

## Mating behavior in *Atheloca subrufella* Hulst, 1887 (Lepidoptera: Pyralidae: Phycitinae)

## Comportamento de acasalamento em *Atheloca subrufella* Hulst, 1887 (Lepidoptera: Pyralidae: Phycitinae)

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### ARTIGO

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### ABSTRACT

The mating behavior of the moth *Atheloca subrufella* Hulst, 1887 (Lepidoptera: Pyralidae) was investigated in laboratory arenas. The descriptions of courtship and copulation in males and females were based on direct observations of virgin couples. The courtship and copulation bioassays results comprise several behavior patterns such as antennation, female searching (walking and/or flying); female encounter; female calling behavior; exhibition of pheromone gland; male approach and copulation. For that, courtship and copulation behavior ethograms were presented. The results also suggest that the mating behavior in *A. subrufella* is mediated by a long-range pheromone and the presence of a short-range pheromone. In overall, this research presents itself as a basis study to further investigations upon *A. subrufella* sex pheromone and to improve the development of new strategies of behavioral control techniques.

### RESUMO

O comportamento de acasalamento da mariposa *Atheloca subrufella* Hulst, 1887 (Lepidoptera: Pyralidae) foi investigado em arenas de laboratório. As descrições de corte e cópula em machos e fêmeas foram baseadas em observações diretas de casais virgens. Os resultados dos bioensaios de corte e cópula compreendem diversos padrões comportamentais tais como, antenação, busca pela fêmea (caminhando e/ou voando), encontro da fêmea, comportamento de chamamento da fêmea, exibição da glândula de feromônio, tentativa de cópula e cópula. Para isso, foram apresentados etogramas comportamentais de corte e cópula. Os resultados também sugerem que o comportamento de acasalamento em *A. subrufella* é mediado por um feromônio de longa distância e pela presença de um feromônio de curta distância. De maneira geral, esta pesquisa apresenta-se como estudo base para futuras investigações sobre o feromônio sexual de *A. subrufella* e desenvolvimento de novas estratégias de controle comportamental.

#### Palavras-chave:

*Cocos nucifera*

Traça do coco

Comportamento de corte

### INTRODUCTION

The study of mating behavior in insects is the first step for the development of sexual behavioral control strategies. Researches regarding calling behavior, presence or absence of pheromone gland, courtship and copulation patterns are being developed and stand as important tools to allow a better understanding and more accurate application of Lepidoptera control techniques (BARRADAS-JUAN et al., 2016; NASCIMENTO et al., 2016; SHI et al., 2018). According to Parra-Pedrazolli and Leal (2006), female moths produce a

mixture of pheromone compounds and the complete bouquet is responsible for male attraction. Nevertheless, control by sex pheromone may be achieved by the use of a single major constituent or by the use of a partial mixture.

The coconut moth *Atheloca subrufella* (Hulst, 1887) [= *Hyalospila ptychis* (Dyar, 1919)] (Lepidoptera: Pyralidae) is an important coconut pests, presenting wide distribution in Brazil (FERREIRA et al. 2002), it was also reported in other countries such as the USA, Mexico, Cuba, and the Virgin Islands (BONDAR, 1940; HEINRICH, 1956; KIMBAL, 1965;

HABECK; NICKERSON, 1982; HODGES et al., 1983; MOORE, 2001). According to FAO (2017) Brazil is the world's fifth largest producer of coconuts, reaching approximately a production of 2.4 million of tonnes in an area of 108,629.00 ha.

The *A. subrufella* damage is performed by the caterpillars that develop inside of the coconut inflorescences and fruits, they feed on the mesocarp and blocking the flow of the sap. Infested fruits and inflorescences are aborted and those that do not fall from the coconut palm tree become deformed and commercially depreciated. The management of this pest by traditional methods is restricted to the collection of infested coconut fruits and the use of chemical insecticides (FONTES; FERREIRA, 2006). Nevertheless, these techniques are often hampered by the caterpillars endophytic habit consequently, studies of new and efficient control measures are required.

Behavioral control strategies is not used for *A. subrufella*. However, some studies were developed to describe biological aspects and calling behavior of this moth (BENTO et al., 2006; NASCIMENTO et al., 2016). In order to contribute as basis to the development of new behavioral management techniques, the objective of this study is to describe the courtship and copulation behavior of *A. subrufella*.

## MATERIAL AND METHODS

### *A. subrufella* rearing

The *A. subrufella* rearing was kept in the Natural Resources Research Laboratory of Federal University of Alagoas (LPQRN/UFAL) under laboratory conditions at  $25 \pm 2$  °C, 80% RH and photoperiod 14L: 10D. The insects were collected in the field, from coconut fruits with signs of infestation by *A. subrufella* caterpillars. The collections were performed in the states of Sergipe and Alagoas, Brazil. The coconut fruits were kept in plastic containers (10 L) covered with voile fabric until the caterpillars reached the pupal stage. Pupae were sexed and after adult emergence, couples were kept in plastic cages (1 L) lined inside with paper towel as oviposition substrate. Eggs were daily collected and kept in Petri dishes with moistened filter paper. The caterpillars were daily fed with mesocarp coconut fruit cube of 2x2cm until reached pupal stage.

### Courtship bioassays

The courtship male behavior was direct observed through bioassays using a rectangular shaped glass arena (20 x 10 x 15 cm) containing a female trapped in a cage (hair roller of 5 cm) exhibiting calling behavior. The cage containing the female was covered by voile fabric to allow the compounds release and physical contact of male and female, but preventing the mating. Ten replicates were performed. For each replicate a different couple of adults was used. Four points were demarcated in the arena and in each replicate the female was allocated at a different point, aiming the elimination of any external physical, chemical or visual influence. *A. subrufella* adults with at least 24 hours age were used in the bioassays, ensuring sexual maturity (BENTO et al., 2006). The bioassays were performed from the first hour of scotophase, according to the period of *A. subrufella* female calling behavior (NASCIMENTO et al., 2016) under laboratory conditions of  $25 \pm 2$  °C and 60% RH. The couples were observed during 10 min.

### Copulation behavior

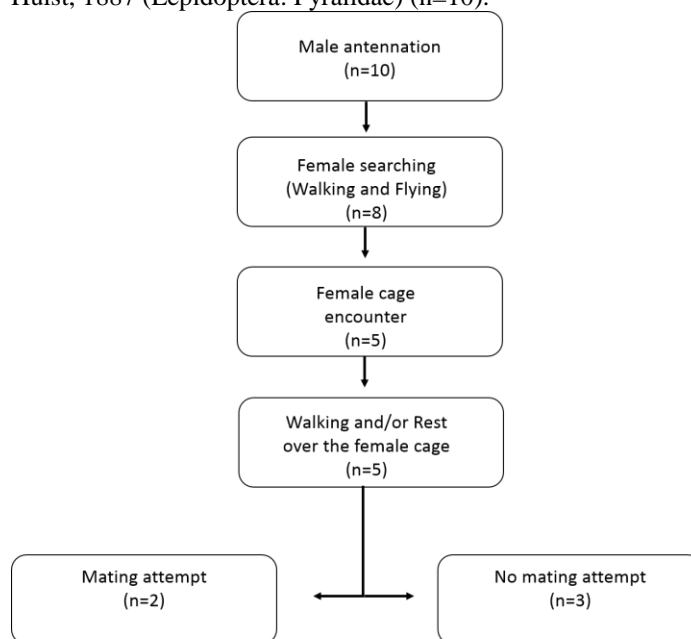
The copulation behavior was direct observed in plastic containers cages (250 mL) containing a virgin couple, the adults used in the bioassays were 24h to 96h of aged. Three replicates were performed. For each replicate a different couple of adults was used. The couple behavior was recorded during the female first scotophase period, according to the female calling behavior (NASCIMENTO et al., 2016). The description of the copulation behavior was performed through a video analyses made under red light conditions. The virgin couple was observed since the moment that the female exhibited the calling behavior until the moment of the copula.

## RESULTS AND DISCUSSION

### Courtship bioassays

During the arena bioassays was observed some behavioral patterns presented by *A. subrufella* adults. Both, males and females showed continuous antennation during courtship. The females performed the calling behavior throughout all the bioassay period. The male courtship behavioral patterns were classified as: antennation, female searching (walking and/or flying on the arena); female encounter; walking over the female cage surface, resting over the female cage surface; antennation on the female cage; mating attempt (flapping wings and arched abdomen); no mating attempt (Figure 1).

**Figure 1.** Courtship bioassays ethogram of *Atheloca subrufella* Hulst, 1887 (Lepidoptera: Pyralidae) (n=10).



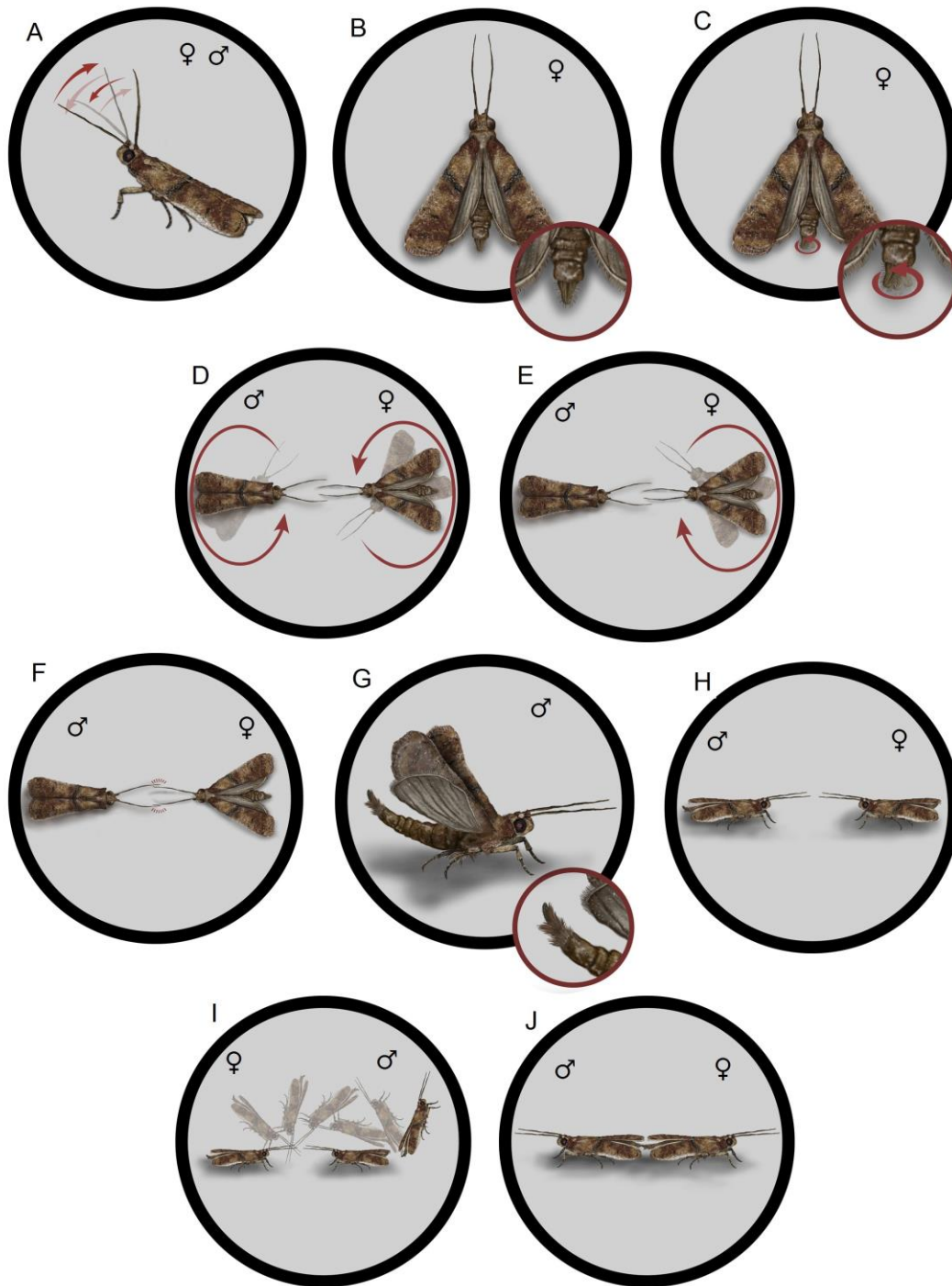
### Copulation behavior

The copulation behavior patterns are described as follow: male and female presented antennation behavior; the female exhibited the calling behavior, with wings slightly apart exposing the pheromone gland at the final portion of the arched abdomen; the female rotated the final portion of the abdomen spinning the pheromone gland 360°; the male rotated its body 360°; the female rotated its body 360° in both directions

(clockwise and anticlockwise); the male approached the female; male and female showed antennation behavior facing each other and touching their antennae; the male lifted its wings exhibiting the hairpencil on the final portion of the abdomen; the male

jumped over the female in a backflip resting on the ground with its abdomen opposite to the female abdomen; finally male and female initiated the copulation (Figure 2).

**Figure 2.** Mating behavior ethogram of *Atheloca subrufella* Hulst, 1887 (Lepidoptera: Pyralidae). Male and female antennation (A), female calling behavior (B, C), male and female body rotation (D, E), male and female touching antennae (F), male lifted its wings exhibiting the hairpencil (G), male jumped over the female in a backflip resting on the ground with its abdomen opposite to the female abdomen (H, I), mating position (J).



Based on the behavioral patterns showed by *A. subrufella* adults in the present study it is possible to assume that courtship and copulation in this species may be mediated by the presence of sex pheromone. *A. subrufella* females seems to release a long-range pheromone to attract the males, which recognize the females through contact, probably mediated by a short-range pheromone and/or cuticular hydrocarbons. This pattern of short distance recognition was also observed in other Pyralidae moth species, the *Amyelois transitella* Walker, 1863 (Lepidoptera: Pyralidae: Phycitinae), which exhibited similar behavior patterns such as antennation indicating a major characteristic of male and female sexual activity, the extrudation of a female abdominal (pheromone) gland and male recognition of female through antennation (PARRA-PEDRAZZOLI; LEAL 2006).

The *Dioryctria abietella* [Denis & Schiffermüller], 1775 (Lepidoptera: Pyralidae: Phycitinae) also presented a very similar behavior to *A. subrufella*, such as the same pattern of female calling behavior; the behavior of the male and female of touching the antennae after assuming the frontal position; and the attempt of male copulation, where the male lifted its wings exhibiting the hairpencil on the final portion of the abdomen and jumped over the female in a backflip resting on the ground with its abdomen opposite to the female abdomen (FATZINGER; ASHER, 1971).

The results of the present study suggest that mating behavior in *A. subrufella* may be mediated by a primary mixture of compounds which acts as long-range sex pheromone and also by a secondary mixture of compounds which acts as a short-range pheromone.

Same as that, the primary sex pheromone of *Choristoneura fumiferana* Clemens, 1865 (Lepidoptera: Tortricidae) is composed of (*E*)- and (*Z*)-11-tetradecenal, produced by the females and are efficient capturing virgin males on baited traps in a ratio 95: 5 (SANDERS and WEATHERSTON 1976; SILK et al., 1980). After cuticular compounds analyses, the presence of unsaturated alkanes in both sexes was noted, but the compounds (*Z,Z,Z*)-3,6,9-tricosatriene and (*Z,Z,Z*)-3,6,9-pentacosatriene were identified only in females. Arena and wind tunnel bioassays using *C. fumiferana* adults showed that males exhibit patterns behavior as flapping wings and arched abdomen in the presence of female body parts extracts and the female unsaturated alkanes. In addition, two female cuticular compounds, (*Z*)-5-tricosene and (*Z,Z,Z*)-3,6,9-tricosatriene, enhanced male responses to the primary sex pheromone components, acting in the male orientation and short-range courtship. (SILK et al., 2017).

Among the subfamilies of Pyralidae, the Phycitinae is one of the most abundant, comprising about 5,000 species distributed throughout the world (LI; REN, 2009). However, only 35 female sex pheromones were identified in this subfamily. The pheromonal mixtures identified to the Phycitinae species are characterized by the presence of aldehydes, acetates, alcohols and hydrocarbons (ANDO; YAMAMOTO, 2018). The number of identified pheromones compared to the number of species in Phycitinae reinforces the requirement and importance of this study.

The use of sex pheromones as a behavioral control strategy offer an environmentally friendly practice, successfully

applied in several techniques such as monitoring, mass control and mating disruption. Nevertheless, for *A. subrufella*, there is no sex pheromone identification until this moment and the control strategies are restricted to collection of infested coconut fruits and the use of chemical insecticides.

In overall, this research shows itself as a basis study to improve the development of new strategies of control, allowing further investigations upon *A. subrufella* sex pheromone, cuticular hydrocarbons and behavioral management techniques, within the principles of the Integrated Pest Management (IPM).

## CONCLUSIONS

Based at the results of this research it is possible to conclude that in the mating behavior of *A. subrufella*, the female produces compounds to attract the male, and the male is responsible by the search, recognition of the female and attempted copulation. This knowledge is essential to apply techniques of population control using pheromone.

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