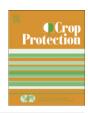


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Differential reproductive maturity between geographically separated populations of *Homalodisca vitripennis* (Germar) in California

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ABSTRACT

The glassy-winged sharpshooter, Homalodisca vitripennis (Germar), is native to the southeastern United States and northeastern Mexico. It was detected in southern California in the late 1980s and in the San Joaquin Valley in 1999, where it transmits the bacterium *Xylella fastidiosa* to grapevines and other crops. The reproductive success of hybrid and pure line H. vitripennis from two geographically separated populations in California (Riverside (RIV) and Bakersfield (BAK)) was evaluated under identical conditions. The RIV and BAK populations had different preoviposition periods that persisted through the second generation of each lineage. From adult molt, the preoviposition period in both female generations was significantly shorter for RIV ($F_0 = 28.2$ days and $F_1 = 62.3$ days) than BAK females ($F_0 = 46.1$ days and $F_1 = 170.4$ days). After a 21-day mating period, F_0 and F_1 females deposited on average 391 (range, 21 -967) and 196 (range, 0-755) eggs, respectively, without significant differences in fecundity among the F₀ and F₁ mating pair treatments. Egg accumulation rates among F₁ treatments showed that females in the RIV groups rapidly deposited their eggs within the first 120 days after adult molt while BAK females maintained a steady accumulation rate during their life. The performance of both hybrid lines was intermediate between the pure lineages. The F_0 mating pairs: $\Re RIV \times \Im RIV \times \Im RIV \times \Im BAK \times \Im RIV$, and $\mathcal{P}BAK \times \mathcal{S}BAK$ produced on average 185, 94, 79, and 0 viable eggs, respectively, which suggested a delayed sexual maturity of BAK males and females. The proportion of viable eggs deposited decreased gradually, which suggests that females completely exhausted sperm reserves. From a management perspective, delayed reproductive maturity and polyandry are weak links in H. vitripennis' biology that may be exploited through mating disruption or insect sterilization strategies to reduce population growth and augment pressure by natural enemies.

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1. Introduction

Pests from different regions may have important biological and behavioral intraspecific variation. Recognition of such biotypes provides the opportunity to adjust pest management programs to take advantage of the local biological and behavioral characteristics of that pest. A classic example is that of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). Twenty biotypes of *B. tabaci* have been identified (Perring, 2001) and studies have determined that biotypes differ in: 1) their susceptibility to insecticides (Byrne and

Devonshire, 1993), 2) invasion success (Reitz, 2007; Delatte et al., 2009), and 3) different host plant preferences (Brown et al., 1995). Clearly taking into account such differences will improve management.

The glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae), is an invasive insect pest native to the southeastern United States and northeastern Mexico (Triapitsyn and Phillips, 2000) that was first discovered in California in the late 1980s (Sorensen and Gill, 1996). The establishment of *H. vitripennis* in California represents a serious threat due to its ability to vector *Xylella fastiodosa* Wells et al., a xylem-limited bacterium that causes Pierce's Disease in grapes (Davis et al., 1978), almond leaf scorch disease (Mircetich et al., 1976; Davis et al., 1980), and many other diseases in economically important woody crops. Since its initial detection, *H. vitripennis* has expanded its range in Southern California and can also be found in southern portions of the San Joaquin Valley (Blua et al., 1999) and Pacific islands such as French Polynesia, Hawai'i, and Easter Island (Grandgirard et al., 2006).

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Various aspects of the biology and behavior of *H. vitripennis* from populations found in Florida, Texas, and California have been studied extensively including its susceptibility to insecticides (Castle et al., 2005; Byrne and Toscano, 2006; Prabhaker et al., 2006a,b; Lauzière and Elzen, 2007), ability to transmit strains of *Xylella fastidiosa* to different plant species (Turner, 1959; Purcell and Saunders, 1999; Costa et al., 2000, 2006; Almeida and Purcell, 2003; Damsteegt et al., 2006), and reproductive biology (Sétamou and Jones, 2005; Hummel et al., 2006a,b; Sisterson, 2008). Biological and behavioral parameters of individuals from different populations and their interaction with the local environment have been studied, but little is understood regarding how these parameters are conserved among populations across the U.S.

de León et al. (2004) analyzed H. vitripennis populations using inter-simple sequence repeat primers and found genetic differences that suggest more than one invasion event has occurred in California. More specifically, in a dendrogram based coancestry analysis (Reynolds et al., 1983), insects collected in Bakersfield (located in the San Joaquin Valley) formed a cluster separate from populations in Southern California which they suggest to be a geographic race or possibly part of a H. vitripennis-species complex. However, Smith (2005) used DNA sequencing analysis to characterize a portion of the mitochondrial cytochrome oxidase I gene from different H. vitripennis populations and suggested that it is a single species, but certainly possible that there may have been more than one founding event in California. As the San Joaquin Valley is separated from Southern California by the San Gabriel and Tehachapi Mountains and the western part of Moiave Desert these populations are isolated from one another and experience different climates. The objectives of this study were: 1) to investigate fertility and fecundity of *H. vitripennis* individuals from geographically separated populations maintained under identical ambient conditions; 2) to evaluate inheritance of reproductive characteristics in hybrid individuals; and 3) investigate the effects of insect size on fecundity. Differences between populations may be important not only from a management perspective but also in determining whether the results from studies on *H. vitripennis* collected in one area extrapolate to the other isolated areas.

2. Materials and methods

2.1. Insect origin

Adult *H. vitripennis* used in the experiments were reared in the laboratory from field-collected egg masses obtained from two Californian populations located 215 km apart, Bakersfield (35° 23′ 48.77″N, 118° 56′ 57.68″W) and Riverside (33° 58′ 23.86″N, 118° 20′ 46.34″W). These areas are separated by mountain ranges and the western part of Mojave Desert. Plant communities in both locations included common host plants such as *Citrus* spp., red-tip photinia (*Photinia fraseri* Dress), crape myrtle (*Lagerstroemia indica* L.), *Eucalyptus* spp., olive (*Olea europaea* L.), and oleander (*Nerium oleander* L.). Egg masses were collected from red-tip photinia plants located in a landscape area in Bakersfield; and from citrus plants located in the Agricultural Operations at University of California at Riverside.

2.2. Establishment and maintenance of insect colonies

Egg masses were collected in mid-March 2008 from both locations and placed in a containment facility located at Fresno, CA. The egg masses (10–20 eggs each) were placed in groups of 10–15 in 10-cm plastic Petri dishes with a moist paper towel on the bottom. The room was maintained at 24–27 $^{\circ}$ C, 22–25% RH, and 16:8 [L:D] h using only artificial light from high pressure sodium vapor bulbs.

Egg masses were checked once or twice a day and emerged nymphs (F₀) were transferred into tent-like cages (Bug Dorm-2[®], BioQuip Products, Rancho Dominguez, CA) using a fine camel's hair brush. Nymphs emerged from eggs of different females were maintained in groups of about 100 per cage containing an assemblage of five cowpea plants, Vigna unguiculata L. Walp. cv. Blackeye (Vermont Bean Seed Co., Randolph, WI): two sorghum plants, Sorghum bicolor (L.) Moench, "TX 7000" (Richardson Seeds Ltd., Vega, TX); one basil plant, Ocimum basilicum L. "Genovese" (Ferry-Morse Seed Co., Fulton, KY); and four sunflower plants, Helianthus annuus L. "American Giant Hybrid" (Ferry-Morse Seed Co., Fulton, KY). Plants were grown in 0.5 l pots using Sunshine Soil Mix 1 (Sun Gro Horticulture, Bellevue, WA). Plants were replaced every 7 days. First instar to adult molt was about 45 days for insects from both populations. Egg masses found in the cages were transferred to plastic Petri dishes and emerged nymphs were used to initiate new cohorts using the methodology described above.

2.3. F₀ hybridization and fecundity

About 100 fifth instar nymphs derived from eggs collected in each location, Bakersfield and Riverside, hereafter BAK and RIV, were randomly selected from the cages described above and separated into groups by gender and origin. Groups of nymphs were kept separately in cages in the same room using the same host plant species as above and checked once a day for newly molted adults. One newly molted (<24 h) adult virgin male and one virgin female were collected from their respective cages and paired on a four-week old cowpea plant using a two-piece (front and back) rectangular plastic cage (10 cm length \times 12 cm width \times 16 cm height) with mesh screen on the front and back walls. Front and back pieces of plastic cage were attached to the top 15 cm of the plant using rubber bands. Cotton was used to seal the gap between the cage and plant stem. Plant and cage assemblage was staked to provide structural support and prevent damage to the plant. Ten replicates of the following mating pairs were established between 17 and 27 May 2008: $PRIV \times PRIV$, $PRIV \times PRIV \times$ were allowed a 21-day mating period, then the male was removed from the cage to exclude uncontrollable variables such as competition for resources that could affect female oviposition.

During the lifetime of a female, plants were changed every 7 days until the end of the mating period and every 3 days thereafter. Plants were checked every day for presence of egg masses until the first egg mass was found and every 3 days thereafter. When an egg mass was found, the plant hosting the eggs was kept in a tent-like cage for 10–15 days to evaluate nymph emergence. The number of eggs per egg mass, proportion of hatched eggs, and female longevity were recorded. Signs of embryo development such as presence of eye spots (Al-Wahaibi and Morse, 2009) were used to determine egg viability in the case of unsuccessful egg hatch. To assess differences in size, the length of one hind tibia of males and females were measured at the end of the experiment.

2.4. F₁ backcrosses and fecundity

Hybrid and pure line nymphs obtained from the F_0 mating pairs (or from the colonies, i.e., pure line BAK) were reared to virgin adults using the same procedure described above. Similarly, ten replicates of each mating pair described above were established, except for the addition of the following female hybrids (i.e., BAK and RIV maternal donors with RIV and BAK paternal donors, respectively) that were backcrossed (paired) with pure line F_1 males of the same maternal origin: P_1 maternal RIV P_2 drilv and P_3 maternal BAK P_4 drilv and egg viability were evaluated as described above for P_0 .

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