FEEDING FREQUENCY, DURATION AND PREFERENCE OF THE SOUTHERN GREEN STINK BUG (HETEROPTERA: PENTATOMIDAE) AS AFFECTED BY STAGE OF DEVELOPMENT, AGE, AND PHYSIOLOGICAL CONDITION

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ABSTRACT

Laboratory studies with the southern green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae) indicated that the number of flanges (stylet sheaths) deposited per day on soaked mature soybean seed increased from 2 to 12 as the nymphs developed from the 2nd to 5th instar. Second and 5th instars fed for longer periods compared with 3rd and 4th instars. Nymphs preferred soybean pods (immature) over raw shelled peanuts, particularly the early instars. Frequency of flanges deposited by adults increased from day 1 to day 4, and decreased thereafter. The number of flanges laid by adults previously deprived of food for 24 hours, did not differ from those fed continuously; but fasted adults fed for longer periods than non-fasted. During 4-hour periods of observation, adults were not observed to feed on emergence day, nor on day 16; feeding time reached a maximum on day 2 and tended to decrease on days 4 and 8. Unlike nymphs, adult *N. viridula* had no preference between soybean pods and peanuts.

KEY WORDS: Insecta, *Nezara viridula*, *Glycine max*, stylet sheath, nutritional ecology.

RESUMO

Frequência, Duração e Preferência Alimentar do Percevejo Verde (Heteroptera: Pentatomidae) Afetadas pelo Estágio de Desenvolvimento, Idade e Condição Fisiológica

Estudos em laboratório com o percevejo verde, *Nezara viridula* (L.) (Heteroptera: Pentatomidae) indicaram que o número de bainhas alimentares depositadas/dia sobre sementes de soja maduras e hidratadas aumentou de 2 para 12 a medida que as ninfas passaram do 2° ao 5° ínstar. Ninfas de 2° e 5° ínstares alimentaram-se por mais tempo que ninfas de 3° e 4° ínstares. As ninfas preferiram vagens de soja (imaturas) a sementes de amendoim, principalmente ninfas jovens. A frequência das bainhas depositadas por adultos aumentou do dia 1 ao dia 4, decrescendo após esse período. O número de bainhas depositadas por adultos que jejuaram por 24 horas e de adultos que se alimentaram continuamente não diferiu.

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Entretanto, adultos jejuados alimentaram-se por mais tempo que adultos não-jejuados. Durante períodos de quatro horas de observação, adultos não se alimentaram durante o dia da emergência ou no 16° dia. O tempo de alimentação atingiu o máximo no dia 2 e tendeu a decrescer nos dias 4 e 8. Diferente das ninfas, adultos de *N. viridula* não demonstraram preferência alimentar entre vagens de soja e sementes de amendoim.

PALAVRAS-CHAVE: Insecta, *Nezara viridula*, *Glycine max*, bainhas, ecologia nutricional.

INTRODUCTION

The southern green stink bug, *Nezara viridula* (L.), is perhaps the most studied of the phytophagous pentatomids feeding on crops of economic importance. Many aspects of its association with soybean [*Glycine max* (L.) Merrill] have been studied (Todd & Herzog 1980, Panizzi & Slanski 1985). Nevertheless, feeding behavior, including the frequency and duration of feeding of nymphs and adults on soybean seeds, has seldom been investigated.

Seed suckers pierce seeds with their stylets, inject a watery saliva containing digestive enzymes, and suck out the liquefied seed contents (Miles 1972). In addition to the digestive saliva, many hemipterans produce a saliva that solidifies to form a stylet sheath or a "flange" (Nault & Gyrisco 1966), which remains in plant tissues and can be used to estimate feeding frequency of these insects (Bowling 1979, 1980). Damage to seeds is a result of frequency of stylet penetration and feeding duration, associated with salivary secretions that can be toxic and cause tissue necrosis (see Slansky & Panizzi, 1987 for further details on seed-suckers feeding behavior).

This study was conducted to compare *N. viridula* feeding frequency and duration among nymphs of different instars and among adults of different ages and physiological condition (deprived of food vs. non-deprived). Moreover, we also wanted to determine if there was a feeding preference by nymphs and adults for the foods used to rear them in the laboratory (i.e., immature soybean pods and raw shelled peanuts, *Arachis hypogaea* L.).

MATERIAL AND METHODS

Egg masses of *N. viridula* were obtained from a laboratory culture maintained in rearing cages (50.0 x 50.0 x 70.0 cm) containing soybean plants cv. 'Paraná', dried soybean seeds and raw shelled peanuts, at the Centro Nacional de Pesquisa de Soja of EMBRAPA in Londrina, Paraná. Egg masses were taken to the rearing facilities of the National Institute of Agro-Environmental Sciences (NIAES) at Tsukuba (import permission 3Y2072, Ministry of Agriculture, Forestry and Fisheries, Japan) and maintained in a walk-in chamber at 25 ± 1° C and a photoperiod of 16:8 (L:D)h.

Feeding Frequency. To study the feeding frequency of nymphs, early 2nd instars (n=40) were placed individually in plastic petri dishes (9.0 x 1.5 cm) with moistened filter paper and with a water soaked mature soybean seed cv. 'Suzuyutaka', placed on a moistened cotton ball. Nymphs were allowed to feed during 24 hours, 1-2 days after the molt. After this period, seeds were submerged in an acid fuchsin solution (1.0%) for 3 hours; excess stain was removed with flowing tap water and the stylet sheaths or the "flanges" formed on the exterior (Nault & Gyrisco 1966) were counted using a stereomicroscope (Bowling 1980). The number of times

each nymph fed per day was calculated based on the number of flanges deposited. Although the presence of a flange is not always continued as a stylet sheath into the substrate (Miles 1972) and do not always mean that food was ingested, we considered its presence as feeding activity. This procedure was repeated for the 3rd (n=40), 4th (n=38), and 5th (n=33) instars.

The feeding frequency of adults ages: 1 day (emergence day) (n=18), 2 days (n=20), 4 days (n=16), 8 days (n=20), and 16 days (n=20) were compared as described for the nymphs. In addition, feeding frequency of adults fasted and non-fasted was compared. Twenty adults (5-11 days old) were allowed to feed on soybean pods and peanuts; these were compared with another 20 adults which were fasted (but were provided water) for 24 hours, before the test. In general, the methodology was the same as described for the nymphal study.

Feeding Duration. To compare the feeding duration, observations were made on the nymphs and on the adults (of different ages and fasted/non-fasted) used in the feeding frequency study. To avoid continuous observation, we followed the methodology used by Simmons & Yeargan (1988) which entailed observing feeding activity at 15 minutes intervals and recording if insects were feeding or not. Each insect was tested for four hours. Based on the frequency of feeding over this period, total feeding duration including all feeding sessions was calculated per nymph for each instar and per adult at each age and fasted/non-fasted. Mean total feeding duration was calculated for 23, 26, 31, and 28 nymphs for the 2nd, 3rd, 4th, and 5th instar, respectively, and for 11, 2, and 8 adults at days 2, 4 and 8, plus 6 fasted and 6 non-fasted adults. The percentage of insects that showed feeding activity was also calculated.

Feeding Preference. Thirty six nymphs and adults were placed individually in plastic petri dishes (9.0 x 1.5 cm) containing one soybean pod at R6 (maximum seed size) stage and one peanut seed, with filter paper and a wet cotton ball. Dead insects were replaced with same age. Twice daily the nymphs and adults were observed and the food feeding on was recorded. The number of observations were 6 (2nd instar), 6 (3rd), 9 (4th), 15 (5th), and 4 (adult). The percentage of cohort of each instar and of adults observed feeding on each food was calculated.

Data from the tests were analyzed with analysis of variance, Duncan's multiple range test (Duncan, 1955), t-test, and chi-square test (Snedecor & Cochran 1980). The level of significance for all tests was $P < 0.05$.

RESULTS

Feeding Frequency. The number of flanges deposited per day by *N. viridula* nymphs increased as the nymphs developed from the 2nd to the 4th instar (from ca. 2 to 11), and leveled off by the 5th instar (ca. 12) (Table 1).

Feeding frequency of adult *N. viridula* estimated for day 1 was greater than for day 4. At adult emergence, there was little feeding frequency (3 flanges deposited). During the following day (day 2), the number of flanges was almost 3X greater, and reached a maximum on day 4 (10); less than 5 flanges/day were recorded on days 8 and 16 (Table 2).

Feeding Duration. Observations of time spent feeding by nymphs (made during a 4-hour period), demonstrated that 2nd instars, despite having a relatively low feeding frequency, feed for longer periods (ca. 130 minutes), which was similar to feeding by 5th instars (ca. 120 minutes). Nymphs of 3rd and 4th instars fed for shorter periods (ca. 80 minutes) (Table 1).

Table 1. Mean (± SEM) number of flanges deposited per day and estimated feeding duration of *Nezara viridula* nymphs when fed a soaked mature soybean seed in the laboratory (number of nymphs in parentheses).

Instar	N° of flanges/day[1,2]	Feeding duration, minutes[1]	% of cohort fed
Second	2.2 ± 0,4 c (40)	130.4 ± 12.8 a (23)	57.5
Third	6.2 ± 0.5 b (40)	76.7 ± 7.8 b (26)	65.0
Fourth	11.3 ± 0.9 a (38)	83.4 ± 7.9 b (31)	81.6
Fifth	11.9 ± 1.1 a (33)	118.9 ± 10.1 a (28)	84.8

[1]Means followed by the same letter in each column are not singnificantly different (P > 0.05) using Dundan's (1955) multiple range test.
[2]Data were transformed to log (0.5 + x) before analysis.

Feeding duration estimated for adult *N. viridula* varied with age. On the day of emergence, adults were not observed to feed. On day 2, insects were observed to feed for about 1.5 hour, during the 4-hour of observation. Feeding duration on days 4 and 8 was about 1 hour. On day

Table 2. Mean (± SEM) number of flanges deposited per day and estimated feeding duration of *Nezara viridula* adults at different ages when fed a soaked mature soybean seed in the laboratory (number of nymphs in parentheses).

Insect age	N° of flanges/day[1]	Feeding duration, minutes[1]	% of cohort fed
Day 1	2.8 ± 0.7 b (18)	0.0	0.0
Day 2	8.0 ± 1.2 a (20)	88.2 ± 13.6 a (11)	55.0
Day 4	10.1 ± 1.1 a (16)	52.5 ± 7.5 a (2)	12.5
Day 8	5.3 ± 0.8 b (20)	54.4 ± 14.2 a (8)	40.0
Day 16	3.0 ± 0.5 b (20)	0.0	0.0

[1]Means followed by the same letter in each column are not significantly different (P > 0.05) using Dundan's (1955) multiple range test. Data of females and males were combined.

16, no adults were observed to feed (Table 2), which is confirmed by observation of the penetration frequency for adults on this day, when few flanges were deposited on soybean seeds. Feeding duration of adult *N. viridula* previously fasted was significantly greater (about 2X) as compared with non-fasted adults (Table 3).

Table 3. Mean (± SEM) number of flanges deposited and estimated feeding duration of *Nezara viridula* adults fasted and non-fasted when fed a soaked mature soybean seed in the laboratory during 1-day (number of adults in parentheses).

Insect feeding condition	N° of flanges/day[2]	Feeding duration, minutes[2]	% of cohort fed
Fasted[1]	5.2 ± 0.8 a (20)	92.5 ± 16.1 a (6)	30.0
Non-fasted	4.1 ± 0.7 a (20)	45.0 ± 9.5 b (6)	30.0

[1]Insects (5-11 days old) were starved for 24 hours in the presence of water before to the test.
[2]Means followed by the same letter are not significantly different ($P > 0.05$) using t test. Data of females and males were combined.

Feeding Preference. More *N. viridula* nymphs fed on soybean pods (immature) than on peanuts (Fig. 1). This was particularly evident for 2nd instars, when over 98% of the nymphs

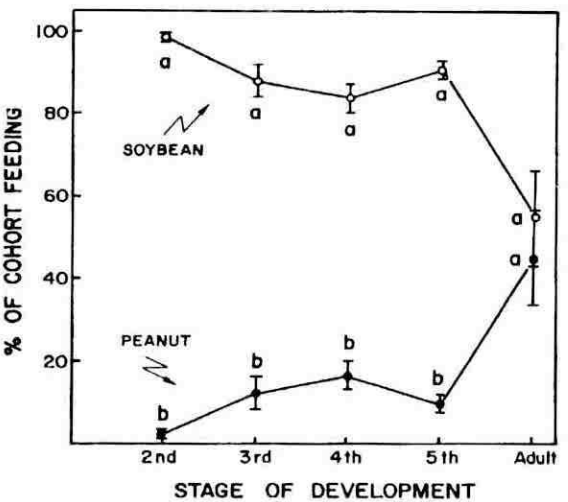

Figure 1. Mean (± SEM) percentage of cohort of nymph and adult *Nezara viridula* feeding on soybean pod or on raw peanut, in the laboratory. Means with the same letter are not significantly different ($P > 0.05$) using chi-square test.

fed on soybean pods rather than on peanuts. This apparent preference decreased somewhat for subsequent instars, but was always greater than 80% of the nymphs. In contrast to the food choice by the nymphs, *N. viridula* adults showed no preference between the two foods (Fig. 1).

DISCUSSION

Feeding activity of *N. viridula* is variable depending on developmental stage, adult age, and physiological condition. Feeding frequency of nymphs generally increased as nymphs developed; this agrees with previous studies with *N. viridula* (Bowling 1980) and with other species of phytophagous pentatomid pests of soybean, such as the green stink bug, *Acrosternum hilare* (Say) (Simmons & Yeargan 1988) and *Edessa meditabunda* (F.) (Panizzi & Machado-Neto 1992). However, feeding duration was longest for the 2nd and 5th instars. This may be related in the first case to the initiation of the feeding process by 2nd instars. Because first instars do not feed, but live on energy stored in the egg, 2nd instars must recover the 'waste' of energy occurred in the previous stage. In the second case, longer periods of feeding may be related to the final molt. Fifth instars that take as much food can produce the best provisioned and presumably fittest adults. Also, at this stage, more critical changes in body structure occur such as the final formation of wings and reproductive organs, which may require extra energy. Simmons & Yeargan (1988) found a greater duration of feeding sessions for 2nd instars of *A. hilare* but not for 5th instars. More studies to elucidate this point are needed.

There was only little evidence of feeding by adults on emergence day which suggests that extra time is needed to harden the stylets before the seed could be pierced. Afterwards, feeding frequency reached a maximum on days 2-4, which may be associated with the need to strengthen body tissue and as a preparation to start reproduction. Usually, female *N. viridula* starts copulation during the second week of adult life (e.g., Panizzi *et al.* 1989). Feeding frequency of adults, which ranged from 3 to 10 flanges/day depending on insect age, may also vary with temperature (Bowling 1980). For *A. hilare*, similar results were reported with the number of flanges deposited/day reaching a maximum (ca. 9) on day 5 and decreasing thereafter (Simmons & Yeargan 1988). A related species, *N. antennata* Scott, was reported to feed 3.9 times/day, but the age of adults was not specified (Kawamoto *et al.* 1987).

The formation of the flange, which involves the secretion of saliva (Hori 1968, Miles 1972) may be energetically costly to *N. viridula*. This may possibly explain why fasted adults compensate by feeding for longer periods than non-fasted adults instead of increasing feeding frequency, which would require the secretion of additional flanges.

The preference of nymphs for soybean pods and the lack of preference of adults for either soybean pods or raw peanuts as food may be related to reproduction. Nymphs do not develop well when fed exclusively on peanuts, but adults increase their fecundity when fed peanuts (Panizzi & Slansky 1991). Laboratory colonies of *N. viridula* are often maintained using mature seed of peanuts (Harris & Todd 1980, Kester & Smith 1984, Corrêa-Ferreira 1985, Jones & Brewer 1987, among others). This and other published data (Panizzi & Slansky 1991 and references therein) suggest different nutritional requirements and different allelochemical tolerance between nymphs and adults.

These results, which indicate differences in feeding frequency, duration, and preference among nymphs at different stages of development, and among adults at different ages and physiological condition, may help us to better understant the feeding behavior of *N. viridula*.

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