Feeding Preference by Stink Bugs (Heteroptera: Pentatomidae) for Seeds Within Soybean Pods[1]

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ABSTRACT Studies were conducted in the laboratory and in growth chambers to determine if the location of particular seeds within soybean pods were preferred by adult *Nezara viridula* (L.), *Euschistus heros* (F.), and *Piezodorus guildinii* (Westwood) (Heteroptera: Pentatomidae). Results demonstrated that feeding frequency, as indicated by the number of punctures (i.e., stylet sheaths), was significantly greater ($P < 0.05$) on the proximal seed, than on other seeds, both for detached pods and pods on plants. In general, the number of punctures on the proximal seed was 2-3X greater than punctures observed in the medial or distal region. Of the three species studied, *N. viridula* showed the greatest feeding activity and *P. guildinii* the least. No significant differences in feeding activity between sexes were observed. *N. viridula* fed more frequently at 22 than at 29°C, while no significant temperature effect was observed for *E. heros*.

KEY WORDS Insecta, stink bug, soybean, feeding behavior, stylet sheath

Among the several insect species that feed on soybean, *Glycine max* (L.) Merrill, stink bugs are major pests in many areas of soybean cultivation in the world (Kogan and Turnipseed 1987). Interactions between stink bugs and soybean, particularly with regard to stink bug impact on seed yield and quality have been investigated (see reviews by Todd and Herzog 1980, Panizzi and Slansky 1985a). However, little research has been conducted on the feeding behavior of stink bugs on soybean pods.

Previous studies have examined feeding frequency, duration, daily rhythm, insect density, seed quality, and seed quantity on the feeding processes of several hemipteran pests of soybean (e.g., Bowling 1980, Kawamoto et al. 1987, Simmons and Yeargan 1988). However, we have not found any reference in the literature regarding feeding preferences of stink bugs on specific seeds within the soybean pod. Therefore, this study was conducted to investigate the feeding preference of adult *Nezara viridula* (L.), *Euschistus heros* (F.), and *Piezodorus guildinii* (Westwood) (Heteroptera: Pentatomidae) by determining adult feeding frequency on seeds located at different positions within the soybean pod. In this study we assumed that the greater number of stylet sheaths the greater the feeding preference, and not unavoidably feeding intensity, because feeding time was not measured.

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Materials and Methods

Laboratory study. To study feeding preferences of stink bugs for seeds within pods, two laboratory tests were conducted in April 1992. Ten pairs of *N. viridula, E. heros* and *P. guildinii* were collected from soybean fields at the EMBRAPA Field Research Station, Warta District, Londrina County, Northern Paraná, Brazil. Insects were transported to the laboratory and placed into plastic rearing boxes lined with filter paper, having a wet cotton ball on a plastic lid, and containing soybean pods 'Paraná, at full bean stage (R6 [Fehr and Caviness 1977]). The following day food was removed, and insects fasted for 24 h. After this period, one insect of each species and sex was placed individually in a Petri dish (9.0 × 3.5 cm) with filter paper and one soybean pod at R6 containing three seeds. Pods were obtained from greenhouse-grown plants and were free of stink bug damage. Dishes were placed at random in an environmental chamber at 25 ± 1°C, 60 ± 5% RH, and 14h:10h (L:D) photoperiod.

Stink bugs were allowed to feed for 48 h. After this time, soybean pods were removed and soaked in an acid fuchsin solution (1%) for 3 h. At the end of this period, pods were washed in running tap water, and the number of punctures (i.e. stylet sheaths) on each region containing a seed was recorded (i.e., proximal = the region closest to the pedicel; medial = the intermediate region; and distal = the more distal region to the pedicel; see Fig. 1). The percentage of punctures made by females and males of each species at each region was calculated. Data were analyzed with analysis of variance (ANOVA) and means transformed to arscine before being compared by Duncan's multiple range test at $P = 0.05$, using SANEST (Zonta and Machado 1992). Comparisons between sexes were made using Student's t test. The total number of punctures per pod also was calculated and compared among species using Duncan's multiple range test, and between sexes within the same species using the t test.

Growth chamber study. Additional studies were conducted during April 1994, using soybean pods attached to the plant. Fifth instars of *N. viridula* and *E. heros* (*P. guildinii* was not available) were field collected at the same location as described for the laboratory study. Nymphs were placed with soybean pods in plastic rearing boxes, similar to the ones used in the laboratory studies, and then placed in an environmental chamber. Upon adult emergence, females and males were placed individually in plastic Petri dishes lined with moistened filter paper, containing soybean pods at stage R6. Adults were allowed to feed for 48 h before they were selected to be used in the test. Potted soybean plants at R6, obtained from the greenhouse, were used for experimentation. Before infestation, the number of pods per plant was reduced to 15, each with three seeds. Plants were individually caged with netting supported by two wire frames to mold the netting into a cylindrical shape. Twelve plants were placed at random in a Percival growth chamber regulated at 22°C and 14:10 h (L:D) photoperiod. Each plant was infested with a pair of *N. viridula* (n = 6) or *E. heros* (n = 6), for 72 h. A second set of 12 plants was placed in a second growth chamber regulated at 29°C and 14:10 h (L:D) photoperiod and infested as in the 22°C chamber. At the end of the infestation period, pods were removed from the plants and transported to the laboratory. Pods were immediately soaked in the acid fuchsin solution for 3 h, washed in running tap water, and the number of stylet sheaths

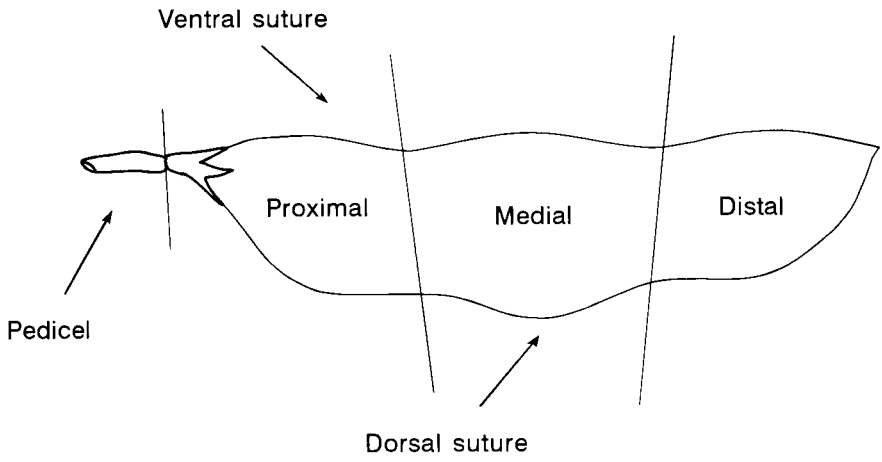

Fig. 1. Schematic representation of a soybean pod with three regions delimited. Proximal = region proximal to the pedicel; Medial = intermediate region; and Distal = distal region.

at each position recorded. The percentage of stylet sheaths in each region was calculated for each pod and averaged across the 15 pods on a plant to obtain a single value. Data were analyzed with analysis of variance (ANOVA) and Duncan's multiple range test, as described for the laboratory study. The total number of punctures per pod also was calculated and compared between species at each temperature and between temperatures within each species using student's *t* test.

Results

Laboratory study. Results of the laboratory studies indicated that, for all three species of stink bugs, the feeding preference was for the proximal seed of the soybean pod. In general, the feeding incidence by female and male *N. viridula* was over two times greater (58% for females and 51% for males) on the proximal seed compared to the medial (20-20%) or distal (22-29%) seeds (Fig. 2). However, no significant differences were observed for this species between seeds on the medial and distal regions. Similar results were obtained with adult *E. heros* and *P. guildinii.* For female and male *E. heros,* 50-49% of the stylet sheaths were on the proximal seed, while the medial seed had 18-21%, and the distal had 31-30%. For female and male *P. guildinii* the percentage stylet sheaths on the proximal seed was 51-62%, decreasing to 15-17% for the medial, and to 33-20% for the distal seed (Fig. 2). Data from all three species indicated the percentage stylet sheaths on the proximal seed ranged from 49 to 62% of the total feeding punctures.

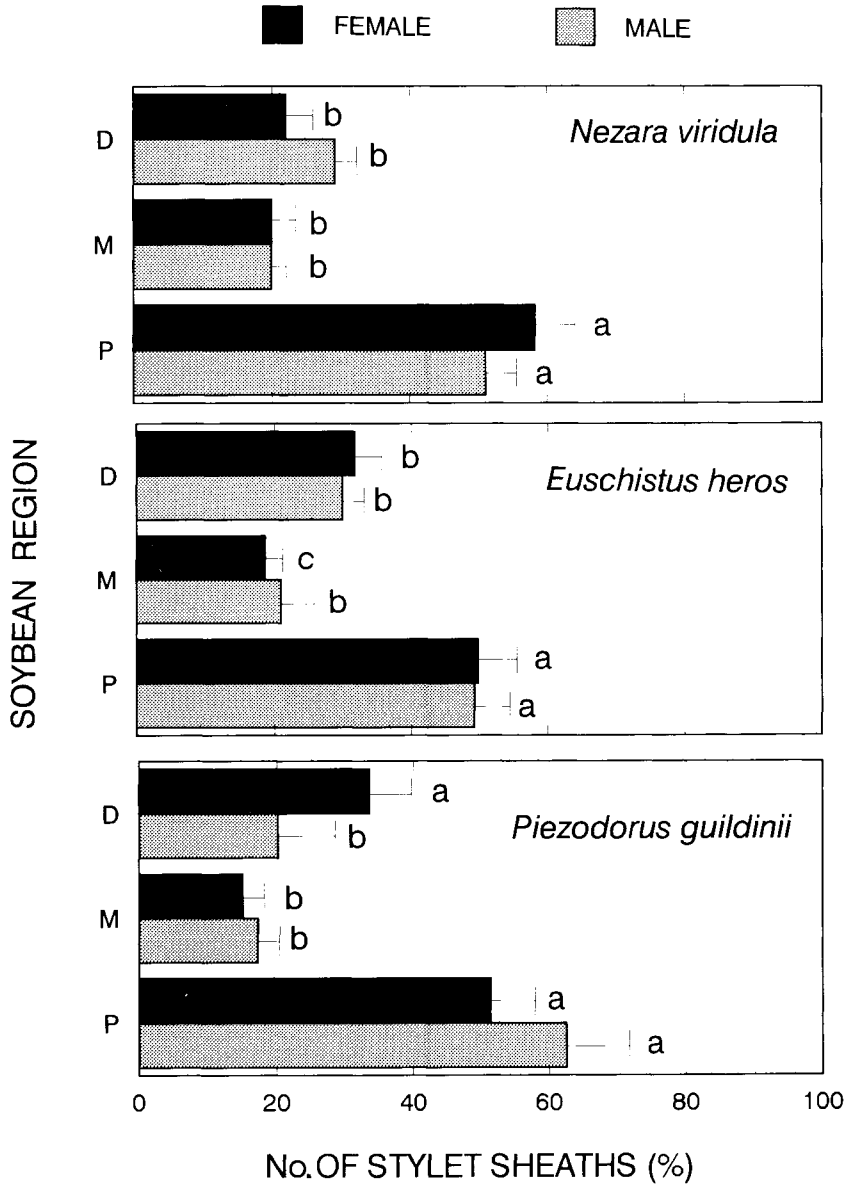

Fig. 2. Mean (+ SEM) percentage of stylet sheaths deposited on different regions of a three-seeded soybean pod by adult *Nezara viridula*, *Euschistus heros,* and *Piezodorus guildinii.* Soybean pods were offered individually and detached from the plant in the laboratory. Means followed by the same letter for each species and sex, are not significantly different ($P = 0.05$) using Duncan's multiple range test.

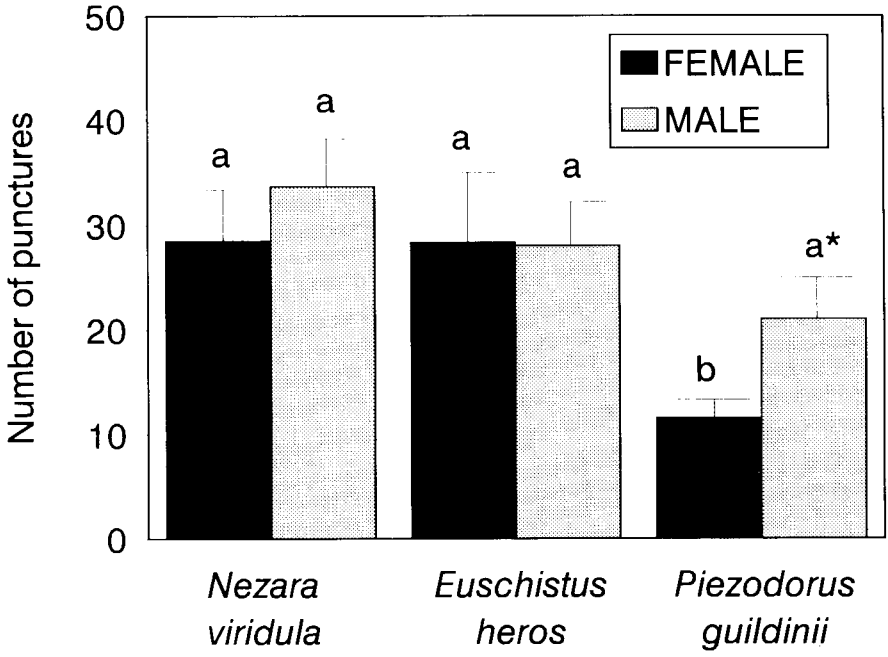

Fig. 3. Mean (+ SEM) number of stylet sheaths deposited on a soybean pod by adult *Nezara viridula*, *Euschistus heros,* and *Piezodorus guildinii.* Soybean pods were offered individually and detached from the plant in the laboratory. Means followed by the same letter are not significantly different ($P = 0.05$) using Duncan's multiple range test. Asterisk indicates significant difference ($P = 0.05$) between sexes and using student's t-test.

Considering the total number of punctures per pod, *N. viridula* and *E. heros* females showed greater feeding activity than *P. guildinii* females (Fig. 3). Males of *N. viridula* and *E. heros* also tended to feed in greater frequency than males of *P. guildinii,* although no significant difference was observed. In general, females and males of the same species demonstrated similar feeding activity, except for *P. guildinii* males which fed with greater intensity than females (Fig. 3).

Growth chamber study. Results of the feeding location on the three regions of the soybean pod by adult *N. viridula* and *E. heros,* using potted soybean plants in growth chambers, were consistent with results obtained in the laboratory using detached pods. For both tests (at 22 and 29°C) with *N. viridula,* over 40% of the total stylet sheaths were observed on the proximal region, with no significant differences between the number of stylet sheaths at the medial and distal regions (< than 30%, except for the medial region at 29°C with 34%) (Fig. 4). Approximately 54% of the punctures by *E. heros* at both temperatures were observed at the proximal region, dropping to less than 25% for each of medial and distal region (Fig. 4).

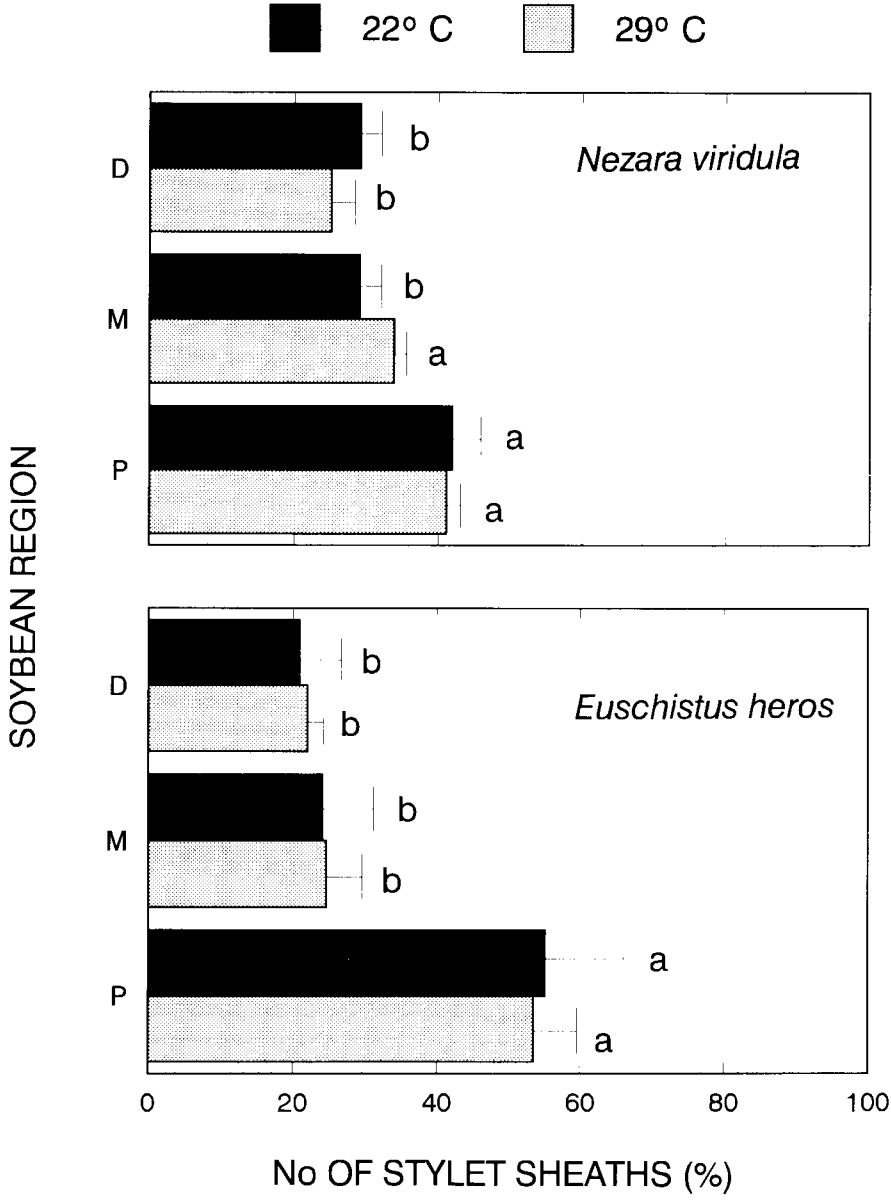

Fig. 4. Mean (+ SEM) percentage of stylet sheaths deposited on different regions of a three-seeded soybean pod by adult *Nezara viridula* and *Euschistus heros*. Soybean pods were obtained from potted caged plants artificially infested. Means followed by the same letter for each species at each test (plants in growth chambers at 22 and 29°C) are not significantly different ($P = 0.05$) using Duncan's multiple range test.

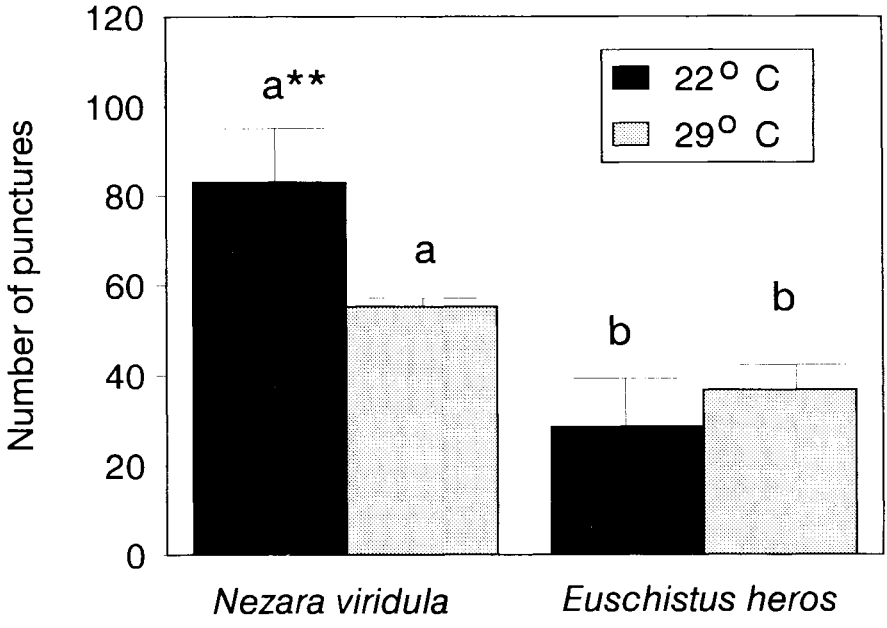

Fig. 5. Mean (+ SEM) number of stylet sheaths deposited on soybean pods by adult *Nezara viridula* and *Euschistus heros*. Soybean pods were obtained from potted caged plants artificially infested in growth chambers. Means followed by the same letter between species at each temperature are not significantly different ($P = 0.05$) using Student's t-test. Asterisks indicate highly significant ($P = 0.01$) differences between temperatures for the same species using Student's t-test.

A significantly greater number of punctures was observed for *N. viridula* than for *E. heros* at both temperatures (Fig. 5). *N. viridula* showed a significantly greater feeding activity at 22 than at 29°C, while no significant temperature effect was observed for *E. heros*.

Discussion

Results of these laboratory and greenhouse studies indicated that *N. viridula, E. heros,* and *P. guildinii* show variable feeding preferences according to the position of the seed in the soybean pod. In pods with three seeds, the seed located closest to the pod pedicel is more preferred than those located on the more distal regions, as suggested by the location of the stylet sheaths remaining after a feeding session. When pods are attached to the plants, this preference may be explained by the observation that bugs walking on the plant and encountering a soybean pod, may start to feed on the first seed found, i.e., the one located closest to the pod pedicel

(Fig. 1). However, results from the laboratory studies with detached pods, indicating a feeding preference for seeds at the proximal region, suggest that feeding preference is based on more than the seed position. In this case seeds should have an equal chance to be pierced by stink bugs, and the involvement of possible physical or chemical factors causing the feeding preference for a particular bean seems remote. Nutrients, water, and allelochemical concentration are variable among soybean seeds of different ages (e.g., Krivoruchco et al. 1979, Rüdiger 1984). If seed filling in soybean occurs proximally to distally, i.e., the proximal seeds fill before the distal seeds, it seems likely that if there are nutrient differences among the seeds, the proximal seeds would have higher levels of nutrients. Consequently, a simple discrimination based on nutrient preference might explain our results. Even in situations where there are no great differences (e.g., late R6 - full seed), there may be some learned feeding behaviors on the part of the stink bugs.

Kawamoto et al. (1987), studying the feeding behavior of the pentatomids *Halyomorpha mista* Uhler, *Nezara antennata* Scott, and *Piezodorus hybneri* Gmelin, and of the alydid, *Riptortus clavatus* Thunberg, on soybean pods, found that beans were randomly selected irrespective of bean size and damage history, except for very small and severely damaged beans. However, possible feeding preferences based on bean position in the pod was not examined. The observed greater feeding frequency of *N. viridula* than the other two species may be a result of its greater body size (fresh body weight of newly-emerged females feeding on soybean pods is approximately 150 mg for *N. viridula* [Panizzi and Slansky 1991], approximately 70 mg for *E. heros* [Pinto and Panizzi 1994], and approximately 55 mg for *P. guildinii* [Panizzi and Slansky 1985b]), and consequently greater need for nutrients. However, greater feeding frequency does not necessarily result in higher levels of food ingested. The decrease in feeding frequency by *N. viridula* at a higher temperature is in agreement with its adaptability to temperate or sub-tropical zones (DeWitt and Godfrey 1972). For the neotropical *E. heros* the reverse was observed. In Brazil, *N. viridula* is better adapted to cooler regions of the south, while *E. heros* prefers the more tropical areas (Panizzi and Slansky 1985, Cividanes 1992). In conclusion, these results demonstrate that stink bugs feeding on soybean pods prefer the seed proximal to the pod pedicel. This information may be useful for more accurate assessments of their damage to soybean or in the decision-making process for their control.

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