SPECIES DIVERSITY, DISTRIBUTION, AND ABUNDANCE OF XYLEM FLUID FEEDING HEMIPTERA IN VINEYARDS THROUGHOUT TEXAS

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ABSTRACT
A survey of xylem fluid feeding insects (Hemiptera: Auchenorrhyncha) exhibiting potential for transmission of *Xylella fastidiosa* (*Xf*) the bacterium causing Pierce's disease (PD) of grapevine was initiated in Texas in 2003 and continues to the present. Twenty five insect species belonging to 4 families and 14 genera were collected from yellow sticky traps and identified. Among these, three species, two leafhoppers and one spittlebug, comprised over 90% of the xylem fluid feeding insects caught. *Homalodisca vitripennis* (formerly *H. coagulata*), the most commonly known vector of PD of grapevine in the U.S. is the most common and abundant insect captured across the state. Insect abundance varied significantly over seasons, with most of captures taking place between June and August, and per location, with 5 to 15 species caught at any given vineyard. Residual populations found overwintering near vineyards will be the focus of future studies. A grape strain and an ornamental strain of *Xf* have been detected in insects processed by real time PCR. These initial observations provide critical information to vineyard managers for timely applications of insecticides prior to insect feeding and vectoring to susceptible grapevines.

INTRODUCTION
Pierce's disease (PD) of grapevine has become the most limiting factor to grape production in Texas, as it has in California during the past decade. It is transmitted by certain insects which feed on xylem fluid of plants where the bacterium develops. During the 1990's, the grape growing region of Central Texas witnessed an increase in the incidence and severity of PD (Texas Pierce's Disease Task Force 2004). The insect vectors were not thoroughly identified but glassy-winged sharp-shooters (GWSS), *Homalodisca vitripennis* (Cicadellidae), were suspected. A modest research program was initiated in Texas, funded by the U.S. Department of Agriculture starting in 2002-2003. Within this program, researchers are provided an opportunity to study glassy-winged sharpshooters in their natural habitat. During the past years, intensive studies to identify the vectors and their vectoring potential have been taking place in a growing multi-disciplinary, multi-institutional research program. 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 well underway.

OBJECTIVES
1. Monitor xylem feeding insect populations in vineyards across Texas. Identify all putative insect vectors of PD. Determine the most common vectors requiring population management, make observations on vector distribution, density 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. Characterize the proportion of insects carrying *Xf* and the pathotypes involved. Investigate evolution of infection levels throughout the year.

RESULTS
Insect populations in the Hemiptera were monitored on a bi-weekly fashion in 45 vineyards for over 3 years. We placed particular interest in those populations of xylem fluid feeding insects that may play a role in the transmission of *Xf*, causal agent of PD in grapevines. Data indicate the presence of xylem fluid feeding leafhoppers-treehoppers (Membracoidea), spittlebugs (Cercopoidea) and cicadas (Cicadoidea). Of 160 Hemiptera species captured, 25 species have been identified so far as xylem fluid feeding insects present in the vineyards and adjacent natural habitat, all with the potential to carry and transmit *Xf* when feeding on susceptible host plants. Captured insects in the family Cicadellidae (leafhoppers) were the most abundant with a total of 15 species recovered; this family contributed to about 75% of all individuals caught. 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. At certain locations, a specific Hemiptera species clearly dominated. Insect species diversity varied from 5 to 15 per location, with an average of 9.18 ± 2.87species per location. Abundance of major xylem fluid feeding Hemiptera species varied greatly throughout time. Monthly variations of the adult populations of xylem fluid feeding Hemiptera well correspond to mean temperatures. These insects' populations increased significantly from April to June when they peaked. After June, insect densities, indicated by trap captures, decreased gradually until they reached their minimal levels between the months of November and April. A similar pattern of

abundance was observed for each of the dominant species recovered. Observations made for *G. versuta* suggest that the host plants found in the habitat outside the vineyards are more suitable than grapevines for feeding and/or reproduction. Initial examination of the three major species by means of Real Time PCR (RT-PCR) indicates that *G. versuta* is more commonly associated with an ornamental strain of *Xf*, rather than the PD strain. *Homalodisca vitripennis* and *C. xanthocephala*, conversely, were both found to be associated with the PD strain of *Xf*. While *C. xanthocephala* is found on sticky traps within the vineyard, there is no evidence yet that it feeds on grape. It may still be important to the epidemiology of Pierce's disease by moving the bacterium from one wild host to another and providing an inoculum source for *H. vitripennis*.

CONCLUSIONS

This study sheds light on the complex system that exists in Texas. These results 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 densities and made up for over 90% of insects collected. Of these 3 species, one, the glassy-winged sharpshooter, is a known vector of *Xf*, while the other two are not confirmed vectors to date. *Graphocephala atropunctata*, the blue-green sharpshooter is a known vector in the western U.S. and so the *Graphocephala* remain suspects by their relationship to this known western vector. At most locations, xylem fluid feeding Hemiptera population densities remained relatively low throughout the vine's vegetative season, except for June when adult counts were the highest. Important information has been gathered while carrying out this large study in Texas. Although the survey was not exhaustive, i.e., only traps were used and they are not equally attractive to all xylem fluid feeding Hemiptera species, we now have a much clearer idea of several, and certainly of the most common, insects species that can be involved in Pierce's disease of grapevine. Extensive molecular analyses are currently being carried out to confirm which of these insects are associated with grape strains 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.

REFERENCES

Texas Pierce's Disease Task Force. 2004. Does Texas hold the key to eradicating Pierce's disease? Wine Business Monthly 11: 34-38.

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ASSOCIATIVE LEARNING OF HOST-PLANT CHEMICAL STIMULI IN IMMATURE GLASSY-WINGED SHARPSHOOTERS

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ABSTRACT

Olfactory conditioning may provide the immature glassy-winged sharpshooter (GWSS) with a mechanism by which they can effectively track their host-plants in space and time. In the presence of vanilla scent, nymphs that ingested vanilla-flavored xylem fluid were significantly more attracted to a neutral visual target than were nymphs that had ingested unflavored xylem fluid. These results are consistent with our previous finding that GWSS response to visual stimuli is enhanced by exposure to host-plant odor.

INTRODUCTION

The nutritional requirements of immature glassy-winged sharpshooters (GWSS) constrain their diet to plants with low amide- and high amino acid concentrations in the xylem fluid (Brodbeck, et al. 1999). To obtain a balanced level of nutrients, nymphs may frequently need to switch host-plants (Brodbeck, et al. 1999, Redak et al. 2004). Locating host-plants that are physiologically-suitable with respect to providing adequate levels of xylem nutrients may require that the nymphs integrate information from several types of host-plant stimuli (Harris and Foster 1995). For example, nymph response to foliar colors (*sensu* Prokopy and Owens 1983, Tipping et al. 2004) is enhanced by the presence of host-plant volatiles (Patt & Sétamou 2006). The ability to learn to recognize stimuli associated with suitable host-plants would facilitate detection and location of host-plants whose distribution varies temporally and spatially within the nymphs' environment (Behmer et al. 2005, Pompilio et al. 2006).

The goals of our ongoing study are to determine whether nymphs can associatively learn to recognize olfactory stimuli produced by host plants, and, if so, to evaluate 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 (Patt & Sétamou 2006) 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. An increased response to the pale green target in the presence of vanilla odor would indicate that the nymphs had developed an attraction to vanilla scent via their previous feeding on vanilla-flavored xylem fluid.

OBJECTIVES

1. Determine whether nymphs can associatively-learn to recognize olfactory stimuli produced by host plants.
2. Evaluate the relative importance of olfactory conditioning in host-plant recognition.

RESULTS

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 (Figure 1). 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 (Figure 2).