step' species-specific molecular diagnostic ISSR-PCR DNA fingerprinting method, we developed an additional 'one-step' molecular diagnostic marker 'gmtx' toward *Gonatocerus morrilli* (Howard) designed toward the ribosomal internal transcribed spacer region 2 (ITS2) to aid in monitoring establishment in California. *Gonatocerus morrilli*, the imported natural enemy from Texas, is very closely related to *G. walkerjonesi*, the native California species. Specificity assays with this newly developed marker and a total of 16 *Gonatocerus* Nees species demonstrated that it was highly specific toward the species that it was designed for (*G. morrilli*), as cross-reactivity was not seen with any of the tested species, including all species belonging to the *morrilli* subgroup of the *ater* species group of *Gonatocerus*. Anaysis of the 'release' 'TX/MX' colony used before the summer of 2005 with this species-specific diagnostic marker confirmed previous results that the 'release' 'TX/MX' colony was not the imported *G. morrilli*, but the native species *G. walkerjonesi*, confirming a colony contamination. Analysis of post-released *G. morrilli* collections with this diagnostic marker detected *G. morrilli* in a site where it was previously released, in accordance with our recent finding using two other diagnostic markers used in combination, ITS2 fragment size and ISSR-PCR DNA fingerprinting. The current results confirm the utility of the newly developed species-specific ITS2 molecular diagnostic marker as an excellent tool to aid in monitoring the establishment of the imported natural enemy of the glassy-winged sharpshooter, *G. morrilli*. These results and molecular tools are critical to the biological control program in California. We now have in our hands the molecular technology to evaluate the *G. morrilli* biological control program in California from start to finish, that is, monitor establishment, dispersal, and efficacy of natural enemies and improve mass rearing.

Reference: See pages 56-66 in Pierce's Disease Research Summaries.

de León, J. H., G. A. Logarzo, and S. V. Triapitsyn. 2006. Genetic studies of *Gonatocerus metanotalis* populations from Argentina uncover divergent clades: A prospective egg parasitoid candidate agent for the glassy-winged sharpshooter in California, pp. 52-55. *In* Proceeding, Pierce's Disease Research Symposium, 27-29 November 2006, San Diego, CA. Complied by Tom Esser, M. Athar Tariq, Raygina Medeiros, Melinda Mochel, and Sean Veling, Sacramento, CA.

Abstract: Two molecular methods were utilized to genetically distinguish geographic

populations of *Gonatocerus metanotalis* (Ogloblin) (Hymenoptera: Mymaridae) from Argentina and to begin to test the possibility that this South American species could exist as a cryptic species complex. *Gonatocerus metanotalis* is a prospective egg parasitoid candidate agent for a neoclassical biological control program in California against the invasive glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar) [=*H. coagulata* (Say)]. Six populations were analyzed: Campo Grande (Misiones Province), Tartagal (Salta), Tafí Viejo (Tucumán), near PROIMI (Tucumán), Santa Clara (Jujuy), Clorinda (Formosa). As a first approach, inter-simple sequence repeat-polymerase chain reaction (ISSR-PCR) DNA fingerprinting was performed with a 5'-anchored ISSR primer. Several distinct banding patterns were identified among the populations with some band sharing and in certain populations (Tartagal and Santa Clara) there was extensive variation. Next, a phylogeographic analysis inferred by the mitochondrial cytochrome oxidase subunit I (COI) gene was performed. A neighbor-joining distance tree clustered the *G. metanotalis* populations into three main distinct clades supported by very strong bootstrap values (100%), uncovering haplotype or phylogeographic structure. With the exception of one population (Campo Grande), all individuals from the populations fell into one of the three clades. Individuals from Campo Grande clustered into the three clades, suggesting that three sympatric strains may be present in this location. A phylogenetic analysis performed by the neighbor-joining algorithmic method along with other named and two unnamed *Gonatocerus* Nees species (15) confirmed species boundaries and again uncovered three main distinct clades in *G. metanotalis*. Very strong bootstrap support (100%) was seen for both the *G. metanotalis* clades and for all of the *Gonatocerus* species. Understanding possible cryptic variation in this prospective GWSS egg parasitoid candidate agent is critical to the biological control program in California.

Reference: See pages 56-66 in Pierce's Disease Research Summaries.

de León, J. H., G. A. Logarzo, and S. V. Triapitsyn. 2006. The utility of the internal transcribed spacer region 2 (ITS2) in confirming species boundaries in the genus *Gonatocerus*: Comparison to the cytochrome oxidase subunit I (COI) gene and taxonomic data: Molecular key based on ITS2 fragment sizes, pp. 56-59. *In* Proceeding, Pierce's Disease Research Symposium, 27-29 November 2006, San Diego, CA. Complied by Tom Esser, M. Athar Tariq, Raygina Medeiros, Melinda Mochel, and Sean Veling, Sacramento, CA.

Abstract: We sequenced the nuclear ribosomal internal transcribed spacer region 2 (ITS2) from several glassy-winged sharpshooter (GWSS) [*Homalodisca vitripennis* Germar (=*H. coagulata* Say)] egg parasitoid species (Hymenoptera: Mymaridae) belonging to the genus *Gonatocerus* Nees to test the utility of this fragment to confirm species boundaries and to define phylogenetic relationships. A total of 35 specimens belonging to 10 named species, one unnamed species, and two specimens from another mymarid genus (*Anagrus erythroneurae*) (outgroup) were analyzed. A phylogenetic tree generated using the neighbor-joining algorithmic method showed that each named *Gonatocerus* species formed its own unique taxonomic unit or clade with very strong bootstrap support (100%), confirming species boundaries. The ITS2 fragment confirmed species boundaries as well as cytochrome oxidase subunit I (COI) sequence data. Furthermore, the phylogenetic relationships among species generated by the ITS2 fragment were in excellent agreement with those delineated by taxonomic data. The current results clearly confirm the utility of the ITS2 fragment in confirming species boundaries of egg parasitoids beloning to the genus *Gonatocerus*. The results showed that the ITS2 fragment appears to be phylogenetically more informative or

valuable than that inferred by COI sequence data. Since several important *Gonatocerus* species were analyzed, a molecular key based on ITS2 sizes was developed. In the event two species (e. g., *G. ashmeadi* and *G. metanotalis* and *G. walkerjonesi* and *G. annulicornis*) were found with similarly sized ITS2 fragments, inter-simple sequence repeat-polymerase chain reaction (ISSR-PCR) DNA fingerprinting was performed to distinguish them. ISSR-PCR very clearly distinguished the aforementioned species, demonstrating that it is an excellent molecular diagnostic tool. The current results are important to the biological control program in California.

Reference: See pages 56-66 in Pierce's Disease Research Summaries.

de León, J. H., G. A. Logarzo, and S. V. Triapitsyn. 2006. Genetic characterization of *Gonatocerus tuberculifemur* from South America uncovers divergent clades: Prospective egg parasitoid candidate agent for the glassy-winged sharpshooter in California, pp. 40-43. *In* Proceeding, Pierce's Disease Research Symposium, 27-29 November 2006, San Diego, CA. Complied by Tom Esser, M. Athar Tariq, Raygina Medeiros, Melinda Mochel, and Sean Veling, Sacramento, CA.

Abstract: In present study we genetically characterized the prospective South American egg parasitoid candidate, *Gonatocerus tuberculifemur* (Ogloblin) of the glassy-winged sharsphooter (GWSS), *Homalodisca vitripennis* (Germar) [=*H. coagulata* (Say)] for a neoclassical biological control program in California. Two molecular methods, inter-simple sequence repeat-polymerase chain reaction (ISSR-PCR) DNA fingerprinting and a phylogeographic approach inferred by the mitochondrial cytochrome oxidase subunit I gene (COI). Five geographic populations from South America were analyzed; in addition, a phylogenetic analysis was performed with several named and two unnamed *Gonatocerus* Nees species. DNA fingerprinting uncovered a fixed geographic banding pattern difference in the population from San Rafael, Mendoza Province, Argentina. The COI analysis uncovered haplotype or geographic structure in *G. tuberculifemur*. A neighbor-joining distance tree clustered the populations into two well-supported distinct clades with very strong bootstrap values (96-100%) with the population from San Rafael clustering into a separate clade than the rest of the South American populations. No haplotype sharing was observed between individuals from the two clades. A phylogenetic analysis performed by the neighbor-joining method of 15 *Gonatocerus* Nees species confirmed species boundaries and again uncovered two distinct clades in *G. tuberculifemur* with very strong bootstrap support (96-100%). The two molecular methods were in accord and the evidence is suggestive of a species level divergence. Because *G. tuberculifemur* is under consideration as a potential biological control agent for the invasive GWSS in California, understanding possible cryptic variation of this species is critical.

Reference: See pages 56-66 in Pierce's Disease Research Summaries.

de León, J. H., G. A. Logarzo, and S. V. Triapitsyn. 2006. Preliminary evidence from reproductive compatibility studies suggests that *Gonatocerus tuberculifemur* exists as a cryptic species complex or a new species is identified: Development and utility of molecular diagnostic markers, pp. 44-47. *In* Proceeding, Pierce's Disease Research Symposium, 27-29 November 2006, San Diego, CA. Complied by Tom Esser, M. Athar Tariq, Raygina Medeiros, Melinda Mochel, and Sean Veling, Sacramento, CA.

Abstract: Recent work uncovered divergent clades or distinct lineages in populations of *Gonatocerus tuberculifemur* from South America. *Gonatocerus tuberculifemur* is a prospective egg parasitoid candidate agent for a neoclassical biological control program in California against the invasive glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar) [=*H. coagulata* (Say)]. In the present study, we developed molecular diagnostic markers by two approaches to distinguish field-collected populations of *G. tuberculifemur* for reproductive compatibility studies. The two diagnostic assays were: polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the mitochrondrial cytochrome oxidase subunit I gene (COI) and inter-simple sequence repeat-polymerase chain reaction (ISSR-PCR) DNA fingerprinting. Clade-specific restriction enzymes generated bands of the correct size with high specificity. Analysis of two isofemales lines created from freshly field-collected populations belonging to clade 1 (Tunuyán) and clade 2 (San Rafael) showed that both of our developed molecular diagnostic markers correctly genotyped these isofemale lines, confirming the utility of our diagnostic markers. Based on our molecular work, we predicted that *G. tuberculifemur* individuals belonging to the two distinct clades would not hybridize. Preliminary mating compatibility studies between these two isofemale lines demonstrated that our prediction was indeed correct. Interspecific crosses produced only male offspring, whereas, the intraspecific control crosses produced both males and females or fertile offspring. Taken together, both our molecular work and the preliminary reproductive compatibility studies strongly suggest that *G. tuberulifemur* either exists as a cryptic species complex or a new species is identified. Since *G. tuberculifemur* is under consideration as a biological control agent against the invasive GWSS in California, understanding cryptic variation of this species is critical.

Reference: See pages 56-66 in Pierce's Disease Research Summaries.

de León, J.H., Fournier, V., Hagler, J.R. and Daane, K.M.. 2006. Development of molecular diagnostic markers for sharpshooters *Homalodisca coagulata* and *H. liturata* for use in predator gut content examinations. Entomologia Experimentalis et Applicata 119: 109-119.

Abstract: To aid in identifying key predators of Proconiini sharpshooter species present in California, we developed and tested molecular diagnostic markers for the glassy-winged sharpshooter (GWSS) *Homalodisca coagulata* (Say) and smoke-tree sharpshooter (STSS) *Homalodisca liturata* (Ball) (Homoptera: Cicadellidae: Proconiini). Two different types of markers were compared, those targeting single-copy sequence characterized amplified regions (SCAR) and mitochondrial markers targeting the multi-copy cytochrome oxidase subunit genes I (COI) and II (COII). A total of six markers were developed, two SCAR and four mitochondrial COI or COII markers. Specificity assays demonstrated that SCAR marker HcF5/HcR7 was GWSS-specific and HcF6/HcR9 was GWSS and STSS (GWSS/STSS)-specific. COI (HcCOI-F/R) and COII (HcCOII-F4/R4) markers were GWSS-specific, COII (G/S-COII-F/R) marker was GWSS/STSS-specific, and lastly, COII marker (Hl-COII-F/R) was STSS-specific. Sensitivity assays using genomic DNA showed the COI marker to be the most sensitive marker with a detection limit of 6 pg of DNA. This marker was 66-fold more sensitive than marker Hl-COII-F/R that showed a detection limit of 400 pg of DNA. In addition, the COI marker was 4.2-fold more sensitive than the COII marker. In predator gut assays, the mitochondrial COI and COII markers demonstrated significantly higher detection efficiency than the SCAR markers. Furthermore, the COI marker demonstrated slightly higher

detection efficiency over the COII marker. Lastly, we describe the inclusion of an internal control (28S amplification) for predation studies performing predator gut analyses utilizing PCR. This control was critical in order to monitor reactions for PCR failures, PCR inhibitors, and for the presence of DNA.

Reference: See pages 56-66 in Pierce's Disease Research Summaries.

de León, J.H., Jones, W.A., Sétamou, M., and Morgan, D.J.W. 2006. Genetic and hybridization evidence confirms that a geographic population of *Gonatocerus morrilli* (Hymenoptera: Mymaridae) from California is a new species: Egg parasitoids of the glassy-winged sharpshooter *Homalodisca coagulata* (Homoptera: Cicadellidae). Biological Control 38: 282-293.

Abstract: We investigated the differentiation and reproductive isolation among different geographic populations of *Gonatocerus morrilli*, egg parasitoids of the glassy-winged sharpshooter (*Homalodisca coagulata*), to confirm previous observations that there may exist a cryptic species complex or a new species. Two mitochondrial genes [cytochrome oxidase subunits I (COI) and II (COII)] and the internal transcribed spacer region 2 (ITS2) of several individuals per population were sequenced. *Gonatocerus morrilli* populations from Texas (TX), Florida (FL), California (CA), and an outgroup (*G. ashmeadi*) were analyzed. For comparison, a population from Argentina (*G. annulicornis*) morphologically similar to *G. morrilli* was also included. For all three sequence fragments, percentage sequence divergence (%D) demonstrated that both the TX and FL populations (TX/FL) were closely related and therefore, determined to be the same species; in constrast, the %D between TX/FL and CA fell within the range of the outgroup, making the CA population a new species or sp. n. Neighbor-joining distance trees also clustered the TX/FL and CA populations or species into two well-supported distinctive clades. The near *G. morrilli* sp.n. was more closely related to *G. annulicornis* than to the TX/FL species. Mating studies demonstrated that the populations or species from CA and TX were reproductively incompatible, producing no female offspring in both direct and reciprocal crosses; whereas, the heterogamic crosses between TX and FL produced fertile offspring and relative compatibility indices similar to the homogamic crosses. Unidirectional cytoplasmic incompatibility was ruled out as a cause for the lack of reproduction since both males and females were infected in equal portions with *Wolbachia*.

Reference: See pages 56-66 in Pierce's Disease Research Summaries.

de León, J. H., V. Fournier, J. R. Hagler, and K. M. Daane. 2006. Development of molecular diagnostic markers for sharpshooters *Homalodisca coagulata* and *H. liturata* for use in predator gut content examinations. Entomologia Experimentalis et Applicata 119: 109-119.

Abstract: To aid in identifying key predators of Proconiini sharpshooter species present in California, we developed and tested molecular diagnostic markers for the glassy-winged sharpshooter (GWSS) Homalodisca coagulata (Say) and smoke-tree sharpshooter (STSS) Homalodisca liturata (Ball) (Homoptera: Cicadellidae: Proconiini). Two different types of markers were compared, those targeting single-copy sequence characterized amplified regions (SCAR) and mitochondrial markers targeting the multi-copy cytochrome oxidase subunit genes I (COI) and II (COII). A total of six markers were developed, two SCAR and four mitochondrial COI or COII markers. Specificity assays demonstrated that SCAR marker

HcF5/HcR7 was GWSS-specific and HcF6/HcR9 was GWSS and STSS (GWSS/STSS)-specific. COI (HcCOI-F/R) and COII (HcCOII-F4/R4) markers were GWSS-specific, COII (G/S-COII-F/R) marker was GWSS/STSS-specific, and lastly, COII marker (Hl-COII-F/R) was STSS-specific. Sensitivity assays using genomic DNA showed the COI marker to be the most sensitive marker with a detection limit of 6 pg of DNA. This marker was 66-fold more sensitive than marker Hl-COII-F/R that showed a detection limit of 400 pg of DNA. In addition, the COI marker was 4.2-fold more sensitive than the COII marker. In predator gut assays, the mitochondrial COI and COII markers demonstrated significantly higher detection efficiency than the SCAR markers. Furthermore, the COI marker demonstrated slightly higher detection efficiency over the COII marker. Lastly, we describe the inclusion of an internal control (28S amplification) for predation studies performing predator gut analyses utilizing PCR. This control was critical in order to monitor reactions for PCR failures, PCR inhibitors, and for the presence of DNA.

Reference: See pages 56-66 in Pierce's Disease Research Summaries.

de León, J. H., W. A. Jones, Mamoudou Sétamou, and D. J. W. Morgan. 2006. Genetic and hybridization evidence confirms that a geographic population of *Gonatocerus morrilli* (Hymenoptera: Mymaridae) from California is a new species: Egg parasitoids of the glassy-winged sharpshooter *Homalodisca coagulata* (Homoptera: Cicadellidae). Biological Control 38: 282-293.

Abstract: We investigated the differentiation and reproductive isolation among geographic populations of the glassy-winged sharpshooter (Homalodisca coagulata) primary egg parasitoid, Gonatocerus morrilli, to confirm previous observations that it may exist in nature as a cryptic species complex. Two mitochondrial genes [cytochrome oxidase subunits I (COI) and II (COII)] and the internal transcribed spacer region 2 (ITS2) of several individuals per population were sequenced. Gonatocerus morrilli populations from Texas (TX), Florida (FL), California (CA), and an outgroup (G. ashmeadi) were analyzed. For comparison, a population from Argentina identified as near G. morrilli (G. annulicornis) was also included. For all three sequence fragments, percentage sequence divergence (%D) demonstrated that both the TX and FL populations (TX/FL) were closely related and therefore, determined to be the same species; in contrast, the %D between TX/FL and CA fell within the range of the outgroup, making the CA population a novel species (nov. sp. G. morrilli). Neighbor-joining distance trees also clustered the TX/FL and CA populations or species into two well supported, distinctive clades. The nov. sp. G. morrilli was more closely related to G. annulicornis than to the TX/FL species. Mating studies demonstrated that the populations or species from CA and TX were totally reproductively incompatible, producing no female offspring in both direct and reciprocal crosses; whereas, the heterogamic crosses between TX and FL produced fertile offspring and relative compatibility indices similar to the homogamic crosses. Cytoplasmic incompatibility was ruled out as a cause for the lack of reproduction since all geographic populations were determined to be infected with the same strain of Wolbachia pipientis

Reference: See pages 56-66 in Pierce's Disease Research Summaries.

de León, J. H., W. A. Jones, M. Sétamou, and D. J. W. Morgan. 2005. Discovery of a cryptic species complex in *Gonatocerus morrilli* (Hymenoptera: Mymaridae), a primary egg parasitoid of the glassy-winged sharpshooter, pp. 302-305. *In* Proceeding, Pierce's Disease

Research Symposium, 5-7 December 2005, San Diego, CA. Complied by M. Athar Tariq, Stacie Oswalt, Peggy Blincoe, Amadou Ba, Terrance Lorick, and Tom Esser, Sacramento, CA.

Abstract: We investigated the differentiation and reproductive isolation among different geographic populations of Gonatocerus morrilli, an egg parasitoid of the glassy-winged sharpshooter (GWSS) (Homalodisca coagulata Say) (Homoptera: Cicadellidae), to confirm previous observations that there may exist a cryptic species complex. Two mitochondrial genes [cytochrome oxidase subunits I (COI) and II (COII)] and the internal transcribed spacer region 2 (ITS2) of several individuals per population were sequenced. Gonatocerus morrilli populations from Texas (TX), Florida (FL), California (CA), and an outgroup (G. ashmeadi) were analyzed. For comparison, a population from Argentina identified as near G. morrilli (=G. annulicornis) was also included. For all three sequence fragments, percentage sequence divergence (%D) demonstrated that both the TX and FL populations (TX/FL) were closely related and, therefore, determined to be the same species; in contrast, the %D between TX/FL and CA fell within the range of the outgroup, making the CA population a novel species (nov. sp. G. morrilli). Neighbor-joining distance trees clustered the TX/FL and CA populations or species into two well supported distinctive clades. The G. morrilli (nov. sp.) was more closely related to G. annulicornis than to the TX/FL species. Mating studies demonstrated that the populations or species from CA and TX were reproductively incompatible, producing no female offspring in both direct and reciprocal crosses, whereas the heterogamic crosses between TX and FL produced fertile offspring and relative compatibility indices similar to the homogamic crosses. These results are important to the PD/GWSS biological control program in California.

Reference: See pages 56-66 in Pierce's Disease Research Summaries.

de León, J. H., and D. J. W. Morgan. 2005. Small scale post-release evaluation of a *Gonatocerus morrilli* program in California against the glassy-winged sharpshooter: Utility of developed molecular diagnostic tools, pp. 306-309. *In* Proceeding, Pierce's Disease Research Symposium, 5-7 December 2005, San Diego, CA. Complied by M. Athar Tariq, Stacie Oswalt, Peggy Blincoe, Amadou Ba, Terrance Lorick, and Tom Esser, Sacramento, CA

Abstract: Previously we discovered a cryptic species complex in Gonatocerus morrilli (Howard) and developed molecular diagnostic markers that distinguished the two cryptic species. In the current study we tested the utility of the two developed molecular diagnostic markers to evaluate the establishment of G. morrilli in California. In the two cryptic species, the size of the internal transcribed spacer 2 region (ITS2) varies by about 212 base pairs; the Texas G. morrilli species is associated with a size of about 851-853 base pairs and the California G. morrilli (nov.) species with a size of about 1063-1067 base pairs. Secondly, the two cryptic species do not share any inter-simple sequence repeat-polymerase chain reaction (ISSR-PCR) bands or markers. Initially, releases were made from what was thought to be a Mexico culture, but contamination was suspected to have occurred from a Texas culture and therefore, the culture was named "TX/MX". Post-released collections from years 2002 and 2003 were made from the following locations: San Juan Capistrano, Glen Ivy, Pauma, Temecula, and San Marcos. Amplification of the ITS2 rDNA fragments demonstrated that all or 100% of the randomly chosen individuals (125 total) were of the California ITS2 genotype and none were of the Texas ITS2 genotype. ISSR-PCR DNA fingerprinting of the TX/MX colony along with native California and Texas G. morrilli species demonstrated that the TX/MX ISSR-PCR banding pattern was superimposable to that of the California G. morrilli (nov.) species. The

results demonstrated that the TX/MX colony was contaminated with the California species, indicating that what was being released in California was California¿s own native species. Therefore, this is why screening with the ITS2 fragment detected only the California ITS2 genotype. The present results confirm the utility of the two developed molecular diagnostic methods in monitoring the success of the G. morrilli biological control program in California. In addition, this molecular technology will allow us to monitor egg parasitoid colonies to eliminate unwanted species.

Reference: See pages 56-66 in Pierce's Disease Research Summaries.

de León, J.H., and Jones, W.A. 2005. Genetic differentiation among geographic populations of *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae), the predominant egg parasitoid of *Homalodisca coagulata* (Homoptera: Cicadellidae). 9pp. Journal of Insect Science, 5:2, Available online: insectscience.org/5.2.

Abstract: The aim of genetically comparing different populations of the same species of natural enemies is to identify the strain that is most adapted to the environment where it will be released. In the present study, Inter-Simple Sequence Repeat-Polymerase Chain Reaction (ISSR-PCR) was utilized to estimate the population genetic structure of *Gonatocerus ashmeadi* (Girault), the predominant egg parasitoid of *Homalodisca coagulata* (Say), the glassy-winged sharpshooter. Six populations from throughout the U. S. and a population from Argentina identified as near *G. ashmeadi* were analyzed. Four populations [California, San Antonio, TX, Weslaco, TX (WTX-2), and Florida] were field collected and two [Louisiana and Weslaco, TX (WTX-1)] were reared. Three ISSR-PCR reactions were pooled to generate 41 polymorphic markers among the six U. S. populations. Nei's expected heterozygosity values (*h*), including the reared population from Louisiana were high (9.01-14.3%) for all populations, except for a reared population from WTX-1 (2.9%). The total genetic diversity value (Ht) for the field populations was high (23%). Interestingly, the Florida population that was collected from one egg mass (siblings) generated the greatest number of polymorphic markers (20) and was observed with the highest gene diversity value (14.3%). All populations, except WTX-2 generated population-specific markers. Comparison of genetic differentiation estimates, which evaluate the degree of genetic subdivision, demonstrated good agreement between G_{ST} and θ values, 0.38 and 0.50, respectively for field populations, and 0.44 and 0.50, respectively for all populations. Genetic divergence (D) indicated that the WTX-1 population was the most differentiated. Average D results from the Argentina population support the taxonomic data that it is a different species. The present results estimate the population genetic structure of *G. ashmeadi*, demonstrating profound genetic divergence and restricted gene flow (Nm = 0.83) among populations. These results are of interest to the Pierce's disease/glassy-winged sharpshooter biological control program because the key to successful biological control may not be in another species, but instead in different geographic races or biotypes.

Reference: See pages 56-66 in Pierce's Disease Research Summaries.

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

Abstract: DNA polymorphisms were detected in *Homalodisca coagulata* (Say) with the

following DNA fingerprinting methods: ISSR-PCR (Inter-Simple Sequence Repeat-Polymerase Chain Reaction) and pp-ISSR-PCR (primer pair-ISSR-PCR), RAMP (Randomly Amplified Microsatellite Polymophisms), SAMPL (Selective Amplification of Microsatellite Polymorphic Loci) and pp-RAPD-PCR (primer pair-Random Amplification of Polymorphic DNA-Polymerase Chain Reaction). But first, a small-scale DNA fingerprinting screening procedure was initiated with these methods with a few individual insects to estimate the most sensitive and efficient method(s). A total of 205 polymorphic markers were generated with the four methods. The efficiency ratio estimated the following order for each method: 1) pp-ISSR-PCR and ISSR-PCR, 2) RAMP, 3) pp-RAPD-PCR, and 4) SAMPL. The screening efficiency ratio estimated that pp-ISSR-PCR and ISSR-PCR were the most efficient methods. DNA polymorphisms were detected in a natural population of 10-30 insects. The number of polymorphic loci ranged from five (pp-RAPD-PCR reaction 6) to 32 (ISSR-PCR primer 13) and percentage polymorphic loci was 100% for most primers tested. DNA fingerprinting methods tested were able to detect geographic variation in populations of *H. coagulata* from Bakersfield and Riverside, CA and Weslaco, TX. Dendrograms based on Nei's genetic distance showed that *H. coagulata* from Bakersfield and Riverside formed a cluster separate from Weslaco in three DNA fingerprinting reactions tested incorporating simple sequence repeats. DNA fingerprinting methods tested were also able to distinguish between three *Homalodisca* sharpshooters: *H. coagulata*, *H. insolita*, and *H. lacerta*. The present results confirmed the utility of the DNA fingerprinting screening procedure and demonstrated, for the first time, genetic variation in natural populations of glassy-winged sharpshooters by PCR-based DNA fingerprinting methods.

Reference: See pages 56-66 in Pierce's Disease Research Summaries.

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

Abstract: In the present study Inter-Simple Sequence Repeat (ISSR) primers (p-13 and p-15) were utilized to estimate the population genetic structure of the glassy-winged sharpshooter, *Homalodisca coagulata* (Say). Eighteen populations from throughout the U. S. and a population from Tahiti, French Polynesia were analyzed. Populations were arbitrarily assigned to three regions: southeastern, southwestern, and western regions. Exact tests for population differentiation indicated highly significant differences in marker frequencies among the 18 populations with both primers. Analyses of molecular variance (AMOVA) also indicated significant geographic structuring with both primers. A dendrogram based on Reynolds coancestry distance performed with p-15 clustered the U. S. populations into two main groups. The southeastern populations were grouped into one cluster and the southwestern and western populations into a second cluster. Within the western region, dendrograms produced with p-13 and p-15 showed in both cases that two populations (Edison and Bakersfield) clustered as outliers. The average divergence (D) among all populations was 0.099. Divergence values of 0.254, 0.103 and 0.102 were observed when comparing Bakersfield and the southeastern, southwestern, and western populations, respectively. Within the western region, D values for Bakersfield were 1.8- (p-13) and 2.4-fold (p-15) higher than the D of the western populations. The present results suggest that a subset of insects in California may have their origins in the southwestern region (Texas); furthermore, these results are suggestive of more than one

founding event in California and/or biotypes or geographic races.

Reference: See pages 56-66 in Pierce's Disease Research Summaries.

de León, J.H., Jones, W.A., and Morgan, D.J.W. 2004. Molecular distinction between populations of *Gonatocerus morrilli*, egg parasitoids of the glassy-winged sharpshooter *Homalodisca coagulata*, from Texas and California: Do cryptic species exist? 7pp. Journal of Insect Science, 4, Available online: insectscience.org/4.

Abstract: Two molecular methods were utilized to distinguish geographic populations of *Gonatocerus morrilli* (Howard) from Texas and California and to test the possibility that this species could exist as a species-complex. Inter-Simple Sequence Repeat-Polymerase Chain Reaction (ISSR-PCR) was performed with a 5'-anchored ISSR primer. Twenty-five markers were generated with four populations (40 individuals) of *G. morrilli*, twenty-three were polymorphic and percentage of polymorphic loci was 92%. Most markers could be considered diagnostic since there was no band sharing between the Texas and California populations. Such differences typically are not found unless the populations are reproductively isolated. Exact tests for population differentiation indicated significant differences in marker frequencies among the populations. Comparison of other genetic differentiation estimates, which evaluate the degree of genetic subdivision, demonstrated excellent agreement between G_{ST} and θ values, 0.92 and 0.94, respectively; indicating that about 92 to 94% of the variance was distributed among populations. Average genetic divergence (D), as measured by genetic distance, was extremely high (Nei = 0.82 and Reynolds = 2.79). A dendrogram based on Nei's genetic distance, separated the Texas and California populations into two clusters, respectively. Amplification of the Internal Transcribed Spacer-1 (ITS-1) region showed no size differences, whereas the ITS-2 DNA fragments varied in size between the two geographic populations. The ITS-2 fragment sizes were about 865 and 1099 base pairs for the California and Texas populations, respectively. The present study using the two molecular methods provides novel data critical to the glassy-winged sharpshooter/Pierce's disease biological control program in California.

Reference: See pages 56-66 in Pierce's Disease Research Summaries.

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

Abstract: The current work was undertaken to develop molecular genetic markers for the glassy-winged sharpshooter, Homalodisca coagulata (Say) by various PCR-based DNA fingerprinting methods for the purpose of estimating the level of genetic variation within and among populations with the aim of achieving genetic information useful for improving the biological control of this leafhopper. These fingerprinting methods included SSR-PCR (Simple Sequence Repeat-Polymerase Chain Reaction), RAMP (Randomly Amplified Microsatellite Polymorphisms), SAMPL (Selective Amplification of Microsatellite Polymorphic Loci) and RAPD (Random Amplification of Polymorphic DNA). A combined total of about 183 polymorphic bands were detected in sharpshooters from Weslaco, Texas with the four methods and three insects per primer combination (48 total), specifically 54, 58, 34 and 37 polymorphic bands were generated by SSR-PCR, RAMP, SAMPL and RAPD, respectively. We then applied

and compared two of these PCR-based DNA fingerprinting methods to geographic populations of glassy-winged sharpshooters from Texas and California. In addition, we compared three Homalodisca sharpshooter species H. coagulata, H. lacerta and H. insolita. Our results demonstrated that the molecular genetic markers that we developed distinguished the three Homalodisca sharpshooter species, but most importantly the markers distinguished geographic populations (from Texas and California) of glassy-winged sharpshooters. This is the first report describing development of molecular genetic markers for the glassy-winged sharpshooter utilizing four different DNA fingerprinting methods.

Reference: See pages 56-66 in Pierce's Disease Research Summaries.

de León, J. H., W. A. Jones, D. J. W. Morgan, and R. F. Mizell, III. 2004. Genetic differentiation among geographic populations of *Gonatocerus ashmeadi*: A primary egg parasitoid of the glassy-winged sharpshooter, pp. 314-317. *In* Proceeding, Pierce's Disease Research Symposium, 8-10 December 2004, San Diego, CA. Complied by M. Athar Tariq, Stacie Oswalt, Peggy Blincoe, Amadou Ba, Terrance Lorick, and Tom Esser, Sacramento, CA.

Abstract: The aim of genetically comparing different populations of the same species of natural enemies is to identify the strain that is most adapted to the environment where it will be released. In the present study, Inter-Simple Sequence Repeat-Polymerase Chain Reaction (ISSR-PCR) was utilized to estimate the population genetic structure of Gonatocerus ashmeadi. Six populations from throughout the U.S. and a population from Argentina identified as near G. ashmeadi were analyzed. Four populations [California (CA), San Antonio, TX (SATX), Weslaco, TX (WTX-2), and Quincy, Florida (QFL)] were field collected and two [Louisiana (LA) and Weslaco, TX (WTX-1)] were reared. Three ISSR-PCR reactions were pooled to generate 41 polymorphic markers among the six U.S. populations. Nei's expected heterozygosity values (h), including the reared population from Louisiana, were high (9.0-14.3%) for all populations, except for a reared population from WTX-1 (2.9%). The total genetic diversity value (Ht) for the field populations was high (23%). Interestingly, the Florida population that was collected from one egg mass generated the greatest number of polymorphic markers (20) and was observed with the highest gene diversity value (14.3%). All populations, except WTX-2, generated population-specific markers. Comparison of genetic differentiation estimates, which evaluate the degree of genetic subdivision, demonstrated good agreement between GST and ' values, 0.38 and 0.50, respectively for field populations, and 0.44 and 0.50, respectively for all populations. Average genetic divergence (D) indicated that the WTX-1 population was the most differentiated. Average D results from the Argentina population support the taxonomic data that it is a different species. The present results estimate the population genetic structure of G. ashmeadi, demonstrating extensive genetic divergence and restricted gene flow (Nm = 0.83) among populations. These results are of interest to the Pierce's disease/glassy-winged sharpshooter biological control program because the key to successful biological control may not be in another species, but instead in different geographic races or biotypes.

Reference: See pages 56-66 in Pierce's Disease Research Summaries.

de León, J. H., W. A. Jones, D. J. W. Morgan, and R. F. Mizell, III. 2004. Sequence divergence in two mitochondrial genes (COI and COII) and in the ITS2 rDNA fragment in geographic populations of *Gonatocerus morrilli*, a primary egg parasitoid of the glassy-winged

sharpshooter, pp. 322-325. *In* Proceeding, Pierce's Disease Research Symposium, 7-10 December 2004, San Diego, CA. Complied by M. Athar Tariq, Stacie Oswalt, Peggy Blincoe, Amadou Ba, Terrance Lorick, and Tom Esser, Sacramento, CA..

Abstract: The aim of the present study was to resolve the genetic relationships of geographic populations of Gonatocerus morrilli, a primary egg parasitoid of the glassy-winged sharpshooter. A phylogenetic approach was implemented by sequencing two mitochondrial genes (COI and COII) and the Internal Transcribed Spacer-2 (ITS2) region of several individuals per population. Two populations from Weslaco, TX (WTX) (collected at different times), one from Quincy, FL (QFL), two from California (CA) (Orange and San Diego counties), and an outgroup (G. ashmeadi) were analyzed. For all three sequence fragments, percentage sequence divergence (%D) (as measured by genetic distance), the results demonstrated that both the WTX and QFL populations were closely related; in constrast, the %D between WTX and CA fell within the range of the outgroup, G. ashmeadi. For all three sequence fragments, Nieghbor-Joining distance trees separated the CA and WTX and QFL populations into two distinctive clades (A and B). The topology of the clades in each case was supported by very strong bootstrap values, 100% in the three sequence fragments (COI, COII, and ITS2). The present molecular phylogenetics results provide strong evidence that G. morrilli from California may be a different species. The findings of the present study are important to the glassy-winged sharpshooter/Pierce's disease biological control program in California.

Reference: See pages 56-66 in Pierce's Disease Research Summaries.

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

Abstract: In the present study compound Inter-Simple Sequence Repeat (ISSR) primers containing CA/GT-repeat motifs in their sequences were utilized to estimate the population genetic structure of Homalodisca coagulata (Say). Eighteen populations from throughout the U.S. and a population from Tahiti, French Polynesia were analyzed. The 18 U.S. populations were arbitrarily assigned to three regions: southeastern, southwestern (Texas), and western (California) regions. A total of 62 and 91 neutral polymorphic markers were identified with p-15 and p-13, respectively. Exact tests for population differentiation indicated significant differences in marker frequencies among the 18 populations with both primers; in addition, significant differences were also observed within each region. Analyses of molecular variance (AMOVA) showed a significant partitioning of gene diversity among regions, 11% with p-15 and a lower value of 3% with p-13. The majority of the variance, however, were distributed within populations, 83 and 88% with p-15 and p-13, respectively. Comparison of other genetic differentiation estimates showed values for GST (8-11%) and ' (7-10%) for among regional variation that were of comparable magnitudes to the AMOVA results. A dendrogram based on Reynolds coancestry distance performed with p-15 clustered the U. S. populations into two main groups. The southeastern populations were grouped into one cluster and the southwestern and western populations into a second cluster. Within the western region, dendrograms produced with p-13 and p-15 showed in both cases that the Edison and Bakersfield populations clustered as outliers. The present results estimate, for the first time, the population genetic structure of H. coagulata and suggest that a subset of insects in California may have their

origins in the southwestern region (Texas); furthermore, these results are suggestive of more than one founding event in California.

Reference: See pages 56-66 in Pierce's Disease Research Summaries.

de León, J. H., V. Fournier, J. Hagler, K. Daane, and W. A. Jones. 2004. Development of molecular diagnostic markers for *Homalodisca* sharpshooters present in California to aid in the identification of key predators, pp. 326-329. *In* Proceeding, Pierce's Disease Research Symposium, 7-10 December 2004, San Diego, CA. Complied by M. Athar Tariq, Stacie Oswalt, Peggy Blincoe, Amadou Ba, Terrance Lorick, and Tom Esser, Sacramento, CA.

Abstract: The aim of the present study was to develop molecular diagnostic markers to identify key predators of *Homalodisca* sharpshooter species present in California, *H. coagulata* (glassy-winged sharpshooter, GWSS) and *H. liturata* (smoke-tree sharpshooter, STSS). RAPD-PCR DNA fingerprinting of several sharpshooter species identified specific bands that were excised, sequenced, and SCAR (Sequenced Characterized Amplified Region) markers were designed. The results demonstrated that both GWSS- and *Homalodisca*-specific markers were specific toward their targets. The GWSS-specific markers amplified only GWSS and the *Homalodisca*-specific markers amplified only GWSS and STSS. The sensitivity limits for both marker sets was at 50 pg of DNA. The mitochondrial cytochrome oxidase subunit gene II (COII)-specific markers that were developed were each specific for GWSS and *Homalodisca* sharpshooters. The development of diagnostic markers designed toward *Homadisca* sharpshooters present in California should aid in finding key predators and therefore enhance biological control efforts against these sharpshooters.

Reference: See pages 56-66 in Pierce's Disease Research Summaries.

Doddapaneni, H., Francis, M., Yao, J., Lin, H. and Civerolo, E.L. 2007. Genome-wide analysis of *Xylella fastidiosa:* implications for detection and strain relationships. African Journal Biotechnology (accepted).

Abstract: The xylem limited plant pathogenic bacterium *Xylella fastidiosa* causes economically important diseases on agronomic, horticultural and landscape plants. This review includes the current status of polymerase chain reaction (PCR) based systems for detection and characterization of *X. fastidiosa*, and presents a genome-wide analysis of strain differentiation. The use of genomics data for strain comparisons will improve the understanding of the genetic determinants of strain specific pathogenicity and virulence. The genome-level analysis can be applied to design new strategies for management and control of *Xylella fastidiosa* associated diseases in a wide range of crops.

Reference: See pages11-12 in Pierce's Disease Research Summaries.

Doddapaneni, H., Yao J., Lin, H., Walker, M.A. and Civerolo, E.L. 2006. Analysis of the genome-wide variation among multiple strains of the plant pathogenic bacterium *Xylella fastidiosa*. BMC Genome. 7:225 (doi:10.1186/1471-2164-7-225).

Abstract: The Gram-negative, xylem-limited phytopathogenic bacterium *Xylella fastidiosa*

isresponsible for causing economically important diseases in grapevine, citrus and many other plantspecies. Despite its economic impact, relatively little is known about the genomic variations amongstrains isolated from different hosts and their influence on the population genetics of this pathogen. With the availability of genome sequence information for four strains, it is now possible to perform genome-wide analyses to identify and categorize such DNA variations and to understand their influence on strain functional divergence. Results: There are 1,579 genes and 194 non-coding homologous sequences present in the genomes of all four strains, representing a 76. 2% conservation of the sequenced genome. About 60% of the *X. fastidiosa* unique sequences exist as tandem gene clusters of 6 or more genes. Multiple alignments identified 12,754 SNPs and 14,449 INDELs in the 1528 common genes and 20,779 SNPs and 10,075 INDELs in the 194 non-coding sequences. The average SNP frequency was 1.08 × 10-2 per base pair of DNA and the average INDEL frequency was 2.06 × 10-2 per base pair of DNA. On an average, 60.33% of the SNPs were synonymous type while 39.67% were non-synonymous type. The mutation frequency, primarily in the form of external INDELs was the main type of sequence variation. The relative similarity between the strains was discussed according to the INDEL and SNP differences. The number of genes unique to each strain were 60 (9a5c), 54 (Dixon), 83 (Ann1) and 9 (Temecula-1). A sub-set of the strain specific genes showed significant differences in terms of their codon usage and GC composition from the native genes suggesting their xenologous origin. Tandem repeat analysis of the genomic sequences of the four strains identified associations of repeat sequences with hypothetical and phage related functions. Conclusion: INDELs and strain specific genes have been identified as the main source of variations among strains, with individual strains showing different rates of genome evolution. Based on these genome comparisons, it appears that the Pierce's disease strain Temecula-1 genome represents the ancestral genome of the *X. fastidiosa*. Results of this analysis are publicly available in the form of a web database.

Reference: See pages 11-12 in Pierce's Disease Research Summaries.

Dugravot, S., E. A. Backus, B. Reardon, and T. A. Miller. 2007. Correlations of cibarial and precibarial muscle activities with EPG waveforms and excretion in sharpshooter (Hemiptera: Cicadellidae: Cicadellinae) vectors of *Xylella fastidiosa*. Journal of Insect Physiology. *Submitted*.

Abstract: Fluid flow in and out of the stylets of xylem-ingesting sharpshooters (Hemiptera: Cicadellidae: Cicadellinae) is powered by muscles of the cibarial pump. Such fluid flow is crucial for transmission of *Xylella fastidiosa*, the Pierce's Disease bacterium, yet has not been rigorously studied via electrical penetration graph (EPG) technology. By electromyographically (EMG) recording cibarial muscle potentials and video-recording movements of the cibarial diaphragm, we correlated those movements with waveforms representing ingestion and excretory droplet production. Results definitively showed that the C waveform represents fluid flow propelled by cibarial muscle contraction, i.e. active ingestion. There is a 1:1 correspondence of each cycle of cibarial muscle contraction/relaxation to each plateau of EPG waveform C. Moreover, the rise portion of each C plateau represents muscular diaphragm uplift, therefore the suction that pulls fluid into the stylets. The top of the plateau represents holding then release of the diaphragm, propelling fluid into the esophagus or the precibarium. Thus, fine structure of the EPG ingestion waveform represents directionality of fluid flow, supporting the role of streaming potentials as the electrical origin of that waveform. Rhythmic bouts of cibarial pumping were strongly correlated with sustained production of

excretory droplets. However, neither the onset nor cessation of ingestion was correlated with onset or cessation of excretion, respectively. Implications for using EPG to understand the mechanism of *X. fastidiosa* transmission are discussed.

Reference: See pages 34-37 in Pierce's Disease Research Summaries.

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

Abstract: Application of PCR for disease diagnosis has been limited in part by the presence of PCR inhibitors. Inhibition can be overcome and sensitivity increased with BIO-PCR by enriching bacteria on agar media, however, Xylella fastidiosa (Xf) grows slowly. We have developed an agar absorbent BIO-PCR method for detecting Xf in grape and citrus plants. Optimum lengths of time for absorption of inhibitors by the agar medium or enrichment of bacteria on the medium were determined for grapes infected by Pierce's disease and citrus variegated chlorosis. Direct PCR assays of grape and citrus samples showed that 13% (4/32) and 33% (2/6) were positive, respectively. In contrast, with agar absorbent-PCR, 97% (31/32) and 100% (6/6) were positive after 2 d and 4 h for grape and citrus, respectively. In a separate experiment, petioles of symptomatic grape and citrus leaves were spotted onto agar media and the spots washed, after various intervals, and assayed for bacteria by real-time PCR. By agar-absorption, 50% (14/28) of the grape spots were positive after 1h or 4h, and 89% (25/28) after 5d. All six citrus samples were positive after only 4h. Viable Xf were recovered from all samples after 14 days. This simple technique should be useful for routine detection of Xf and other slow-growing bacteria in the presence of PCR inhibitors.

Reference: See page 15 in Pierce's Disease Research Summaries.

Fournier, V., Hagler, J.R., Daane, K.M., de León, J.H., Groves, R.L., Costa, H.S., and Henneberry, T.J.. 2006. Development and application of a glassy-winged and smoke-tree sharsphooter egg-specific predator gut content ELISA. Biological Control 37: 108-118.

Abstract: The recent invasion of southern California by the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae), has triggered a statewide control effort. Management of GWSS will include biological control using resident and imported natural enemies. Currently, very little information is available on the role of generalist predators in suppression of GWSS eggs, nymphs or adults. We have developed a sharpshooter egg-specific monoclonal antibody (MAb) for use as a diagnostic tool for predator gut content analysis. The MAb was tested by an indirect enzyme-linked immunosorbent assay (ELISA) for specificity to the different life stages of GWSS, smoke-tree sharpshooter (STSS), *H. liturata* Ball (Hemiptera: Cicadellidae), and various life stages of 27 other arthropod species. We found that the MAb only reacted to the egg stage of both sharpshooters and, to a lesser extent, to the adult stage of gravid GWSS and STSS females. Moreover, the ELISA was more responsive to younger GWSS eggs than older ones. Laboratory trials were conducted to determine how long GWSS egg antigen remained detectable in the guts of the lacewing, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) and ladybird beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) using both an indirect and sandwich ELISA format. We found that GWSS egg antigen was detectable for up to 30 h and 12 h in the guts of

C. carnea and *H. axyridis;* respectively, and that the sandwich ELISA was much more sensitive than the indirect ELISA. Finally, 98 field-collected lacewings were examined for sharpshooter remains using our sharpshooter-specific sandwich ELISA. The assay detected sharpshooter egg antigen in 8.2% of the lacewings examined. This work represents a first step towards identifying the GWSS predator complex.

Reference: See page 24 in Pierce's Disease Research Summaries.

Fournier, V., J. Hagler, K. Daane, and J. H. de León. 2006. Identifying key predators of the glassy-winged sharpshooter in a citrus orchard, pp. 64-66. *In* Proceeding, Pierce's Disease Research Symposium, 27-29 November 2006, San Diego, CA. Complied by Tom Esser, M. Athar Tariq, Raygina Medeiros, Melinda Mochel, and Sean Veling, Sacramento, CA.

Abstract: Over 1,500 predators were screened for glassy-winged sharpshooter (GWSS) remains using a GWSS egg-specific monoclonal antibody (MAb) and several GWSS-specific genetic markers. Specimens were collected in 2002 and 2003 from a citrus orchard (Riverside, CA) harboring high densities of GWSS. We found that 6.2% of all specimens examined tested positive for GWSS remains. The most frequent predators to test positive included the assassin bug, Zelus renardii (Kolenati) (Hemiptera: Reduviidae) and the spiders Trachelas pacificus Chamberlin and Ivie (Araneae: Corinnidae) and Olios sp. (Araneae: Sparassidae) with 41, 22, and 19% of the specimens testing positive with either ELISA and/or PCR, respectively.

Reference: See page 24 in Pierce's Disease Research Summaries.

Fournier, V., Hagler, J.R., Daane, K.M., de León, J.H, Groves, R.L., Costa, H.S. and Henneberry, T.J. 2006. Development and application of a glassy-winged and smoke-tree sharsphooter egg-specific predator gut content ELISA. Biological Control 37: 108-118.

Abstract: The recent invasion of southern California by the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae), has triggered a statewide control effort. Management of GWSS will include biological control using resident and imported natural enemies. Currently, very little information is available on the role of generalist predators in suppression of GWSS eggs, nymphs or adults. We have developed a sharpshooter egg-specific monoclonal antibody (MAb) for use as diagnostic tool for predator gut content analysis. The MAb was tested by an indirect enzyme-linked immunosorbent assay (ELISA) for specificity to the different life stages of GWSS, smoke-tree sharpshooter (STSS), *H. liturata* Ball (Hemiptera: Cicadellidae), and various life stages of 27 other arthropod species. We found that the MAb only reacted to the egg stage of both sharpshooters and, to a lesser extent, to the adult stage of gravid GWSS and STSS females. Moreover, the ELISA was more responsive to younger GWSS eggs than older ones. Laboratory trials were conducted to determine how long GWSS egg antigen remained detectable in the guts of the lacewing, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) and ladybird beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) using both an indirect and sandwich ELISA format. We found that GWSS egg antigen was detectable for up to 30 h and 12 h in the guts of *C. carnea* and *H. axyridis;* respectively, and that the sandwich ELISA was much more sensitive than the indirect ELISA. Finally, 98 field-collected lacewings were examined for sharpshooter remains using our sharpshooter-specific sandwich ELISA. The assay detected sharpshooter egg antigen in 8.0% of the lacewings examined. This work represents a first step towards

identifying the GWSS predator complex.

Reference: See page 24 in Pierce's Disease Research Summaries.

Fournier, V., Hagler, J.R., Daane, K.M., de León, J.H, Groves, R.L., Costa, H.S. and Henneberry, T.J. 2006. Development and application of a glassy-winged and smoke-tree sharsphooter egg-specific predator gut content ELISA. Biological Control 37: 108-118.

Abstract: The recent invasion of southern California by the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae), has triggered a statewide control effort. Management of GWSS will include biological control using resident and imported natural enemies. Currently, very little information is available on the role of generalist predators in suppression of GWSS eggs, nymphs or adults. We have developed a sharpshooter egg-specific monoclonal antibody (MAb) for use as diagnostic tool for predator gut content analysis. The MAb was tested by an indirect enzyme-linked immunosorbent assay (ELISA) for specificity to the different life stages of GWSS, smoke-tree sharpshooter (STSS), *H. liturata* Ball (Hemiptera: Cicadellidae), and various life stages of 27 other arthropod species. We found that the MAb only reacted to the egg stage of both sharpshooters and, to a lesser extent, to the adult stage of gravid GWSS and STSS females. Moreover, the ELISA was more responsive to younger GWSS eggs than older ones. Laboratory trials were conducted to determine how long GWSS egg antigen remained detectable in the guts of the lacewing, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) and ladybird beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) using both an indirect and sandwich ELISA format. We found that GWSS egg antigen was detectable for up to 30 h and 12 h in the guts of *C. carnea* and *H. axyridis;* respectively, and that the sandwich ELISA was much more sensitive than the indirect ELISA. Finally, 98 field-collected lacewings were examined for sharpshooter remains using our sharpshooter-specific sandwich ELISA. The assay detected sharpshooter egg antigen in 8.0% of the lacewings examined. This work represents a first step towards identifying the GWSS predator complex.

Reference: See page 24 in Pierce's Disease Research Summaries.

Francis, M., Lin, H., Cabrera-Rosa, J., Doddapaneni and Civerolo, E.L. 2006. Genome-based PCR primers for specific and sensitive detection and quantification of *Xylella fastidiosa*. European Journal of Plant Pathology 225:203-213.

Abstract: *Xylella fastidiosa* is an important pathogen in many commercial crops. With the recent introduction of the glassy-winged sharpshooter vector into California, there is an increased threat of spread of the pathogen to grapes, almonds, and possibly other horticultural crops. Detection of X. fastidiosa is difficult due to low concentrations of the bacteria in insects and asymptomatic plant tissue and non-uniform distribution in infected plants. A dual purpose conventional PCR and quantitative PCR (TaqMan) system was developed for the generic detection of X. fastidiosa strains. Primers HL5 and HL6, designed to amplify a unique region common to the genomes of the sequenced Xylella strains, amplified a 221bp fragment from strains associated with Pierce¿s disease of grapes, almond leaf scorch, and oleander leaf scorch disease and from DNA from X. fastidiosa associated with citrus variegated chlorosis. Standard curves were obtained using concentrations of Xylella ranging from 5 to 100,000 cells per reaction in water and grape extracts and 10 to 100,000 cells in insect DNA. Regression curves

were similar, with correlation coefficients of R2>0.97. In quantitative PCR analyses, Ct values ranged between 20-36 cycles for 5 to 100.000 bacterial cells per reaction. No amplicons were obtained with several non-Xf bacteria tested including related plant pathogenic, grape endophytic bacteria and endosymbiotic bacteria isolated from glassy-winged sharpshooters. The method was evaluated for clinical diagnosis of X. fastidiosa in grapes, almonds and insect vectors. The procedure described is reliable for detection of the pathogen with a high degree of sensitivity and specificity.

Reference: See pages 11-12 in Pierce's Disease Research Summaries.

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

Abstract: Almond leaf scorch (ALS) disease has emerged as a serious threat to almond (Prunus amygdalus) production areas throughout California's San Joaquin Valley. This disease is caused by the xylem-limited bacterium Xylella fastidiosa, and this pathogen is transmitted by xylophagous insects including sharpshooter leafhoppers (Hemiptera: Cicadellidae) and spittlebugs (Hemiptera: Cercopidae). Among four orchards surveyed, enzyme-linked immunosorbent assay (ELISA) and bacterial isolation followed by polymerase chain reaction (PCR) were equally effective in detecting X. fastidiosa from ALS-symptomatic trees. Disease incidence varied among almond cultivars in each orchard, with the highest mean incidence and most severe symptoms frequently encountered in 'Sonora'. X. fastidiosa isolates consisted of mixtures of grape or "G-genotype" and almond or "A-genotype" strains present in surveyed orchards. The X. fastidiosa G-genotypes characterized from each orchard were associated with the most severely affected 'Sonora' trees in three of the four orchards. Both ordinary runs and simple randomization analyses revealed aggregations of ALS in three of the four orchards. Clusters of ALS affected trees frequently occurred in the outermost orchard rows. Plots of semivariance in ALS incidence over distance varied in shape and magnitude among cultivars. Semivariance increased over distance in 'Sonora' and 'Carmel', indicating spatial dependence or aggregations of incidence best fit by a combination of spherical and linear models. These results document both random and aggregate patterns of ALS spatial distribution in selected orchards and further illustrate how cultivar susceptibility influences the distribution patterns of ALS incidence. Following the recent introduction and establishment of the glassy-winged sharpshooter, Homalodisca coagulata, the impact upon the epidemiology and spread of ALS is unknown.

Reference: See pages 6, 13, and 14 in Pierce's Disease Research Summaries.

Huang, H., Lu, J., and Hunter, W. 2006. Comparative Sequence and Functional Analysis of Stilbene Synthase Genes among *Vitis* Species. Acta Horticulturae.

Abstract: Pierce disease (PD), anthracnose and downy mildew are the limiting factors in the production of European grapes (V. vinifera) in the southeastern United States, especially in Florida where annual precipitation is high. Native American grape species such as V. shuttleworthii, V. aestivalis and V. rotundifolia have evolved strong resistance to these diseases. In order to understand the genetic variation and expression of stilbene synthase, StSy, a phytoalexin, in grape species/varieties and their correlation to disease resistance, 32 sequences

of the StSy among grape Vitis species were compared and analyzed. The StSy transcripts from V. shuttleworthii were also analyzed by BLAST comparison against 260 known StSy genes from plants retrieved from NCBI; this list included StSy identified as either full-length or partial sequences. Two V. shuttleworthii, StSy transcripts were represented in low abundance in both V. vinifera and V. shuttleworthii cDNA libraries. Interestingly, one of the StSy contigs was found to have significantly higher transcriptional, expressed sequence tag (EST) abundance in the V. shuttleworthii library while being less represented in the V. vinifera libraries. While differences may be in part to variation in library construction or plant biology when samples were taken examine the differential StSy expression of these three transcripts during challenge by disease pathogens there appeared to be a correlation. Homologous relationships among the StSy multigene family were analyzed by multiple alignments of protein sequences (BLASTP and PAUP). Single nucleotide polymorphism (SNP) sites in the coding region were discovered within the multigene family. Amino acid analyses showed that some of the single nucleotide substitution resulted in amino acid changes. Correlations between single nucleotide substitutions /amino acid changes and disease resistant phenotypes were identified. Analysis of the cSNPs among the StSy appears to be a useful approach for identification of disease resistance in grape phenotypes and is being further evaluated for application to screen current and newly selected grape cultivars for increased disease resistance.

Reference: See page 19-20 in Pierce's Disease Research Summaries.

Hunnicutt, L.E., Hunter, W.B., Cave, R.D., Powell, C.A., and Mozoruk, J.J. 2006. Complete Genome Sequence and Molecular Characterization of *Homalodisca coagulata virus-1*, a novel virus discovered in the glassy-winged sharpshooter (Hemiptera: Cicadellidae). Virology 350:67-78.

Abstract: Few viral pathogens of leafhoppers have been discovered or examined as potential microbial control agents. The recent discovery of HoCV-1 a virus which infects and kills glassy-winged leafhoppers, GWSS, Homalodisca coagulata, (Hemiptera: Cicadellidae) provides a unique opportunity to better understand viral/leafhopper interactions. GWSS is an important vector of Xylella caused diseases. The best known example is Pierce¿s Disease of grapes. However, the bacterial complex of Xylella fastidiosa, also causes many different diseases which affect fruit trees and woody crops. The complete nucleotide sequence of this novel single-stranded RNA virus, Homalodisca coagulata virus-1 (HoCV-1) has been determined. In silico analysis of HoCV-1 revealed a 9321 nt polyadenylated genome encoding two large open reading frames (ORF1 and ORF2) separated by a 179 nt intergenic region (IGR). The deduced amino acid sequence of the 5'-proximal ORF (ORF1, nt 420-5807) exhibited conserved core motifs characteristic of the helicases, cysteine proteases, and RNA-dependent RNA polymerases of other insect-infecting picorna-like viruses. A structural model created using M-FOLD exposed a series of stem-loop (SL) structures immediately preceding the second ORF which are analogous to an internal ribosome entry site (IRES), suggesting that ORF2 begins with a noncognate GCA triplet rather than the canonical AUG. This 3' ORF2 (nt 5987-8740) showed significant similarity to the structural proteins of members of the family Dicistroviridae, particularly those belonging to the genus Cripavirus. Evidence demonstrating relatedness of these viruses regarding genome organization, amino acid sequence similarity, and putative replication strategy substantiate inclusion of HoCV-1 into this taxonomic position. HoCV-1 as the first virus reported from GWSS is being evaluated for potential use as a microbial biological control agent to augment current management strategies against the GWSS.

Reference: See page 19-20 in Pierce's Disease Research Summaries.

Hunter, W.B., Katsar, C.S., and Chaparro, J.X. 2006. Nucleotide sequence of 3′ -end of *Homalodisca coagulata Virus-1*. A new leafhopper-infecting virus from the glassy-winged sharpshooter. Journal of Insect Science 6.28. Available online: insectscience.org/6.28/.

Abstract: A new virus that infects and causes increased mortality in leafhoppers was isolated from the glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae). The virus, named *Homalodisca coagulata virus -1*, HoCV-1, was associated with increased mortality of cultured 5th instar *H. coagulata*. To identify the presence of *H. coagulata* viral pathogens, cDNA expression libraries were made from adult and nymphs. Analysis using reverse transcriptase PCR demonstrated that the virus was present in midgut tissues. As the viral capsid proteins are commonly used in classification of newly discovered viruses, the capsid proteins (CP) of the virus discovered in *H. coagulata* was examined. The order of the polyprotein subunits of HoCV-1 capsid proteins was determined to be CP2, CP4, CP3, and CP1. The CP4/CP3 (AFGL/GKPK) cleavage boundary site was clearly identified when the sequences were aligned. The putative CP3/CP1 (ADVQ/SAFA) cleavage site and the putative CP2/CP4 (VTMQ/EQSA) cleavage site of HoCV-1, respectively, were located in the same region as that of the other viruses. After alignment, the CP3/CP1 cleavage sites and CP2/CP4 cleavage sites of the viruses analyzed fell within 50 amino acids of one another. As with the *cricket paralysis virus*, HoCV-1 was found to be mainly comprised of β-sandwiches in CP1-3 with a jelly roll topological motif. CP4 of HoCV-1 appeared to be mainly α-helical in structure. CP1-4 domains are most homologous to insect picorna-like virus coat proteins as was demonstrated by the Results of the BLASTP and PSI-BLAST tests, and is strongly supported by the structural modeling. While sequence homology between the *cricket paralysis virus* and HoCV-1 was low, the global structure of the proteins was conserved. Sequence identities were analyzed by *in silico* comparison to known genes in the public database, NCBI. Phylogenetic analysis performed using the optimized protein alignment generated a phylogram containing 5 clades. Clade 1 consisted of *Drosophila C virus*, Clade 2 consisted of *cricket paralysis virus*, Clade 3 of *Triatoma virus*, *Plautia stali intestine virus*, *Himetobi P virus*, *black queen cell virus*, and HoCV-1. Clade 4 encompassed *acute bee paralysis virus* and *Kashmir bee virus*, and Clade 5 consisted of *Rhopalosiphum padi virus*. Analysis of the capsid protein of this new leafhopper virus provided significant evidence that it is related to other ssRNA insect viruses within the Family, *Dicistroviridae.* The HoCV-1, capsid protein sequence has been deposited in GenBank, Accession number: DQ308403.

Reference: See pages 19-20 in Pierce's Disease Research Summaries.

Joost, P. H., E. A. Backus, D. J. W. Morgan, and F. Yan. 2006. Specific stylet activities by the glassy-winged sharpshooter, *Homalodisca coagulata* (Say), are correlated with AC EPG waveforms. Journal of Insect Physiology. 52: 327-337.

Abstract: Glassy-winged sharpshooer (GWSS), *Homalodisca coagulata* (Say), is an efficient vector of *Xylella fastidiosa* (*Xf*), the causal bacterium of Pierce's disease, and leaf scorch in almond and oleander. Acquisition and inoculation of *Xf* occur sometime during the process of stylet penetration into the plant. That process is most rigorously studied via electrical penetration graph (EPG) monitoring of insect feeding. This study provides part of the crucial

biological meanings that define the waveforms of each new insect species recorded by EPG. By synchronizing AC EPG waveforms with high-magnification video of GWSS stylet penetration in artifical diet, we correlated GWSS stylet activities with three previously described EPG pathway waveforms, A1, B1 and B2, as well as one ingestion waveform, C. Waveform A1 occured at the beginning of stylet penetration. Subtypes of this waveform were correlated with salivary sheath trunk formation, repetitive stylet movements involving retraction of both maxillary stylets and one mandibular stylet, extension of the stylet fascicle, and the fluttering-like movements of the maxillary stylet tips. Waveform B1 was ubitquious, interspersed throughout the other waveforms. B1 subtype, B1w, was correlated with salivation followed by maxillary tip fluttering. This tip fluttering occurred before and during B1 subtype B1s, but was not directly correlated with either the occurrence or frequency of this waveform. Waveform B2 was correlated with sawing-like maxillary stylet movements, which caused salivary sheath branching. Waveform C was correlated with ingestion. Fluid outflow was also observed as a mechanism to clear the maxillary tips from debris during waveform C. This detailed understanding of stylet penetration behaviors of GWSS is an important step toward identifying the instant of bacterial inoculation which, in turn, will be applied to studies of disease epidemiology and development of host plant resistance.

Reference: See pages 34-37 in Pierce's Disease Research Summaries.

Joost, P. H., E. A. Backus, D. J. W. Morgan, and F. Yan. 2006. Specific stylet activities by the glassy-winged sharpshooter, *Homalodisca coagulata* (Say), are correlated with AC EPG waveforms. Journal of Insect Physiology. 52: 327-337.

Abstract: Glassy-winged sharpshooer (GWSS), *Homalodisca coagulata* (Say), is an efficient vector of *Xylella fastidiosa* (*Xf*), the causal bacterium of Pierce's disease, and leaf scorch in almond and oleander. Acquisition and inoculation of *Xf* occur sometime during the process of stylet penetration into the plant. That process is most rigorously studied via electrical penetration graph (EPG) monitoring of insect feeding. This study provides part of the crucial biological meanings that define the waveforms of each new insect species recorded by EPG. By synchronizing AC EPG waveforms with high-magnification video of GWSS stylet penetration in artifical diet, we correlated GWSS stylet activities with three previously described EPG pathway waveforms, A1, B1 and B2, as well as one ingestion waveform, C. Waveform A1 occured at the beginning of stylet penetration. Subtypes of this waveform were correlated with salivary sheath trunk formation, repetitive stylet movements involving retraction of both maxillary stylets and one mandibular stylet, extension of the stylet fascicle, and the fluttering-like movements of the maxillary stylet tips. Waveform B1 was ubitquious, interspersed throughout the other waveforms. B1 subtype, B1w, was correlated with salivation followed by maxillary tip fluttering. This tip fluttering occurred before and during B1 subtype B1s, but was not directly correlated with either the occurrence or frequency of this waveform. Waveform B2 was correlated with sawing-like maxillary stylet movements, which caused salivary sheath branching. Waveform C was correlated with ingestion. Fluid outflow was also observed as a mechanism to clear the maxillary tips from debris during waveform C. This detailed understanding of stylet penetration behaviors of GWSS is an important step toward identifying the instant of bacterial inoculation which, in turn, will be applied to studies of disease epidemiology and development of host plant resistance.

Reference: See pages 34-37 in Pierce's Disease Research Summaries.

Katsar, C.S., Hunter, W.B., and Sinisterra, X.H. 2006. Phytoreovirus-like sequences from glassy-winged sharpshooter salivary glands. Florida Entomologist (accepted, in press, March 2007).

ABSTRACT: The salivary glands of the Glassy-winged sharpshooter (GWSS), Homalodisca vitripennis Germar 1821, (syn. H. coagulata, Hemiptera: Cicadellidae) were collected and used to produce a cDNA library. Examination by BLASTX analyses identified two viral sequences, one a 610 base pair fragment and a second 839 base pair fragment, both of which had significant homology to viruses within the genus, Phytoreovirus. Resequencing of the fragments confirmed sequence validities, which were used for in silico protein translation and BLASTP analysis to the Phytoreoviruses. While the GWSS is the primary vector of Pierce's disease of grapes, this is the first report that GWSS may be a vector of a phytoreoviruses. Phylogenetic and homology comparisons using BLASTX, BLASTP and PAUP analyses indicated that the viral sequences were closely related to the viruses in the Family, Reoviridae, Genus, Phytoreovirus, specifically Rice Dwarf Phytoreovirus (RDV). RDV is the only plant reovirus that is not limited to the phloem. The GWSS, although considered to feed primarily from the xylem, ingests from other plant tissues, such as the phloem and mesophyll during probing similar to other leafhoppers. Phytoreoviruses are transmitted in a propagative manner by cicadellid leafhoppers (Hemiptera:
Cicadellidae), being acquired and transmitted during feeding. Phytoreoviruses have been reported from Agallian, Agalliopsis, Nephotettix, and Recilia, genera of leafhoppers, with evidence for transovarial transmission. The Phytoreovirus, wound tumor virus, WTV has been reported to occur in North America and infects the phloem of dicotyledonous plants, thus causing leaf and root galling. The feeding behavior and wide host range of the GWSS which will feed from grasses as a transitory host, and on herbaceous and woody plants as primary hosts, provides an overlapping condition for these two organisms, leafhopper and virus, such that it may favor the acquisition and transmission of Phytoreovirus by the GWSS. Monitoring for an increase of Phytoreovirus spread in graminaceous crops that are in close proximity to vineyards or tree crop orchards, where GWSS occurs, such as in southern California, will provide a better understanding of the potential role of the GWSS as a disease vector in the spread of phytoreoviruses and other plant pathogens.

Reference: See page19-20 in Pierce's Disease Research Summaries.

Lin, H., Civerolo, E.L., Hu, R., Barros, S., Francis, M. and Walker, M.A. 2005. Multilocus Simple Sequence Repeat Markers for Differentiating Strains and Evaluating Genetic Diversity of *Xylella fastidiosa.* Applied and Environmental Microbiology 71: 4888-4892.

Abstract: A genome-wide search was performed to identify simple sequence repeat (SSR) loci among the available sequence databases from four strains of *Xylella fastidiosa* (strains causing Pierce's disease, citrus variegated chlorosis, almond leaf scorch, and oleander leaf scorch). Thirty-four SSR loci were selected for SSR primer design and were validated in PCR experiments. These multilocus SSR primers, distributed across the *X. fastidiosa* genome, clearly differentiated and clustered *X. fastidiosa* strains collected from grape, almond, citrus, and oleander. They are well suited for differentiating strains and studying *X. fastidiosa*

epidemiology and population genetics.

Reference: See pages 11-12 in Pierce's Disease Research Summaries.

Lin, H., Civerolo, E.L., Hu, R., Barros, S., Francis, M. and Walker, A.M. 2005. Multilocus simple sequence repeat markers for differentiating strains and evaluating genetic diversity of *Xylella fastidiosa*. Applied and Environmental Microbiology 71:4888-4892.

Abstract: A genome wide search was performed to identify simple sequence repeat (SSR) loci among the available sequence databases for four strains of Xylella fastidiosa (strains causing Pierce's disease, citrus variegated chlorosis, almond leaf scorch and oleander leaf scorch). Thirty-four SSR loci were selected for SSR primer design. These primers detected varying levels of polymorphism among 43 X. fastidiosa isolates derived from grape, citrus, almond and oleander. These multi-locus SSR primers are distributed across the X. fastidiosa genome and are useful for differentiating among and within host-associated X. fastidiosa isolates and for genetic diversity and population structure studies.

Reference: See pages11-12 in Pierce's Disease Research Summaries.

Logarzo, G.A., de León, J.H., Triapitsyn, S.V., Gonzalez, R.H., and Virla, E.G. 2006. First report of a Proconiine Sharpshooter *Anacuerna centrolinea* (Hemiptera: Cicadellidae) in Chile, with Notes on its Biology, Host Plants, and Egg Parasitoids. Annals of the Entomological Society of America 99: 879-883.

Abstract: The first representative of the leafhopper tribe Proconiini (subfamily Cicadellinae), *Anacuerna centrolinea* (Melichar) is reported herein from Tarapacá Region in northern Chile. This species was discovered at high elevation (ca. 4000 m) in the course of a survey conducted in South America by the Agricultural Research Service of the United States Department of Agriculture for the neoclassical biological control program against the glassy-winged sharpshooter, *Homalodisca coagulata* (Say), in California, USA. New data are given on the biology and host plants of *A. centrolinea*. Information is also provided on its egg parasitoid, *Gonatocerus tuberculifemur* (Ogloblin) (Hymenoptera: Mymaridae), which was also the first time recorded from Chile. This discovery encourages further exploration for leafhopper egg parasitoids in northern and central regions of Chile to identify new perspective biological control agents that are more adapted to California-like climates. In addition, it is possible that *G. tuberculifemur* may be a good candidate for the biological control of the recently discovered *H. coagulata* in Easter Island, Chile.

Reference: See pages 56-66 in Pierce's Disease Research Summaries.

Leopold, R.A., Freeman, T.P., Buckner, J.S., and Nelson, D.R. 2003. Mouthpart morphology and stylet penetration of host plants by the glassy-winged sharpshooter, *Homalodisca coagulata* (Homoptera: Cicdellidae). Arthropod Structure & Development 32(2-3):189-19.

Abstract: The ultrastructural morphology of the mouthparts of the glassy-winged sharpshooter, *Homalodisca coagulata*, and method of plant penetration was examined using light microscopy, SEM, and TEM methods. The gross morphology of the labrum, labium, and

stylet fascicle was consistent with what has been described for other plant-sucking homopterans. The ultrastructural examination of the mouthparts revealed unique details that have previously gone unreported. Several types of sensilla-like structures having the form of pegs and multi-lobed objects were identified on the outer surfaces of the labrum and within the labial groove. Dendritic canals terminated in an extensive network of smaller canals at the distal tip of the maxillary stylets below a series of surface denticles suggesting that this area may have a sensory function associated with locating xylem elements of host plants. Examination of salivary sheath pathways established that 65% of the plant penetrations by this insect terminated in the xylem vessels of the host plant. Probing by the insect was largely intracellular and terminal branching of a single probe site was common. Plant surface feeding sites varied with the stage of development which correlates with the depth of the xylem vessels and the length of the maxillary stylets of the various instars.

Reference: See pages 25-26 in Pierce's Disease Research Summaries.

Lin, H., Walker, M.A., Hu, R. and Granett. J. 2006. New Simple Sequence Repeat Loci for the Study of Grape Phylloxera *(Daktulosphaira vitifoliae)* Genetics and Host Adaptation. American Journal of Enology and Viticulture. 57(1) 33-39.

Abstract: Vineyard damage due to grape phylloxera, *Daktulosphaira vitifoliae* Fitch, has been controlled by resistant rootstocks for over 100 years. There are now a wide range of rootstocks used in California vineyards since the collapse of AXR#1. To study the effect of this rootstock diversity on phylloxera genetics and possible host adaptation, a set of microsatellite primers were developed to augment the four produced by Corrie and colleagues (2002). In order to develop more microsatellite loci to improve the sensitivity and effectiveness of these markers for use in genetic diversity and rootstock adaptation studies, a subtractive-based hybridization strategy was used to construct microsatellite enriched genomic libraries from grape phylloxera DNA. Fifty loci were identified for primer design. Nineteen produced good PCR products, seven of which reliably detected polymorphisms across the 32 grape phylloxera populations tested. These seven SSR loci were used to distinguish genetic diversity in California and European grape phylloxera populations. Results confirm the utility of these loci for analyzing genetic diversity, "finger-printing" strains, and studying host associations. A significant deviation from the Hardy-Weinberg equilibrium for the tested California populations suggests that parthenogenesis is perhaps the primary, if not only, reproductive system in California.

Reference: See pages 46-49 in Pierce's Disease Research Summaries.

Lu, J., and Hunter, W.B. 2007. Moving towards Disease Resistance in Grapes. pp 10-15. Wine East January.

Abstract: We produced the North American Native Grape Genome database to host grape research data for the U.S. viticulture industry. Within this database we have produced and identified hundreds of genetic markers (~800) which are being screened to expedite the selection of new grape varieties with increased disease and pest resistance. The need and development for disease and insect resistant grape varieties has become more intense with the spread of the glassy-winged sharpshooter, GWSS, (Homalodisca vitripennis) into southern California. The GWSS has a wider host range, prefers to feed in cultivated vineyards, and tree crops, and can disperse long distances up to five miles at a time. The changing dynamics of the

epidemiology of Pierce¿s disease, has thus focused on the management of the insect vector more intensely. As new grape varieties are being produced, grape breeders can now also include grape characteristics that may reduce the feeding and/or preference of leafhoppers such that Pierce¿s disease becomes less of an economic problem. Discussed are the successful approaches being used to develop new grape varieties which includes: 1. Traditional breeding: hybridization and seedling selection: parental selections, seed production, selection for desired traits among the seedlings. 2. Induced mutations and selection. 3. Clonal selection. 4. Molecular breeding: using molecular methods to speed up the process of traditional plant breeding. These methods result in the movement of desirable grape genes from one grape variety into another, and reduces the amount of time and costs normally associated with traditional plant breeding, seed production, and variety selection. We have produced and sequenced nearly 25,000 expressed sequence tags, EST¿s, which are small genetic sequences of the genes being expressed inside the growing grapevines (V. shuttleworthii, and Noble). These sequences were analyzed in silico using computers, to identify the proteins that are produced. The protein sequences are then compared to the GenBank database (http://www.ncbi.nlm.nih.gov/. By isolating these sequences from the plants, we have identified over 800 sequences for use as genetic markers, and have identified specific genes associated with disease resistance, fruit quality, drought tolerance, insect resistance and more. Over 25,906 cDNA clones were sequenced, resulting in ~19,200 high-quality ESTs. After sequence assembly the cDNAs resulted in 12,440 total sequences, including both contiguous sequences and singlets. The putative protein transcript of each assembled sequence was annotated based on the biochemical function of matching gene sequences using BLASTX, TBLASTX, and BLASTN analyses, GenBank, nr ESTdb. The subsequent unigene set produced ~6,000 sequences which had significant identities with homologous genes in the GenBank¿s database. The remaining ~4000 of the cDNA¿s showed `no significant homology¿ in either the non-redundant protein or nucleic acid databases, demonstrating that de novo EST sequencing projects still provide new information to the scientific community. The grape gene expression data set advances current research efforts, and provide the focus for the identification of genes and physiological processes of North American grapes. The genomic information and database is also being used as a teaching tool in one of the first Bioinformatics programs at an 1890¿s University aimed at increasing minority participation in the fields of Bioinformatics and Genomics.

Reference: See pages 19-20 in Pierce's Disease Research Summaries.

Lu, J., Huang, H., Ren, Z., Bradeley, F., and Hunter, W. 2006. Comparative Genomics Analysis between *V. shuttleworthii* and *V. vinifera* grapes. Acta Horticulturae.

Abstract: Vitis shuttleworthii is a grape species native to the southeastern United States that is known for its resistance to major grape diseases and pests. To identify and isolate the genes linked to disease resistance in V. shuttleworthii, a clone was used to produce a cDNA library derived from leaves and flowers during anthesis. The expressed sequence tags, ESTs, were then used to conduct comparative, in silico analysis. Phase I of this project analyzed 12,936 ESTs which generated a set of 12,008 quality scored ESTs. These produced a set of 5,766 unigenes after assembly. Among these 5,766 V. shuttleworthii unigenes, 157 produced full-length protein sequences. The 157 contigs were compared by BLASTX analyses against the V. vinifera unigene set which contains a total of 23,871 unigenes generated from 139,380 ESTs (TIGR database). Of these, 153 had significant similarity to known Vitis proteins (E value -30). Then the 5,766 V. shuttleworthii unigene set was compared to the current set of genes from the wine

grape; of the 23,871 V. vinifera unigenes using BLASTN, roughly 27%, or 1,588 of these sequences did not have a significant identity match (E value ' -10). Comparison of the translated protein sequences identified 1,086 sequences which did not have homologs in the Vitis unigene set (BLASTX, E value ' -10). Furthermore, when these1,086 unique sequences were compared by BLASTX against the 119,971 Arabidopsis protein database, a unique set of only 428 proteins from V. shuttleworthii was identified. These had no significant matches (E value -20). Further comparison of these unique proteins by BLASTX analyses to the grape V. viniferea unigene set, to our surprise, showed that none of them were found to be homologous to any of the 23,871 sequences in the grape database. Comparing the unique set of 428 sequences to known disease resistance gene families in other crop plants identified 54 putative candidates significantly linked to disease resistance. These candidates are being further characterized for their potential usefulness as genetic markers linked to disease resistance for grape breeding programs, and to identify their role(s) in disease resistance pathways. A unique microarray has been produced (LuVs) and which is being used to examine expression of these genes in relation to disease resistance in grapes.

Reference: See pages 19-20 in Pierce's Disease Research Summaries.

Monteiro-Vitorello, C.B., de Oliveira, M.C., Zerillo, M.M., Varani, A.M., Civerolo, E. and Van Sluys, M.-A. 2005. *Xylella* and *Xanthomonas* mobil'omics. OMICS 9:146-159.

Abstract: *Xylella fastidiosa* is a xylem-dwelling, insect-transmitted, gamma-proteobacterium that causes diseases in many plants, including grapevine, citrus, periwinkle, almond, oleander, and coffee. *X. fastidiosa* has an unusually broad host range, has an extensive geographical distribution throughout the American continent, and induces diverse disease phenotypes. Previous molecular analyses indicated three distinct groups of *X. fastidiosa* isolates that were expected to be genetically divergent. Here we report the genome sequence of *X. fastidiosa* (Temecula strain), isolated from a naturally infected grapevine with Pierce's disease (PD) in a wine-grape-growing region of California. Comparative analyses with a previously sequenced *X. fastidiosa* strain responsible for citrus variegated chlorosis (CVC) revealed that 98% of the PD *X. fastidiosa* Temecula genes are shared with the CVC *X. fastidiosa* strain 9a5c genes. Furthermore, the average amino acid identity of the open reading frames in the strains is 95.7%. Genomic differences are limited to phage-associated chromosomal rearrangements and deletions that also account for the strain-specific genes present in each genome. Genomic islands, one in each genome, were identified, and their presence in other *X. fastidiosa* strains was analyzed. We conclude that these two organisms have identical metabolic functions and are likely to use a common set of genes in plant colonization and pathogenesis, permitting convergence of functional genomic strategies.

Reference: See pages 3-4 in Pierce's Disease Research Summaries.

Mozoruk, J., Hunnicutt, L.E., Cave, R.D., Hunter, W.B., and Bausher, M.G. 2006. Profiling transcriptional changes in *Citrus sinensis* (L.) Osbeck challenged by herbivory from the xylem-feeding leafhopper *Homalodisca coagulata* (Say) by cDNA macroarray analysis. Plant Science 170:1068-1080.

Abstract: The molecular mechanisms underlying plant defense to sap-feeding insects are slowly being uncovered. In large part, past research has focused on interactions between

phloem-feeding insects and their annual host plants with little emphasis on xylem-feeders or woody perennials¿especially fruit trees. Using nylon filter cDNA arrays, we analyzed the transcriptional changes of 1731 non-redundant citrus transcripts that resulted from herbivory by a xylem-feeding leafhopper, the glassy-winged sharpshooter (GWSS), Homalodisca coagulata (Say) (Hemiptera: Cicadellidae). In addition, herbivory-elicited changes were compared to those of mechanical damage to better identify GWSS-specific responses. GWSS feeding led to a significant expression change in 50 transcripts. Of these, 14 were also changed by mechanical damage; however, the magnitude was in many cases reduced, suggesting transcriptional modification by GWSS-derived elicitors. Sequence similarity searches with the public database GenBank indicated that the responsive transcripts broadly function in direct defense, defense signaling, ROS scavenging, transport, cell wall modification, photosynthesis and abiotic stress. In particular, GWSS feeding resulted in a transcript profile that resembled wounding, likely through jasmonic acid-independent pathways as well as an association with dehydration stress. In contrast to similar studies with aphids, salicylic acid-dependent pathogenesis¿related genes were weakly induced. Interestingly, six of the GWSS-responsive transcripts failed to significantly match any public protein sequence signifying their potential as novel genes functioning in plant defense, wound response or abiotic stress.

Reference: See pages 19-20 in Pierce's Disease Research Summaries.

Patt, J. M., and M. Sétamou. 2007. Associative learning of host-plant chemical cues in immature glassy-winged sharpshooter, *Homalodisca vitripennis* (Hemiptera: Cicadellidae). In Preparation for Journal of Insect Behavior.

Abstract: We determined whether *Homalodisca vitripennis* nymphs can associatively-learn to recognize olfactory stimuli produced by host plants, and evaluated the relative importance of olfactory conditioning in host-plant recognition. To provide nymphs for testing, second- to fourth instars were placed on cowpea (*Vicia unguiculata*) sprigs for 1.5 days. The cut-ends of the sprigs were immersed either in hydroponic solution containing a low concentration of vanilla extract, or, as a control, in hydroponic solution alone. After removal from the sprigs, the nymphs' responsiveness to a pale green disk in the presence of vanilla extract odor was tested in an olfactometer using no-choice tests. In preliminary tests with blank air, 44% of nymphs from the control group jumped to the pale green target, demonstrating that innate attraction to this color is low. Vanilla extract constituents were detected by gas chromatography-mass spectrometry analysis of ethanolic extractions made from vanilla-treated cowpea sprigs. Nymphs that fed on plant sprigs with vanilla-flavored xylem fluid were significantly more attracted to the pale green target than nymphs that fed on control sprigs with non-flavored xylem fluid. However, there was no difference between individuals in the experimental and control groups with respect to the amount of time they required to orient- and jump to the visual target. The finding that nymph response to a non-attractive color was enhanced following ingestion of a novel flavor indicated that immature GWSS are capable of olfactory conditioning. Rapid population growth of GWSS may depend on the close proximity of host plants suitable for successful juvenile development. Therefore, 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.

Reference: See pages 27-28 in Pierce's Disease Research Summaries.

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

Abstract: The relative effects of visual and olfactory stimuli on host-plant detection in immature and adult *Homalodisca coagulata* Say (Homoptera: Cicadellidae) were studied using a novel olfactometer and factorial experimental designs. Colored, gray, and white cards were used as visual targets. Each card was attached to a glass thistle tube, from which host-plant odor (from *Vigna unguiculata* L.) or blank, humidified air was dispensed. Visual + odor stimuli combinations were presented in no-choice tests. Nymphs were released onto a perch stick downwind from the target. Nymph response to color + odor treatments was measured by the duration of orientation behavior, residence time on the perch, and percentage of individuals that jumped to the target. The assay was modified so that adults crawled from the perch onto the target. Adult response was measured by the duration of individual behaviors (e.g., foraging), and by their position and residence time on the target. Both main effects and interactive effects of the stimuli were observed. Nymphs showed a decrease in orientation- and residence times in the colored target + host odor treatments and increased jumping response in the gray + host odor treatment. When adults were exposed to host odor, the duration of foraging behavior increased while crawling- and phototactic behaviors decreased. Although nymphs and adults responded to visual stimuli + blank air treatments, host odor enhanced their responses. The primary effect of host odor on host-detection behavior may be to enhance *H. coagulata* responsiveness to visual cues.

Reference: See pages 27-28 in Pierce's Disease Research Summaries.

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

Abstract: Xylella fastidiosa (Xf), a fastidious bacterium causing disease in over 100 plant species, is classified as a single species, although genetic studies support multiple taxons. To determine the taxonomic relatedness among strains of Xf, we conducted DNA-DNA relatedness assays and sequenced the 16S-23S intergenic spacer (ITS) region using 26 strains from 10 hosts. Under normal stringency (Tm-15C), the DNA relatedness for most Xf strains was = 70%. However, at high stringency (Tm-8C), three distinct genotypes (A, B, and C) were revealed. Taxon A included strains from cultivated grape, alfalfa, almond, and maple, interrelated by 86%; taxon B included strains from peach, elm, plum, pigeon grape, sycamore, and almond, interrelated by 82%; and taxon C included only strains from citrus, interrelated by 87%. The mean reciprocal relatedness between taxons A and B, A and C, and B and C, were 56, 39, and 45%, respectively. ITS results generally agreed with the DNA relatedness data; taxons A and B, A and C, and B and C had identities of 98.7, 97.9, and 99.2 %, respectively. Previous and present phenotypic data supports the molecular data. Taxon A strains grow faster on PD2 agar whereas B and C strains grow more slowly. Taxon B strains are susceptible to penicillin and resistant to carbenicillin whereas A strains are opposite. Each taxon can be differentiated serologically as well as by structural proteins. We propose taxons A, B, and C be named X. fastidiosa subsp. piercei, subsp. nov, subsp. agglomeri, subsp. nov., and subsp. idiotraposa, subsp. nov., respectively

Reference: See page 15 in Pierce's Disease Research Summaries.

Schaad, N.W., Postnikova, E., Lacy, G., Fatmi, M., and Chang, C.J. (2004b). *Xylella fastidiosa* subspecies: *X. fastidiosa* subsp. *fastidiosa* subsp. nov., *X. fastidiosa* subsp. *multiplex* subsp. nov., and *X. fastidiosa* subsp. *pauca* subsp. nov.. Systematic and Applied Microbiology 27:763.

Abstract: In the description of *Xylella fastidiosa* subsp. *piercei* on page 297, the proposed type strain is ATCC 35879 which is also the type strain of *Xylella fastidiosa*. According to Rule 13d of the International Code of Nomenclature of Bacteria (1990 Revision), "A subspecies that includes the type of the species must bear the same epithet as the species (see also Rules 45 and 46)." Therefore, the newly proposed subspecies for the taxon "A" strains must be named *Xylella fastidiosa* subsp. f*astidiosa*. The name "*piercei*" should be changed to "*fastidiosa*" throughout the manuscript.

Reference: See page 15 in Pierce's Disease Research Summaries.

Shatters, R.G., Jr., Bausher, M.G., Hunter, W.B., Chaparro, J.X., Dang P.M., Niedz, R.P., Mayer, R.T., McCollum, T.G., and Sinisterra, XH. 2003. Putative protease inhibitor gene discovery and transcript profiling during fruit development and leaf damage in grapefruit (Citrus paradise Macf.). GENE 326: 77-86.

Abstract: Seven putative protease inhibitor (PPI) cDNAs, representing four protein families, were isolated from a grapefruit (Citrus paradisi Maef. Cv. Marsh) immature fruit flavedo cDNA library. Clones represented: legume Kuntiz inhibitors (LkiL-1, LkiL-2, LkiL-3), potato trypsin inhibitor I (PtiIL-1), serpins (SerpL-1), cystatins (CysL-1), and gamma thionins (GthL-1). Response of transcript abundance to fruit development and leaf wounding was determined for all but LkiL-3 using real-time RT-PCR. Immature leaves had the highest transcript levels for all PPIs. The GthL-1 transcript in immature leaves was the most abundant transcript but was absent from healthy mature leaves. Transcripts for all PPIs were most abundant in flavedo of youngest fruit (<15mm dia. fruit), and declined during development. Mechanical or Diaprepes root weevil feeding damage to leaves caused a <10-fold reduction or had no effect on transcript level with the exception of GthL-1 which as a result of damage increased >50-fold in mature leaves and decreased >1400-fold in immature leaves. Except for GthL-1, the PPI transcripts were more responsive to development than to wounding. Changes in PPI transcript levels suggest diverse roles for the products of these genes in citrus, with only GthL-1 responding in a defense-like manner.

Reference: See pages 19-20 in Pierce's Disease Research Summaries.

Takiya, D.M., Cavichioli, R.R., & McKamey, S.H. 2006. Sharpshooters of the genus *Homalodisca* Stål, 1869 (Hemiptera, Cicadellidae) in Brazil: notes, new records, and key to species, and descriptions of the male of *H. ignota* Melichar, 1924 and a new Northeastern species. Zootaxa 1249: 23-36

Abstract: A new species of Homalodisca, H. potti, implicated as a possible vector of Citrus Variegated Chlorosis disease, in described from Brazil. The previously unknown male of H.

ignota Melichar and the first undistorted view of the female sternum, are described. A key, illustrations, new distribution records are provided for the four Homalodisca species now known to occur in Brazil.

Reference: See page 30 in Pierce's Disease Research Summaries.

Takiya, D.M., S. H. McKamey, & R. R. Cavichioli. 2006. Fixation of the type-species of *Homalodisca* Stål as *H. vitripennis* (Germar), the oldest name for the glassy-winged sharpshooter (Hemiptera: Cicadellidae: Cicadellinae). Annals of the Entomological Society of America 99(4): 648 – 655.

Abstract: A male lectotype of Tettigonia vitripennis Germar — deposited in the allegedly lost Germar Hemiptera collection, but recently found in the Ivan Franko National University (Ukraine) — is herein designated and assumed to be erroneously labeled from Brazil. Homalodisca vitripennis is considered a senior synonym of Tettigonia coagulata syn. nov., and should therefore used as the new scientific name for the glassy-winged sharpshooter, a major vector of the bacterial Pierce´s disease of grapes, phony peach disease, plum leaf scald, and oleander leaf scorch in southern US and northern Mexico. Furthermore, due to a misidentification of the previous type-species of Homalodisca Stål by the subsequent designator, Tettigonia vitripennis is herein fixed as the new type-species of this economically important genus. The previously designated type-species of Homalodisca, Cicada triquetra F., is herein transferred to Propetes Walker. Propetes triquetra comb. nov., represented previously only from an unknown locality in South America, is herein newly recorded from Brazil (Mato Grosso and Pará states).

Reference: See page 30 in Pierce's Disease Research Summaries.

Tipping, C., Mizell III, R., Brodbeck, B.V., Andersen, P.C., Hunter, W.B., and Lopez-Gutierrez, E.R. 2005. A novel method to induce oviposition of the glassy-winged sharpshooter, *Homalodisca coagulata* (Hemiptera: Auchenorryncha: Cicadellidae). Journal of Entomological Science 40(2): 246-249.

Abstract: Research on the main vector of Pierce's Disease of grapes, Homalodisca coagulata, the glassy-winged sharpshooter, is often hindered by lack of the supply of leafhoppers or their eggs. To solve this problem, gravid Homalodisca coagulata females were induced into ovipositing a significantly greater proportion of their eggs 24h after a desiccation treatment with a directed flow of warm air (40°C, 5.0 meters per second) for 15 m than untreated females. Treated and untreated females oviposited 54.5% and 28.2% of their eggs, respectively, regardless of host plant. Use of induced oviposition will aid studies currently examining sharpshooter development. The California Department of Food and Agriculture; and the University of California, Davis provided funding towards this research. Contribution of the Florida Agricultural Experiment Station Journal Series number R-10335.

Reference: See pages 19-20 in Pierce's Disease Research Summaries.

Tubajika, K.M., E. L. Civerolo, G.J. Puterka, J.M. Hashim and D.A. Luvisi. 2006. The effects of kaolin, harpin, and imidacloprid on development of Pierce's disease in grape. Crop Protection 26:92-99.

Abstract: Incidence of Pierce's disease (PD), caused by the bacterium Xylella fastidiosa, continues to increase in many vineyards in California due to the establishment and spread of the vector Homalodisca coagulata (Say) (Glassy-winged sharpshooter). Commercially available materials [particle file (Surround WP) containing 95% kaolin, systemic acquired resistance inducer (Messenger) containing 3% harpin], and Admire 2F (imidacloprid) were evaluated in greenhouse and field experiments, for their effects on reducing X. fastidiosa transmission, and reducing X. fastidiosa infections and PD development. In the greenhouse tests, PD incidence was reduced by 30%, 42%, and 42% with Admire 2F, harpin and Surround WP, respectively. Treatment of grapevines with harpin, Surround WP, and Admire 2F reduced transmission of X. fastidiosa by 11%, 9%, and 11%, respectively, in treated grape plants maintained in the greenhouse. Infield studies, PD incidence was 6% in Surround WP treated plots and 14% in convential insecticide treated plots. PD development in harpin treated plots was 13%, 7%, and 6% when 160, 320, and 460 g harpin a.i./ha were used respectively. PD incidence in untreated control plants was 19%. harpin treated plants grew more vigorously than plants treated with Surround WP and Admire 2F, and untreated control plants. Higher GWSS mortality rates were observed on plants treated with Surround WP. Results from both greenhouse and field studies show that harpin, Surround WP, and Admire 2F would be useful in reducing transmission of X. fastidiosa by GWSS and in reducing infections and PD development.

Reference: See pages 51, 69, and 70 in Pierce’s Disease Research Summaries.

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

Abstract: The incidence of Pierce's disease caused by Xylella fastidiosa, was monitored in 11 naturally- infested commercial vineyards to examine the spatial patterns of the disease, elucidate possible influences of surrounding environments, and presence of a X. fastidiosa vector, Homalodisca coagulata (Say), (glassy-winged sharpshooter). Disease incidence ranged from <1% in field 1 in 2001 to 71% in field two in 2002. Disease incidence doubled in most vineyards during the 2002 production season. The lack of evidence for a gradient or presence of at most a weak gradient relative to GWSS sources (like citrus) was observed. Spatial patterns of symptomatic vines in 2001 and 2002, as determined by ordinary runs analysis, showed strong evidence for within and across-row aggregation of infected vines. Two-dimensional distance class analyses revealed a higher frequency of infected pairs of vines and a greater degree of clustering of diseased vines as disease incidence increased. In most fields, they were no disease gradients observed relative to GWSS source (e.g. citrus). Within fields, however, disease incidence displayed strong spatial dependence and a high degree of anisotropy, indicating strongly aggregated patterns of disease with distinct directional orientation. The within-row (0o) and across-row (90o) orientation generally were the predominant directions of increased disease incidence, consistent with vine-to-vine spread of X. fastidiosa. The predominant vector species observed within commercial vineyards was H. coagulata. Presumably, the distribution of PD in the field should reflect the feeding pattern of infectious vectors carrying the bacteria. Based on these results, effective PD management is likely to be based on practices that reduce significant insect vector populations, remove infected vines as soon as identified, and use of resistant cultivars.

See pages 69 and 70 in Pierce's Disease Research Summaries.

Valles, S.M., Strong, C.A., Dang, P.M., Hunter, W.B., Pereira, R.M., Oi, D.H., Shapiro, A.M., Williams, D.F. 2004. A picorna-like virus from the red imported fire ant, *Solenopsis invicta*: Initial discovery, genome sequence, and characterization. Virology 328: 151-157.

Abstract: We report the first discovery and genome sequence of a virus infecting the red imported fire ant, Solenopsis invicta. The 8,026 nucleotide, polyadenylated, RNA genome encoded two large open reading frames (ORF1 and ORF2), flanked and separated by 27, 223, and 171 nucleotide untranslated regions, respectively. The predicted amino acid sequence of the 5' proximal ORF1 (nucleotides 28 to 4,218) exhibited significant identity and possessed consensus sequences characteristic of the helicase, cysteine protease, and RNA-dependent RNA polymerase sequence motifs from picornaviruses, picorna-like viruses, comoviruses, caliciviruses, and sequiviruses. The predicted amino acid sequence of the 3' proximal ORF2 (nucleotides 4390 to 7803) showed similarity to structural proteins in picorna-like viruses, especially the acute bee paralysis virus. Electron microscopic examination of negatively stained samples from SINV-1-infected fire ants revealed isometric particles with a diameter of 31 nm, consistent with Picornaviridae. A survey for the fire ant virus from areas around Florida revealed a pattern of fairly widespread distribution. Among 168 nests surveyed, 22.9% were infected. The virus was found to infect all fire ant caste members and developmental stages, including eggs, early (1st-2nd) and late (3rd-4th) instars, worker pupae, workers, sexual pupae, alates (' and '), and queens. The virus, tentatively named Solenopsis invicta virus (SINV-1), appears to belong to the picorna-like viruses. We did not observe any perceptible symptoms among infected nests in the field. However, in every case where an SINV-1-infected colony was excavated from the field with an inseminated queen and held in the laboratory, all of the brood in these colonies died with 3 months.

Reference: See pages 19 and 20 in Pierce's Disease Research Summaries.

Van Sluys, et al. 2003. Comparative analyses of the complete genome sequences of Pierce's disease and citrus variegated chlorosis strains of *Xylella fastidiosa*. Journal of Bacteriology 185:1018-1026.

Abstract: *Xylella fastidiosa* is a xylem-dwelling, insect-transmitted, gamma-proteobacterium that causes diseases in many plants, including grapevine, citrus, periwinkle, almond, oleander, and coffee. *X. fastidiosa* has an unusually broad host range, has an extensive geographical distribution throughout the American continent, and induces diverse disease phenotypes. Previous molecular analyses indicated three distinct groups of *X. fastidiosa* isolates that were expected to be genetically divergent. Here we report the genome sequence of *X. fastidiosa* (Temecula strain), isolated from a naturally infected grapevine with Pierce's disease (PD) in a wine-grape-growing region of California. Comparative analyses with a previously sequenced *X. fastidiosa* strain responsible for citrus variegated chlorosis (CVC) revealed that 98% of the PD *X. fastidiosa* Temecula genes are shared with the CVC *X. fastidiosa* strain 9a5c genes. Furthermore, the average amino acid identity of the open reading frames in the strains is 95.7%. Genomic differences are limited to phage-associated chromosomal rearrangements and deletions that also account for the strain-specific genes present in each genome. Genomic islands, one in each genome, were identified, and their presence in other *X. fastidiosa* strains was analyzed. We conclude that these two organisms have identical metabolic functions and are likely to use a

common set of genes in plant colonization and pathogenesis, permitting convergence of functional genomic strategies.

Reference: See pages 3-4 in Pierce's Disease Research Summaries.

Yuan, J., X. Yang, J. Lai, Lin, H., Cheng, Z. M., Nonogaki, H. and Chen. F. 2007. Comparative Genomic Analysis of Endo-ß-Mannanase Gene Families in Arabidopsis, Rice and Poplar. Functional and Integrative Genomics. 7:1-16.

Abstract:
Mannans are widespread hemicellulosic polysaccharides in plant cell walls. Hydrolysis of the internal β-1, 4-D-mannopyranosyl linkage in the backbone of mannans is catalyzed by endo-β-mannanase. Plant endo-β-mannanase has been well studied for its function in seed germination. Its involvement in other plant biological processes, however, remains poorly characterized or elusive. The completed genome sequences of Arabidopsis (Arabidopsis thaliana), rice (Oryza sativa), and poplar (Populus trichocarpa) provide an opportunity to conduct comparative genomic analysis of endo-β-mannanase genes in these three species. In silico sequence analysis led to the identification of eight, nine and 11 endo-β-mannanase genes in the genomes of Arabidopsis, rice, and poplar, respectively. Sequence comparisons revealed the conserved amino acids and motifs that are critical for the active site of endo-β-mannanases. Intron/exon structure analysis in conjunction with phylogenetic analysis implied that both intron gain and intron loss has played roles in the evolution of endo-β-mannanase genes. The phylogenetic analysis that included the endo-β-mannanases from plants and other organisms implied that plant endo-β-mannanases have an ancient evolutionary origin. Comprehensive expression analysis of all Arabidopsis and rice endo-β-mannanase genes showed divergent expression patterns of individual genes, suggesting that the enzymes encoded by these genes, while carrying out the same biochemical reaction, are involved in diverse biological processes.

Reference: See pages 11-12 in Pierce's Disease Research Summaries.