A METAPOPULATION ANALYSIS OF GLASSY-WINGED SHARPSHOOTER IN TEXAS

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ABSTRACT
An understanding of the metapopulation dynamics of the glassy-winged sharpshooter (GWSS; *Homalodisca vitripennis*) and its interaction with associated bacteria, most notably *Xylella fastidiosa (Xf)*, is essential for detecting and predicting shifts in Pierce's disease (PD) dynamics and the development of long term and effective management strategies for PD. With this study we attempt to understand how GWSS populations are laid out across Texas vineyards and what factors affect how those populations interact with associated bacteria, most importantly *Xf*. GWSS were collected on sticky traps from nine vineyards across Texas. Wings collected from the insects were use to determine relative ages based on red pigmentation in the wings. In future work, the presence and relative quantities of PD strain *Xf* contained in the insects will be determined and analyzed along with relative age data to attempt to identify any correlation between these two factors. For 3-4 insects from each vineyard, total genomic DNA was extracted, and used for amplified fragment length polymorphism analysis (AFLP) and 454 pyrosequencing of DNA fragments generated. The AFLP/sequencing data will be used to determine if there are genetically distinct populations of GWSS across Texas vineyards and the structure of those populations.

LAYPERSON SUMMARY
Because of its ability to cause Pierce's disease (PD) by transporting *Xylella fastidiosa* to grapevine, the glassy-winged sharpshooter (GWSS; *Homalodisca vitripennis*) is, economically, one of most important insect pests to the wine and table grape industries in Texas, California, and Florida. By looking at samples of GWSS from vineyards across Texas, an effort is being made to understand where distinct populations of GWSS exist in the state, how those populations interact with another, and how they change over time. The information gained from this study may help to provide a forecast for the future of PD in vineyards across Texas, as well as, provide information that may prove useful in the development of management strategies to combat PD. In this work, relative age was determined for GWSS collected from vineyards across Texas and genetic analysis was performed to identify distinct populations of GWSS in Texas.

INTRODUCTION
The glassy-winged sharpshooter (GWSS) is a potential vector of a number of plant pathogens including those that affect oleander, almond, and grapevine. This insect has been identified as the primary vector of *Xylella fastidiosa (Xf)*, the causal agent of Pierce's Disease (PD) in grapevine (Davis et al. 1978). Within *Xf*, a single strain group has been identified to contain all PD causing strains of the species (Chen et al. 2002). With new techniques being developed in the study of the both the GWSS and *Xf* it is important to utilize those techniques to track the population dynamics the insect vector and how the organisms interact with one another at the population and metapopulation level.

In 2010, Hail et al. identified three GWSS carrying PD strain *Xf* in vineyards that had no previous history of *Xf* positive GWSS. A state-wide population study incorporating this information could help answer how likely an event like this would be to occur again and if it is already occurring. Molecular markers have been utilized in the past to study GWSS populations. In 2004, using inter-simple sequence repeat (ISSR) analysis, de León et al. identified genetically distinct populations across the United States and divided these populations into the western (California) and southwestern (Texas and Florida) groups. This study was; however, was unable to resolve specific details of population structure within Texas. It also suggested that distinct biotypes may be present within GWSS (deLeon et al. 2004). Additional population analysis specific to Texas may be able to resolve population structure within the state as well as provide clues to the origin(s) of Texas populations and the presence of distinct biotypes within those populations. Amplified fragment length polymorphism (AFLP) analysis which, in many studies, has outperformed other molecular markers (Meudt and Clarke 2006), may provide the information needed to answer these questions. Direct sequencing of AFLP fragments may increase the information provided by these markers and allow for more robust analysis.

The age structure of a GWSS population may prove an important factor in the population's ability to spread PD. Once an adult GWSS has been colonized by *Xf*, infectivity is sustained throughout the lifetime of the insect as the bacteria multiply within the foregut of the sharpshooter. This gives GWSS the ability to infect a host at any time after colonization during its adult life (Severin 1949; Hill and Purcell 1995). Also, the age of GWSS has been shown to be correlated with the number of sensilla of mouthparts of the insect. This may have some effect on the vectoring ability of the sharpshooter (Leopold et al. 2003). Timmons et al. 2011 showed that it is possible to accurately estimate the age of a GWSS by using digital photography to measure the amount of red pigmentation in the wings of the sharpshooter. If the ages of glassy-winged sharpshooters

could the correlated with the quantity of *Xf* housed within those insects, this could provide information regarding the risk of PD infection. Along with population structure analysis, this could provide valuable insight on the probability of PD becoming more prevalent and spreading to new areas in the near future.

OBJECTIVES

1. Successfully determine relative ages of GWSS collected from vineyards across Texas for use in additional analysis incorporating insect associated *Xf* data.
2. Complete amplified fragment length polymorphism (AFLP) and direct sequencing of AFLP fragments for use in population analysis.

RESULTS AND DISCUSSION

The average relative ages from GWSS collected from seven vineyards were shown in **Figures 1 and 2**. It can be seen from **Figure 2** that the results suggest that both Post Oak Vineyard and Oak Creek Vineyard show significantly but slightly higher predicted population mean of GWSS relative ages than Delaney Grapevine Vineyard. Despite these noted differences, the sample sizes used for this portion of the study, especially for Oak Creek Vineyard and relatively small variation seen from this dataset, it is difficult to state any conclusions concerning differences average ages of sharpshooters across Texas vineyards. Future work will focus on the collection of larger sample sizes of GWSS from vineyards for aging at later dates and the estimation of actual ages of GWSS collected. This will allow for comparisons between vineyards based on a more informative data set as well as information regarding the change in age structure of populations of GWSS over large segments of time (seasons and years). Additional future work will attempt to detect any correlation between GWSS age and presence and quantity of PD strain *Xf* present in the foregut of the insect.

The AFLP and 454 pyrosequencing analysis generated a total of 31,593 sequence fragments from 25 GWSS genomic DNA samples from collected from seven vineyards (**Table 1**) with sequence sizes ranging from to 124 to 467 bp. **Figure 3** shows a subset of sequences from sample 10D from Post Oak Vineyard. Future analysis of the AFLP sequencing data will focus on clustering the data into identical sequences within samples and performing metapopulation structure population structure analysis using a variety of techniques.

REFERENCES CITED

Chen J, J.S. Hartung, C. Chang , and A. Vidaver. 2002. An evolutionary perspective of Pierce's disease of grapevine, citrus variegated chlorosis, and mulberry leaf scorch diseases. Curr Microbiol 45: 423–428.

Davis M.J., A.H. Purcell, and S.V. Thomson. 1978. Pierce's Disease of grapevines: Isolation of the causal bacterium. Science 199: 75-77.

de León J.H., W.A. Jones, 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.

Hail D., F. Mitchell, I. Lauziere, P. Marsall, J. Brady, and B. Bextine. 2010, Detection and analysis of the bacterium, *Xylella fastidiosa*, in glassy-winged sharpshooter, *Homalodisca vitripennis*, populations in Texas. Journal of Insect Science. 10: 1-11.

Hill B.L., A.H. Purcell. 1995. Acquisition and retention of *Xylella fastidiosa* by an efficient vector, *Graphocephala atropunctata*. Phytopathology 85: 209-212.

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

Meudt H.M. and A.C. Clarke. 2006. Almost Forgotten or Latest Practice: AFLP application, analyses and advances. Trends in Plant Science 12:106-117.

Severin, H.H.P. 1949. Transmission of the virus of Pierce's disease of grapevines by leafhoppers. Hilgardia 19:190–206.

Timmons C., A. Hassell , I. Lauziere, and B. Bextine. 2011. Age determination of the glassy-winged sharpshooter, *Homalodisca vitripennis*, using wing pigmentation. Journal of Insect Science 11:78 available online: insectscience.org/11.78

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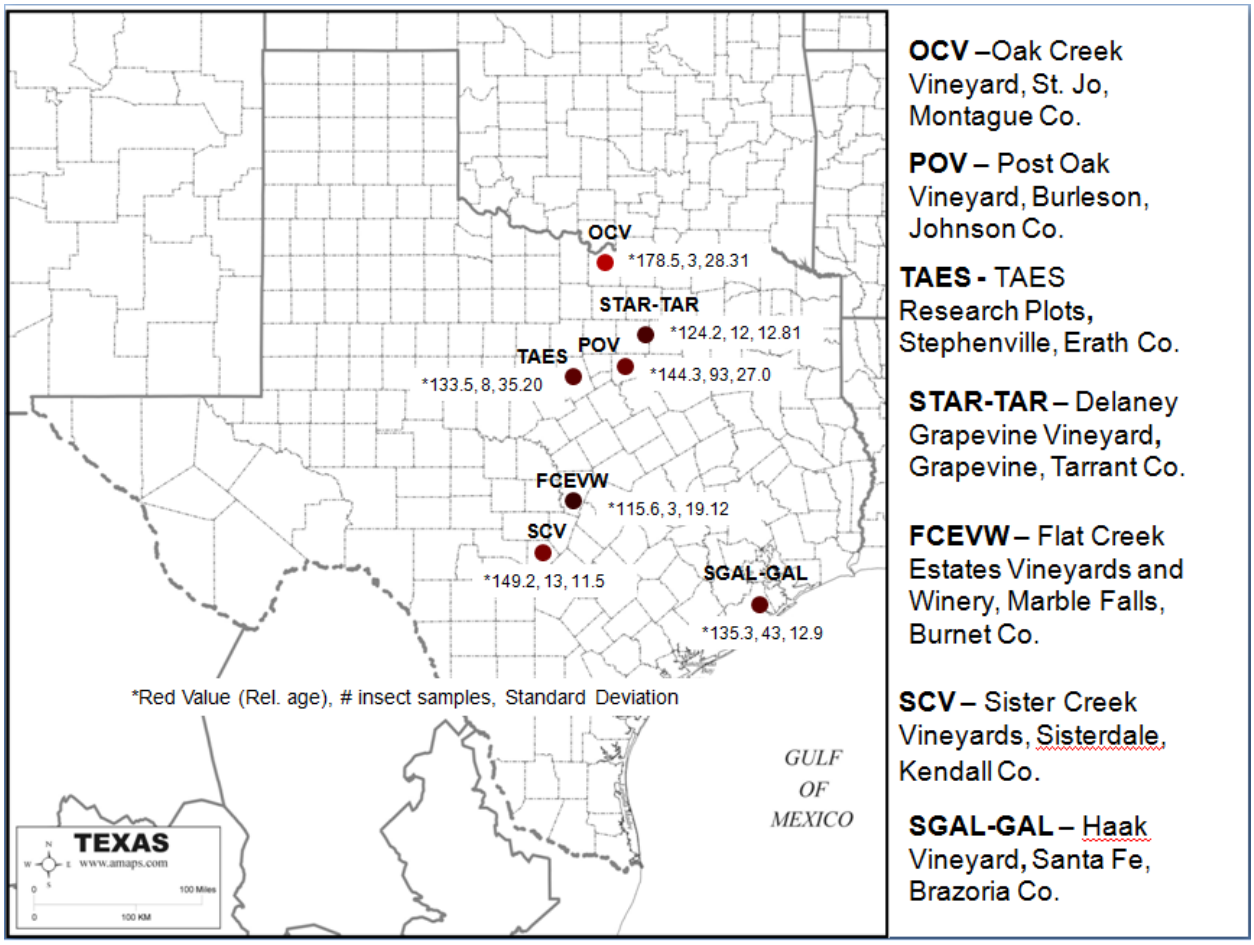

Figure 1. The average relative ages of GWSS from seven vineyards across Texas. Relative ages of GWSS collected from Texas vineyards between the dates of 07/31/2007 and 09/04/2007 were determined by measuring red pigmentation in the wings, averaged for each vineyard, and representing those ages as circle with amounts of red proportional to the average amount of red pigment found in the wings. Lower red pigmentation values represent greater age and vice versa.

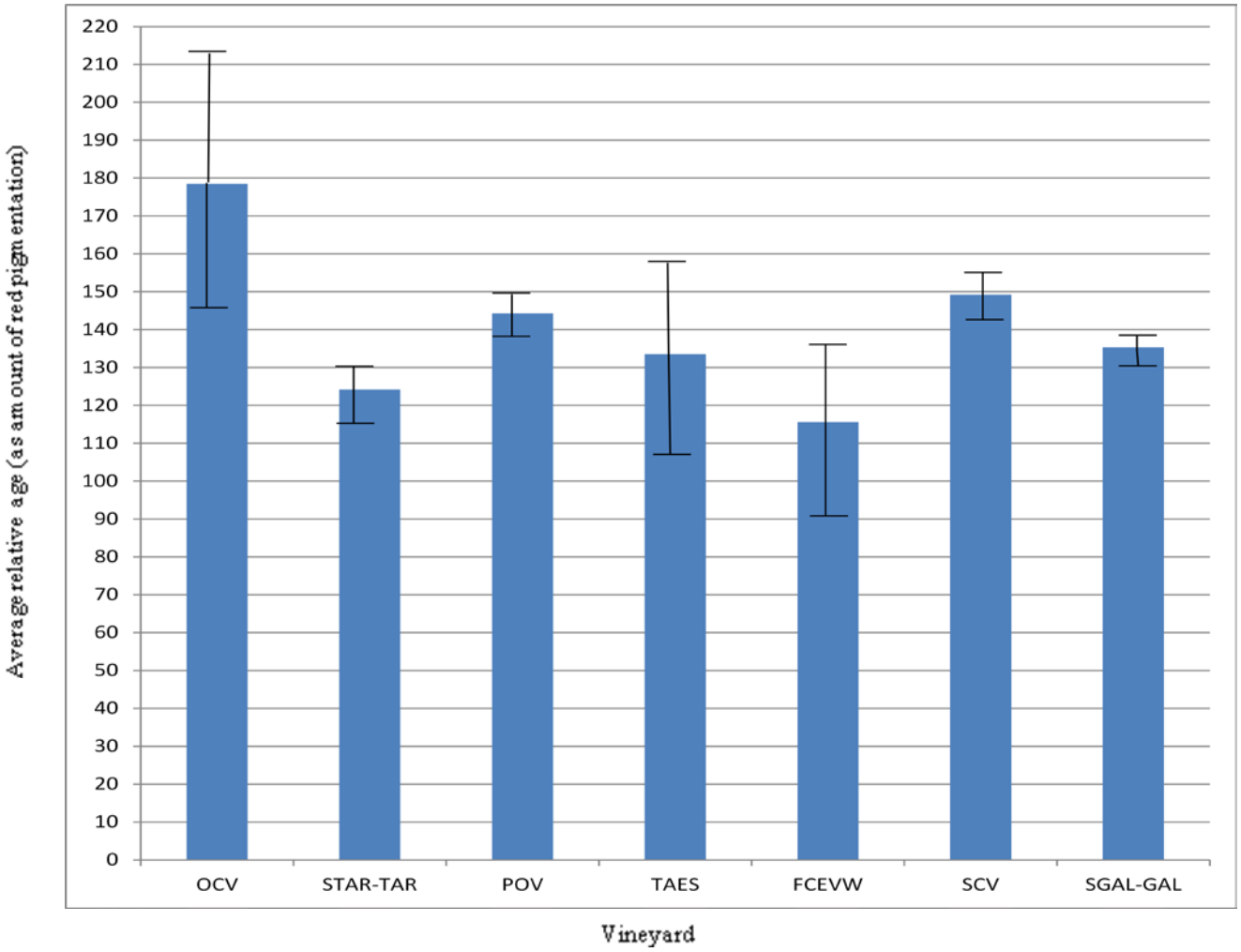

Figure 2. The average relative ages of GWSS from seven vineyards across Texas. Relative ages of GWSS collected from Texas vineyards between the dates of 07/31/2007 and 09/04/2007 were determined by measuring red pigmentation in the wings and averaged for each vineyard. Lower red pigmentation values represent greater age and vice versa. Error bars represent confidence intervals of 95% for the population mean.

Table 1. Quantity of AFLP sequence fragments generated from AFLP PCR and sequencing analysis. Total genomic DNA was extracted from 25 GWSS from seven different vineyards and amplified with amplified fragment length polymorphism (AFLP) PCR. Fragments generated from AFLP were sequenced with 454 pyrosequencing.

Vineyard	Sample #	# of AFLP sequence fragments
Flat Creek Estates Vineyards and Winery, Marble Falls, Burnet Co.	32A	1037
Flat Creek Estates Vineyards and Winery, Marble Falls, Burnet Co.	33A	496
Flat Creek Estates Vineyards and Winery, Marble Falls, Burnet Co.	34A	923
Haak Vineyard**,** Santa Fe, Brazoria Co.	53A	1049
Haak Vineyard**,** Santa Fe, Brazoria Co.	57G	1353
Haak Vineyard**,** Santa Fe, Brazoria Co.	57J	1913
Haak Vineyard**,** Santa Fe, Brazoria Co.	57V	1463
Oak Creek Vineyard, St. Jo, Montague Co.	23A	1136
Oak Creek Vineyard, St. Jo, Montague Co.	23B	1286
Oak Creek Vineyard, St. Jo, Montague Co.	24A	1327
Post Oak Vineyard, Burleson, Johnson Co.	10D	1095
Post Oak Vineyard, Burleson, Johnson Co.	12E	1638
Post Oak Vineyard, Burleson, Johnson Co.	40I	1661
Post Oak Vineyard, Burleson, Johnson Co.	43D	1153
Sister Creek Vineyards, Sisterdale, Kendall Co.	26B	1095
Sister Creek Vineyards, Sisterdale, Kendall Co.	29A	1903
Sister Creek Vineyards, Sisterdale, Kendall Co.	30B	1539
Sister Creek Vineyards, Sisterdale, Kendall Co.	31A	1026
TAES Research Plots**,** Stephenville, Erath Co.	17A	1169
TAES Research Plots**,** Stephenville, Erath Co.	18A	1063
TAES Research Plots**,** Stephenville, Erath Co.	21B	1214
TAES Research Plots**,** Stephenville, Erath Co.	22A	1102
TJ	27C	1071
TJ	27E	976
TJ	27JK	1905
Total		31593

Figure 3. A subset of sequences generated from amplified fragment length polymorphism (AFLP) and sequencing analysis from GWSS DNA sample 10D from Post Oak Vineyard, Burleson, Johnson Co. TX. Total genomic DNA was extracted from 25 GWSS from seven different vineyards and amplified with amplified fragment length polymorphism (AFLP) PCR. Fragments generated from AFLP were sequenced by 454 pyrosequencing.

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