N O T E

Response by *Lygus hesperus* (Hemiptera: Miridae) Adults to Salivary Preconditioning of Cotton Squares [1]

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Despite being an important pest of fruit, vegetable, and field crops in the western United States, many aspects of *Lygus hesperus* Knight (Hemiptera: Miridae) feeding behavior are poorly understood. *Lygus hesperus* use a cell-rupture feeding strategy. The insects lacerate and macerate host tissues by vigorous movements of the stylets and discharge of lytic salivary enzymes such as polygalacturonases, then ingest the liquefied products (Backus et al. 2007, Ann. Entomol. Soc. Am. 100: 296 - 310). The salivary polygalacturonases of *L. hesperus* are resilient and remain active in host tissues long after the cessation of feeding (Strong and Kruitwagen 1968, J. Insect Physiol. 14: 1113 - 1119). Because salivary enzymes continue to digest plant tissues after a feeding event, it may be beneficial for *L. hesperus* to return to feeding locations that have been preconditioned by salivary enzymes discharged during earlier stylet-probing activities. We have observed adult *L. hesperus* revisiting previously inhabited floral buds (squares) on caged cotton (*Gossypium hirsutum* L.) plants (WRC, personal observations), but we did not specifically assess feeding behaviors in relation to previous stylet-probing activities. A better understanding of the influence of salivary preconditioning on *L. hesperus* feeding preference could facilitate interpretation of results from assays investigating *Lygus* feeding injury. The purpose of our study was to investigate whether *L. hesperus* adults exhibit a preference for squares that are preconditioned by prior stylet-probing activities, compared with previously unvisited squares.

Adult *L. hesperus* were obtained from a laboratory colony maintained on green bean pods (*Phaseolus vulgaris* L.) and raw sunflower seeds (*Helianthus annuus* L.). Laboratory rearing results in relatively rapid changes in *L. hesperus* physiology and behavior, compared with native insects (Spurgeon 2012, Environ. Entomol. 41: 415 - 419).

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Therefore, experimental insects were <3 generations removed from field populations. The experimental design was simplified by using only female insects. Prereproductive adult *Lygus* (<5-d old at 27°C) feed more than reproductive adults (>6-d old at 27°C) (Cooper and Spurgeon 2011, Environ. Entomol. 40: 367 - 373). Therefore, only prereproductive adults were used in our study.

Before each assay, 6-mm diam squares were collected from Acala cotton (*G. hirsutum*, 'Phytogen 725', Dow AgroSciences, Indianapolis, IN) grown in a greenhouse (20 - 30°C, 14:10 [L:D] h photoperiod). Squares were rinsed with deionized water before assays, and bracts were removed from the squares to facilitate unimpeded views of insect behaviors. Video arenas were prepared by partially embedding two 6-mm diam squares in paraffin wax within the lid of a 100-mm diam plastic Petri dish (BD Falcon, Franklin Lakes, NJ). Squares were aligned opposite each other with their apices pointing toward the center of the Petri dish. Each square was centered on a position ≈23 mm (25% of the lid diameter) from the edge of the Petri dish lid. At least 1 extrafloral nectary at the base of each square was left exposed.

Insect behaviors were recorded using 2 high definition video cameras (Sony Handycam HDR-CX100, Sony Corporation, Tokyo, Japan) at rates of 30 frames/sec (Cooper and Spurgeon 2011, Environ. Entomol. 40: 367 - 373). Unobstructed views of behaviors were obtained by recording from opposing sides of the Petri dish, with each camera elevated to point downward at an angle of 45° from horizontal. An overhead incandescent lamp and 2 small fluorescent lamps provided light. Cameras, lights, and the experimental arena were cloaked with a white cloth to minimize disturbance to the insects from activities within the laboratory. Each of 10 assays included 2 separate 2-h steps: (1) square preconditioning, and (2) a choice assay. Insect behaviors were recorded during both steps. During square preconditioning, the bottom portion of a 100-mm diam two-section compartmentalized Petri dish (part no. 351003; BD Falcon) was placed over the squares so that each Petri dish compartment enclosed a single square. A single prereproductive adult female released into 1 of the Petri dish compartments was confined to a single square for 2 h. Immediately after the 2-h preconditioning step, the compartmentalized Petri dish was replaced with a standard 100-mm diam Petri dish bottom (BD Falcon) to provide the insect uninhibited access to both squares during the 2-h choice assay. Before the choice assay was initiated, the insect was disturbed from the preconditioned square to enforce a choice between the 2 test squares.

Recorded videos were viewed using Adobe Premiere Pro CS4 (Adobe Systems, Mountain View, CA). If the insect did not settle on the square within the first hour of the preconditioning step, the assay was discarded and repeated with fresh squares and a new insect. Data collected from choice assays included the numbers of visits and stylet-probing events to each square. A visit was defined as any continuous period during which the insect physically contacted the square. The numbers of square visits and stylet-probing events were examined in separate analyses using logistic regression (PROC GLIMMIX, SAS 9.2). In the analysis of square visits, the dependent variable was the number of visits to the respective square treatments (preconditioned or not preconditioned) divided by the total number of visits to both squares occurring during each 2-h choice assay. Similarly, the dependent variable for analysis of feeding events was the number of stylet-probing events on each square divided by the total number of probing events occurring within the Petri dish during the 2-h choice assay. In both analyses, treatment was the fixed effect, and replication was the random variable.

The mean (±SE) total time the insects stylet-probed during the 2-h preconditioning step was 74 ± 10.6 min. The mean (±SE) number of square visits and stylet-probing events per adult during the 2-h choice assay was 1.5 ± 0.27 and 10.4 ± 2.01, respectively. The logistic regression analysis of square visits indicated the probability of an *L. hesperus* adult visiting the preconditioned square was significantly greater than the probability of the insect visiting the control square (Table 1). The patterns observed for stylet-probing activities were identical to those observed for square visits. The probability of an insect stylet-probing the preconditioned square was significantly greater than the probability of an insect stylet-probing the control square (Table 1). These results indicate that *L. hesperus* adults prefer to feed on squares that have been preconditioned by prior stylet-probing events compared with squares that have not been previously stylet-probed.

Results of this study demonstrate a previously unrecognized behavior of *L. hesperus* adults and will facilitate further elucidation of *Lygus* behavior and feeding injury to cotton. Additional study is required to determine whether *Lygus* locate previous feeding sites using host-plant volatiles released by stylet-probing activities (Rodriquez-Saona 2002, J. Chem. Ecol. 28: 1733 - 1747) or by other mechanisms such as attraction to salivary constituents. Further work is also needed to determine the ecological implications of square preconditioning and feeding preference on intact plants. For example, the propensity for *L. hesperus* to visit and feed upon previously stylet-probed squares may contribute to within-plant or within-field spatial aggregation. The tendency to revisit previously stylet-probed squares may also influence rates of feeding injury and square abscission. Responses to *Lygus* feeding differ among cotton cultivars (Laster and Meredith 1974, J. Econ. Entomol. 67: 686 - 688; Meredith and Laster 1975, Crop Sci. 15: 535 - 538); specifically, injured squares are more readily shed by some cultivars than by others. Crop management practices may also influence rates of square shed (Guin 1982, USDA Technical Bulletin No. 1672). Given that *L. hesperus* return to previously fed upon squares, factors that promote retention of injured squares may reduce overall crop injury by concentrating *Lygus* feeding on fewer squares. A better understanding of the mechanisms and ecological implications related to the propensity for *L. hesperus* to return to previous feeding locations could improve the design and interpretation of laboratory and greenhouse investigations of *Lygus* feeding injury.

Table 1. Probabilities and 95% confidence intervals corresponding to visits and stylet-probes by prereproductive *Lygus hesperus* female adults on control squares versus squares preconditioned by previous stylet-probing.

Square treatment	Propensity to visit squares	Propensity to stylet-probe squares
Control	0.27, 0.09 - 0.58	0.21, 0.14 - 0.32
Preconditioned *	0.73, 0.42 - 0.91	0.79, 0.68 - 0.87
	$F = 6.0$; df = 1, 9; $P = 0.04$	$F = 60.1$; df = 1, 9; $P < 0.01$

*Squares were stylet-probed by an *L. hesperus* adult during the 2-h period before choice assays.

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