ECOLOGY, BEHAVIOR AND BIONOMICS

Calling Behaviour and Evaluation of Sex Pheromone Glands Extract of *Neoleucinodes elegantalis* Guenée (Lepidoptera: Crambidae) in Wind Tunnel

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Comportamento de Chamamento e Avaliação do Extrato de Glândula de Feromônio Sexual de *Neoleucinodes elegantalis* Guenée (Lepidopera: Crambidae) em Túnel de Vento

RESUMO – Foram determinados o horário de emergência e o comportamento de chamamento de *Neoleucinodes elegantalis* Guenée em condições de laboratório (23 ± 1ºC, 12 h fotofase e 70% UR). As fêmeas iniciaram a emergência na primeira hora após o início da escotofase até a oitava hora, com um pico observado na quarta hora. Os machos emergiram após a segunda hora até a 11ª hora com um pico também observado na quarta hora da escotofase. Os acasalamentos foram precedidos de vibração de asas dos machos antes e durante a aproximação. Os acasalamentos ocorreram somente durante a escotofase entre a quarta e a 10ª hora, com um pico observado na sétima hora. Casais recém-emergidos raramente acasalaram (2,8%) enquanto casais com idades entre 48 e 72 horas apresentaram maior proporção de acasalamentos, 26,3% e 27,5% respectivamente. Extratos de glândula do feromônio sexual de fêmeas virgens de 48-72 h foram avaliados em túnel de vento e demonstraram ser mais atrativos do que as fêmeas virgens.

PALAVRAS-CHAVE: Insecta, broca-pequena-do-tomate, emergência, cópula.

ABSTRACT- Adult emergence and mating behavior of *Neoleucinodes elegantalis* Guenée were studied under laboratory conditions (23 ± 1ºC, 12 h photophase and 70% RH). Adult emergence occurred only at scotophase period. Female began to emerge from the 1st hour to 8th hour with a peak occurring at 4th hour of scotophase. Male showed similar trend, but emerging from 2nd to 11th with a peak also at 4th hour of scotophase. Mating was preceded by wing vibration in the male prior to and during walking approach. Mating occurred only during the scotophase period between the 4th and 10th hour of scotophase, with the peak occurring at the 7th hour. Newly emerged *N. elegantalis* couples rarely mated (2.8%) whereas 48 and 96 h old couples mated 26.3% and 27.5% respectively. Glands extracts from abdominal tips of 48-72 h virgin female moths evaluated in a wind tunnel were more attractive than virgin females.

KEY WORDS: Insecta, tomato-fruit borer, emergence, copulation.

Tomato, *Lycopersicon esculentum* Mill, is one of the most important vegetable crops in Brazil, with 55,000 ha under production, yielding 1.5 million ton/year (FAO 1993). The average production is about 50 ton/ha. However, in Rio de Janeiro State, the tomato production average is higher (70 ton/ha) and represents 80% of the vegetable crops on the market.

The tomato-fruit borer *Neoleucinodes elegantalis* Guenée (Lepidoptera: Crambidae) is the most important pest in several tomato growing regions of Central and South America, including some Caribbean Islands (Salas *et al.* 1992). The larvae feed inside the tomato fruit, causing estimated losses of up to 45%. After the larvae leave the tomato fruit, they pupate in the soil or enfolded in leaves (Marcano 1991). After mating, females deposit their eggs on small fruits (23 mm diam) and under the calyx (Blackmer *et al.* - in press). Each moth is capable of laying 160 eggs (Marcano 1991). The majority of eggs hatch during the first hour of scotophase and approximately one hour later the larvae enter in the fruit (Blackmer *et al.* in press). Current control strategies include two to three applications of pesticides per week (Reis & Souza 1996), and cultural practices such as the destruction of infested fruits or wild solanaceous hosts (Gallo *et al.* 1988). Once the larvae enter the fruit, pesticides are ineffective and sublethal doses of pesticides can lead to insecticide resistance problems. Therefore, new strategies for monitoring and controlling *N. elegantalis* need to be developed such as the use of a sex pheromone (Jutsum & Gordon 1989, Ridgway *et al.* 1990).

Synthetic sex pheromone in Lepidoptera have been widely used for monitoring, timing spray and controlling methods (eg. lure & kill, mass trapping or matting disruption) (Roelofs & Cardé 1987, Jutsum & Gordon 1989, Ridgway *et al.* 1990). No information has been published on the mating behavior and sex pheromone of *N. elegantalis,* although it has been know that virgin females of Lepidopteran attract male for matting from long distances. Therefore, this paper aimed to study the emergence of adults and mating behavior of female of the tomato-fruit borer, *N. elegantalis.*

Extraction and evaluation of the sex pheromone glands were also evaluated within a wind tunnel in order to confirm the sex pheromone components.

Material and Methods

Insects. Tomato fruits showing the typical scared entrance hole of the larva were collected from São José de Ubá and Itaperuna, state of Rio de Janeiro, Brazil. They were maintained in the laboratory until the larvae left the fruit to pupate. Pupae were sexed according to Butt e Cantu (1962). Pupae and adults were kept in transparent perspex cages (30x30x30 cm) at 23 ± 1ºC, on a 12L:12D photoperiod and 70% RH. Male adults were maintained in separate BOD chambers to avoid contamination from the female sex pheromone.

Timing of Adult Emergence. Male (N=100) and female (N=100) pupae were kept in perspex cages and hourly observed for adult emergence during 24 hours. As adults emerged, they were recorded and removed from the cage. The observations during the scotophase were made with a torch that had the light source covered with red cellophane.

Calling Behavior. The observations of calling behavior were carried out at 23±1°C and 64±3% RH, beginning one hour before scotophase. Approximately 20 couples were placed in screened cages (30x30x30 cm) two hours prior to the observations. The calling and mating behavior of newly emerged (<6 h) (N=108), 24 h (N=69), 48h (N=85), 72 h (N=80) and 96 h (N=80) old couples were observed throughout the scotophase using a torch as already described. Once copulation occurred, the pair was removed from the cage and placed in a 8.5x2.5 cm glass tube in order to have the duration of the copulation recorded.

Gland Extracts. Abdominal tips of virgin females were extracted for 10-15 min. in hexane and kept in glass microcapillary tubes in a freezer (-20ºC) until behavioral tests could be conducted.

Wind Tunnel Tests. Behavioral tests were carried out within a flight tunnel (80x30x30 cm) calibrated to 25 cm s^{-1}, 5 lux (light intensity), 24±1ºC and 65% R.H. The airflow was produced by an electrical fan and was purified by a 5 cm layer of activated charcoal filter. The odor source was placed at 80 cm upwind from the release platform (37 cm lenght x 4 cm diam.) of the male moths. Groups of three males were placed in a screened cage (5x5x10 cm) and allowed to acclimatize to the tunnel condition for at least two hours prior to testing. The males were tested once, during the female calling period. The odor source consisted of either two virgin females placed within a 3x5 cm diam. screened cage or a gland extract containing two female equivalents (2 FE) or hexane as control. Gland extracts or hexane were placed on separated 1x1 cm filter paper and tested after the solvent had evaporated. The number of males taking off, performing upwind flight behavior towards the odor source, landing at the source and attempting to copulate with the odor source were recorded. A smoke plume of hydrochloric acid and ammonium hydroxide was regularly visualized to ensure that the structure of the plume was conical and that it passed over the platform containing the males.

Results and Discussion

Emergence of Adults. The emergence of adults of *N. elegantalis* is governed by a circadian rithym and this behavior is very common among the lepidopteran (Matthews & Matthews 1988, Horodyski 1996). Both sexes emerged only during the scotophase. The females began to emerge during the 1st hour of scotophase, whereas the males began to emerge in the 2nd hour. The highest emergence rate occurred during the 4th hour for both sexes (Fig.1). Adult emergence was periodic, occurring mainly early evening until 7th hour of scotophase. The results suggest that there is a synchronized pattern of emergence of adult moths.

Calling Behavior and Duration of Copulation. During the photophase (the adult resting period), both sexes curved their abdomens upward and kept their antenna motionless. This position is typical for calling behavior of several species of moths such as the tomato leaf miner *Tuta absoluta* (Meyrick) (Hickel *et al.* 1991), the tomato pinworm *Keiferia lycopersicella* (Walsingham) (McLaughlin *et al.* 1979) and the pink bollworm *Pectinophora gossypiella* (Saunders) (Tóth 1985). During the calling behavior, *N. elegantalis* females maintained their abdomen straight in an horizontal position while moving their antennae up and down vertically. The males during activity also performed such position and movements of antennae. The courtship behavior began when males flew near a calling female (usually 1-2 cm) and walked towards her while rapidly vibrating his wings and opening the hair brushes. Occasionally, a female would fly off when approached.

In general, the percentage of mating of *N. elegantalis* in laboratory was low. Such results could be a reflect on the difficulty of rearing *N. elegantalis* in laboratory conditions (Alvaro E. Eiras & José R. P. Parra, unpublished). Newly emerged (<6 h) *N. elegantalis* couples rarely mated (2.8%) whereas 48 and 96 h old mated in higher proportion, 17.4% and 17.5% respectively (Fig. 2). The highest number of moths mated at age of 48 and 72 h, 26.3% and 27.5% respectively. Mating of *N. elegantalis* in the tomato plantation was observed between the 6th and 9th hour of scotophase when the humidity was over 95% (Alvaro E. Eiras, unpublished data). Thus, the effect of humidity on mating behavior in this insect pest should be evaluated in order to facilitate its rearing in laboratory conditions. All matings, regardless the insect age, occurred between the 4th and 10th hour of scotophase, with a peak occurring at the 7th

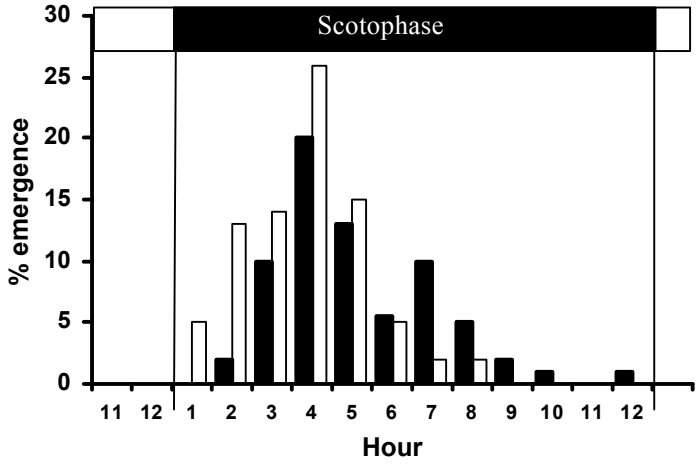

Figure 1: Emergence time for female ( )□nd male (■) *N. elegantalis* during a 12 L:12D cycle (scotophase : photophase) in the laboratory (N = 100).

hour (27.5%) (Fig. 3). Thus, these results suggest that the best time to extract the sex pheromone glands would be between the 5th and 9th hour of scotophase, using 48-72 h old virgin female *N. elegantalis*.

The majority of Lepidoptera mate during

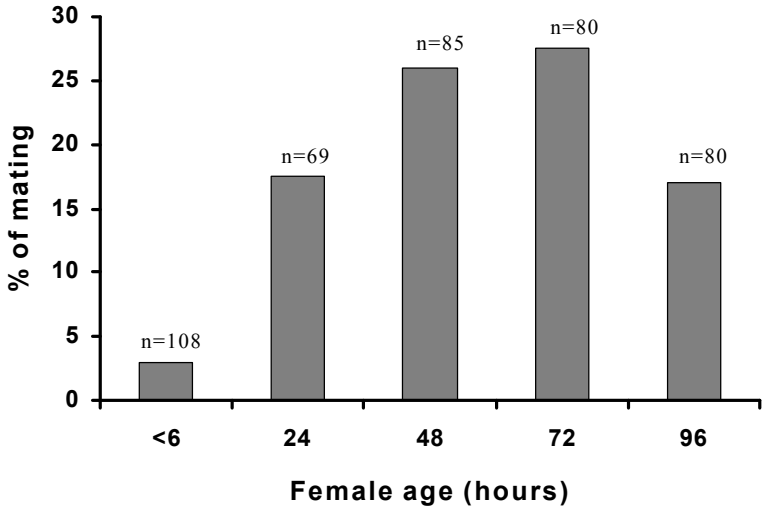

Figure 2**:** Mating of *N. elegantalis* couples of different ages in the laboratory.

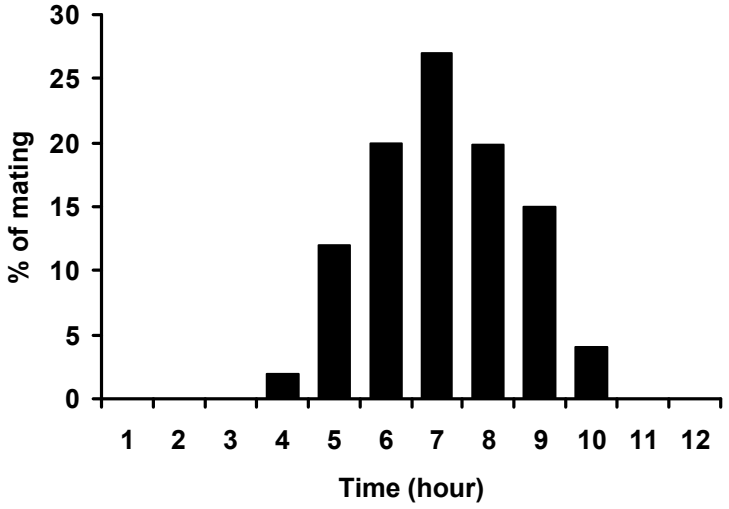

Figure 3. Percentage of mating of *N. elegantalis* under laboratory conditions during scotophase (N=422) (23°C; 12L:12D, 65% RH).

the scotophase (Roelofs & Cardé 1987) and the periodicity of the calling behavior has been considered one of the main mechanism ensuring reproductive isolation (Cardé & Roelofs 1973). One of the characteristics of insects that have a short life span is that mating occurs shortly after emergence (Matthews & Matthews 1988).

There is no published data on the lifespan of *N. elegantalis* in the field, but observations of caged virgin female held in a trap in the field, survived up to 14 days (Alvaro Eiras, unpublished data). Adults reared in the laboratory with different liquid diets survived up to 15 days (mean 9.9 ± 1.5 days for males) and 14 days (8.8 ± 2.3 for females) (Andrade *et al.,* unpublished). There is no evidence that whether this species is migratory. However, this moth has the ability to move between tomato fields, which can be separated by quite a distance.

The duration of the copulation ranged from 70 min. (24 h old) up to 224 min. (96 h old). The longest period of copulation was observed for 48 h, although there was no difference between the ages (Fig. 4).

Wind Tunnel. The observations of the upwind flights of *N. elegantalis* in the wind tunnel showed that males exhibited significantly higher response levels to the gland extract than to control (Fig. 5). Only the take-off rate was similar when the source was either a virgin female or the gland extract. However, the upwind flights, flights close to the source, landing rates and attempts to copulate at the source were significantly greater for the gland extract than for the virgin female. Very few upwind flights to the control treatment were observed. Although the virgin females exhibited the calling behavior position when they were presented as the odor source, the behavior of males suggested that the females were not releasing sex pheromone. The low proportion of couples mating (Figs. 2 and 3) under laboratory conditions suggests that not all virgin females engage in calling behavior when conditions (age and hour of scotophase) seem

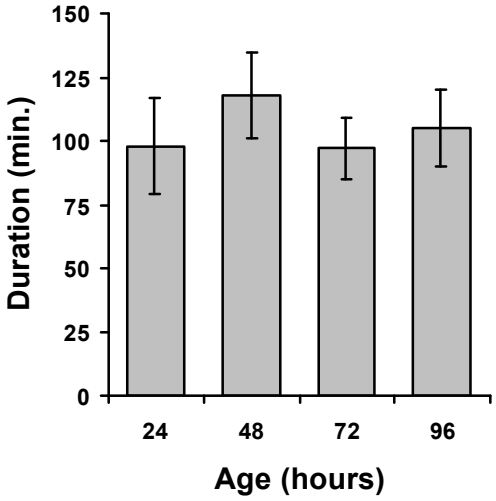

Figure 4: Mean duration (±s.e.) of *N. elegantalis* copulating at different ages.

otherwise favorable. Many factors could influence the females to elicit upwind flight (wind tunnel) and mating (calling behavior). It is likely that the physiological state of this insect and the environmental conditions (i.e., temperature, relative humidity, wind speed) influence calling behavior. As *N. elegantalis* is a specialist on solanaceous plants, host plant

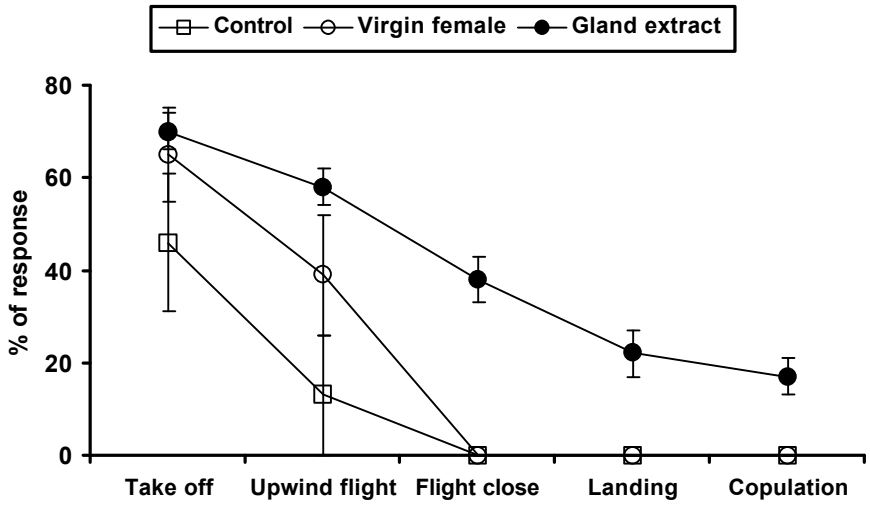

Figure 5: Behavioral responses of the *N. elegantalis* males to virgin females (n=35), control (n=15) and gland extracts (n=72) in the wind tunnel. Bars mean standard error.

volatiles may act as additional cues that stimulate calling behavior. The fact that mating occurs on host plants is important in sex selection and mating finding. In many species of herbivorous insects, both sexes are attracted to the host plant odors. In some species, males are attracted to females in the presence of host plant odor. For example, the males of the leek moth, *Acrolepiopsis assectella* (Zeller) are attracted in the presence of the odors of leek (Bernays & Chapman 1994).

At the moment, sex pheromone gland extracts of 48-72 h virgin female *N. elegantalis* is being submitted to chemical analysis in order to identify the active compounds.

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