



# HOST PLANT AFFECT CALLING BEHAVIOUR IN *NEOLEUCINODES ELEGANTALIS* ?

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## INTRODUCTION

In most species of moths, female produce and release sex pheromones to attract conspecific males for mating (Raina *et al.*, 1989). Some works had demonstrated the host influence on the sexual behavior of herbivorous insects (Herrebout & de Water, 1982; Hendrikse & Vos - bunnemeyer, 1987; McNeil & Delisle, 1989b). However, there are some studies indicating that the host plant is not directly involved in pheromone production (Gomez & Rojas, 2006; Groot & Visser, 2001; Cardé, 1984). This difference could be explained as a consequence of the host plant range, so the kairomonal interaction would be most likely to occur in species that are monophagous or oligophagous, as postulated by (Cardé, 1984).

The insect studied is the tomato fruit borer, *Neoleucinodes elegantalis* (Lepidoptera: Crambidae), a specialist insect in solanaceous plant (Gallo, 2002). The larvae of *N. elegantalis* after eclosion penetrate and feed inside the green fruit where it will develop and stay until pupation (Blackmer *et al.*, 001; Jaffe *et al.*, 007), so the host selection by gravid females is a crucial step because the larvae can not move to long distances and thus, depends on the choice made by the adult female. Once the larvae of *N. elegantalis* does not locomote until locate suitable sites to feed and depends on the female choice to acquire food for its development it is important to female mate and deposit its eggs in an adequate plant to its descendents development. So, understanding the effects of host plants on the sexual behaviour of this insect is important in both evolutionary and ecological perspectives and may help improving the programs of pest management.

## OBJECTIVES

The purpose of this study is to determine whether the calling behavior of *N. elegantalis* is affected by the presence and absence of the host plant volatiles.

## MATERIAL AND METHODS

### Insects and Experimental conditions

*N. elegantalis* used in our experiment were reared in the laboratory at the Federal University of Viçosa, Minas Gerais, Brazil. Larvae were reared on *Solanum gilo* until pupation. Pupae were sexed and separated in cages (50 x 50 x 50cm) until adult emergence. After emergence they were held separately in plastic containers, so that females did not have contact with males. After emergence, newly emerged virgin males and females were supplied with a honey - water solution of 10 %. Observation of calling behaviour was carried out during the scotophase. Tomatoes for preparation of extracts to use in these experiments were collected from commercial crop *Lycopersicon esculentum* Mill. (Solanaceae, cv. Santa Clara).

### Observations of calling behavior

The experiments started during the first complete scotophase after emergence. The observation was performed in a dark room at temperature  $25 \pm 10^{\circ}\text{C}$  and  $71,2 \pm 10\%$  RH. Newly emerged females were individualized in numbered plastic cages and in each one there was a piece of filter paper inserted in a bottom of the cage treated with (i)  $10 \mu\text{l}$  of an hexanic tomato extract and (ii)  $10 \mu\text{l}$  containing only hexan, as control. A drop of cotton with natural honey was placed daily to ensure that females had food *ad libitum*. Two groups of fifty five females were maintained separately in two rooms with the same conditions to avoid interference of plant volatiles. All females were observed every 10 min during all scotophase period of five successive days to determine the pattern of calling behaviour. Moths were considered calling if the ovipositor was extended. The following parameters measured each day for the first five days of calling: the time at which calling was initiated (MOTC, expressed as min. after the onset of the scotophase) and the time spent calling (MTSC), the number of calling bouts (NCB) and the duration of calling bout (DCB).

### Statistical analysis

The calling behaviour was observed in two treatments, a total of hundred ten females. All statistical analyses were per-

formed in R statistical system (R Development Core Team, 2006), using generalized linear models followed by an anova with test - F (Crawley, 2007). The models were built by including all variables and their interactions according to term complexity ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

The number of females that initiated calling varied considerably with age in both, treatment and control ( $F=76.9712$ ,  $df=1,7$   $P < 0.01$ ). The large number of females calling for the first time occurred at the first day of age. The age at which females calling was not significantly influenced by the presence or absence of host plant volatiles ( $F=1.0052$ ,  $df=1,8$   $P=0.3547524$ ). Females provided with extract ( $n=47$ ) or solvent ( $n=47$ ) called  $1.27 \pm 0.452$  and  $1.23 \pm 0.559$  days following emergence, respectively. Because not all females initiated calling at the same chronological age, the calling age was used for comparing calling behavior (Turgeon & McNeil, 1982). Thus, the calling patterns were compared among individuals that started calling on the same day, and similar comparisons were made for subsequent calling days. Our results showed that the presence of host plant volatiles affected only the time that females spending calling. The initiation and termination of some reproductive events often depend on environmental factors, such as temperature, humidity, photoperiod, and availability of food or a suitable egg - laying site. Furthermore, internal factors like nutritional condition and state of maturation of the oocytes may modify those external conditions (Gullan & Cranston, 2005). The age was a parameter that influenced the number of females calling for the first time, with the largest number of females calling occurred at one day of age.

The influences of plants on calling behaviour are modulated by availability of host plant in yield and this can induces the insects in strategies that optimize feeding, mating and reproduction (Landolt & Phillips, 1997). Plant volatiles are widely known to stimulate pheromone production and oviposition in some phytophagous insects (Robert, 1986; Hendrikse & Vos - bunnemeyer, 1987; Raina, 1988; McNeil & Delisle, 1989b; Landolt & Phillips, 1997). Nevertheless in *N. elegantalis* it's not seems true, once this insect find constantly fruits in field in order to synchronize reproduction and oviposition in a way that food are available for its descendents.

The calling activity was initiated at the sixth hour of the scotophase for females in presence or absence of tomato volatiles. The mean onset time of calling was not significantly different between treatments ( $F=0.0123$ ,  $df=1,179$   $P=0.91169$ ) and nor with calling age ( $F=0.4139$ ,  $df=1,178$   $P=0.52085$ ).

In this study, we found that only some reproductive behaviour is modulated by the presence of host plant volatiles suggesting that there is an interaction between *N. elegantalis* and tomato fruits. Compared to control conditions, the presence of host volatiles had effects on duration of calling. Nonetheless, it did not affect the other reproductive parameters evaluated.

Several studies have shown that the presence of the host plant or its volatile chemicals stimulate the production,

releasing and perception of the sex pheromone in several moths species (Hendrikse & Vos - bunnemeyer, 1987; Raina, 1988; McNeil & Delisle, 1989b; Raina et al., 1992, 1997; Pittendrigh & Pivnick, 1993; Sadek & Anderson, 2007). However, *Zamagiria dixolophella* (Gomez & Rojas, 2006), *Lygocoris pabulinus* (Groot & Visser, 2001) and *Antheraea polyphemus* (Cardé, 1984) did not require the host plant for the production and release the pheromone and this seems to be the case in *N. elegantalis*.

The time spent calling showed significantly differences between treatments and age. Females in presence of tomato fruit extract spent significantly less time calling ( $F=5.2906$ ,  $df=1,179$   $P < 0.01$ ). The mean time spent calling differs in terms of female's age ( $F=0.007724$ ,  $df=1,178$ ,  $P < 0.01$ ) which was higher in the second and first day to extract treatment and control ( $83.33 \pm 12.92$  min and  $115.95 \pm 17.78$  min, respectively). The reduction of the time spent calling in the presence of host volatiles may reflect the lesser time spending to find a pair to mate and subsequently their descendents successfully exploiting resource, once the host plant represent a suitable site to meet, mating and feeding (Visser, 1986; Schoonhoven et al., 2005; Bruce et al., 2005). Summarizing all results, we conclude that plant volatiles are not directly involved in pheromone production (calling behaviour) in *N. elegantalis* females as suggested by (Eiras, 2000). The number of calling bouts don't differ in neither treatments ( $F=0.7062$ ,  $df=1,179$ ,  $P=0.12883$ ). The females in presence of tomato fruit extract showed lower mean of time duration in each bout than control ( $26.64 \pm 0.9579$  min and  $35.54 \pm 3.006$  min, respectively).

## CONCLUSION

We conclude that plant volatiles are not directly involved in pheromone production (calling behaviour) in *N. elegantalis* females as suggested by (Eiras, 2000). The presence of host volatiles had effects on duration of calling. Nonetheless, it did not affect the other reproductive parameters evaluated. Acknowledgements: We thank FAPEMIG and CNPq for funding.

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