

External Visibility of Spermatophores as an Indicator of Mating Status of *Lygus hesperus* (Hemiptera: Miridae) Females¹

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Abstract Mated females of the western tarnished plant bug, *Lygus hesperus* Knight, are distinguished from unmated females by the presence of one or more spermatophores. The presence of a spermatophore is normally determined by dissection. A simple and nondestructive method to distinguish mated *L. hesperus* females from unmated females would facilitate laboratory studies that require mated insects. Spermatophores are visible through the abdominal sternites of recently mated *L. hesperus* females, but the consistency and persistence with which spermatophores are externally visible has not been previously documented. The overall objective of this study was to evaluate whether examination for the presence of externally visible spermatophores is a reliable method to determine whether *L. hesperus* females have mated. The presence of externally visible spermatophores correctly discerned 99% of recently mated (≤ 24 h) females from unmated females. None of the unmated females were misclassified as mated. The appearance of visible spermatophores decreased with increasing time after mating until the spermatophores were no longer visible. The period during which spermatophores were externally visible decreased linearly with increasing temperature from 15.6 - 29.4°C. Females continued to oviposit fertile eggs after spermatophores were no longer externally visible. Results indicate that examination for the presence of externally visible spermatophores is a reliable method to discern mated female *L. hesperus* from unmated females in controlled laboratory studies. Because spermatophores become less apparent with increasing time after mating, this method is not suitable for the classification of mating states of field-collected insects or others of unknown history.

Key Words Western tarnished plant bug, mating status, *Lygus*, spermatophore, seminal depository

Lygus hesperus Knight (Hemiptera: Miridae) is a key agricultural pest in the western United States (Wheeler 2001), but certain aspects of *L. hesperus* biology are poorly understood. The reproductive biology of *Lygus* is one of these aspects that has received considerable recent attention (Villavaso 2007, Brent 2010a, 2010b, Spurgeon and Brent 2010, Cooper and Spurgeon 2012a). In addition to the physiological aspects of *Lygus* reproduction, it is important to understand how adult reproductive state (prereproductive, reproductive and unmated, reproductive and mated) influences *Lygus* behavior. Previous studies identified variations among *Lygus* adults of different reproductive states in feeding behavior (Cooper and Spurgeon 2011, 2012b) and propensity to initiate flight (Stewart and Gaylor 1994, Blackmer et al. 2004). In each of

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these studies, mated females were discerned from unmated females by dissecting insects to assess the presence of a spermatophore, or by monitoring the development of eggs oviposited by the females.

A simple and nondestructive method to distinguish mated female *Lygus* from unmated females would be useful for future studies that require mated insects, including the further study of reproductive biology and adult behavior. Strong et al. (1970) reported that spermatophores were visible through the abdominal sternites of recently mated females. However, the consistency with which spermatophores are externally visible through the sternites of mated females has not been documented. Examination for externally visible spermatophores may provide a nondestructive method to identify female mating status if (1) spermatophores are consistently visible among different mated females and (2) visibility of spermatophores persists after mating. The objective of this study was to determine whether mated *L. hesperus* females could be reliably distinguished from unmated females by the presence of an externally visible spermatophore.

Materials and Methods

Experimental insects. *Lygus hesperus* adults were obtained from a laboratory colony maintained on green bean pods (*Phaseolus vulgaris* L.) and raw sunflower seeds (*Helianthus annuus* L.). Experimental insects were no more than 3 generations removed from field populations and were obtained from the colony within 24 h after adult eclosion. To ensure experimental insects were reproductively mature, adults were held in same gender groups of 100 - 150 insects for 9 d within an environmental chamber (Percival Scientific, Perry, IA, Model I30BLL) maintained at 26.7°C with a 14:10 (L:D) h photoperiod (Strong et al. 1970). Groups of adults were held in 4-L plastic rearing buckets provisioned with shredded paper. Green bean pods were provided as a food source.

Observation of spermatophores. Three mixed-gender groups of 9-d-old adults, each composed of 15 males and 15 females, were held for 24 h to facilitate mating. Groups of insects in rearing buckets provisioned with shredded paper and green bean pods were held in an environmental chamber maintained at 26.7°C with a 14:10 (L:D) h photoperiod. After 24 h, living females were aspirated from the buckets, anesthetized with CO₂ and positioned ventral-side up on the laboratory bench. The ventral surface of the abdomen of each female was examined under an illuminated bench-top magnifier at $\approx 2.25\times$ (Luxo Lamp, Elmsford, NY). Each female was classified as mated if a white spot was visible through the sternites anterior to the ovipositor indicating the presence of a spermatophore (Fig. 1A). Each female was then pinned through the thorax with the ventral-side up, submersed in saline (0.7% NaCl, w:v), and dissected to verify mating status. Mating was indicated by the presence of a spermatophore in the seminal depository (Fig. 1B). The mean percentage (\pm SE) of mated females that were classed correctly based on external examination was calculated for each group. This experiment was conducted 3 times using 3 separate groups of insects in each repetition (a total of 9 groups of insects).

Temperature-dependent persistence of externally visible spermatophores. Nine-day-old reproductive male and female adults were paired in 18.5-ml clear plastic vials closed with ventilated lids (Thornton Plastics, Salt Lake City, UT). Each vial was provisioned with a segment of a green bean pod with the cut ends sealed with

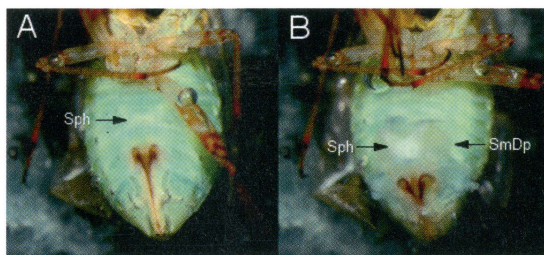


Fig. 1. Spermatophore (Sph) observed (A) through the abdominal sternites of a recently mated *Lygus hesperus* female, and (B) exposed by dissection. The seminal depository (SmDp) is also visible in the dissected female.

paraffin wax. Vials were maintained at room temperature on the laboratory bench for 6 h to allow mating. After 6 h, males were removed from vials, and each female was examined through the vial for external evidence of a spermatophore (Fig. 1A). Examinations for visible spermatophores were conducted under an illuminated bench-top magnifier. Females that did not exhibit externally visible spermatophores were discarded. Each female exhibiting an externally visible spermatophore was randomly assigned to an environmental chamber maintained at 15.6, 21.1, 26.7, or 29.4°C ($\pm 1^\circ\text{C}$) with a 14:10 (L:D) h photoperiod. Temperature within each chamber was monitored using a HOBO Data Logger (Onset Computer Corporation, Pocasset, MA).

Each female was examined every 24 h for external evidence of a spermatophore until spermatophores were no longer visible, at which time they were dissected to verify mating. The period (days) during which spermatophores were externally visible was calculated for 15 females at each temperature. Linear regression (PROC REG; SAS Institute 2008) was used to model the relationship between temperature and the length of the period during which spermatophores were visible through the sternites of mated females. The study was conducted 3 times beginning with 100 pairs of adults in each of 2 repetitions and 38 pairs in the third repetition.

Relationship between female fertility and spermatophore visibility. Four mixed-gender groups of 9-d-old adults, each composed of 10 males and 10 females, were held for 24 h to facilitate mating. After 24 h, each female was individually aspirated into an 18.5-ml vial and examined under an illuminated bench-top magnifier for external evidence a spermatophore (Fig. 1A). Females that did not exhibit an externally visible spermatophore were discarded. Each of 15 females that exhibited an externally visible spermatophore was provided a section of a green bean pod with cut ends sealed with paraffin wax as food and oviposition substrate. Each female was examined through the vial for a visible spermatophore every 24 h until the second consecutive day after the spermatophore was no longer externally visible. Green bean sections were replaced concurrently with examinations of females. Each bean section, which contained *L. hesperus* eggs oviposited over a 24-h period, was transferred to a new vial labeled with the female identification and day of oviposition. Vials were monitored daily for the appearance of one or more first instars, the presence of which indicated egg fertility.

Results and Discussion

Observation of spermatophores. Dissections revealed that $58 \pm 8.5\%$ of 10-d-old *L. hesperus* females were mated after being maintained in mixed-gender groups for 24 h at 26.7°C. Based on the presence or absence of an externally visible spermatophore mating status was correctly assigned to 74 of 75 mated females ($99 \pm 0.01\%$) and to all 60 unmated females. The single spermatophore that was not externally visible was shriveled and small compared with other spermatophores observed. The apparency of externally visible spermatophores appeared to vary with spermatophore size; spermatophores that were more apparent through the sternites were larger compared with those that were faintly visible through the sternites (WRC personal observation). These results indicate that recently mated (≤ 24 h at 26.7°C) *L. hesperus* females can be reliably discerned from unmated females based on the presence of spermatophores externally visible through the abdominal sternites (Fig. 1A).

Temperature-dependent persistence of externally visible spermatophores. The apparency of externally visible spermatophores decreased each day after mating until the spermatophores were no longer visible. The period during which spermatophores were externally visible decreased linearly with increasing temperature from 15.6 to 29.4°C ($F=78.5$; $df=1, 58$; $P < 0.001$; Fig. 2). At 29.4°C, spermatophores were visible for periods ranging from <24 h to 3 d after mating, whereas at 15.6°C spermatophores were visible from 4 to 12 d after mating (Fig. 2). Dissection of females after spermatophores were no longer externally visible revealed the aged spermatophores were shriveled and small compared with the externally visible spermatophores observed within 24 h after mating.

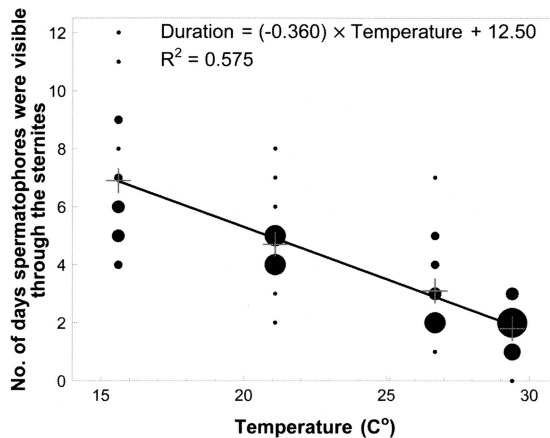


Fig. 2. Linear regression relating temperature to the length of the period during which spermatophores were visible through the abdominal sternites of mated *Lygus hesperus* females. Sizes of plotted points are proportional to the number of females, and gray crosses (+) indicate the mean duration at each temperature.

Relationship between female fertility and spermatophore visibility. Fertile eggs were oviposited by each female that exhibited an externally visible spermatophore regardless of the amount of time since mating. In addition, fertile eggs were oviposited by $96 \pm 4.2\%$ of mated females during the 2-day period after spermatophores were no longer externally visible. Strong et al. (1970) reported that examination of females for the presence of a spermatophore externally visible through the abdominal sternites may provide a method to distinguish mated from unmated females in field collections. However, observations from the current study demonstrate that mated females could be misclassified as unmated using this method if enough time has passed since mating to allow the spermatophore contents to be partially depleted.

Conclusions. Results of this study indicate that although spermatophores are readily visible through the sternites of recently mated *L. hesperus* females, they become less apparent with increasing time after mating. The length of the period after mating during which spermatophores were externally visible was negatively correlated with the temperature at which the females were maintained. In fact, at the highest observed temperature (29.4°C) the spermatophore of one mated female was no longer detected by 24 h after mating (Fig. 2). This change in spermatophore appearance indicates that presence or absence of an externally visible spermatophore is not a suitable criterion for determining mating status of *L. hesperus* females of unknown history, such as those from field collections. However, this method of detecting mating, when used with care, should be useful for obtaining recently mated females in the laboratory. In those cases, errors in detecting mating can be avoided by limiting the mating period to a few hours, or by maintaining mating insects at a temperature <29.4°C.

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