Diel Periodicity of *Euschistus conspersus* (Heteroptera: Pentatomidae) Aggregation, Mating, and Feeding

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ABSTRACT The diel periodicity of pheromone-mediated aggregations and mating and feeding cycles of *Euschistus conspersus* Uhler (Heteroptera: Pentatomidae) was investigated in a series of laboratory and field experiments. The aggregation response of adult *E. conspersus* to lures loaded with the male-produced aggregation pheromone component methyl *E*2,*Z*4-decadienoate placed on common mullein, *Verbascum thapsus* L., peaked in the early hours of the scotophase, at ≈2100 hours. Peak mating occurred at 2300 hours, with 78.5% of the insects in aggregations mating at this time. Aggregations dispersed and declined to 30% of their maximum level by dawn on pheromone-baited plants. Equal numbers of males and females were found within aggregations. Aggregations continued to form nightly and disperse by morning throughout a 72-h observation period on pheromone-baited and unbaited mullein plants. A laboratory analysis of feeding periodicity indicated that although feeding occurred throughout the 24-h observation period, the percentage of feeding insects was significantly higher during the scotophase. There were no differences between feeding rates of males and females. The implications of these findings for monitoring and management of *E. conspersus* are discussed.

KEY WORDS aggregation, *Euschistus conspersus*, mating, feeding, diel activity patterns

Euschistus conspersus Uhler (Heteroptera: Pentatomidae) is endemic to western North America and has been reported as a pest of a variety of tree fruits (Madsen 1950, Borden et al. 1952). In Washington state, this insect is an occasional pest of apple and pear (Beers et al. 1993), and control measures have met with mixed success (McGhee 1997, Krupke 2000), leading researchers to search for additional information regarding the life history and habits of this insect.

Observations of *E. conspersus* pheromone response, mating, and feeding patterns have been conducted primarily during daylight (Alcock 1971, Krupke et al. 2001). For example, Krupke et al. (2001) showed that *E. conspersus* aggregations form within 24 h of pheromone lure placement but the diel cycle of such aggregations was not investigated. Current control measures consist of spraying contact insecticides in orchards where stink bugs have historically been a problem or when damage is observed. A more detailed understanding of the daily temporal patterns of stink bug behavior, especially their responses to pheromone sources or potential food resources found in commercial orchards, would be helpful in the development of a holistic pest management approach for this pest. The following series of observational studies were performed to develop a diel activity profile of *E. conspersus* to help improve current integrated pest management programs and provide baseline information for further basic behavioral studies.

Materials and Methods

Aggregation Formation and Peak Mating Survey. These experiments were conducted in the field between 1 and 14 June during both 2002 and 2003, by using natural populations of stink bugs present during the late spring–early summer mating period. Common mullein, *Verbascum thapsus* L., identified by Krupke et al. (2001) as mating sites for field populations of *E. conspersus* in Washington, plants were used to document the aggregations described in this experiment. All specimens of *E. conspersus* were removed from the plants before the start of this experiment.

Lures consisted of hollow polyethylene caps (No. 60975D-3, Kimble Glass Co., Vineland, NJ) that were loaded with 0.5 ml of *E*2,*Z*4-decadienoate, the primary *E. conspersus* pheromone component (Aldrich et al. 1991) (hereafter referred to as pheromone). Polyethylene caps were a two-piece design consisting of a hollow reservoir of ≈1-ml capacity and a plastic lid that snapped into place over the top of the reservoir. The pheromone was 85.5% pure and was synthesized by Dr. Jocelyn Millar (Department of Entomology, University of California, Riverside, CA). The release rate was governed by diffusion of pheromone through

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the sides of the polyethylene cap and was found in a previous study to be ≈140 μg/h (Krupke et al. 2001). Lures were aged at room temperature in a fume hood (flow rate 0.5 m/s) for 24–48 h before placing them in the field to allow any pheromone contamination on the outside of the lure to dissipate.

Twenty mullein plants, 10 pheromone baited and 10 unbaited controls, were surveyed at each of two sites for each year of the study. Mullein was plentiful at both sites, and the plants chosen were spaced out evenly along an ≈1,000-m transect so that they were separated by a minimum of 40 m. The experiment began with the placement of a pheromone lure on a mullein plant at 0700 hours. Control (unbaited) and pheromone-baited plants were monitored hourly for a 24-h period by walking along a transect, so that each plant was surveyed at approximately the same time within an hour. Data collected during this phase of the experiment included the number of adults of *E. conspersus* on the plant and the number of mating pairs on the plant. This phase of the experiment was strictly observational, and no aggregations or individual insects were handled or otherwise disrupted during the course of the experiment, other than the necessary movement of leaves to ensure an accurate count. All observations conducted at night were done using night vision goggles (Bushnell 1 by 20 Night Vision Goggle, Bushnell Inc., Lenexa, KS) to minimize disruption to the insects. Mating data were analyzed using analysis of variance (ANOVA) to determine whether there were significant differences in the numbers of insects mating on pheromone-baited verses unbaited plants. Analysis was performed on square-root transformed percentage of mating data to satisfy conditions of normality (Zar 1999).

Sex Ratio. This experiment was conducted on 15 May 2002 and 11 June 2002 and followed similar protocols to those described in the previous section. Mullein plants were baited with pheromone and paired with unbaited controls as described above. Selected plants were a minimum of 10 m apart to prevent interference between treatments (C.H.K., unpublished data). Sites were located in the same general area as the previous experiments, but different plants were used. Twenty plants were used (10 baited and 10 unbaited). Pheromone lures were placed on 10 plants at 0800 hours, and all plants were sampled every 4 h over the next 24 h (i.e., 1200 hours, 1600 hours, 2000 hours, 000 h, 0400 hours, and 0800 hours). At each sample interval, all bugs were removed from the plants, counted, sexed, and then removed from the study area. Each plant was used only once in this study, as this was a form of destructive sampling. A chi-square test was used to determine whether the percentage of females captured differed significantly from 50% at any of the sample intervals.

Aggregation Stability. This experiment used a similar protocol to the sex ratio experiment described above. However, counts were observational, and no bugs were removed from plants during the course of the study. Experiments were conducted on 17–19 May and 11–13 June 2002. During each of these periods, 60 mullein plants were used, 30 pheromone baited and 30 unbaited. Pheromone-baited and unbaited plants were paired and placed a minimum of 10 m apart. Lures were placed on the 30 pheromone-baited mullein plants at 0800 hours, and all plants were then monitored at 12-h intervals for a total of 72 h after the start of the experiment. The total numbers of adults of *E. conspersus* present on each plant were recorded at each interval.

Feeding. Specimens of *E. conspersus* were field collected in early to mid-August 2003. These individuals were collected as adults and were not reproductively active (McGhee 1997, Krupke et al. 2001). It is at this time of year that damage begins to occur in orchards (C.H.K., unpublished data). Field-collected insects were held in a greenhouse in screen cages without artificial light to preserve the insects natural diel cycle. No supplemental heating or cooling was used in the greenhouse. These screen cages contained potted mullein plants as a primary food source. Ambient air was allowed to circulate freely (passive) through the open-sided greenhouse at all times. Twelve hours before the start of the experiment, 20 individuals of *E. conspersus* of each sex were selected at random from the field-collected individuals. These insects were placed in a 1-liter plastic cup (Solo Cup Co. Urbana, IL) that had 1-cm-diameter hole drilled in the bottom. The hole allowed for the placement of a mullein leaf into each cup, the stem of which would protrude through the bottom and into a water-filled reservoir. Each cup also contained a single 'Red Delicious' apple that had been picked the same day from an orchard at the Washington State University Tree Fruit Research and Extension Center. Apples had not been sprayed with insecticide during the entire season. Each cup had a fine mesh lid to prevent escape of insects while allowing airflow. After the 12-h acclimation period, insects were monitored hourly for 48 h. Data collected at each interval included whether the insect was feeding, and whether it was feeding on the mullein leaf or the apple. This experiment was conducted three times, on 19 August, 28 August, and 12 September 2003, using different insects each time. Feeding observations were partitioned into photophase and scotophase and analyzed using a chi-square test to determine whether there were significant differences in feeding between the two periods.

Results

Aggregation Formation and Peak Mating Survey. Aggregations on pheromone-baited and unbaited plants peaked at 1900–2000 hours and 2200 hours, respectively (Fig. 1). Both aggregation curves displayed similar patterns, with aggregations on unbaited plants lagging slightly behind those of pheromone-baited plants. Aggregations increased steadily throughout the late photophase and peaked early in the scotophase. Aggregations dispersed to 30 and 20%

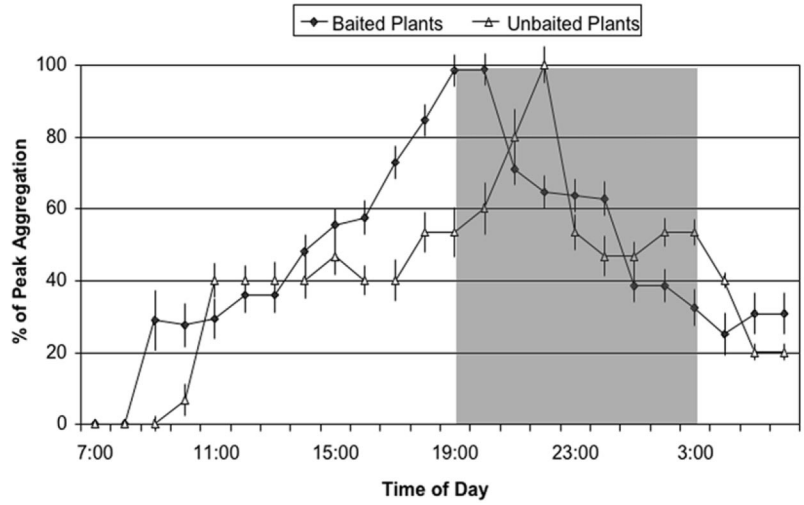

Fig. 1. Twenty-four-hour aggregation curves of *E. conspersus* adults on mullein plants, showing both pheromone-baited and unbaited plants. Shaded areas indicate hours of scotophase. Data are expressed as mean ± SEM percentage of maximum aggregation during the 24-h period (n = 10 plants).

of their maximum levels by dawn the following day on pheromone-baited and unbaited plants, respectively. The mean percentage of *E. conspersus* mating in the aggregations reached peak levels between 2100 and 0100 hours on pheromone-baited plants and between 2200 and 0300 hours on unbaited plants (Fig. 2). On both the pheromone-baited and unbaited plants mating occurred primarily during the scotophase. The proportion of *E. conspersus* mating on unbaited plants was significantly lower than on pheromone-baited plants (F = 13.23; df = 1, 16; $P < 0.01$).

Sex Ratio. The sex ratio did not deviate significantly from 50% at the 4-h intervals observed except in one of the 20 comparisons. In that case, at 16 h postbaiting, 74% of the adults on the plants were female (χ^2 = 4.34, df = 1, $P < 0$ 0.05) (Fig. 3). Analysis over all time periods showed no significant differences in sex ratio between pheromone-baited and unbaited plants (χ^2 = 8.31, df = 1, P = 0.21) (Fig. 3).

Aggregation Stability. When aggregations were observed every 12 h throughout a 72-h period in May and June, a general pattern emerged with the highest numbers occurring at night (2000 hours), and only a fraction of that number occurring during the day (0800 hours) (Fig. 4). The pattern of night–day aggregation was similar on pheromone-baited and unbaited mul-

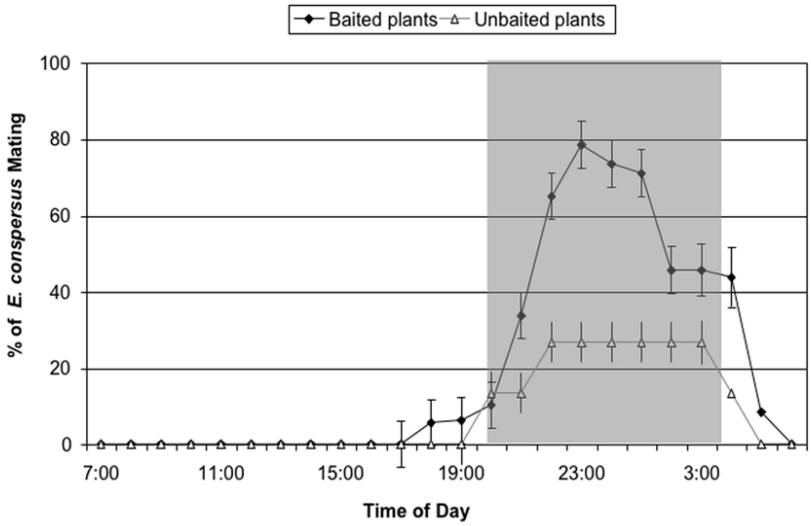

Fig. 2. Initial 24-h mating curves of *E. conspersus* adults in aggregations on mullein plants, showing both baited and unbaited plants. Shaded areas indicate hours of scotophase. Data are expressed as mean ± SEM percentage of adults mating (n = 10 plants).

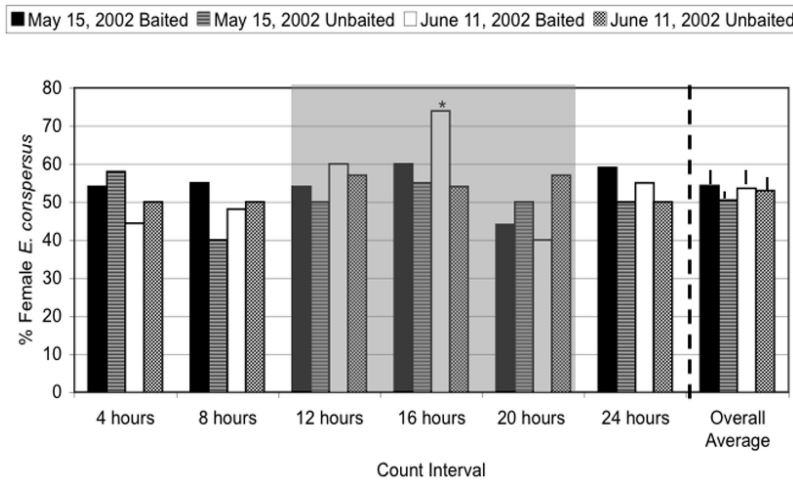

Fig. 3. Mean percentage of female *E. conspersus* adults in aggregations on mullein plants sampled at 4-h intervals, showing both baited and unbaited plants. Shaded areas indicate hours of scotophase. Bar marked with asterisk (*) denotes significant departure from a 50:50 sex ratio by using chi-square analysis ($P < 0.05$) ($n = 10$ plants).

lein plants. Daytime samples averaged 42% lower (range 28–64%) than the nighttime samples.

Feeding. Feeding occurred throughout the 24-h observation period (Fig. 5). However, the percentage of insects feeding was significantly higher during the scotophase ($\chi^2 = 106.09, \text{df} = 1, P < 0.001$). Of the total feeding events recorded, 75% were feeding on mullein. Although both sexes fed preferentially on mullein, females were more likely than males to feed on apple (32% of female feeding events versus 20% of male feeding events; $\chi^2 = 7.01, \text{df} = 1, P < 0.01$). There were no differences between the number of males or females feeding at any time period ($\chi^2 = 0.11, \text{df} = 1, P = 0.73$).

Discussion

Results of these experiments have implications for both basic studies of the behavior of this insect and for its potential management. The formation of regular and nightly aggregations of *E. conspersus* agrees with reports documenting regular aggregation formation for other phytophagous stink bug species, including *Plautia stali* Scott (Moriya and Shiga 1984), *Biprorulus bibax* Breddin (James 1990), and *Thyanta pallidovirens* Stal (Wang and Millar 1997). For all of these species, the diel periodicity of the aggregation cycle was relatively constant, although the actual time of aggregation peak differed among species. For a pest such as *E.*

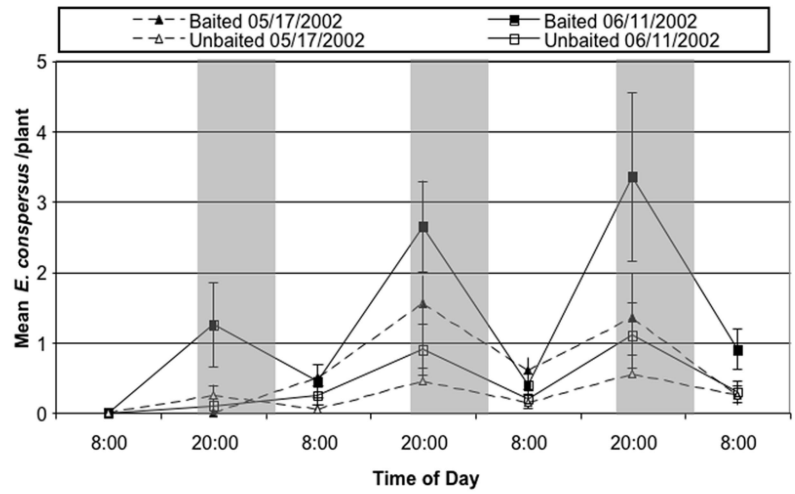

Fig. 4. Initial 72-h aggregation curves of *E. conspersus* adults on mullein plants, showing both baited and unbaited plants. Shaded areas indicate hours of scotophase. Data were taken at 12-h intervals and are expressed as mean ± SEM numbers of *E. conspersus* adults found per plant during the 72-h period ($n = 30$ plants).

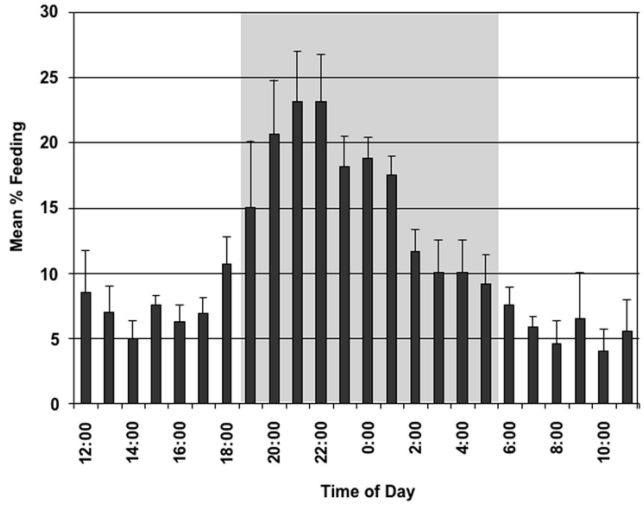

Fig. 5. Mean ± SEM percentage of *E. conspersus* adults observed feeding on mullein leaf or apple over 24-h period. Shaded areas indicate hours of scotophase. (n = 60 males, 60 females).

conspersus, knowing that aggregations are a nightly occurrence (and not an isolated event within the season) allows for the possibility of developing monitoring methods that accurately assess *E. conspersus* populations over the course of the season. For example, it may be important to assess when overwintering populations decline or when new summer adults begin to increase (Krupke et al. 2001), which may in turn indicate when damage could be expected to become visible in orchards. It should be possible without further refinement to use daytime sampling of pheromone-baited mullein plants as a means of assessing the density of *E. conspersus* adults in an orchard. Coupled with an effective trapping system to retain night-time responders, however, this strategy could be made even more useful and possibly expanded to a mass-trapping approach. Alternately, insecticides could be applied to pheromone-baited mullein plants to create killing stations along orchard borders.

The finding that sex ratios were approximately equal on pheromone-baited plants throughout a 24-h study period supports a hypothesis that the pheromone acts as a bisexual attractant. Male-produced aggregation pheromones have been shown in several other stink bug species, including *Plautia stali* Scott (Moriya and Shiga 1984), *Podisus maculiventris* (Say) (Aldrich et al. 1984), *Biprorulus bibax* Breddin (Aldrich 1995) and *Piezodorus hybneri* (Gmelin) (Leal et al. 1998). For practical purposes, a bisexual attractant represents a potentially more powerful management tool than a sex pheromone (Pedigo 2002), which usually attracts only one sex (most often males). For monitoring purposes, counting females may give more clues about the size of future populations. By the same token, reducing both male and female numbers by mass trapping or pheromone-based attract-and-kill methods can potentially have a larger impact upon populations than a male-targeted approach, as illustrated by the success of a pheromone-based management system for the boll weevil, *Anthonomus grandis grandis* Boheman (van Emden and Service 2004).

The periodicity of aggregations over 72 h on baited plants is particularly noteworthy in light of the fact that baited plants represent a constant source of attractive pheromone (i.e., there is no periodicity to the pheromone release rate from these sources). The maintenance of diel periodicity in the response of adult males and females suggests that this behavior is modulated by some other cue(s), most likely abiotic in nature, and this type of entrained pheromone response periodicity has been demonstrated previously in other insect species (Liang and Schal 1990, Zhukovskaya 1995). The diel, repetitive cycle of *E. conspersus* raises interesting questions about the membership in these aggregations and how consistent it may be over time; for example, do aggregations on subsequent nights have the same membership, given the high rate of mating within aggregations?

Finally, the finding that *E. conspersus* feeds mainly at night adds to the picture of an insect that, although it is sometimes observed during daylight, is primarily nocturnal. This is similar to another phytophagous stink bug, *Nezara viridula* L., that has been shown in laboratory (Shearer and Jones 1996) and field studies (Lockwood and Story 1986) to feed more actively during the scotophase. Although our studies quantifying feeding is strictly a laboratory study, the results of this experiment coupled with the results of field aggregation and mating experiments have definite implications for orchard pest management. For example, grower-applied control measures such as contact insecticides may be more efficacious if applied near the end of the day or at dusk, when the largest percentage of *E. conspersus* adults are present on the plants. Current grower practices involve application of insecticides in the morning or at midday, so a shift in application timing may result in improved control of stink bugs.

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