Spodoptera frugiperda (Lepidoptera: Noctuidae) Females Can Detect the Sex Pheromone Emitted by Conspecific Females

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Spodoptera frugiperda (Lepidoptera: Noctuidae) females can detect the sex pheromone emitted by conspecific females

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Abstract

The sex pheromones emitted by *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) females attract males for copulation, but no studies to date have investigated if conspecific females also are attracted. Here, the attraction of females of *S. frugiperda* to their sex pheromone in flight tunnel laboratory bioassays and field trapping is reported. Genitalia of females and males captured in the field were dissected for taxonomic identification and studied with an environmental scanning electron microscope to know the mating status of the females. In wind tunnel attraction bioassays, virgin females flew upwind and landed on the stimulus, likewise the males, whereas mated females, although they headed for the stimulus, showed fewer landings. The sex ratio of captured insects in the field was 1 female to 4 males. The presence of spermatophores allowed the separation of mated and virgin females using the genitalia; both were found in the traps throughout the sampling period. This study demonstrated that *S. frugiperda* females autodetect their sex pheromone, and its implications on the management strategy for these moths are discussed.

Key Words: autodetection; females; sex pheromone; wind tunnel; traps

Resumen

Las feromonas sexuales emitidas por las hembras de *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae) atraen a los machos para la cópula, pero no hay estudios que investiguen si las hembras conespecíficas son atraídas. En este estudio, se informa de la atracción de las hembras de *S. frugiperda* a su feromona sexual en bioensayos de laboratorio en túnel de vuelo, y trampas de campo. Se disectaron las genitalias de las hembras y los machos capturados en el campo para su identificación taxonómica y también se estudiaron con el microscopio electrónico de barrido ambiental para conocer el estado de apareamiento de las hembras. En los bioensayos de atracción en el túnel de viento, las hembras vírgenes volaron contra el viento y se posaron en el estímulo, al igual que los machos, mientras que las hembras apareadas, aunque se dirigieron al estímulo, mostraron menos aterrizajes. En el campo, la proporción sexual de los insectos capturados fue de una hembra por cada cuatro machos. Las genitalias de las hembras apareadas y vírgenes se distinguieron por la presencia del espermatóforo. Este estudio demuestra que las hembras de *S. frugiperda* autodetectan su feromona sexual y se discuten sus implicaciones en la estrategia de manejo de estas palomillas.

Palabras Clave: autodetección; hembras; feromona sexual; túnel de viento; trampas

Spodoptera frugiperda J.E. Smith (Lepidoptera: Noctuidae) is native to the Americas and recently has spread to different countries of Europe (Rwomushana 2019), Africa (Goergen et al. 2016; Cock et al. 2017; Brévault et al. 2018), and Asia (Ganiger et al. 2018; Deshmukh et al. 2018; Bajracharya et al. 2019; Chormule et al. 2019). Producers use chemical insecticides, mainly organophosphates and pyrethroids, for *S. frugiperda* control in sorghum, rice, cotton, and corn, which can affect the health of both producers and consumers (Nicholson 2007; Cazmuz et al. 2010). Furthermore, these compounds are contaminants of water and soil, and they also cause death of beneficial insects (Téllez-Rodríguez et al. 2014). Therefore, the use of alternatives for the management of *S. frugiperda* using insect behavior modifiers aims to reduce population density and the survival of progeny; the use of the synthetic sex pheromone has been the most successful (Gut et al. 2004; Witzgall et al. 2010), because it exploits the pest reproductive behavior. There is growing evidence that female Lepidoptera can detect their sex pheromone and modify their behavior accordingly (Holdcraft et al. 2016). This phenomenon is known as autodetection, and research is carried out for the benefit of trapping systems because a trap is more efficient when it targets both sexes of the pest.

The sex pheromone of female *S. frugiperda* consists of (Z)-7-dodecenyl acetate ([Z]-7-12: Ac), (Z)-9-tetradecenyl acetate ([Z]-9-14: Ac), and (Z)-11-hexadecenyl acetate ([Z]-11-16: Ac) (Tumlinson et al. 1986; Gaona 2015), and is produced in glands located in the abdominal 8th and 9th segments (Fig. 1) (Jurenka 2003). The behavior shown by the males to the sex pheromone typically consists of a zigzag-oriented flight with short or long angles depending on the trajectory to be covered. Different authors have reported the capture of conspecific males

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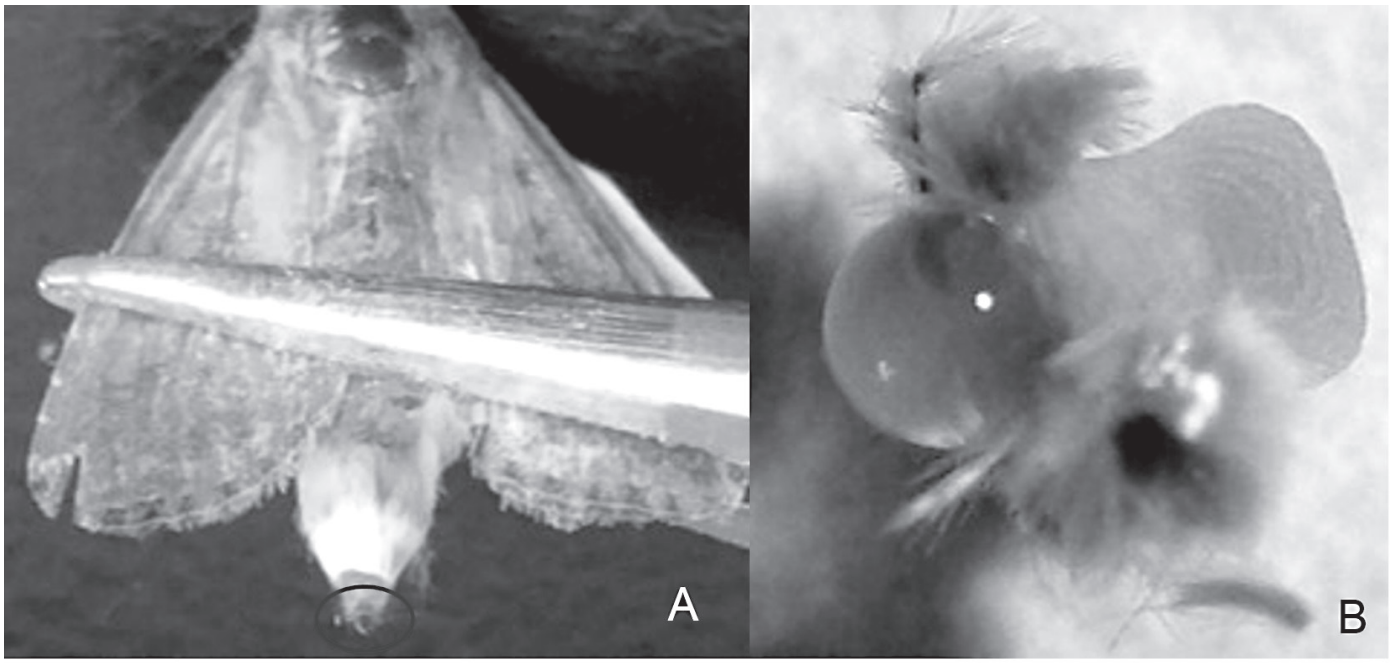

Fig. 1. (A) Virgin female abdomen 3 to 5 d old *Spodoptera frugiperda* females, black circle is location of sex pheromone gland; (B) sex pheromone-producing gland in female *S. frugiperda*.

with the sex pheromone released by the female (Howse et al. 1998; Malo et al. 2001, 2018). However, there is evidence that females of certain Lepidoptera species can autodetect their sex pheromone, unlike those females considered anosmic. It has been reported that female *Trichoplusia ni* Hübner (Grant 1970), *Spodoptera exigua* Hübner (both Lepidoptera: Noctuidae) (Yang et al. 2009), and *S. frugiperda* (Malo et al. 2004) respond to their sex pheromone at the antennal level. However, the response intensities are lower than those of males. Changes in behavior have been observed in moths when exposed to the sex pheromone of conspecific females. Virgin females of *S. exigua*, *Heliothis armigera* Hübner, and *Helicoverpa zea* Boddie (both Lepidoptera: Noctuidae) are not only not attracted to the stimulus but had a repellent response, whereas mated females showed attraction to the sex pheromone of the glandular extract (Saad & Scott 1981). The mechanism mediating the response of mated females to their sex pheromone is unknown. However, pre-exposure of females to the conspecific sex pheromone at the moment of copulation could generate a kind of "olfactory learning" and thus facilitate changes in their behavior (Stelinski et al. 2014).

Autodetection of the sex pheromone can improve pheromone-based trapping systems since a trap is more efficient if both sexes are caught (Holdcraft et al. 2016). Thus, if the females of a species of Lepidoptera can autodetect their sex pheromone, they may be able to locate sites for more successful mating, so that pheromone could function as an aggregation pheromone. In this regard, female moths also can perceive when there are too many females, with little chance of mating (Stelinski et al. 2014; Holdcraft et al. 2016). Nonetheless, autodetection of conspecific pheromone by females also can induce a repellent response to avoid competition for the host plant (Stelinski et al. 2014).

Antennal receptivity of *S. frugiperda* females to their sex pheromone has been studied by electro-antennography bioassays (Malo et al. 2004). However, there are no reports of the behavioral response in flight tunnels of virgin and mated females to their sex pheromone, nor are there reports of field captures of females using the conspecific sex pheromone complemented with the description of the genitalia of the *S. frugiperda* female. Therefore, in the present study, we evaluated the attraction of males, and virgin and mated *S. frugiperda* females to the conspecifics sex pheromone in wind tunnel laboratory bioassays and field traps. This study provides the knowledge needed to improve the pheromone-based management strategy for this insect.

Materials and Methods

INSECTS

Larvae of *S. frugiperda* were collected from a maize field located in the community of Tlatenchi, Jojutla of Juarez, Morelos, Mexico (18.596389°N, 99.186389°W; 900 masl). They were transported to the Insect Chemical Ecology laboratory of the Centro de Desarrollo de Productos Bióticos del Instituto Politécnico Nacional, Yautepec, Morelos, Mexico, and placed in quarantine to mitigate the presence of parasitoids or diseases. Offspring were reared under controlled conditions with a photoperiod of 12:12 h (L:D) at 25 ±2 °C and 65 ± 2% RH. They were fed on an artificial diet especially developed for lepidopterans (Burton & Perkins 1987). Neonatal larvae were placed individually into plastic containers with a lid (4 × 3 cm) and were reared until they reached the adult stage. Adults emerged within the containers, then later they were grouped according to their sex. Some adults were used to form pairs and were fed on a 50% sugar solution daily. Adults were placed in acrylic boxes (20 × 20 cm) for mating; females were allowed to oviposit on the leaves of a maize seedling so that the eggs could be collected. Leaves with the clusters of eggs were placed carefully in plastic containers (ULINE, Monterrey, Nuevo León, México) of 946 mL until the eclosion of the neonatal larvae.

OLFACTORY STIMULI

Extraction of the Sex Pheromone from Female Glands

Extraction of the sex pheromone from the glands of 4-d-old virgin females that had presented sex calls was conducted at the start

of the scotophase (7:00 PM–9:00 PM). Twenty-five dissected glands were placed in a 2 mL amber glass vial (Merck KGaA, Hesse, Darmstadt, Germany) containing 1 mL of methanol. Subsequently, the vial was stirred for 2 min in a vortex agitator (Genie II Mixer SI-0236, Scientific Industries, Bohemia, New York, USA) to homogenize the sample. Tissue, scales, and solid residues were removed under the light of a stereomicroscope (Zeiss Stereo Discovery V8, Oberkochen, Baden-Württemberg, Germany) with forceps or dissecting needle. Samples were concentrated to 250 µL with a nitrogen stream and stored in a brown vial at −4 °C until use in the bioassays.

Extraction of the Sex Pheromone from the Commercial Septum

The commercial septum of the sex pheromone (Pherocon Cap, Trécé Inc., Adair, Oklahoma, USA) was used for the extraction of the sex pheromone because its synthetic components, ([Z]-7-12: Ac; [Z]-9-14: Ac; and [Z]-11-16: Ac), are the same as those identified and characterized in *S. frugiperda* of the state of Morelos, Mexico (Gaona 2015).

The extraction of synthetic sex pheromone compounds was done using 5 mL of methanol and stirring in a vortex for 2 min (Genie II Mixer SI-0236). Two methanolic dilutions of the extract were prepared, 1:10 (dilution A) and 1:20 (dilution B). Dilutions were stored at −4 °C until use.

CHEMICAL ANALYSIS OF EXTRACTS

Analyzes to confirm the chemical profile of the glandular extract and the commercial septum sex pheromone were conducted in a gas chromatograph coupled with a mass spectrometer (GC-MS; HP 6890/5972, Agilent, Palo Alto, California, USA). Samples were analyzed using a non-polar column HP 5MS (30 m, 250 µm internal diam, 0.25 µm film thickness) (Agilent, Palo Alto, California, USA); hydrogen was used as a carrier gas, at a constant flow of 2 mL per min. Mass spectrometry was used with the electron ionization (70 eV) SCAN mode, and a mass interval of 35 to 550 atomic mass units. The injector temperature was 250 °C (splitless mode for 18 s), the auxiliary temperature of 280 °C, and the initial oven temperature was 50 °C for 2 min followed by 15 °C per min increments until reaching 280 °C for 10 min. Compounds were identified by their retention times. A comparison was made of the mass spectra obtained from the Wiley 175 and NIST libraries with those produced by synthetic standards in the gas chromatograph-mass spectrometer. In all the extracts used in the bioassays, we verified the presence of (Z)-7-12: Ac, (Z)-9-14: Ac, and (Z)-11-16: Ac (Gaona 2015).

Aliquots (2 µL), equal to 1.2 female equivalents of the glandular extract and 1 µL of the septum extract (dilution B), were injected for gas chromatograph-mass spectrometer analysis. The synthetic standards were: (Z)-7-12: Ac (purity 98%), (Z)-9-14: Ac (purity 99%), and (Z)-11-16: Ac (purity 95%) (Sigma Aldrich®, Toluca, Mexico).

WIND TUNNEL BIOASSAYS

The wind tunnel bioassays with moths exposed to different olfactory stimuli were performed in a Plexiglas wind tunnel (180 cm L × 80 cm H × 80 cm W). The airstream was produced using an extractor (Frequency Inverter CFW-08 Software 4.1x, WEG Electric Corp., Minneapolis, Minnesota, USA) and purified through an activated carbon filter. The stimulus was directed at the moth with a stream of air at a speed of 0.4 m per s and measured with an anemometer (Sper Scientific 840003, Scottsdale, Arizona, USA). The bioassays were performed at 25 ± 2 °C and 65 ± 2% RH, at the start of scotophase, between 7:00 PM and 10:00 PM, using 3 red lights (20 watts, Philips®, Naucalpan de Juarez, Mexico).

Mated females and virgin females and males of 4-d-old were used for bioassays. Each day, 20 moths were evaluated to 1 of the extracts from the female glands or sex pheromone septum. Different dilutions of the extracts were tested on subsequent d. Care was taken to make sure that each group of 20 moths had the same age and mating status.

The moth was placed close to the extractor while the glandular extracts (0.3 female equivalents = 3 µL, 0.5 female equivalents = 5 µL, 0.7 female equivalents = 7 µL, and 0.9 female equivalents = 9 µL), the extracts of the sex pheromone septum (3 µL, 5 µL, 7 µL, and 9 µL dilution B), and methanol used as a control, were placed on a 2 × 2 cm piece of filter paper (Whatman #1®, 2V, Merck KGaA, Darmstadt, Germany), which was placed at the upwind end of the tunnel and replaced in each test.

In each bioassay, a moth was released 20 s after the stimulus was positioned in the tunnel, and its behavior was observed for 180 s. After each bioassay, the wind tunnel was cleaned with a non-stimulus airflow for 300 s. The percentage of moths that flew and landed on the emission source was recorded, and the response between them was compared (Robledo et al. 2018).

The landing behavior of the females and males is the same, and consists of an unfolding of wings with rapid movements that stop when the insect lands. The female maintained a slight vibration in the wings and squeezed the sex pheromone-producing gland during landing on the emitting source.

THE CAPTURE OF *SPODOPTERA FRUGIPERDA* WITH FIELD TRAPS

The trapping system was implemented in a maize field located in Tlatenchi, "Jojutla de Juarez," Morelos, Mexico (18.596389°N, 99.186389°W; 900 masl). The traps were set up 1 wk after the maize (*Zea mays* L.; Poaceae) seeds were sown. Four traps (Traps 1–4) were distributed over 1 ha of maize and were placed at 25 m from the edge of the field and 50 m from each other. Each trap was baited with the same bait, a sex pheromone releasing septum (Pherocon Cap, Trécé Inc., Adair, Oklahoma, USA); each baited trap had its control trap (water with neutral soap) 1 m away.

Each trap consisted of a green plastic jar (Visapack, Tlalnepantla, Estado de México, México) of 10 L with 25 cm^2 openings on 3 sides to allow the entrance of insects. A wooden stake supported the trap, always held above the maize canopy (Barrera et al. 2006). The releasing device with the olfactory stimulus (commercial sex pheromone septum) was held in the upper internal part of the trap, and in the lower internal part was a water retention system containing neutral soap (1:1 proportion). This retention system was changed daily.

Captured insects were taken to the Insect Chemical Ecology laboratory of the Centro de Desarrollo de Productos Bióticos del Instituto Politécnico Nacional, Yautepec, Morelos, Mexico, for taxonomic identification, using taxonomic keys based on morphological characters of the wings and genitalia (Rizzo & La Rosa 1993; Quimbayo et al. 2010). Counts of the captured *S. frugiperda* adults were recorded using the index of captured insects per trap per night for 40 d. Each septum had a useful life of about 34 d according to the manufacturer Pherocon (Pherocon Cap, Trécé Inc., Adair, Oklahoma, USA). After this time, the septum was replaced.

DISSECTION OF THE GENITALIA OF *SPODOPTERA FRUGIPERDA*

Insects captured in the field traps were collected daily from the traps to count the catches per d and were separated by sex. Males were identified by the presence of claspers, and females by the termination of the abdomen in a V-shape (Rizzo & La Rosa 1993; Quimbayo et al. 2010).

Female insects were collected randomly from the 4 traps during the capture period. Subsequently, insects were placed in a Scheerperts mixture (60% water, 1% acetic acid, and 39% glycerin) to keep their joints soft, avoid abdominal distension, and thus facilitate the manipulation and removal of the genitalia (Barrientos 2004). To determine if the captured females had mated, the bursa copulatrix was dissected, and the presence of spermatophores was assessed (Ramos 2015). Genitalia of 240 females were dissected using dissecting forceps and scissors in a stereomicroscope (Nikon, C-DSD115 1003012, Melville, New York, USA) according to Ramos (2015). For mounting, genitalia were placed on a concave slide (frontal view), and a drop of Hoyer's solution was added on top (Anderson 1954); slides were covered with a coverslip and sealed with transparent enamel (Ramos 2015). Additionally, genitalia of a male and a female of *S. frugiperda* reared in the laboratory and separated by sex at their emergence (using the morphological characteristics of their wings as criteria) were used as the reference for the genitalia of the species.

Finally, all genitalia were observed using an environmental scanning electron microscope (ESEM) (Zeiss, Evo LS10, Oberkochen, Baden-Württemberg, Germany). A sample of the genitalia of 10 females was observed under a confocal scanning laser microscope (Zeiss, LSM 800, Oberkochen, Baden-Württemberg, Germany). The presence of spermatophores was documented, and the genital structures were compared in detail with references (Klots & Tuxen 1970).

STATISTICAL ANALYSIS

The percentage of moths that flew and landed on the emission source was recorded in the wind tunnel and analyzed with a Chi-squared test with the Yates correction. A Mann-Whitney test was performed to analyze the total male and female catches per d during the trapping period. The median and interquartile (Q1 to Q3) were determined for each group. Sigma Plot 12 (Systat Software, Inc., San Jose, California, USA) was used for all statistical analyzes.

Results

WIND TUNNEL RESPONSES

Sex Pheromone from Female Glands

The results of the response of virgin and mated females and virgin males to the glandular extract in wind tunnel bioassays showed that virgin females and males landed with the same frequency on the stimulus of 5 µL (χ^2 = 24.661; df = 1; *P* = 0.001), compared with mated females who had a lower landing response on the stimulus of glandular extract (Fig. 2).

The males' landing to the 7 µL of glandular extract was significantly higher than the landing exhibited by the virgin (χ^2 = 23.178; df = 1; *P* = 0.001) and mated (χ^2 = 30.420; df = 1; *P* = 0.001) females. There were no significant differences in landing between virgin and mated females to the 7 µL and 3 µL of extract glandular.

The males' landing to the 9 µL of glandular extract was significantly higher than the landing exhibited by the virgin (χ^2 = 70.323; df = 1; *P* = 0.001) and mated (χ^2 = 99.187; df = 1; *P* = 0.001) females. Likewise, there was a significant difference in the landing of virgin and mated females on the glandular extract, with a higher landing frequency of virgin females (χ^2 = 4.500; df = 1; *P* = 0.034) (Fig. 2).

Virgin females did not respond to the methanol stimulus (control) and there was a significant difference between the landing of males and mated females; the males presented more landings to this stimulus (χ^2 = 8.96; df = 1; *P* = 0.003) (Fig. 2).

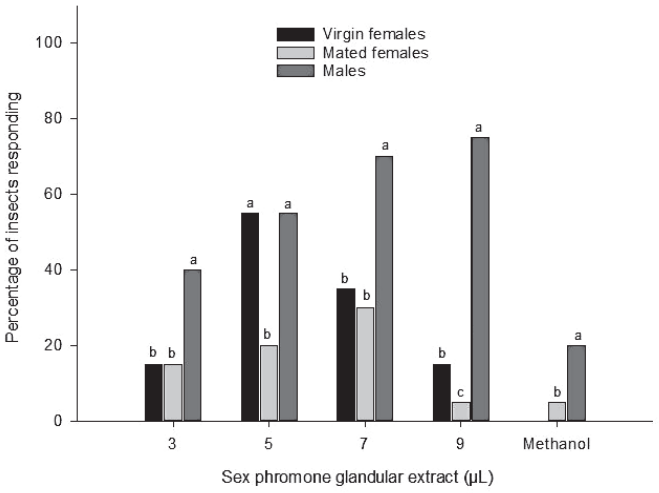

Fig. 2. Percentage of female and male *Spodoptera frugiperda* that landed on the female glandular extract. Bars of different colors with different letters for the same extract concentrations indicate a significant difference, *n* = 20 (χ^2; *P* < 0.05).

Sex Pheromone from the Commercial Septum

In the landing response on the extract of the sex pheromone septum, virgin *S. frugiperda* males present more landings to 3, 5, 7, and 9 µL of stimulus compared to virgin and mated females. However, it is important to point out that virgin females displayed a higher response to 5 and 7 µL of the septum sex pheromone than the mated females (χ^2 = 12.288; df = 1; *P* = 0.001 and χ^2 = 9.627; df = 1; *P* = 0.002, respectively) (Fig. 3).

Virgin females did not have any landings on the 9 µL of stimulus, whereas mated females had landings, although in a lower proportion than males (χ^2 = 116,821; df = 1; *P* = 0.001) (Fig. 3). No moths landed on the methanol stimulus (control).

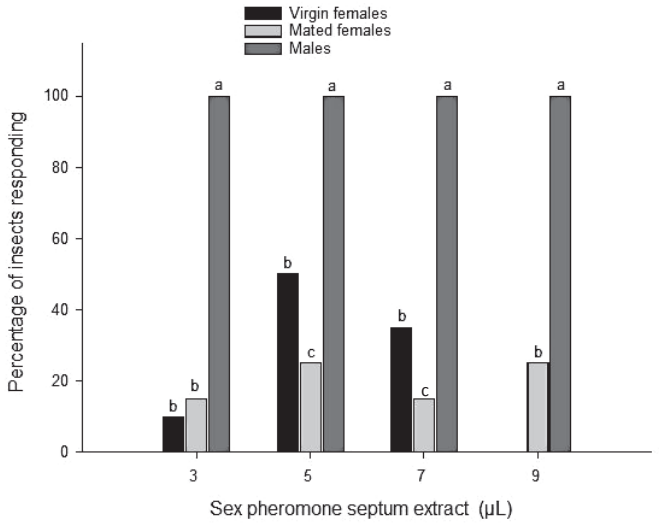

Fig. 3. Percentage of female and male *Spodoptera frugiperda* that landed on different concentrations of the extract of the sex pheromone septum. No moths landed on the control (methanol). Bars of the same color with different letters indicate that there is a significant difference, *n* = 20 (χ^2; *P* < 0.05).

FIELD CAPTURE OF *SPODOPTERA FRUGIPERDA*

Total captures were 11,221 *S. frugiperda* adults out of which 2,392 were female. A constant fluctuation in the capture of both sexes was observed; however, males showed higher variation. When analyzing the complete data of the total captures, between the captures by sex, 4 males were captured by 1 female. In all traps baited with sex pheromone septa placed in the maize field, there was a higher capture of males compared to females: Trap 1 – U = 224; n = 34; P = 0.001; Trap 2 – U = 203.5; n = 34; P = 0.001; Trap 3 – U = 186.5; n = 34; P = 0.001; and Trap 4 – U = 197.5; n = 34; P = 0.001 (Fig. 4). No moths were captured in the control traps.

DISSECTION OF THE GENITALIA OF *SPODOPTERA FRUGIPERDA*

The genital structure of the female of *S. frugiperda* is formed by the corpus bursae (Fig. 5A) and the bursal duct (Fig. 5A, B). Spermatophores were observed within the corpus bursae of mated females (Fig. 5A). The spermatophore produced by the male, which contains the spermatozoids, is stored in the bursa copulatrix, sometimes with 'teeth' or signum; the sperm must travel through the ductus seminalis to reach the ovaries. The bursa copulatrix presents a diverticulum, denominated bursae appendix (Fig. 5C). The sac is a flexible structure, lightly opaque and colorless, whereas the duct is hard and dark brown; this characteristic allows us to define the specimens' reproductive state (Cordero & Baixeras 2015).

The genitalia has a pair of anal papillae that surround the anus and form part of the ovipositor tube; the postvaginal and antevaginal lamella protect the ostium bursae orifice, which is associated with fecundation and differs from the ovipore (Fig. 5D) (Cordero & Baixeras 2015; García-Barros et al. 2015).

Seventy-two females that were dissected (30% of 240) had mated, 51 females had 2 spermatophores, 20 females had 1 spermatophore, and 1 female had 3. In the case of males, the genital armor was observed to confirm that the species captured corresponded to *S. frugiperda*.

Discussion

In this study, it was confirmed that *S. frugiperda* females can detect their sex pheromone. In the wind tunnel bioassays, virgin and mated females landed on the stimulus of sex pheromones. It has been reported that in some moths such as *H. armigera* and *H. zea*, lack of attraction and repulsion to the sex pheromone occurred due to intrasexual competition; this behavior keeps moths away from other pheromone emitting sources to avoid intraspecific competition (Pearson et al. 2004). In other cases, like in the females of *Eupoecilia ambiguella* Hübner (Stelinski et al. 2006), *Pandemis pyrusana* Kearfott (Kuhns et al. 2012), and *Lobesia botrana* Denis and Schiffermüller (all Tortricidae: Lepidoptera) (El-Sayed & Suckling 2005), the exposure to the sex pheromone did not influence the behavior of virgin and mated females.

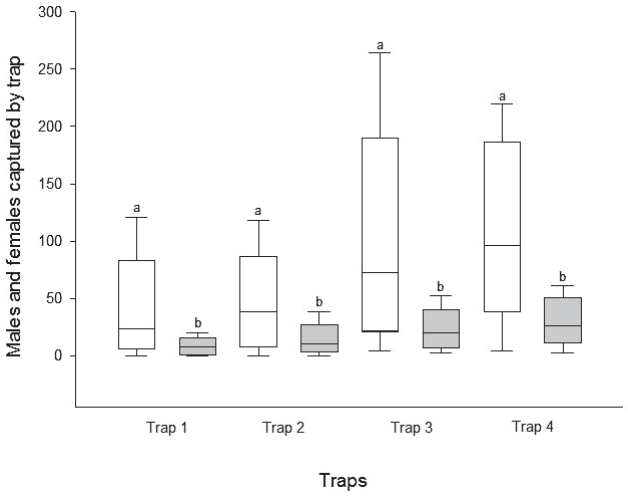

Fig. 4. Male (white bars) and female (gray bars) *Spodoptera frugiperda* caught by traps with sex pheromone septa (Q1 < Median < Q3). Different letters for Trap 1, Trap 2, Trap 3, or Trap 4 indicate significant differences (Mann-Whitney Test U; n = 34; P < 0.05).

On the one hand, the responses of mated females to the sex pheromone could be related to locating sites where there are more females of the same species nearby or on a host plant (Holdcraft et al. 2016). On the other hand, exposure to the pheromone before copulation would move them away from areas of high population density, reducing the competition for resources between their progeny and that of conspecific females (Trematerra & Battaini 1987; Stelinski et al. 2014). Some moths, such as the mated females of *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae), behave similarly to *S. frugiperda* (Ellis et al. 1980).

In field studies, *S. frugiperda* captures were higher consistently at the beginning of the trapping experiment and decreased as the d passed. It is probably because pheromone release from the septum decreases as time goes by and with it the attraction of insects; moreover, there were no captures when there was a storm. Every time the traps were emptied, they contained either virgin or mated females. This kind of behavior is reported in other moths, like *T. ni*. A significant amount of virgin female moths of this species was captured in traps baited with cis-7-dodecenol acetate, the main compound of the sex pheromone; this behavior was unusual, as documented by Mitchell et al. (1972).

The genitalia of *S. frugiperda* females caught in the maize field coincides with the descriptions of *S. frugiperda* from other continents (Rojas et al. 2004; Ganiger et al. 2018). However, in previous studies, several genital structures were not described. Only the external parts that comprise the female abdominal termination were reported; this was likely because they facilitate the rapid identification and differentiation between males and females, but moths can lose these external characteristics in trapping systems. In previous descriptions, the ostium bursae is situated in the middle of the ductus bursae; however, when observing lepidopterans' general anatomy, the orifice of the ostium bursae is located at the end of the ductus bursae, in contact with the exterior. The bursa copulatrix is also a defining structure for identification because, although being similar morphologically, its size varies noticeably between several species.

Previous field studies on moths have not been directed at investigating autodetection or anosmia (Holdcraft et al. 2016). Autodetection of the sex pheromone can have benefits and implications in managing insect pests, as is the case of *S. frugiperda*, in which virgin and mated females have an attraction response to the sex pheromone of conspecifics. Like males, virgin *S. frugiperda* females could be captured using low concentrations of sex pheromone, or the strategy of interrupting copulation could benefit from the antennal saturation of males and also females (Malo et al. 2004) with sex pheromone. If females mated before the capture of males, the incidence of their offspring on crops would be less significant, because mated females would also respond to and land on the conspecific sex pheromone stimulus as observed in this study. The fact that virgin and mated females represented 21% of the trapped insects is a promising result, meaning that 21% fewer females can mate or lay eggs, translating to a reduction in crop damage; moreover, many males also were removed from the population

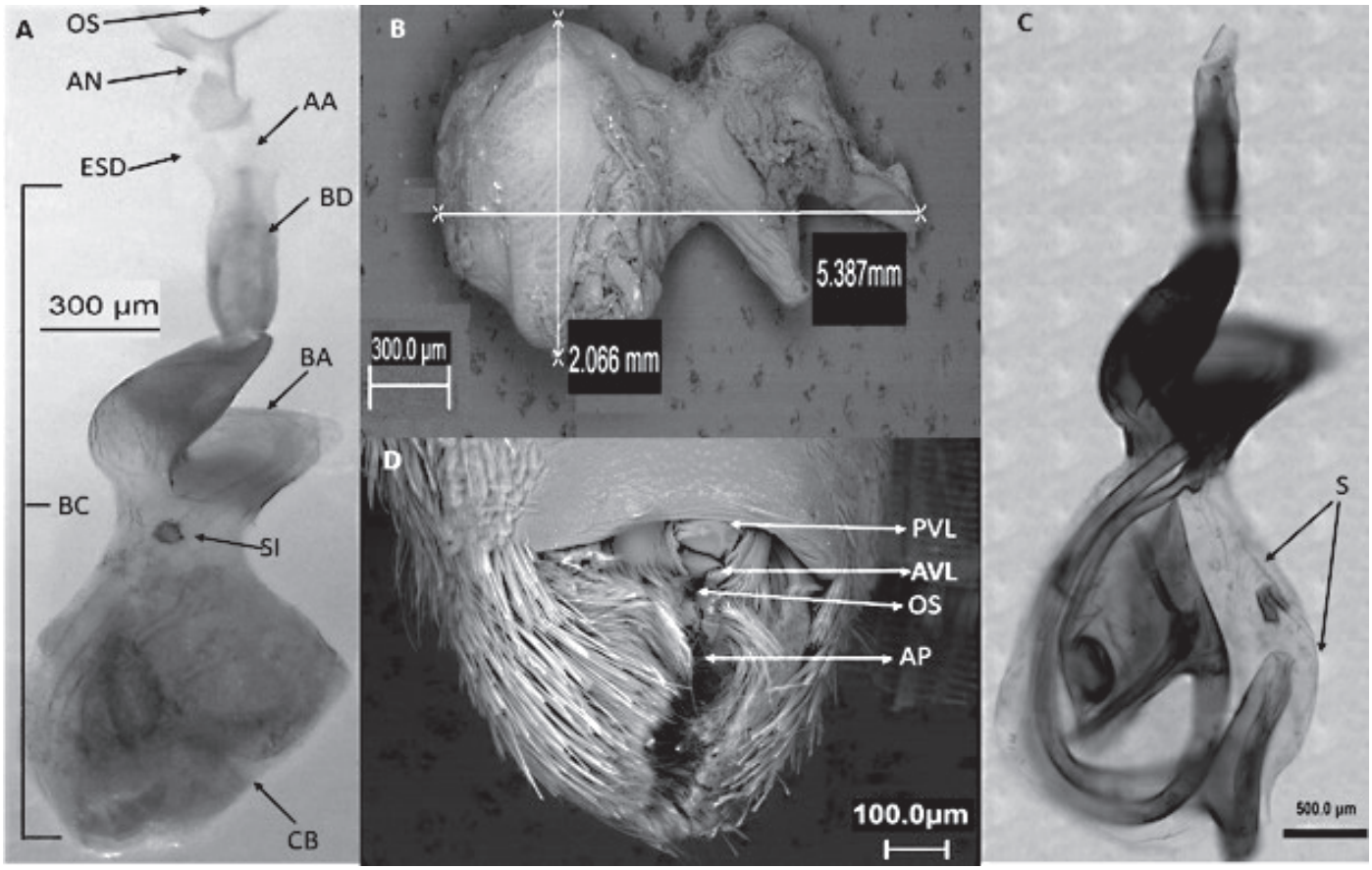

Fig. 5. Genital structure of female *Spodoptera frugiperda*. (A) Confocal image of the bursa copulatrix, frontal view. View of spermatophores within the corpus bursae (BC = bursa copulatrix; SI = signum; CB = corpus bursae; BA = bursae appendix; OS = ostium (exit); ESD = exit to a seminal duct; AA = anterior apophysis; AN = antrum; BD = bursal duct). (B) Micrograph of bursa copulatrix in zenith angle, observing the length and width measurements of the structure (length = 5.38 mm; width = 2.066 mm). (C) Stereoscopic image presenting a frontal view of the genital structure (S = spermatophores). (D) Micrograph of the terminal abdominal (PVL = postvaginal lamella; AVL = antevaginal lamella; OS = ostium; AP = anal papilla).

and with them the possibility of reproduction. The sex pheromone, as it was initially known, can have an aggregation function for this species because it attracts males and females, which could influence the efficiency of the management strategy of this pest species in the field.

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