FEEDING INJURY TO COTTON CAUSED BY *LYGUS HESPERUS* (HEMIPTERA: MIRIDAE) ADULTS OF DIFFERENT REPRODUCTIVE STATES

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Abstract

Lygus hesperus Knight is a key pest of cotton (*Gossypium* spp.) in the western United States. *Lygus*-induced injury is considered to be highly variable, and no clear relationship between *Lygus* population level and plant injury has been established. A likely explanation for this variability is failure to account for important sources of biological variation among experimental insects. Recent video-based laboratory studies indicated that feeding behaviors and trivial movements differed among classes of adult *Lygus* of different gender and reproductive state (pre-reproductive, reproductive and unmated, and reproductive and mated). Therefore, we assessed the feeding injury to greenhouse-grown cotton plants caused by *L. hesperus* adults of different gender and reproductive states. Cotton plants were grown in single rows within 680-l planters (blocks) to the 'match-head' square stage. The experimental design was an incomplete block with gender assigned to blocks and reproductive state assigned to plants within blocks. Individual plants, enclosed within acrylic cages with nylon mesh windows, were each infested with one adult *L. hesperus*. Insects were removed from the cages after 1 wk. Feeding injury was evaluated by counting the numbers of missing squares and squares with injured anthers. Pre-reproductive adults caused significantly more square abscission and square injury than did reproductive adults, but feeding injury did not differ by mating status or gender. These results are consistent with results of our previous video-based assays, and indicate adult reproductive state represents a source of variation that must be controlled in studies to evaluate *Lygus*-induced injury to cotton.

Introduction

Damage to cotton (*Gossypium* spp.) caused by *Lygus hesperus* Knight is perceived as being highly variable because observed fruit losses are often inconsistent with estimates of *Lygus* populations (Rosenheim et al., 2006). Previous studies that investigated *Lygus* stage-dependent injury to cotton have reported conflicting conclusions (Scales and Furr, 1968; Gutierrez et al., 1977; Mauney and Henneberry, 1984; Leigh et al., 1988). Whereas some studies indicate that adults cause more damage than nymphs (Gutierrez et al., 1977; Zink and Rosenheim, 2005), other studies indicate the opposite (Jubb and Carruth, 1971) or indicate no difference in damage caused by nymphs and adults (Rosenheim et al., 2006). Because of the inconsistencies in results among published studies, it is not possible to explicate clear patterns corresponding to *Lygus* stage-dependent square loss.

Behavioral variations among adults represent a possible explanation for the inconsistencies among published studies of *Lygus*-induced square loss. We recently determined that feeding behaviors and trivial movements varied among *L. hesperus* adults of different gender and reproductive state (pre-reproductive, reproductive and unmated, and reproductive and mated) (Cooper and Spurgeon, 2010). In general, pre-reproductive adults fed more and exhibited fewer trivial movements compared to reproductive adults. It is not known, however, whether this observed variation corresponds with variation in feeding injury to intact plants. Therefore, our objective was to compare feeding injury to squaring cotton caused by *L. hesperus* adults representing combinations of gender and reproductive state.

Materials and Methods

We obtained *Lygus* adults of different gender and reproductive states (pre-reproductive, reproductive and unmated, and reproductive and mated) from a laboratory colony maintained on green beans (*Phaseolus vulgaris* L.) and sunflower seeds (*Helianthus annuus* L.). Experimental insects were ≤4 generations removed from the field population, and were obtained from the colony within 24 h of adult eclosion. *Lygus* adults do not complete reproductive development until about 5-d after adult eclosion (Strong et al. 1970; DWS and WRC personal observations), thus adults ≤4 days old were considered pre-reproductive. Most *Lygus* adults kept in mixed-gender colonies mate by 7 d after adult eclosion (Strong et al. 1970; Brent 2010; DWS and WRC personal observations). Therefore, adults >7 d old were considered reproductive. Once newly emerged adults were obtained, those designated as unmated and reproductive were held for 7-10 d in same-gender groups of <100 insects within 4-l clear

plastic rearing containers provisioned with green bean pods. Adults designated as mated and reproductive were similarly held in mixed-gender groups. Pre-reproductive adults were obtained from the colony on the same day they were released on plants.

Acala cotton plants (Phytogen 72, Bayer CropScience, Research Triangle Park, NC) were grown in single rows within 680-l greenhouse planters. When plants reached the 'match-head' square phenological stage (largest squares 3-6 mm diameter), three plants per planter were chosen based on similarity of plant size. Each experimental plant was enclosed within an acrylic cage (91 × 91 × 156 cm, L × W × H) ventilated by nylon mesh windows. The experimental design was an incomplete block. Each planter was considered a block and was randomly assigned an insect gender. Each cage within a block was randomly assigned an insect reproductive state.

A single insect was released into each cage before noon. Insects were monitored daily for mortality, and dead *L. hesperus* were immediately replaced. All insects were replaced after the first 3 d to maintain the assigned reproductive status. After 7 d, insects and cages were removed and the plants were treated with Imidacloprid (Provado 1.6F, Bayer CropScience) to eliminate any nymphs hatching in cages occupied by reproductive females. Seven days after *L. hesperus* were removed, each plant was assessed for missing or blasted squares. Preliminary tests indicated that squares >3 mm diameter that were fed upon by *Lygus* may not exhibit external signs of feeding despite containing discolored and shriveled anthers. Therefore, each square >3 mm in diameter was removed from the plant and dissected to assess the anthers for *L. hesperus* feeding injury.

The experiment was conducted 3 times (replications). One replication was conducted in mid-July 2010 (n=6 blocks/gender, N=36 total plants) and another in early October 2010 (n=5 blocks/gender, N=30 total plants) within a greenhouse under supplemental lighting provided by 1000-W high-pressure sodium lamps. The third replication (n=6 female blocks, 5 male blocks, N=33 total plants) was conducted in mid-July 2010 in a separate greenhouse under supplemental lighting provided by 300-W LED grow lights (GrowPanel Pro 300, SunShine Systems, Wheeling, IL). In each replication, artificial lighting ensured a day length of at least 14 h.

Dependent variables for respective analyses of *L. hesperus* feeding injury were 1) the number of squares >3 mm in diameter exhibiting injured anthers, and 2) the number of abscised squares. Both analyses were conducted using PROC GLIMMIX (SAS ver. 9.2, SAS Institute, Cary, NC) with insect gender, reproductive state, and their interaction as independent variables, and replication, block, and rep×block×gender as random variables. Data were examined for heterogeneity of variance and non-normality of errors by inspecting residual and normal quantile-quantile plots, respectively. Based on these plots, untransformed data were used for each analysis.

Results and Discussion

L. heperus gender did not significantly influence the number of squares with injured anthers (F=0.3; df=1, 34.6; P=0.61). However, the number of squares >3 mm in diameter with injured anthers was greater for cotton plants exposed to pre-reproductive adults than for plants exposed to either mated or unmated reproductive adults (F=6.5; df=2, 69.6; P<0.01) (Figure 1A). The lack of a significant gender × reproductive state interaction (F=0.5; df=2, 69.6; P=0.60) indicated that the effect of *Lygus* reproductive state on anther injury was similar for both genders. Gender also did not influence square abscission (F=1.2; df=1, 27.3; P=0.28), and the gender × reproductive state interaction for square abscission was not significant (F=1.5; df=2, 62.8; P=0.24). However, the number of squares that abscised was greater for plants exposed to pre-reproductive adults than for plants exposed to either unmated or mated adults (F=17.0; df=2, 62.8; P<0.01) (Figure 1B). In preliminary studies, none of 21 control plants grown in greenhouse conditions exhibited injured anthers or square abscission. We therefore assume that square abscission observed in our study was attributed to *Lygus* feeding. When numbers of squares that abscised were added to numbers with injured anthers, pre-reproductive adults damaged a total of nearly 3× more squares (6.5/plant) compared with reproductive adults (2.3/plant), regardless of gender or mating status.

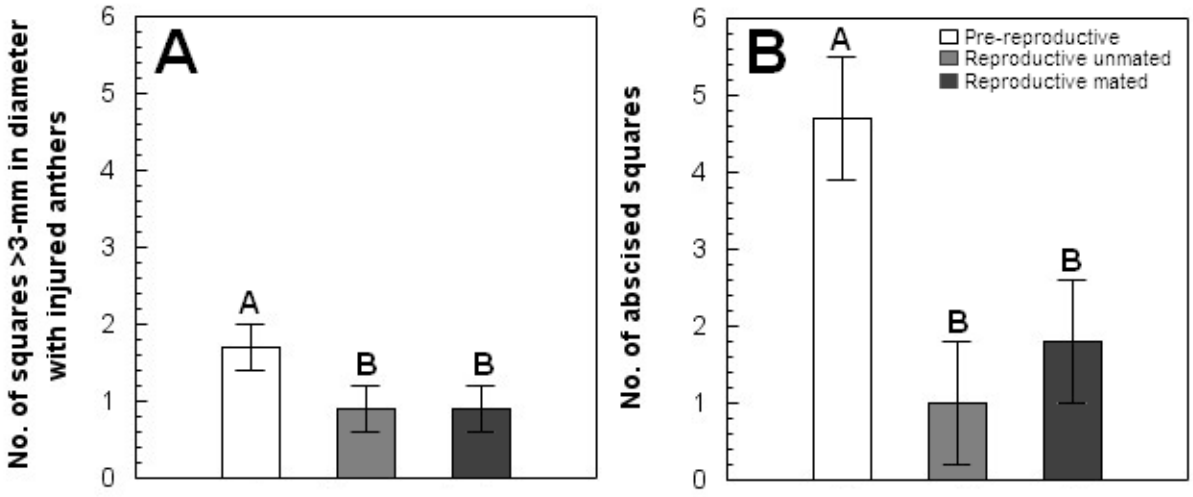

Figure 1. (A) The mean number of squares >3 mm in diameter with injured anthers per plant and (B) the mean number of abscised squares per plant. Values with different letters are significantly different (α=0.05), and error bars reflect standard errors.

The greater amount of feeding injury caused by pre-reproductive adults compared with reproductive adults in our greenhouse assays was consistent with results from our previous video-based behavior assays, which indicated that pre-reproductive adults generally fed more and were less active compared to reproductive adults (Cooper and Spurgeon, 2010). These video-based assays also indicated that mated females exhibited more short duration stylet-probes than did adult *L. hesperus* of other gender × reproductive state combinations (Cooper and Spurgeon, 2010). Despite exhibiting numerous short duration stylet-probes in laboratory behavior assays, mated females did not cause more injury to cotton squares on intact plants compared with other gender × reproductive state combinations. The short duration stylet-probes by mated females are likely associated with oviposition behaviors (Romani et al., 2005; Cooper and Spurgeon, 2010; Cooper and Spurgeon, unpublished). Because *Lygus* typically oviposit on plant stems and leaf peduncles (Benedict, 1983), it is likely that the numerous ovipositional stylet-probes typical of reproductive females were made to tissues other than squares in our greenhouse assays.

Dissected squares that exhibited internal feeding injury to the developing anthers generally did not exhibit external symptoms of feeding 7 d after *L. hesperus* were removed from the plants. These injured squares would likely have continued development despite the occurrence of *Lygus* feeding. The potential for this type of injury to reduce cotton yield or impact fiber quality has not been conclusively determined. Square abscission, however, may cause direct yield losses or may lead to delayed crop maturity in the absence of sufficient plant compensation. Of the total plant injury (abscised plus injured squares) caused by pre-reproductive adults, 64% was represented by square abscission compared with only 36% for reproductive adults. These observations suggest that pre-reproductive adults not only cause more total injury to cotton plants than do reproductive adults, but the type of injury inflicted by pre-reproductive adults has greater potential to cause yield reductions than does the injury inflicted by reproductive adults. This observed trend warrants further investigation.

Summary

Our study confirms our previous video-based behavior assays indicating that 'adult' *Lygus* is a heterogeneous classification (Cooper and Spurgeon, 2010). Collectively, these studies indicate that compared to reproductive adults, pre-reproductive adults feed more and exhibit fewer trivial movements while inflicting 3× more damage to cotton plants. Previous studies of *Lygus*-induced square loss did not account for variations in adult age, and produced highly variable and conflicting conclusions. Our results clearly demonstrate adult reproductive state represents a source of variation that must be controlled in greenhouse and laboratory studies to facilitate a meaningful evaluation of *Lygus*-induced host injury.

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