Research Article

Calling behavior in virgin females of *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) in laboratory

Conducta de llamado en hembras vírgenes de *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) en laboratorio

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Abstract. The stemborers of the genus *Diatraea* are the pest with the greatest impact on sugarcane production systems in Colombia. To understand the calling behavior of virgin females of *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae), two preliminary tests were established during twenty-four continuous hours in the laboratory. These were set up using females of one and two days old respectively under controlled conditions of temperature and relative humidity (25 °C - 50%) and photoperiod 12h (L): 12h (D). The variables evaluated were onset hours, call duration, and sexual gland exposure number. With previous test results, we determined the slot time when females call and thus, we were able to establish a new trial to evaluate the same variables in females one, two, and three days old (n=30), with inverted photoperiod. The calling behavior was evidenced from the fifth to the eleventh hour of scotophase. Calling duration varied between 30 and 210 min, with the percentage of females calling during 30 min being higher. 37% of the one day old females called only once. These basic studies allow the understanding of sexual behavior of this, which leads to creating tools that can be included on *D. saccharalis* integrated pest management.

Key words: Borer; sexual behavior; sexual gland; sugarcane.

Resumen. Los barrenadores del tallo pertenecientes al género *Diatraea* son la plaga de mayor impacto en los sistemas productivos de caña de azúcar en Colombia. Para conocer el horario de llamado de las hembras de *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae), se realizaron dos ensayos preliminares en laboratorio donde, durante 24 horas continuas se evaluó el comportamiento de llamado de hembras vírgenes de uno (n=26) y dos (n=29) días de edad, bajo condiciones controladas de temperatura y humedad relativa (25 °C – 50% respectivamente) y fotoperiodo de 12h (L): 12h (D). Con los resultados obtenidos en los ensayos anteriores se determinó la franja horaria del comportamiento de llamado, la cual fue empleada para establecer un nuevo ensayo con hembras de uno, dos y tres días de edad, empleando 30 hembras por edad en fotoperiodo invertido. Las variables evaluadas fueron: hora de inicio, duración del llamado, número de exposiciones de la glándula sexual. El comportamiento de llamado se evidenció desde la quinta hasta la decimoprimera hora de la escotofase. La duración del llamado varió entre 30 y 210 min, siendo mayor el porcentaje de hembras que llamaron durante 30 min. El 37% de las hembras de un día llamaron una única

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vez. Estos estudios básicos permiten entender el comportamiento sexual de la plaga para generar herramientas que puedan ser incluidas en los planes de manejo integrado de *D. saccharalis.*

Palabras clave: Barrenador; caña de azúcar; comportamiento sexual; glándula sexual.

Introduction

Diatraea spp. are cane borers moths belonging to the family Crambidae which includes genera of agricultural importance such as *Chilo* Zincken, 1817, *Crambus* (Fabricius, 1798) and *Diatraea* Guilding, 1828 (Solis & Metz 2016). Solis & Metz (2016) reports 41 species of *Diatraea* for the American continent. The borers belonging to this genus represent great economic importance due to the host´s diversity they affect and larval stage habits.

The sugarcane borer *Diatraea saccharalis* (Fabricius, 1794) is a wide distribution specie in the American continent and one of the most limiting pests in grasses including the sugar cane crop, bringing about losses in young and mature crops (Pérez & Martínez 2011; Sidhu *et al*. 2013; Solis & Metz 2016). The damage carried out by the larvae in early crop stages causes dead heart symptoms, in mature canes the larvae develop inside the stems creating galleries and feeding of them, causing biomass loss and tissue fermentation (Lopez 2015).

Due to the larvae habit and the producer´s lack of knowledge about the insect impact on production, it is necessary to control it integrating strategies into management plans (Quecine *et al.* 2014; Reguilón *et al.* 2014). The use of commercial pheromones for monitoring or control of different pest insects has been included in the integrated management programs of some agricultural importance species such as *Tecia solanivora* Povolný, 1973 (Bosa *et al*. 2008), *Phthorimaea operculella* (Zeller, 1873) (Salas 2007), *Ceratitis capitata* (Wiedemann, 1824) (Ros *et al*. 1996), *Cydia pomonella* Linnaeus, 1758 (Minarro & Dapena 2000), *Anthonomus eugenii* Cano (Muñiz-Merino *et al*. 2014), *Rhynchophorus palmarum* (Linnaeus, 1758) (Chinchilla *et al*. 1996), among others.

Sexual communication in Lepidoptera is mediated by pheromones, volatile compounds that are released by females to attract males and induce pre-copulation or mating (Cañas-Hoyos *et al*. 2017; Roelofs & Carde 1977). The pheromone emission is mediated by a typical behavior in females known as "calling", which occurs when they expose the sexual gland located in the caudal region, releasing the sexual pheromone as a stimulus to attract the male (Cardé & Haynes 2004).

Osorio and Cibrián (1997), studied *Diatraea considerata* Heinrich, 1931 females calling behavior and carried out the sexual pheromone extraction, finding that 100% of females called from the first emergency night. Palacio-Cortés *et al*. (2010, 2014) conducted studies to establish the geographic variation of *D. saccharalis* sexual pheromone, calling behavior and major components of chemical structure of *Diatraea indigenella* sex pheromone in the laboratory, finding a variation in the pheromone's composition for different populations evaluated.

In 2010, Palacio and collaborators carried out studies to know the *D. saccharalis* sexual pheromone composition on populations from Brazil, Colombia, Uruguay, Mexico, Venezuela, Jamaica and the USA, finding a variation in the genetic population´s composition and in the sex pheromone compounds quantification. Oliveira *et al*. (2018) studied the synthesis and evaluation of *D. saccharalis* sexual pheromone compounds to improve its efficiency in field. In Colombia, Barreto-Triana *et al*. (2018) evaluated two commercial pheromones for this species, obtaining low capture percentages compared to captures made with virgin females, showing the low attraction and efficacy for Colombian populations borer. Silva *et al*. (2021) identified two additional constituents of *D. saccharalis* female sex glands, these new compounds improve wind tunnel attraction.

Basic studies like this, allow for further knowledge regarding the age and time of greatest female sexual activity and also the most efficient moment to extract pheromones to know their concentration and characterization as a tool which can be potentially integrated to management plans. Therefore, we aim to establish the calling behavior of *D. saccharalis* in the laboratory.

Materials and Methods

All the studies were carried out in the entomology laboratory of Agrosavia C.I. Tibaitatá, in the municipality of Mosquera (Cundinamarca, Colombia) (latitude: 4.695066, longitude: -74.203953, altitude: 2575 m), with controlled conditions of temperature 25 °C, 50% relative humidity and 12h light: 12h dark. The insects and biological material analyzed for this study were collected under the collection frame permission 1466 of 2014 issued by the Autoridad Nacional de Licencias Ambientales to the Corporación Colombiana de Investigación Agropecuaria.

To evaluate *D. saccharalis* females calling behavior, virgin females from breeding in the entomology area were used. Once the pupae were obtained, they were separated by sex and individualized in acrylic containers.

Source of insects

Diatraea saccharalis breeding was established in the entomology laboratory of Agrosavia. The larvae were placed in acrylic containers with a plastic lid, which contained modified meridic diet proposed by Hensley & Hammond (1968) and used in the Biology Laboratory of Insects in the Escola Superior de Agricultura. Luiz de Queiroz Piracicaba - SP, Brazil and kept at 25 °C, 50% RH, and 12h Light (photophase): 12h Darkness (scotophase), following the breeding protocol established by this laboratory (Parra 1999).

Calling behavior

Two preliminary tests were conducted for 24 continuous hours to determine females peak activity time. Twenty-six one-day-old and 29 two-day old females were evaluated, and observations were performed with 30-minute intervals. Females calling process was recognized by wings raising and the partial or total exposure of sexual gland, following the methodology proposed by Osorio and Cibrián (1997).

Utilizing previous trial's results, the scotophase time slot (nighttime activity time between 18 hs and 6 hs when the females were the most active) was established and this was used to perform the behavioral evaluations of the call, making use of inverted photoperiod. Subsequently, the same variables of the previous tests were evaluated in 30 virgin female, one, two, and three days old respectively, at 30 minutes intervals between the fifth and twelfth hour of scotophase, using red light lamps (approximate wavelength of 645 -700 nm) according to Osorio and Cibrián (1997).

Statistical analysis

To determine significant differences between the variables onset, duration, and call number compared to females age, a completely randomized design with 3 treatments (ages) and 30 repetitions were implemented. The data was analyzed by generalized linear models using the negative binomial distribution, to determine differences in means, an LSD Fisher comparison was performed ($p < 0.05$), with Bonferroni correction (to avoid type I errors). All analyzes were done using the statistical software R, version 3.6.0 (R Core Team 2017).

Results and Discussions

Temporal pattern of calling behavior exhibited by *D. saccharalis* females

For preliminary trials, it was observed that virgin females between one and two days old presented calling behavior during the scotophase. It was established that slot time with the highest activity of one-day virgin females is between the sixth and tenth hour and between the sixth and ninth hour for two-day-old females (Fig. 1). This behavior wasn´t observed during the photophase.

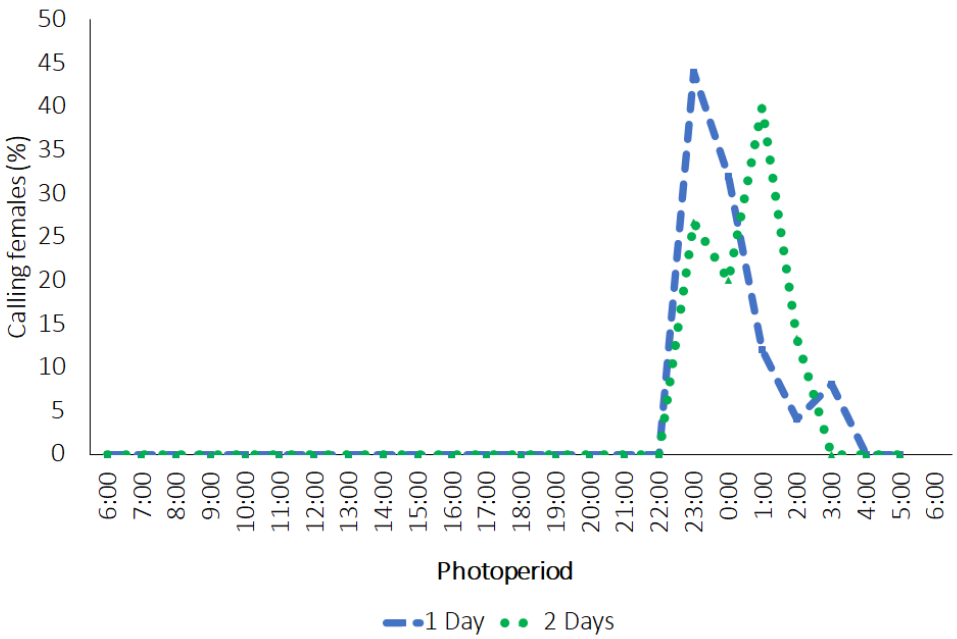

Figure 1. Temporal pattern of calling behavior exhibited by *Diatraea saccharalis* females. / Patrón temporal de llamado de hembras de *Diatraea saccharalis*.

Virgin female calling, was observed in females from the first emergence day, it suggests sexual maturity and could also be related to greater male attracting probability for copulation (Batista-Pereira *et al*. 2004).

D. saccharalis virgin females onset, duration, and calls number

D. saccharalis females calling behavior started from the fifth hour of scotophase (22:00 hs - 23:00 hs) and extended until the eleventh hour (04:00 hs - 05:00 hs) for one-day-old females. This time decreased as age increased, registering females call between the sixth hour (23:00 hs - 00:00 hs) and the tenth hour (03:00 hs- 04:00 hs) for two-day-old females and between the seventh (00:00 hs - 01:00 hs) and the ninth (02:00 hs- 03:00 hs) for three-day-old females (Fig. 2). No significant differences were found ($F=0.97$ $p = 0.3851$) between the three ages evaluated for the female call time variable.

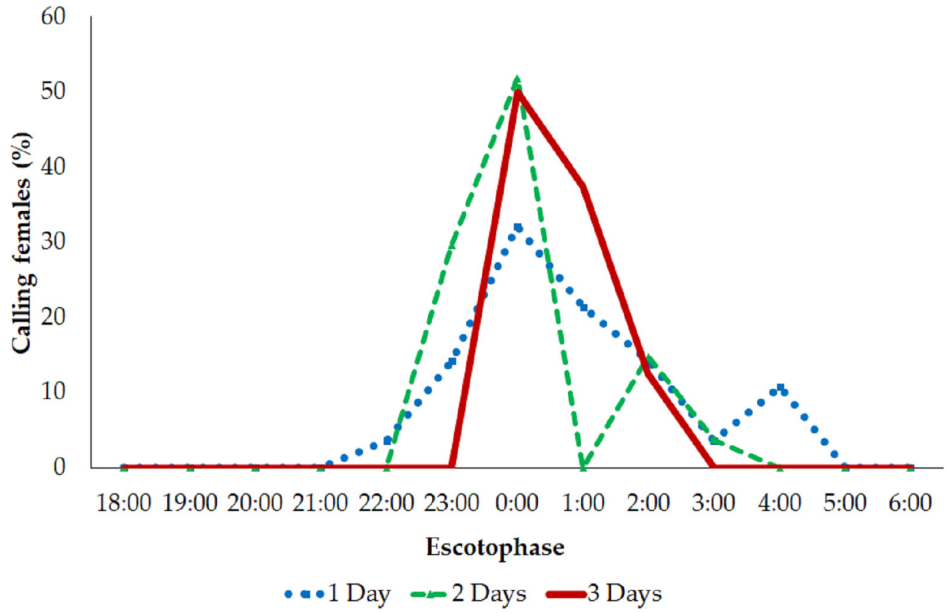

Figure 2. *Diatraea saccharalis* calling temporal pattern exhibited by one, two and three-days-old females. / Horario de llamado de hembras de *Diatraea saccharalis* de uno, dos y tres días de edad en laboratorio.

The time of greatest activity for three ages corresponded to the seventh hour, indicating that the calling behavior of evaluated insects is governed by the circadian rhythm. According to these results, it can be established that sexual glands extraction from virgin females of one, two, and three-days-old can be performed at this time of scotophase.

Osorio and Cibrián (1997), studied *D. considerata* females calling behavior, finding that call initiated from the first night and was decreasing as age increased, showing activity between the sixth and tenth scotophase time. Palacio-Cortés *et al.* (2014) report the onset of *D. indigenella* females calling behavior from the first day of emergence and between the fifth and eleventh hour. Female cabbage looper *Trichoplusia ni* (Hübner, 1803) were observed calling from the eighth hour of scotophase (Sower *et al*. 1970). Swier *et al*. (1976) relates calling behavior with sexual maturity, which would allow inferring that *D. saccharalis* females are sexually receptive since emergence.

Calling behavior was characterized by sexual gland exposure, this behavior has been observed in other insect species (Barrer & Hill 1977; Barth 1958; Sower *et al*. 1970). Kanno (1979) reports the same phenomenon in rice stem borer *Chilo suppressalis* (Walker, 1863), as did Pires *et al*. (1994) for *Elasmopalpus lignosellus* (Blanchard, 1852). Sandoval-Cáceres *et al*. (2022) reports the sexual gland exposure as the triggering factor of copulation behavior in *D. saccharalis* adults.

For the variable call duration, there is a variation between 30 min and 210 min, pointing out the highest percentage of 2-day-old females (37.9%) called for 30 minutes, while 20.7% of one-day females extended the call up to 60 minutes. About 60% of 3-day-old females did not call during the evaluations (Fig. 3). The reduction in calling time of two-day-old females can be explained as a strategy used by individual to have greater copulation probability than young females due to the decrease in available resources as these have been used to perform the previous call (Swier *et al*. 1976). Were observed significant differences between the ages and the duration of the call ($F= 3.20$, $p = 0.049$).

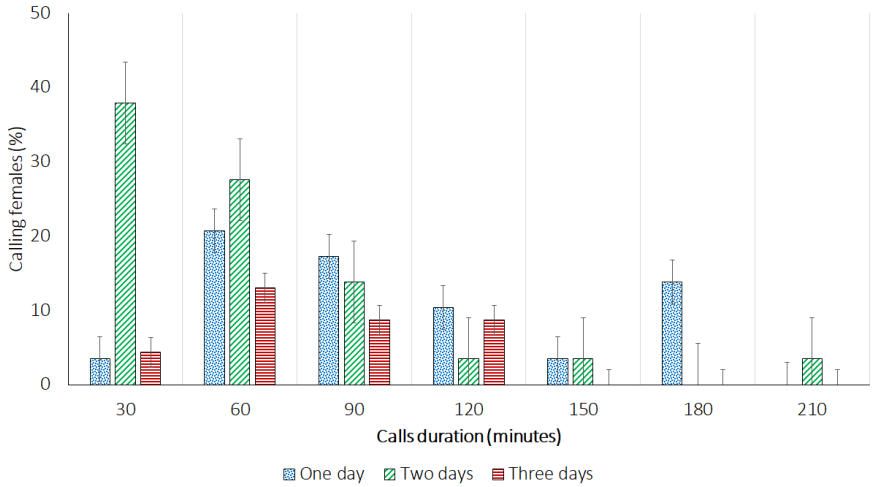

Figure 3. Call duration of one, two and three days old *Diatraea saccharalis* females. / Duración de llamado de hembras de uno, dos y tres días de edad de *Diatraea saccharalis* en laboratorio. * Error bars indicate standard error.

Osorio and Cibrián (1997) studied the virgin females call duration of *D. considerata*, finding an average between 117, 175 and 172 minutes for one, two and three -days females, respectively. The call time decreased with age increasing, coinciding with this study. Other authors have observed a similar behavior such as those presented by Pires *et al*. (1994) for *E. lignosellus* and Kanno (1979) for *Chilo suppressalis* Walker, 1863, who documented the initiation of a call from the first night after the emergency.

Sexual gland exposed time varied between zero and three times. The highest percentage of one-day-old females called once (37%), while two-day-old females (40%) called twice and about 70% of three-day-old females did not call (Fig. 4). No significant differences were observed for this variable between one and two- days-old females (F=8.60, $p = 0.0004$), but in three-days-old females respect to the other two groups was observed.

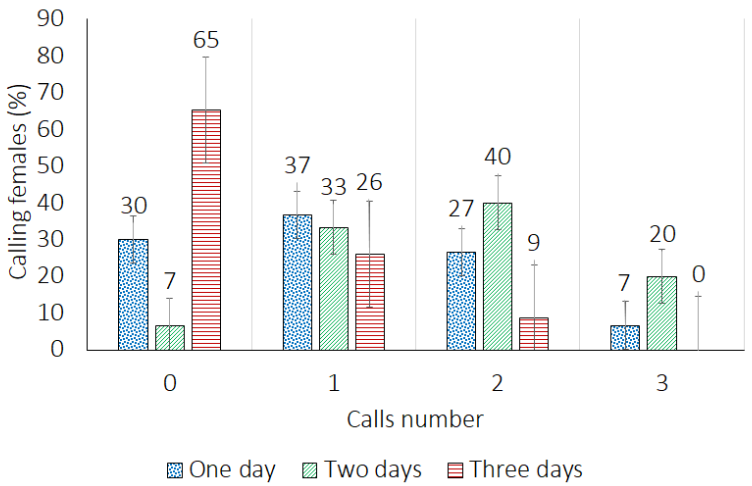

Figure 4. Calling number of one, two and three- days- old *Diatraea saccharalis* virgin females. / Número de llamados de hembras vírgenes de *Diatraea saccharalis* de uno, dos y tres días de edad. * Error bars indicate standard error.

Obtained results show that two-day-old virgin females increased their calls number, this occurs as a females strategy to expand the copulation probability, where they release large pheromone amounts in short periods, to increase the impregnation surface and later in prolonged periods the pheromone emission is reduced (Osorio and Cibrián 1997).

Tillman *et al.* (1999) attribute this behavior to insect physiological parameters that can influence pheromone concentration in the sexual gland. Conner *et al*. (1980) indicate that this behavior may be an important adaptive strategy, used by insects to avoid losing chemoreceptor sensitivity and avoiding sensory adaptation.

Our findings allowed us to establish that the optimal time for the extraction of *D. saccharalis* sexual gland is between the seventh and ninth hour of scotophase, because at this time the highest female's percentage of the three ages evaluated presented the calling behavior. The better understanding of adults' sexual behavior mediated by infochemicals allows predicting the form and conditions in which the insects produce the compounds and their response to stimuli highlighting its potential for the further development of semiochemical-based insect control methods.

Conclusions

The time of greatest activity (gland exposure) occurred between the sixth and eighth hour of scotophase, being seventh hour the most appropriate for sexual glands extracting.

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