ECOLOGY, BEHAVIOR AND BIONOMICS

Oviposition Behavior of *Neomegalotomus parvus* (West.) (Hemiptera: Alydidae): Daily Rhythm and Site Choice

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Comportamento de Oviposição de *Neomegalotomus parvus* (West.) (Hemiptera: Alydidae): Ritmo Diário e Escolha do Local

RESUMO – Foi o estudado o comportamento de *Neomegalotomus parvus* (West.) relacionado à escolha de fendas em vagens de guandu, *Cajanus cajan* (L.) Millsp., para deposição de ovos. O bloqueamento de antenas, tarsos, olhos, abdome ou todas as estruturas simultaneamente não afetou a escolha do local em vagem de guandu (fendas). A oviposição ocorreu principalmente no período da tarde. A maior quantidade de ovos foi depositada das 16 às 18 h. As fêmeas moveram as antenas alternadamente para baixo e para cima; o tateamento/antenação foi realizado, primeiramente com antena e depois com a extremidade do labium e o ovipositor foi evertido e esfregado na superfície da vagem determinando a oviposição. Os ovos foram depositados com uma substância adesiva. Mecanoreceptores foram observados no ovipositor.

PALAVRAS-CHAVE: Insecta, ritmo cicardiano, *Cajanus cajan.*

ABSTRACT – *Neomegalotomus parvus* (West.) behavior related to choosing crevices of pigeon pea, *Cajanus cajan* (L.) Millsp., pods to lay eggs were studied. Blocking antennae, tarsi, eyes, abdomen, or all structures simultaneously, did not affect the site of the pod (crevices) chosen to lay eggs. Oviposition occurred mainly in the afternoon. Most eggs were laid from 4 to 6 pm. Females moved antennae alternately up and down. Dabbing/antennation was accomplished, first with antennae and then with antennae and labial tip and the ovipositor was exposed and swept over the surface of the pod, leading to oviposition. Eggs were held together with an adhesive substance. Mechanoreceptors were observed on the ovipositor.

KEY WORDS: Insecta, cicardian rhythm, *Cajanus cajan.*

The broad-headed bug *Neomegalotomus parvus* (Westwood) is a pest of legumes, such as common bean, *Phaseolus vulgaris* L., and soybean, *Glycine max* (L.) Merrill in Brazil. The importance of *N. parvus* as a pest is increasing with the expansion of these crops in the West-Central region of the country (Panizzi 1997).

Studies on its biology have been accomplished with several host plants (Panizzi 1988, Santos & Panizzi 1998). In relation to oviposition behavior, Panizzi *et al*. (1996) studied the sites of egg allocation on soybean plants, comparing leaves vs. pods and, considering the leaves, adaxial vs. abaxial surfaces, and internal (close to the midrib) vs. external (close to the border) areas. In the laboratory, it was noted that *N. parvus* lays eggs preferentially in crevices, such as those between seeds in vessels (M.U. Ventura, unpublished). On non-food substrates, the crevices are also preferred, such as cadavers of dead insects, where eggs are laid between two legs; or breaches in vessels of food or water. On pigeon pea pods, *Cajanus cajan* (L.) Millsp., eggs were laid in the rifts of pods among the seeds (M.U. Ventura, unpublished).

To elucidate how *N. parvus* localize the sites (crevices) for egg deposition, behavioral studies were carried out to verify the possible role of organs related to host selection in the choice of these sites. The occurrence of oviposition rhythms was also studied to determine the period of the day in which the insects lay most of the eggs. Females were observed in order to determine the sequence of behaviors preceding oviposition, and the presence of sensilla on the ovipositor.

Material and Methods

Bioassays were carried out in environmental chambers maintained at 25+1°C, 65±5% RH and a photoperiod of 12:12 L:D. Insects were obtained from the colony kept at EMBRAPA Soja, Londrina county, PR, South Brazil, fed with pigeon pea seeds and pods. Females were at the time of onset of reproduction (10 days after adult emergence). Acrylic cages (12.0 x 12.0 x 3.8 cm) were used in all bioassays.

Structures Blocking. A blocking test was carried out to verify the influence of sensilla of the abdomen, antennae, tarsi, or eyes in the selection of the site for oviposition. Sensilla of abdomen, antennae, tarsi and the compound eyes were blocked. All structures were also blocked simultaneously.

Adult females, including controls, were maintained in a freezer (-12°C) for five minutes to decrease movement. Shellac was used for sensilla blocking (Levin & Anfinogenova 1981) and black acrylic resin ink for insect blinding (Numata *et al*. 1986). Five pairs per treatment were kept in cages with five replications. Ten mature pods of pigeon pea were supplied as food, plus water. Seventy hours after the start of the experiment, the number of eggs deposited in the rifts of the pods (external space among the seeds) and in other sites were counted.

Oviposition Rhythm. Thirty pairs were individualized in cages with pigeon pea pods and water. Laid eggs were recorded every 2h, from 6:00 am to 6:00 pm. The test was repeated five times. Dead insects were replaced with individuals of the same age.

Oviposition Behavior. One female and a pigeon pea pod were placed in opposite corners of a cage. Females were observed during two hours at the time of greater oviposition to record behaviors related to oviposition. Thirty females were observed.

Ovipositor Morphology. Squeezing the abdomen, the ovipositor was exposed, observed and photographed using a stereomicroscope. Ovipositors were dissected and fixed on stubs with doubled-face adhesive tape, coated with gold, and observed with a scanning electronic microscope (Zeiss, DMS 940). To test ovipositor sensilla permeability, ovipositors were treated with silver nitrate (0.1%) for 30 minutes in the dark and dehydrated through graded ethanol, then mounted in Canada balsam (Peregrine 1972).

Experimental Design and Statistical Analysis. Assays were conducted in a randomized complete block design. Percent data were transformed using arc sine $(x/100)^{1/2}$ constant. ANOVA was performed. Duncan's multiple range test was used to compare individual means (P < 0.05) (SAS Institute 1989).

Results and Discussion

Structures Blocking. No statistical differences were found among the treatments for percent eggs placed in rifts of pigeon pea pods (Fig. 1). The hypothesis that eyes, antennae, abdomen, or tarsi drive the choice of sites for oviposition was shown not to be true. In all treatments, eggs were laid mostly on rifts, and a small percentage was laid elsewhere. Thus, the presence of breaches did not induce oviposition in *N. parvus* females, but it influenced the choice of the site of egg allocation. The same was verified for *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (Justus & Mitchell 1996). The choice of depressions for deposition of the eggs has probably evolved as a defense strategy to provide greater protection. In another alydid, *Riptortus dentipes* F., the oviposition occurs mostly on the base of the pods or on the brown stem of the pods, thus, the eggs stay hidden (Aina 1975). For *R. linearis* (F.), 3% and 14% of the eggs were placed on branches or on inflorescences, respectively, where they are hardly noticed (Talekar *et al.* 1995). Egg masses of *Chilo partellus* (Swinhoe) (Lepidoptera: Noctuidae) are generally laid in depressions at the side of the midrib of leaves or in crevices of dead leaves (Chadha & Roome 1980).

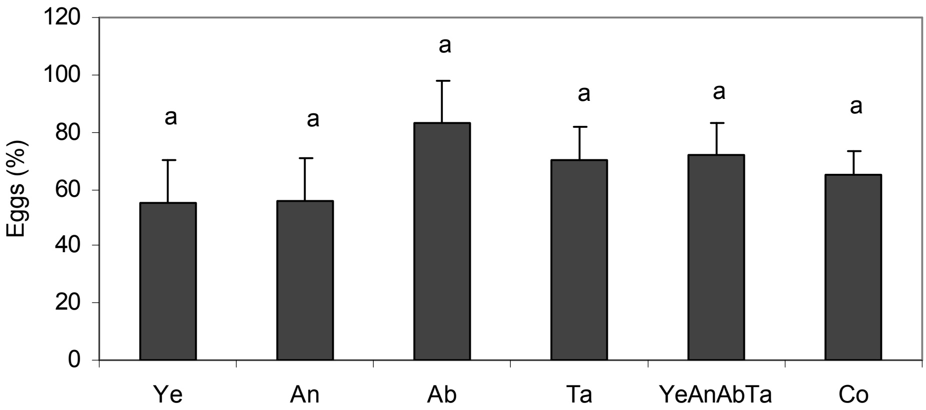

Figure. 1. Mean (± SEM) number (%) of eggs placed on rifts of pigeon pea pods by *N. parvus* females with organs related to host selection blocked by application of shellac or black ink in cages in the laboratory. Means with different letters are significantly different according to the Duncan multiple range test (P < 0.05), n = 5.
Ye = eyes; An = antennae; Ab = abdomen; Ta = tarsi; Co = control.

Oviposition Rhythm. Oviposition by *N. parvus* increased at 2:00 PM, and concentrated in the afternoon period. Most eggs were laid from 4:00 to 6:00 PM, and eggs were laid fewest from 10:00 to 12:00 AM. (Fig.2). A

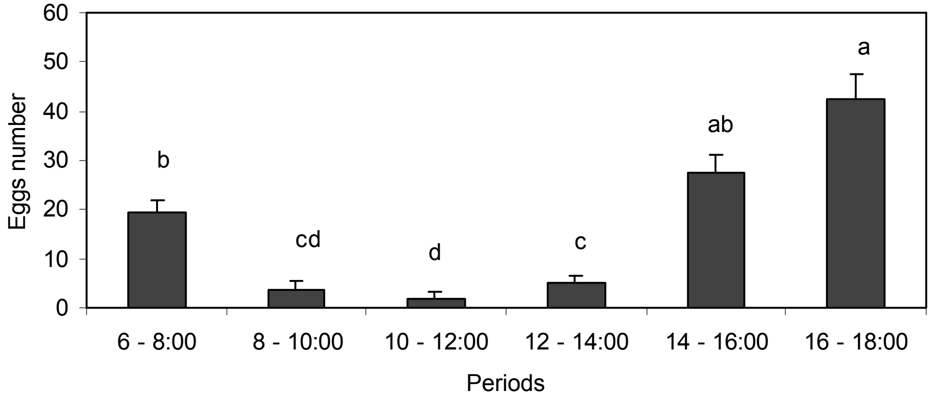

Figure. 2. Mean (± SE) number (%) of eggs laid by *N. parvus* during different periods of the day in cages in the laboratory. Means with different letter are significantly different according to the Duncan multiple range test ($P < 0.05$), $n = 5$.

circadian rhythm of oviposition was also verified for the pentatomid *Thyanta pallidovirens* (Stål) (Schotzko & O'Keeffe 1990) and for the alydid *R. clavatus* Thunb. (Numata & Matsui 1988). For these insects, maintained in different photoperiods than used in this study, oviposition was also greater during the afternoon.

Oviposition Behavior. The main steps and their sequence in the oviposition behavior were described (Fig. 3). Selected behaviors are presented in Figs. 4a–f. Before contact with the pod, *N. parvus* carried out behaviors that are also related to food location. The female was initially still (Fig. 4a), and then moved the antennae alternately up and down (Fig. 4b). In *P. xylostella*, the antennae rotates (Justus & Mitchell 1996). In spite of differences in the shape of the movements, it is likely that the function is the same, i.e., increase the volume of air and create a wind vortex around the antenna, facilitating contact of odorous molecules with the antennae multiporous sensilla (Justus & Mitchel 1996).

The dabbing/antennation was done first with the antennae and immediately after with the antennae and the labial tip (Figs. 2c and d). This behavior is called "plant surface exploration" (Backus 1988). In Heteroptera, dabbing/antennation is also related to the choice of food (Miles 1958, Hatfield & Frazier 1980, Ventura *et al.* in press). Therefore, they are not specific in the choice of the site of oviposition. It is rather a mechanism by which bugs taste a potential substrate for feeding and subsequent oviposition. Antennation was also verified for *P. xylostella* (Justus & Mitchell 1996) and *C. partellus* (Chadha & Roome 1980).

In the next step, the ovipositor was exposed and swept over the surface of the pod (Fig. 4e) or the surface of the cage. On pods, sweeping of the ovipositor quickly leads to oviposition. After the female swept the ovipositor a few times, eggs are laid immediately. On the cage surface, female swept her ovipositor ca. 100 times, but oviposition did not occur. The action of sweeping the ovipositor on the surface of the pod (Fig. 4e) is specific; therefore, this behavior is a mechanism to locate an oviposition site. Information on the site is probably provided by mechanosensilla located on the ovipositor (Fig. 5). On soybean,

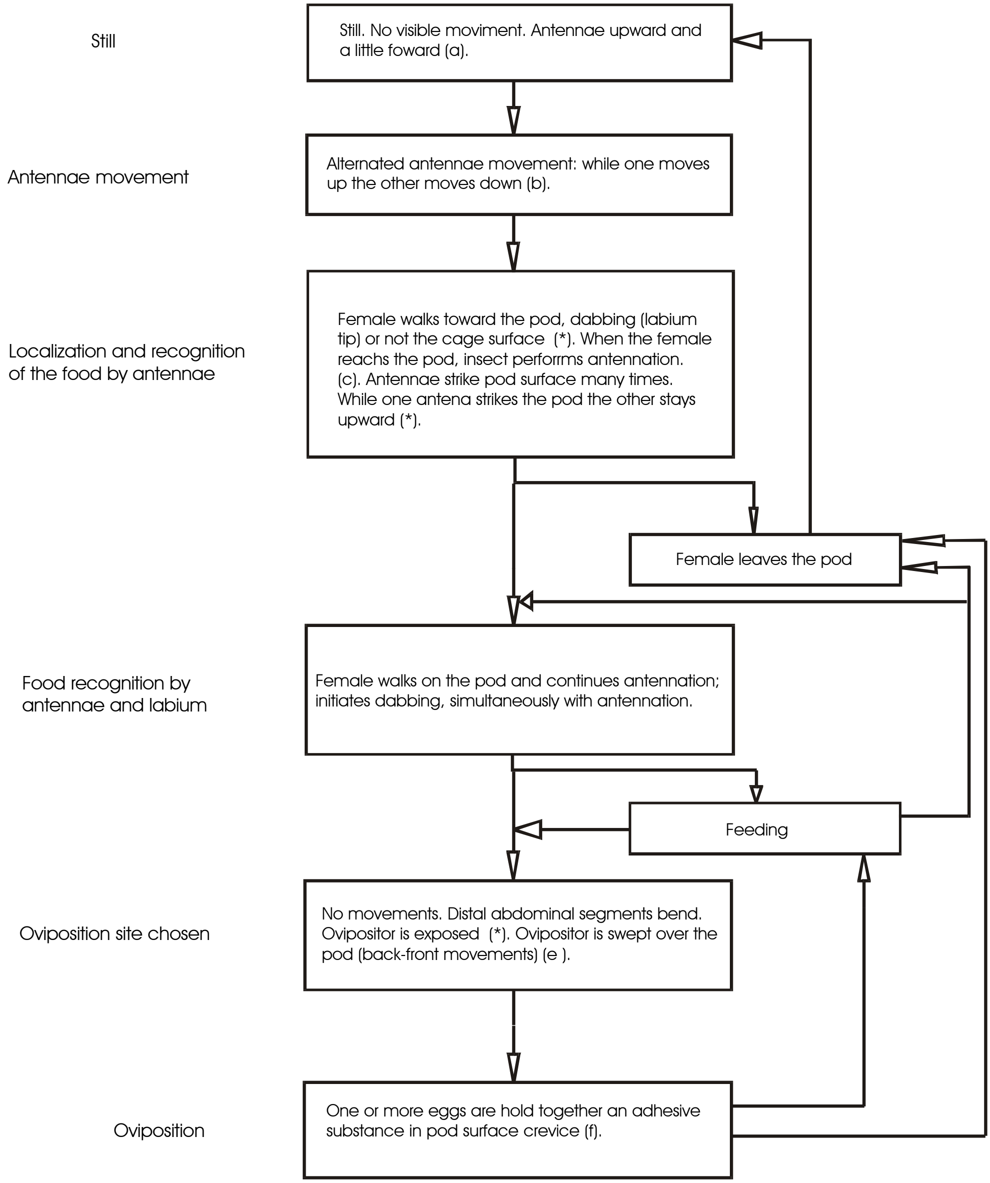

Figure. 3. Description of the sequence of behaviors performed by females of *N. parvus* during oviposition (n = 30). (*) Behavior not showed in Fig. 4.

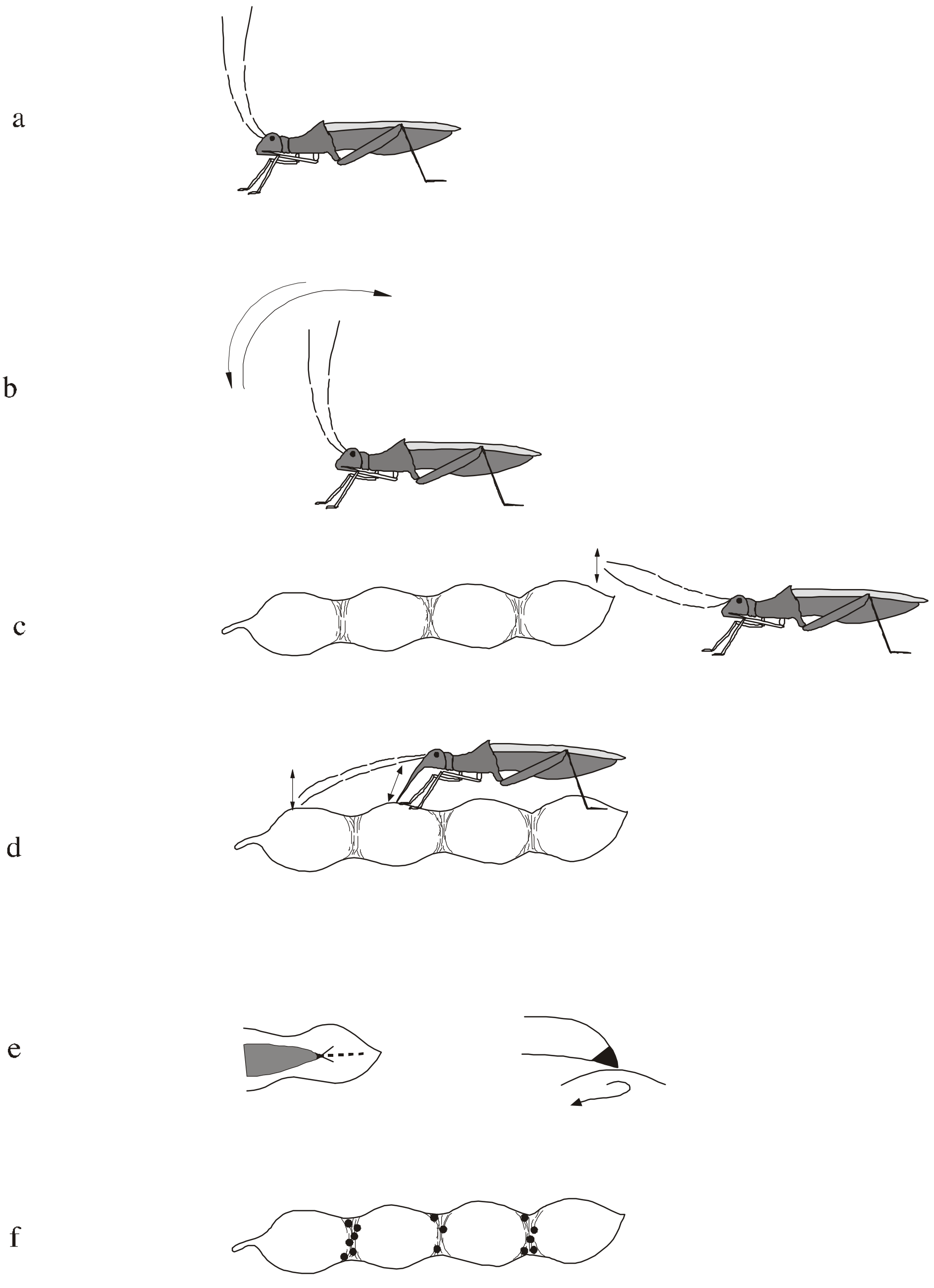

Figure. 4. Behaviors related to the choice of the oviposition site by *N. parvus*. A = still; B = antennae moving; C = antennation–labial tip strikes pod surface; D = dabbing/antennation–labial and antennal tips strike pod surface; E = ovipositor is swept backward and forward; and F = eggs are laid and glued in the breaches of the pods (n = 30).

the eggs of *N. parvus* are laid mainly on the leaves (Panizzi *et al.* 1996); and on pigeon pea, the eggs are mainly deposited on the pods (M.U. Ventura, unpublished). Probably the depressions (among seeds) of the soybean pods are not sufficiently conspicuous to stimulate the sensilla and then initiate oviposition. Also on common bean, eggs are laid mainly on leaves (Yokoyama 1998). Like soybean, the rifts on common bean pods are less pronounced than those on pigeon pea. On soybean, the abaxial surface of the leaves is preferred over the adaxial surface (Panizzi *et al*. 1996). Again, it is probable that the greater saliency of the veins on the abaxial surface determines the choice. Another alydid, *Leptocorisa acuta* (Thunberg), lays its eggs on lines, close to the midrib, on the adaxial surface (Rai 1981). Other insects, such as the lepidopterans *P. xylostella* and *C. partellus,* that place their eggs in rifts or crevices also sweep the abdomen on the substratum (Chadha & Roome 1980).

Eggs were held together with an adhesive substance, as occurs in other species of alydids, such as *L. oratorius* (Fabricius), *L. acuta* (Cobblah & Den Hollander 1992), and *R. dentipes* (Aina 1975).

Figure. 5. Ovipositor of *N. parvus* exposed (41 X).

Ovipositor Morphology. The exposed ovipositor of *N. parvus* is shown in Fig. 5. The sensilla are located on its distal end. Electronic microscopy showed long sensilla containing flexible sockets in the base (Fig. 6). Stimulated these sensilla would experience differentiated pressure, allowing perception of crevices. In *C. partellus*, mechanosensilla on the ovipositor are related to location of rifts for eggs deposition. *C. partelus* and *Spodoptera littoralis* (Boisd.), besides using mechanosensilla for location of the oviposition site, also use them to organize the eggs in arrays (Chadha & Roome 1980). In these insects, chemoreceptors are associated with the ovipositor. No depositions of silver nitrate were detected in the *N. parvus* ovipositor sensilla, suggesting no contact chemoreception function. In Heteroptera, oviposition selection is made after the selection of the food by the female (Figs. 4a-f), because adults feed on the same food as nymphs. In consequence, oviposition occurs preferably on the females-feeding substrates. The chemical perception of the host plant occurs through the dabbing/antennation and probing that are accomplished before each feeding session.

These data and observations showed that

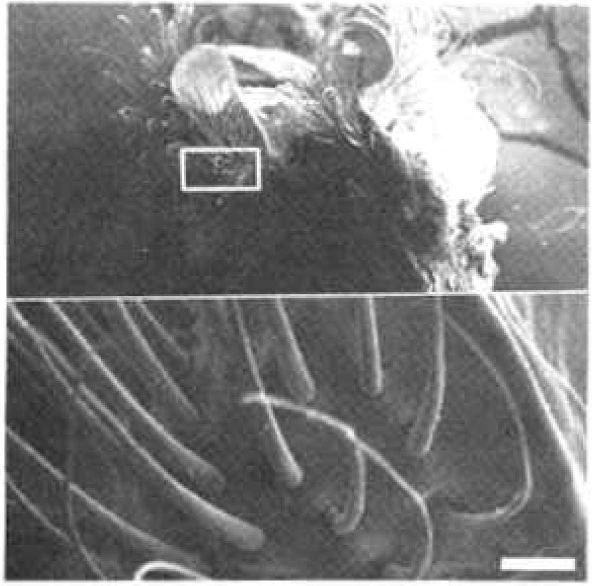

Figure. 6. Mechanoreceptors located on the ovipositor of *N. parvus* (Bar = 12,5 mm).

N. parvus females have cicardian rhythm of oviposition, most eggs being laid during the afternoon. Eggs were preferably laid in the rifts of the pigeon pea pods and preference was not affected by vision or abdominal sensilla, antennae and tarsi blocking; the rifts on the pods among the pigeon pea seeds were chosen by a back-front sweeping movements of the ovipositor, where the mechanosensilla are located.

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