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Authors: Barroso-Aké, Hanzel J., Cibrián-Tovar, Juan, Segura-León, Obdulia L., and Azuara-Domínguez, Ausencio

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Pre-courtship behavior and the effect of age on its duration in *Diatraea magnifactella* (Lepidoptera: Crambidae)

*Hanzel J. Barroso-Aké[1], Juan Cibrián-Tovar[1], Obdulia L. Segura-León[1], and Ausencio Azuara-Domínguez[2,]**

Abstract

To understand the chemical interaction of *Diatraea magnifactella* Dyar (Lepidoptera: Crambidae) with its conspecifics, the aim of the present paper was to describe and determine the effect of age on the duration of pre-courtship behavior in the female of *D. magnifactella*. To do so, we established a colony of *D. magnifactella* in the laboratory and placed 60 newly emerged females individually in 15 × 15 cm (height × width) acrylic cases. Then, we observed the behavior and effect of age on the duration of pre-courtship in the females for 11 d. The pre-courtship behavior of *D. magnifactella* was described based on the pre-courtship behavior of *Diatraea considerata* Heinrich, whereas the relationship between the duration of the pre-courtship behavior and the age of the female was determined through a non-linear regression analysis. The pre-courtship behavior of *D. magnifactella* females began with the separation of the wings from the body and the rhythmic movement of the antennae. After this, the female rotated its body on the surface where it was located, flexed its body, and exposed its sex gland. Pre-courtship started 6 h after the onset of darkness. It lasted longest on days 3 to 6 after it began. The duration decreased from day 5 until it ended on day 10. This pattern indicated that the age of the female plays a negative role in the duration of the pre-courtship activity in *D. magnifactella*.

Key Words: stem borer; *Saccharum officinarum*; chemical interaction; sex gland; pheromone

Resumen

Con la finalidad entender la interacción química de *Diatraea magnifactella* Dyar (Lepidoptera: Crambidae) con sus congéneres, el presente trabajo tuvo como objetivo describir y determinar el efecto de la edad en la duración del precortejo de la hembra de *D. magnifactella.* Para ello, en el laboratorio se estableció una cría de *D. magnifactella*. Enseguida, 60 hembras de cero días de edad fueron confinadas de manera individual en cajas de acrílico de 15 cm x 15 cm (altura y anchura). Posteriormente, la conducta y el efecto de la edad en la duración del precortejo de las hembras fue observada por 11 días. La conducta de precortejo de *D. magnifactella* fue descrita con base en el precortejo de *D. cosiderata* Heinrich. Mientras que, la relación entre la duración de la conducta de precortejo y la edad de la hembra fue determinada a través de un análisis de regresión no lineal. La conducta de precortejo de las hembras de *D. magnifactella* inicio con la separación de las alas del cuerpo y el movimiento rítmico de las antenas. Enseguida, la hembra giró el cuerpo sobre la superficie donde se encontraba situada, flexionó el abdomen y expuso la glándula sexual. El precortejo ocurrió seis horas después de comenzar la fase oscura. Este tuvo una mayor duración tres días después del inicio. Posteriormente, la duración comenzó a disminuir hasta finalizar en el día 10. Lo anterior indicó que la edad de la hembra participa de manera negativa en la duración de la actividad de precortejo de *D. magnifactella*.

Palabras Clave: barrenador del tallo; *Saccharum officinarum*; interacción química; glándula sexual; feromona

Diatraea magnifactella Dyar (Lepidoptera: Crambidae) feeds on the apical meristem, pods, and leaves and penetrates the knots to feed on the stem of the sugarcane plant (*Saccharum officinarum* L.; Poaceae) (Rodríguez-del-Bosque & Pantaleón-Paulino 2012). The larvae remain within the stem for 18 to 24 d in the summer and up to 60 d in the winter (Rodríguez-del-Bosque & Pantaleón-Paulino 2012). This behavior makes the chemical control of *D. magnifactella* inefficient (de Freitas et al. 2007). Through the study of pre-courtship and courtship behavior, a sex pheromone was described and used to monitor the *Diatraea* and *Eoreuma* genera (David & Birch 1989; Osorio & Cibrián-Tovar 2000a; Hummel et al. 2010; Rodríguez-del-Bosque & Pantaleón-Paulino 2012). David & Birch (1989) mentioned that the behavioral pattern of the insect could be studied to determine the effect of the pheromone on the reproductive behavior. In this regard, Osorio & Cibrián-Tovar (2000a) determined the pre-courtship and courtship behavior of *Diatraea considerata* Heinrich. It begins when the female flexes its abdomen upwards. After this, it exposes its gland and releases sex pheromones. As a response, the male shakes its antennae and wings, exposes its genitalia, and flies towards the female. This information sets the basis of understanding the chemical communication of *D. considerata* with its conspecifics. In the case of *D. magnifactella*, the pre-courtship behavior of the females and the factors involved in its development are unknown. Therefore, the objective was to describe and determine the effect of age on the duration of the pre-courtship behavior in *D. magnifactella* females.

[1]Colegio de Postgraduados, Montecillo, Estado de México, 56230, México; E-mail: hanzel.barroso@gmail.com (H. J. B.-A.), jcibrian@colpos.mx (J. C.-T.), sleon@colpos.mx (O. L. S.-L.)

[2]Instituto Tecnológico de Cd. Victoria, Tamaulipas, 87010, México; E-mail: azuarad@gmail.com (A. A.-D.)

*Corresponding author; E-mail: azuarad@gmail.com (A. A.-D.)

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Materials and Methods

INSECT REARING

For the pre-courtship behavior experiment, a colony of *D. magnifactella* was established in the Insect Chemical Ecology Laboratory of the Colegio de Postgraduados, Texcoco, Estado de México. Pupae to initiate this colony had been donated by the Biotechnology Research Center of the Autonomous University of Morelos. The brood was established at 25 ± 1 °C, 70 ± 10% relative humidity, and a photoperiod of 12:12 h L:D, following the method described by Osorio & Cibrián (1997).

PRE-COURTSHIP BEHAVIOR

The experiment on pre-courtship behavior was completed from Apr to Jun 2013. Sixty newly hatched females were used. These were individually confined in 15 × 15 cm (height × width) acrylic boxes, with a 10% sucrose solution as food. The boxes with the females were immediately placed in a breeding chamber, and the pre-courtship behavior was analyzed at 25 ± 1 °C temperature and 70 ± 10% relative humidity in an inverted 14:10 h L:D photoperiod (dark started at 4:00 am). The pre-courtship behavior was described based on that of *D. considerata* as reported by Osorio & Cibrián-Tovar (2000b). The behavior was assigned to 6 steps: activation (movement of the antennae or the wings), the female moves, the female flexes its body, the female does not flex its body, the female exposes its sex gland, and the female does not expose its sex gland. Observations were taken after beginning of the dark phase for 11 d. In each data collection, the test was finished if the female did not move for 5 min or at the time when the female retracted its sex gland. In addition, the duration of the pre-courtship behavior was recorded and defined as the time elapsed from the activation of the pre-courtship behavior until the female retracted its sex gland.

DATA ANALYSES

The numbers of females that completed the pre-courtship steps were added to a first-order Markov's contingency table (Fagen & Young 1978). In each cell of this table, the number of females that, taking a particular step (columns), had taken a previous step (lines), was noted considering that the transition from one step to the other depends solely on the identity of the previous step (Fagen & Young 1978; Liimatainen & Hoikkala 1998). The probabilities of transition were obtained by dividing the frequencies of each cell by the total number of females in each line. To evaluate the degree of general dependency between the steps that make up the pre-courtship sequence in females, the Fisher exact test was used. Finally, the probabilities of transition were used in the construction of a flux ethogram that shows the sequence of steps and routes that the pre-courtship behavior in the *D. magnifactella* females can follow (Birch et al. 1989; Osorio & Cibrián-Tovar 2000b; Tomislav & Muñoz 2011). The effect of age on the duration of the pre-courtship behavior was analyzed using a non-linear regression in the statistical software SAS version 9.1 (SAS Institute 2004).

Results

The routes and probabilities of the pre-courtship steps in the *D. magnifactella* females are shown in Fig. 1. The pre-courtship began with the separation of the wings from the body and the rhythmic movement of the antennae (activation step; Fig. 2a). Immediately, 85% of the females rotated their body 45° on the surface they were on, 89% flexed their abdomen, and finally, 98% of the females exposed their sex gland (Fig. 2b). By contrast, 3% of the females that separated their wings and moved their antennae flexed their abdomen without rotating their body 45°. Furthermore, 8% of the females that rotated their body and 1% of the females that only presented the activation step did not flex their abdomen. Finally, 56% of the females that did not flex their abdomen did not expose their sex gland.

Pre-courtship happened 6 h after the beginning of darkness. The duration peaked 5 d after its initiation (57.2 min). Later, the duration decreased until it stopped on day 10 (3 min). The relationship between the duration of the pre-courtship behavior and the age of the females was adjusted to a polynomial second-degree regression model (R^2 = 97.4). The model indicated a negative relationship; the age of the female played a negative role in the duration of the pre-courtship activity of *D. magnifactella* (Fig. 3).

Discussion

The pre-courtship behavior of *D. magnifactella* was similar to that observed in *D. considerata*, *Dioryctria abietella* Denis & Schiffermüller (Lepidoptera: Pyralidae), and *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) (Fatzinger & Asher 1971; Barrer & Hill 1977; Osorio & Cibrián-Tovar 2000a). In *E. cautella*, the position of the antennae plays an important role in the average number of successful courtships (Barrer & Hill 1977). The females of *D. abietella* and *E. cautella* facilitate the exposure of the gland and the mating process by flexing their abdomen (Phelan & Baker 1990; Osorio & Cibrián-Tovar 2000a). However, the females of *D. considerata* release volatile compounds when exposing their gland, which attract the males and assure that mating occurs (Osorio & Cibrián-Tovar 2000a).

We determined that the age of the female plays an important role in the duration of the pre-courtship behavior. Osorio & Cibrián-Tovar (2000b) reported that the duration of the pre-courtship behavior is related to the courtship activity. For *D. considerata* and *Copitarsia decolora* (Guenée) (Lepidoptera: Noctuidae), the highest percentage of pre-courtship and courtship activity was observed in the first 3 d after female emergence (Rojas et al. 1993; Osorio & Cibrián-Tovar 2000b).

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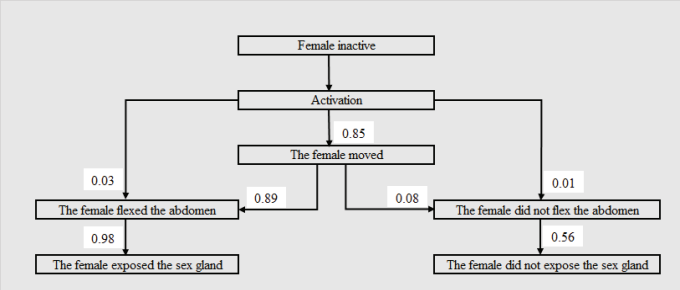


Fig. 1. Routes and probabilities of the transition between successful steps of pre-courtship behavior in *Diatraea magnifactella* females ($P < 0.05$; $n = 60$). The values indicate the probability of the occurrence of the next step.

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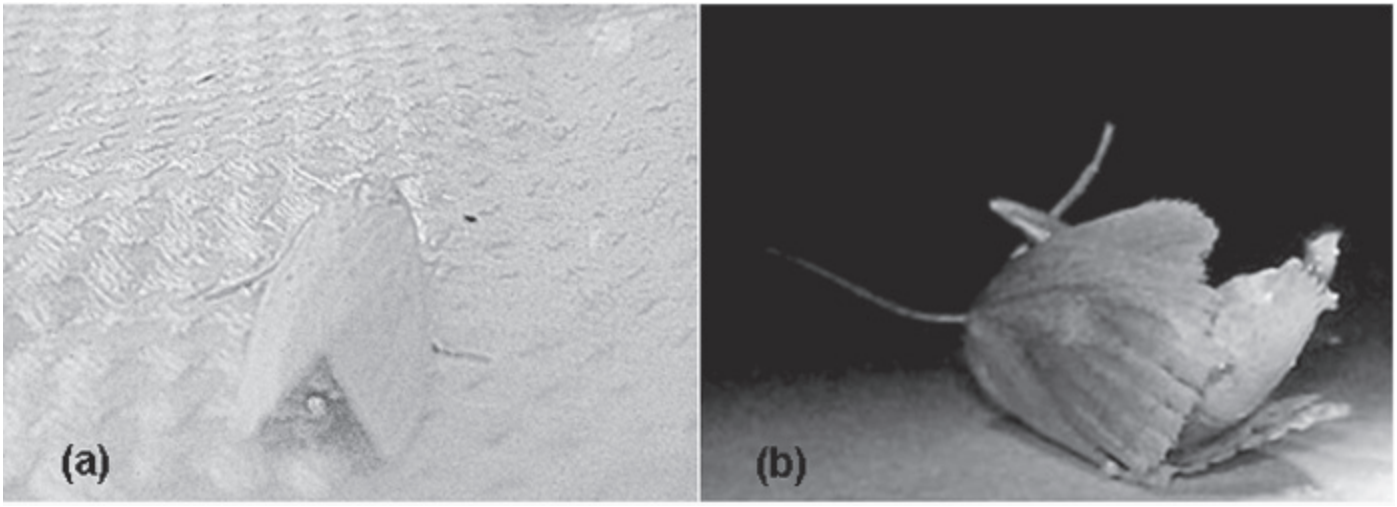


Fig. 2. Position of the female at the beginning of pre-courtship (a). Flexing of the abdomen and exposure of the sex gland of *Diatraea magnifactella* (b).

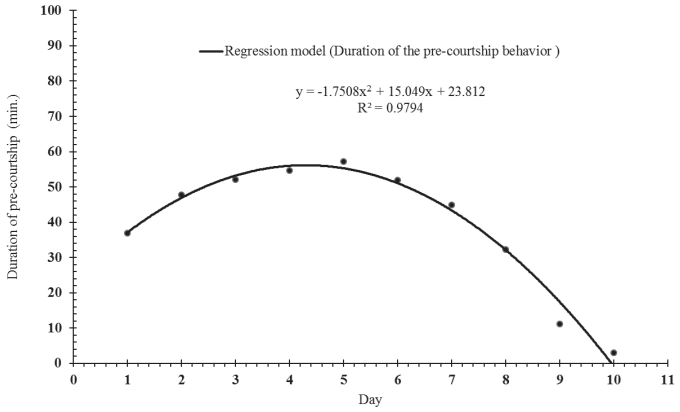


Fig. 3. Analysis of the effect of age on the duration of pre-courtship in *Diatraea magnifactella* females.

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