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Reproduction of the western tarnished plant bug, *Lygus hesperus*, in relation to age, gonadal activity and mating status

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ABSTRACT

Understanding the basic life history and underlying regulatory mechanisms for a pest insect is essential for developing targeted control strategies, but for many insects relatively little is known. Although the western tarnished plant bug, *Lygus hesperus* Knight (Heteroptera: Miridae) has a substantial negative impact in the western U.S., its basic biology is poorly characterized. To elucidate the regulation of *L. hesperus* reproductive dynamics, the onset times of gonadal activation and mating behavior were examined in young adults. Newly emerged adults reared under laboratory conditions at 25 °C were monitored daily for changes in gamete production and willingness to mate. Males matured more quickly than females. Sperm was present at emergence and a small proportion of males were willing to mate as early as 2 days post-emergence. Females were unwilling to mate until at least 5 days post-emergence, although many produced choriogenic oocytes by 4 days. Males appeared to discriminate female age and were more likely to attempt mating with females >5 days post-emergence than with younger females. Males were also able to detect previous mating and attempted to mount virgins more often than recently inseminated females. Collectively these results indicate that the changes in the mating behaviors of *L. hesperus* are linked to reproductive status, although there is a lag between gamete production and willingness to mate. The results also suggest that interactions of the sexes are chemically mediated.

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1. Introduction

The regulation of reproduction results from the translation of various exogenous and endogenous stimuli into appropriate physiological and behavioral responses, a process that is mediated by the central nervous system and neuroendocrine organs. The general flexibility of this process promotes the optimal timing of gonadal activity and reproductive behaviors to maximize individual fitness. For many insects, key determinants of reproduction include gonadal and nutritional status, mate availability, and recency of mating (reviewed in Ringo, 1996; Gillot, 2003). Although knowledge of reproductive regulation is crucial for understanding the life history and population dynamics of a given species, and for devising targeted control strategies, there is a dearth of such information for many economically important insects.

One such pest species is the western tarnished plant bug, *Lygus hesperus* Knight (Heteroptera: Miridae), which damages numerous cultivated crops in the western United States (Jackson et al., 1995). Despite its economic importance, relatively little is known about the specific factors influencing the dynamics of *L. hesperus* reproduction. Previous work on reproduction in this species is limited (Leigh, 1963; Beards and Strong, 1966; Strong et al., 1970; Strong and Sheldahl, 1970) and principally descriptive, often relying on modest sample sizes. A more accurate and detailed assessment is necessary to facilitate future studies of the underlying regulatory mechanisms of reproduction in *L. hesperus*, and to ensure appropriate comparisons with data collected from other mirid species.

In *L. hesperus* (Strong et al., 1970), and other mirids (Wheeler, 2001; Castañé et al., 2007), there is a pre-mating period after adult eclosion during which sexual maturation is completed. The first objective of this study was to characterize the progression of gonadal activation in *L. hesperus* and to determine whether male and female reproductive behavior was coordinated with these physiological processes. Beginning at adult emergence, changes in gonad development and sexual attractiveness and receptivity were tracked in both females and males. Because reproductive behavior is also influenced by mating status in many insects (Ringo, 1996; Simmons, 2001; Gillot, 2003), including some mirids (Wheeler, 2001; Gemeno et al., 2007), post-mating changes to female attractiveness and sexual receptivity were also determined.

2. Methods and materials

2.1. Insects

The *L. hesperus* used in this study were obtained from a laboratory colony maintained at the USDA-ARS Arid Land

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Agricultural Research Center (Maricopa, AZ, USA). The individuals in this colony are periodically outbred with locally caught conspecifics. The stock insects were given unrestricted access to a supply of green beans and an artificial diet mix (Debolt, 1982) packaged in Parafilm (Patana, 1982). Both food sources were replenished as needed. Insects were reared at 25 °C, 20% relative humidity, under a L14:D10 photoperiod.

Adults were produced from groups of nymphs reared in 1890-ml waxed chipboard cup (Huhtamaki, De Soto, KS) at a density known to have minimal effect on *L. hesperus* development (≤100 nymphs/container; Brent, in press). Nymphs in each container were provided approximately 20 g of fresh green beans and 12 g of artificial diet, which was replaced every 48 h. Rearing cups were covered with a nylon mesh to ensure adequate air circulation and light exposure. Daily monitoring allowed adults to be collected within 24 h of emergence. Cohorts of adults of the same age and sex were reared under conditions matching those for nymphs, but with population densities ranging between 50 and 120 adults/container.

2.2. Gonadal activity

Gonadal activity was assessed in 25 female and 25 male adults when <24-h-old and on each of the subsequent 10 days. Sampled individuals were preserved in a −80 °C freezer until dissected in Ringers solution under a stereomicroscope. Based on a previously defined pattern of *Lygus* oocyte development (Ma and Ramaswamy, 1987), a three stage scale was used to rate ovarian activity: pre-vitellogenic oocytes only; vitellogenic oocytes present; choriogenic oocytes present. Male gamete production was assessed by homogenizing one testis per male in 20 μl of distilled water. A 10 μl aliquot of the homogenate was placed on a hemocytometer and developing spermatozoa were counted under a compound microscope to calculate sperm number per testis. Because the seminal vesicles rather than the testes of *L. hesperus* store mature sperm (Strong et al., 1970), this measure provides a rough estimate of the relative rates of spermatogenesis among males. It should be noted that all of the testes examined in this experiment were composed of seven lobes, not five as reported by Strong et al. (1970).

In addition to sperm, male *L. hesperus* provide a large volume of other material in the spermatophore transferred to the female (Strong et al., 1970). Spermatophores were dissected from females within 30 min of insemination, and weighed on a microbalance (Sartorius TE153S, Goettingen, Germany). Between 17 and 22 spermatophores were collected for each male age except for males younger than 2 days post-emergence, which did not mate. Although composition of the spermatophore is unknown, this mass originating from the medial and lateral accessory glands and seminal vesicles (Strong et al., 1970) may influence female fecundity, mating receptivity, and ovipositional behavior. Because the ability to produce a spermatophore of a threshold mass may influence male mating behavior, the effect of age on accessory gland condition was determined. At the time of emergence and for 7 days following, the condition of both sets of glands and the seminal vesicles was visually assessed. Because the compounds produced in the lateral accessory glands are often translucent, visual assessment of status was verified by opening the glands. A simple qualitative scale, based on descriptions by Spurgeon (2009), was used to rate condition: *empty*, *filling*, *filled* or *distended*. *Empty* organs were translucent, colorless and smaller than active organs. *Filling* glands were more opaque with white material at either their basal or terminal ends, but not throughout the entire lumen. Filling seminal vesicles had traces of white material throughout, but the lumen was not fully occupied. *Filled* glands and seminal vesicles had material throughout the lumen and tended to have a uniform width, except at their gently tapered ends. *Distended* glands and seminal vesicles had bulbous basal ends that tapered sharply, giving them a club-like shape.

2.3. Reproductive behavior

Mating behaviors were assessed by pairing male and female *L. hesperus* of known age in mating arenas for one hour of observation. All observations were conducted during the morning hours, when mating activity is most commonly observed (Blackmer and Brent, unpublished data). Arenas consisted of glass Petri dishes measuring 1.5 cm × 5.0 cm. Individuals were only used once. Three specific male mating behaviors were recorded. A *Court* was scored when a male moved toward a female and shook his body (Strong et al., 1970). A *Mount* was scored when a male climbed onto a female from behind, curling his abdomen to present his aedeagus. A *Mate* was scored when a male maintained copulation for at least 30 s (less time generally meant failure to achieve full intromission). The duration of each copulatory event was recorded, and mating was confirmed by dissecting the female to ensure a spermatophore was present. The mass of each spermatophore was also recorded. Not all males that courted females attempted to mount, and not all that mounted successfully mated. Female behavior consisted of either rejection (moving away or kicking) or acceptance of males for mating. Although group reared *L. hesperus* females are less likely to mate than those reared individually (Ho, 2000), a clear pattern in changing receptivity was observable. Rarely, females approached males and solicited copulation (brief head bobbing followed by moving in front of the male with raised abdomen), but occurrence of this behavior was too infrequent for analysis. However, it almost always resulted in immediate copulation.

The effect of a male's age on his willingness to mate was assessed by placing individual virgin males of known age (newly eclosed to 7 days post-eclosion) in mating arenas along with a virgin 7-day-old female. For each male age group, 65–81 trials were conducted.

To determine if female age influences male reproductive behavior, individual virgin males that were 7 days post-eclosion were each confined with two virgin females in a mating arena. One female was 7 days old, and the other was younger (1–6 days post-eclosion). Females of each pair were distinguished by marks of enamel paint on the pronotum. Paints were switched between trials to ensure that the males were not attracted by a particular color. For each age combination, there were 37–51 trials.

Male response to female mating status was assessed by confining individual virgin males with two females. One female was a virgin whereas the other was mated 16–18 h before the test. One hour before testing, both females were individually marked with enamel paint, with colors switched between trials to compensate for any potential male color bias. All individuals used were aged 7 days post-eclosion. A total of 95 trials were conducted to determine the frequency with which the males approached, mounted and mated with the females. Following each assay, females were dissected to recover any spermatophores. This was done to verify initial mating status prior to the trial and to confirm mating during the assay.

The influence of male stimuli and spermatophore resources on female ovipositional behavior was determined by placing newly emerged females in 355-ml rearing cups with artificial diet and an oviposition substrate (a Parafilm coated packet containing 15 g/l agar solution). Two groups of ten cups each were monitored daily for 10 days, counting both the number of females alive and the number of eggs oviposited during the previous 24 h. Ten females were placed in each cup of one group, while cups in the other group each contained ten females and ten males. All individuals were