XYLEM SPECIALISTS COLONIZING TEXAS VINEYARDS: ABUNDANCE AND DISTRIBUTION STUDIED THROUGH GENERALIZED ADDITIVE MODELS

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ABSTRACT
Xylem specialists in the Hemiptera: Auchenorrhyncha feed on xylem fluid and exhibit therefore the potential for transmission of Xylella fastidiosa (Xf), the causal agent in Pierce’s disease of grapevine. An increasingly larger survey was initiated in Texas in 2003 and remains ongoing with 45 locations currently monitored. Over 25 insect species belonging in this group were collected from yellow sticky traps. Among these, two leafhoppers and one spittlebug, comprised over 90% of the xylem specialists caught. The glassy-winged sharpshooter Homalodisca vitripennis, the most commonly known vector of Pierce’s disease of grapevine in the U.S. is the most common and abundant insect captured across the state. Insect abundance varied significantly over seasons, and among locations. Residual populations were found overwintering near vineyards, but also in a variety of habitats including urban and vegetation found near natural sources of water. Generalized additive models which were originally developed to analyze complex population data were used with our Central Texas dataset as a tool to identify changes in the distribution of leafhopper and spittlebug species near vineyards. These models helped understanding insect distribution through time.

INTRODUCTION
Pierce’s disease of grapevine is one of the most important limiting factors to grape production in Texas. During the 1990’s, the grape growing region of Central Texas witnessed an increase in the incidence and severity of Pierce’s disease (Texas Pierce’s disease Task Force 2004). A research program was initiated in Texas in 2002 with funding from the U.S. Department of Agriculture. Within this program, researchers are provided an opportunity to study glassy-winged sharpshooters in their native habitat, and their interaction with cultivated vines. Exploration for insect species involved, their ecology, host plants used, molecular characterization of Xf, vectoring capacity of the Hemiptera captured, natural enemies and population dynamics are all underway.

OBJECTIVES
1. Monitor xylem feeding insect populations in vineyards across Texas. Identify all putative insect vectors of Pierce’s disease. Determine the most common vectors requiring population management, make observations and analyze vector distribution, abundance and seasonality.
2. Explore for host plants used as breeding sites by insect vectors throughout the year, assess the reproductive state of adult females and determine the age structure composition of the vectors
3. Determine the extent of primary and secondary spread in vineyards.

RESULTS
Insect populations in the Hemiptera have been monitored (mostly) on a bi-weekly fashion in 45 vineyards statewide for over three years. A large database was assembled for analyses using a two-year dataset which included all insect counts per species (from trap data) and a layer of geographical data. Scientists and staff at Fredericksburg and Stephenville worked at organizing the different fields of this database. Predominant species were Homalodisca vitripennis, Graphocephala versuta and Clastoptera xanthocephala, two leafhoppers and a spittlebug. These species together comprised over 90% of all xylem fluid feeding insects identified. Homalodisca vitripennis and C. xanthocephala were present at each of the surveyed locations. Relatively new (new to entomology) statistical analyses, generalized additive models (GAMs) were tested using one of our first Central Texas trap dataset. The power of those analytical methods is both impressive and very instructive and useful. This modeling technique is a semi-parametric extension of the more traditional general linear models (GLMs). The statistical models identify non-linear and non-monotonic relationships between the response and the set of explanatory variables. They are a useful tool to identify factors responsible for the distribution of species, and help in understanding changes that occur through time via a model element known as a smooth function, similar in concept to a moving average. A distinctive feature of the data we collected was an over-abundance of zero counts, or zero xylem specialists on one given trap, at a given location, and for a given time period. Using a Poisson based GAM, we were able to better describe particular factors of interest affecting insect populations, and the degree to which factor(s) is/are important to the presence of sharpshooters and disease. Because of the extensive multyear dataset, this powerful approach will better explain variations in insect populations through time and between locations in a large ecological setting.
Because of its abundance in vineyards, diverse plant host range, high degree of association with *Xf* in Texas and the economic problems it causes in California due to its vector status, the glassy-winged sharpshooter (GWSS) is a high priority target for management. Populations of the GWSS can be very high in Texas depending on where the data are collected. In Central Texas, populations have been consistent both in abundance and time of appearance. Observational and census data indicate that secondary spread of Pierce’s disease in vineyards is in play here.

Figure 1 summarizes GWSS census data from traps collected during 2003 – 2006 in Central Texas. Insects were selected from traps on multiple dates during these years and subjected to PCR to detect the presence of *Xf*. *Xf* is absent from the earliest GWSS and accumulates over the course of the year, ending in nearly 70% prevalence within the insects. Peak bacterial prevalence does not coincide with the population peak in the four years examined. Had primary spread been the main mode of transmittal, one would expect the incoming insects to have a higher load of bacteria. This graph alone is insufficient to defend the point however, and other data are needed.

![Central Texas Homalodisca vitripennis Population Density vs Xylella Positive Insects](image)

**Figure 1.** Of the vineyards under census, six have been in the program since 2003. Three of these vineyards are seriously impacted by Pierce’s disease, three are not. When the *Xf* prevalence data for GWSS are compared between these vineyards, the following summary in Table 1 results.

<table>
<thead>
<tr>
<th>Vineyard Status</th>
<th>Species</th>
<th>Total Tested</th>
<th># Positive</th>
<th># Negative</th>
<th>% Positive</th>
<th>% Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impacted</td>
<td><em>H. vitripennis</em></td>
<td>490</td>
<td>115</td>
<td>375</td>
<td>23.47%</td>
<td>76.53%</td>
</tr>
<tr>
<td>Unimpacted</td>
<td><em>H. vitripennis</em></td>
<td>404</td>
<td>10</td>
<td>394</td>
<td>2.48%</td>
<td>97.52%</td>
</tr>
</tbody>
</table>

This supports the hypothesis that the increase in bacterial prevalence found within the GWSS arises from within the vineyards and perhaps its vicinity. Other vector species will likely play roles. Data from the second most abundant putative vector species, *Graphocephala versuta*, presents similar results (not shown).

**CONCLUSIONS**

Over the past months, we have worked on a two-year field database that was built with insect counts (of Central Texas alone) and one layer of geographical data. GAMs are one approach being used to analyze this complex population data. They are more flexible and for this reason are sometimes more suitable than GLMs for some types of analysis. More details can soon
be found in our recently accepted paper at Environmental Entomology. We demonstrated a rich fauna of xylem fluid feeding insects where three species, *H. vitripennis*, *G. versuta* and *C. xanthocephala*, stood out from the others in terms of population abundance and made up for over 90% of insects collected. Extensive molecular analyses are being carried out to confirm which of these insects are associated with the grape strain of *Xf*. A number of leafhopper species have been examined by RT-PCR and many are contaminated with *Xf*, indicating an association and a possible vector relationship much broader than first suspected. We also are now in the process of building a much larger database that holds more sampling years and sampled locations, along with more geographical and some environmental data to further test this approach.

Secondary spread of Pierce’s disease in Central Texas vineyards may be the main mode of transmission. The data are not yet analyzed and experimental detail is needed to confirm this, but should it be the case then management protocols for this type of spread (vector management within the vineyard, roguing infected vines at the earliest opportunity) will be appropriate.

REFERENCES

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