REPRODUCTIVE BEHAVIOR OF A KEY VECTOR OF *XYLELLA FASTIDIOSA* IN TEXAS

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ABSTRACT
The glassy-winged sharpshooter (*Homalodisca vitripennis;* GWSS) is a xylem specialist in the Hemiptera Auchenorrhyncha which has the potential to transmit *Xylella fastidiosa*, the causal agent in Pierce's disease of grapevine. It is the most common leafhopper associated with vineyards in Texas. Using wild GWSS adults collected in Central Texas parks and vineyards, egg loads and biometrics were obtained and used to determine the seasonality of reproduction in females of this insect species. Captive adults reared in the greenhouse during the summer and fall months were studied throughout their lifespan. During this study, caged females produced large numbers of eggs in the same seasons as wild females do. Fall females delayed oviposition by about 75 days, with nymphal emergence being postponed then until late winter. More suitable rearing conditions are needed to reduce the duration of the pre-oviposition period in fall emerged females.

INTRODUCTION
Between 1995 and 2004, (fresh) grape production in the United States averaged 6.5 million tons annually (NASS, Crops Branch, 2005). With citrus and commercial apples, grapes are the most important fruit crop in the country. In recent years, grapes have been a very popular fruit crop in Texas, the fifth largest wine producing state in the nation. There are currently over 220 family-owned vineyards and about 3,700 acres under production; this industry contributes over $1 billion a year to the state's economy (Texas Wine and Grape Growers Association). The leading grape varieties are the French/European hybrids of *Vitis vinifera* traditionally associated with the highest quality wines. These varieties are susceptible to Pierce's disease (PD), an incurable and fatal bacterial infection of disseminated by xylem specialist insects such as the glassy-winged sharpshooter (*Homalodisca vitripennis;* GWSS; Hemiptera: Cicadellidae). These insect pests are often designated as vectors. Certain grape varieties such as Blanc du Bois, Le Noir, Champanel or the muscadines may be better adapted for Texas and are considered fairly immune to this disease, whereas the most popular *V. vinifera* are susceptible to diverse degrees. PD of grapevine is the most important limiting factor to grape production in Texas (Texas Pierce's Disease Task Force 2004). A research program was initiated in 2002 with funding from the U.S. Department of Agriculture. Within this program, researchers are provided an opportunity to study the vectors in their natural habitat and their interaction with cultivated vines and other vegetation.

OBJECTIVES
1. Wild GWSS populations have been monitored in Central and North Central Texas since 2003-2004. A number of adult females were sorted out to assess biometrics and evaluate their reproductive status through time based on egg load.
2. Using GWSS reared in captivity, total fecundity, daily oviposition pattern and longevity of GWSS females produced in summer and fall months were determined. Embryonic and nymphal survival and sex ratio of the first generation were measured in both seasons.

RESULTS
GWSS populations are monitored using sticky traps in Texas vineyards and *in situ* in urban areas of Central and North Central Texas. Wild adult females used in this study were from a subsample of insects harvested from July 2005 to October 2008 and stored at 10°C until processed. Dissections of the ovaries were carried out under stereomicroscope and egg loads were assessed individually. Only mature eggs were counted. Over 3,000 adult GWSS females were sorted out, with numbers varying from 44 in the combined months of January 2006-2008 to 700 in the months of June 2006-2008. These data reflected well the seasonal abundance of GWSS in our area (Lauzière et al. 2008). Females exhibiting active egg production (vitellogenesis) were harvested from February to September. Eggs loads varied significantly between months ($F = 60.0$; $df = 11, 3152$; $P < 0.0001$). Highest egg loads were observed in March (13.8 ± 7.2 eggs/female; $n = 155$). The length of the left hind tibia was measured individually and used as an indicator of adult size. Tibia are relatively well conserved in trap collected adults and therefore a suitable choice for this study. The size of the tibia in wild females varied significantly between months ($F = 36.8$; $df = 11, 3069$; $P < 0.0001$). The largest females were caught in May, June and July (4.1 ± 0.2 mm; $n = 1605$), the smallest ones in December (3.9 ± 0.2 mm; $n = 44$).

GWSS adults were reared to adulthood under greenhouse conditions using black-eyed peas and hibiscus as hosts. Newly emerged females ($n = 30$) were selected and individually introduced into cages, each with five adult males. Adults and plants (leaves) were monitored once daily. The duration of the pre-oviposition period (time elapsed between adult emergence and first egg laid) was determined. Oviposition was recorded as the number of eggs per day. Host plants were renewed every

two weeks and observations continued until females died. After their death, tibias were individually measured under stereomicroscope. Females reared during the summer months initiated oviposition within 13 days of the last molt to adulthood and deposited on average 187 eggs (range: 36-457) in approximately one month. The duration of the pre-oviposition period in females reared during the fall months was 5.6-fold longer than in summer individuals ($F = 48.12$; $df = 1, 48$; $P < 0.0001$). Both the oviposition ($F = 2.30$; $df = 1, 48$; $P < 0.0001$) and post-oviposition ($F = 1.21$; $df = 1, 48$; $P < 0.0001$) periods were not affected by the season. The mean number of eggs laid by females in confinement did not vary significantly with seasons ($F = 2.2$; $df = 1, 49$; $P = 0.2$). In both seasons, a high proportion of females (≥ 80%) deposited at least one egg during their lifespan and season did not affect the egg laying ability of captive females ($\chi^2 = 1.2$; $df = 1$; $P = 0.3$). Three of the summer females and six of the fall females never laid eggs in captivity. The mean size of an egg mass was 5.4 and 5.7 eggs in the summer and fall, respectively. The largest egg mass contained 39 eggs in the summer and 37 eggs in the fall group. A mixed model analysis showed that in greenhouse-reared individuals, both the size of the female ($F = 0.1$; $df = 1, 54$; $P = 0.8$) and the season ($F = 0.2$; $df = 1, 54$; $P = 0.7$) did not affect total fecundity. However, the total number of eggs laid by GWSS females was significantly and positively affected by their longevity ($F = 5.5$; $df = 1, 54$; $P = 0.02$). Unlike many insect species where longevity is positively determined by size, in captive *H. vitripennis*, we observed that longevity was strongly dependent upon the season in which the adults emerged ($F= 124.0$; $df = 1, 176$; $P < 0.001$). Mated summer adult females lived on average 49.3 ± 2.9 days, as compared to 104.0 ± 7.9 days in the fall counterparts. The viability of *H. vitripennis* eggs exceeded 77% and there was no significant difference observed between seasons ($F = 0.8$; $df = 1, 49$; $P = 0.4$). However, nymphal survival to adulthood was season dependent ($F = 5.5$; $df = 1, 49$; < 0.001). The ultimate percentage of adult emergence (from egg to adult) was also affected by the season with significantly more adults obtained from eggs laid by the fall emerged females. Of all the F1 adults emerged, the sex ratio was near 50:50 in both seasons ($F = 0.3$; $df = 1, 49$; $P = 0.6$).

CONCLUSIONS

In captivity, the onset of the oviposition period in summer females occurred two weeks after emergence, whereas in fall emerged females, oviposition was delayed for about 75 days, which in turn postponed nymphal emergence to mid February-early March of the following year. Under the greenhouse conditions tested, i.e., warm temperatures and supplemented artificial lighting maintained throughout the winter, adult fall females behaved as they would have under field conditions (mortality due to harsh weather and predators excluded) where no reproduction was observed between November and January. Additional research is underway to better understand reproductive behaviors in *H. vitripennis,* stimulate mating and reproduction in captivity off season so that a reliable rearing procedure that would allow fall-winter production of GWSS immatures needed for other research activities can be developed.

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