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Evaluation of sex pheromone formulations to attract *Spodoptera frugiperda* (Lepidoptera: Noctuidae) adult males in Argentina

BRATOVICH, Celina[1,2,*], SALUSO, Adriana[3,4], MURÚA, María G.[5] & GUERENSTEIN, Pablo G.[1,2]

[1] Facultad de Ingeniería, Universidad Nacional de Entre Ríos. Entre Ríos, Argentina.
* E-mail: cbratovich@ingenieria.uner.edu.ar

[2] Laboratorio de Estudio de la Biología de Insectos (LEBI), CICyTTP (CONICET-Prov. de ER, UADER). Entre Ríos, Argentina.

[3] Grupo Ecofisiología Vegetal y Manejo de Cultivos. Estación Experimental Agropecuaria INTA Paraná. Entre Ríos, Argentina.

[4] Facultad de Ciencias Agropecuarias, Universidad Nacional de Entre Ríos. Entre Ríos, Argentina.

[5] Instituto de Tecnología Agroindustrial del Noroeste Argentino (ITANOA)-CONICET, Estación Experimental Agroindustrial Obispo Colombres. Tucumán, Argentina.

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Evaluación de formulaciones de feromona sexual para la atracción de machos de *Spodoptera frugiperda* (Lepidoptera: Noctuidae) en Argentina

RESUMEN. *Spodoptera frugiperda* (Smith) es la plaga más importante del maíz en Argentina. En América su monitoreo se realiza con trampas cebadas con feromona sexual femenina sintética. Tal monitoreo tiene éxito variable, principalmente, porque la composición de la feromona sexual de poblaciones de áreas geográficas distantes es diferente y no se ha desarrollado un cebo sintético específico para cada área. Se desconoce la composición de la feromona sexual de las poblaciones encontradas en Argentina. El objetivo de este trabajo fue probar un cebo comercial (Hercon) y tres formulaciones experimentales (ChemTica) de la feromona, para establecer si alguna de esas formulaciones es mejor que la que se usa actualmente en el país (es decir, el comercial ChemTica). La atracción por las feromonas sintéticas se probó en un túnel de viento utilizando machos de diferentes regiones de Argentina. Además, se realizaron pruebas de campo en dos regiones distantes del país. Una de las formulaciones experimentales de ChemTica y la formulación comercial de Hercon provocaron la mayor atracción en las pruebas de túnel de viento. En las pruebas de campo, solo el cebo de Hercon capturó un número significativo de machos. Por lo tanto, si bien se hallaron cebos que evocan mayor atracción que el cebo comercial de ChemTica, la atracción y la captura, incluso con esos cebos, no alcanzaron valores altos.

PALABRAS CLAVE. Cebo de olor. Manejo de plagas. Monitoreo. Plaga de cultivo. Túnel de viento.

ABSTRACT. *Spodoptera frugiperda* (Smith) is the most important pest of corn in Argentina. Its monitoring in the Americas is carried out by traps lured with a synthetic version of its female sex pheromone. Such monitoring has variable success mainly because the composition of the sex pheromone of populations from distant geographical areas is different, and a synthetic lure has not been developed for each area. The composition of the sex pheromone of the populations found in Argentina is not known. The objective of this work was to test one commercial (Hercon lure) and three experimental formulations (from the manufacturer ChemTica) of the pheromone to establish if any of those formulations is better than the one currently used in the country (*i.e.*, the commercial ChemTica lure). Attraction to the synthetic pheromones was tested in a wind tunnel using males from different regions of Argentina. In addition, field tests were carried out in two distant regions of the country. One of the experimental formulations from ChemTica and the commercial formulation from Hercon evoked the highest attraction in wind tunnel tests. In field tests, only the Hercon lure captured a significant number of males. Although lures evoking higher attraction than the commercial ChemTica lure were found, attraction and capture, even with those lures, did not reach high values.

KEYWORDS. Crop pest. Monitoring. Odor lure. Pest management. Wind tunnel.

INTRODUCTION

The lepidopteran *Spodoptera frugiperda* (Smith), also known as "fall armyworm", is one of the major agricultural pests in the Western Hemisphere, infesting maize (*Zea mays* L.), sorghum, turf grasses (*Sorghum* spp.), and a number of other crops such us cotton (*Gossypium hirsutum* L.) and soybean (*Glycine max* (L.) Merr.) (Luginbill, 1928; Willink et al., 1993; Casmuz et al., 2010). This species is the most important pest of maize in the Americas (Sparks, 1979) and was cited on 190 host plant species (Casmuz et al., 2010; Bohnenblust & Tooker, 2012). Therefore, it is highly desirable to develop methods to monitor and control this pest.

At present, the most widely used tool for *S. frugiperda* control is genetically modified maize hybrids expressing *Bacillus thuringiensis* (*Bt*) insecticidal proteins (Blanco et al., 2010; Okumura et al., 2013; Huang et al., 2014). However, to complement this management, it would be necessary to develop efficient monitoring methods to detect these insects early enough, in case additional control measures (*e.g.*: spraying with insecticides) are required. Thus, it will be possible to assess when and where to carry out control measures (Cruz et al., 2012). While light traps are not highly sensitive to monitor *S. frugiperda*, sex pheromone traps are more efficient (Capinera, 2014). Thus, monitoring of the pest should be carried out by capturing males using synthetic sex pheromone as attractant (Andrade et al., 2000; Malo et al., 2001; Hall et al., 2005; Batista-Pereira et al., 2006; Unbehend et al., 2014). Despite the advantages of the use of pheromone traps, including targeted capture of adults even at low population densities, this method is not often in use in Argentina for this species. *Spodoptera frugiperda* adults have high dispersal capacity (Pair et al., 1986), possibly covering long distances (Mitchell, 1991; Hendrix & Showers, 1992; Nagoshi & Meagher, 2003; Nagoshi et al., 2009). For example, it is believed that part of the Argentine populations of this insect originate from an annual migration from Brazil and Bolivia (Murúa et al., 2008; Nagoshi et al., 2017). There are two different strains of *S. frugiperda*, called rice-strain and corn-strain according to their preferred host, although they are sympatric (Pashley et al., 1985, Pashley, 1986; Nagoshi & Meagher, 2003). In Argentina both strains have been found (Juárez et al., 2012, 2014; Nagoshi et al., 2012; Murúa et al., 2015).

The female sex pheromone of *S. frugiperda* has a principal component ((Z)-9-tretadecenyl acetate [Z9-14:OAc]) and many secondary components ((Z)-11-hexadecenyl acetate [Z11-16:OAc] and (Z)-7-dodecenyl acetate [Z7-12:OAc], among others) in different proportions (Tumlinson et al., 1986). However, differences in the composition of the pheromone between geographically distant populations were reported. This is usually due to variations in the proportions of the components, although the secondary constituents are not always the same (Descoins et al., 1988; Batista-Pereira et al., 2006). Responses of males to sex pheromone differ between geographically distant populations (Mitchell et al., 1985; Andrade et al., 2000; Malo et al., 2001; Batista-Pereira et al., 2006; Unbehend et al., 2014) although this is not the case within Mexico (Cruz-Esteban et al., 2018). Few studies on differences in sex pheromone composition respect to *S. frugiperda* host strain were carried out and some differences were found (Groot et al., 2008; Cruz-Esteban et al., 2018). However, no differential responses to sex pheromone according to strain were found (Unbehend et al., 2014).

The sex pheromone of *S. frugiperda* has been characterized in different regions of the Americas (Sekul & Sparks, 1976; Andrade et al., 2000; Batista-Pereira et al., 2006), although no information is available on the pheromone composition of females from Argentina. On the other hand, responses of males from Argentine populations to two synthetic 4-component blends based on pheromone compositions identified in females from Florida (USA) were assessed and some capture was obtained (Unbehend et al., 2014). In addition, both Argentine farmers and researchers reported some capture of *S. frugiperda* males using traps lured with the commercially available lure from the manufacturer ChemTica® (Murúa & Virla, 2004; see Material and Methods section).

In order to provide farmers with information useful to efficiently monitor *S. frugiperda* in Argentina at the short term, it would be necessary to study the attraction of Argentine males towards different synthetic formulations of the available sex pheromone. Here, we present a study, in lab and field, on the responses of males of *S. frugiperda* from geographically distant populations of Argentina to different formulations of their sex pheromone.

MATERIAL AND METHODS

Collection of insects

Larvae of *S. frugiperda* were collected from December 2014 to January 2015 from maize fields located in three Argentine provinces. The collections were made in Tafí Viejo county (26° 44' S; 65° 16' W) (Tucumán province), in Marcos Juárez county (32° 42′ S; 62° 06′ W) (Córdoba province), and in Federal (30° 57′ S; 58° 48′ W) and Paraná (31° 49′ S; 60° 31′ W) counties (Entre Ríos province). Insects from each location were treated as a single population. At each collection site, a minimum of 300 larvae (instars 2-5) were collected and placed individually in glass tubes (12 cm high and 1.5 cm diameter) with pieces of artificial diet (Murúa et al., 2003). Collected larvae were taken to the laboratory and placed in breeding chambers under controlled ambient conditions (27 ± 2 °C, 70-75% relative humidity, 14:10 light:dark cycle) until adult emergence. All adults that emerged in the laboratory were examined using male genitalia as criterion to confirm the species.

In addition, adults of a fifth population (the "Laboratory population") were obtained from a colony maintained in the Laboratorio de Estudio de la Biología de Insectos (LEBI, CICyTTP, Entre Ríos) from individuals collected in Paraná county (Entre Ríos) in January 2014.

Insect Rearing

Colonies were started with *ca*. 200 adults raised from the collected larvae. Within 24 h after emergence, groups of four females and four males were placed in cylindrical polyethylene-terephthalate oviposition cages (30 cm high, 10 cm diameter). For aeration, top of each cage was covered with a nylon mesh cloth, and a hole was made on one side. The cages contained pieces of paper that females could use as substrate for oviposition. Food was provided via a cotton plug saturated with honey and water (1:1) mixture, which was renewed every day. Cages were checked daily for oviposition and adult mortality. Approximately 15 egg masses were collected *per* cage and introduced in the glass tubes mentioned above. Once emerged, 15 neonate larvae from different egg masses were selected at random and placed individually in the glass tubes with artificial larval diet (Murúa et al., 2003), which was renewed every two to three days. As larvae pupated, they were sexed and males were separated before emergence for lab experiments. The rest of the pupae were placed in cylindrical cages until adult emergence to produce a new generation of moths. Colonies of each county were reared in isolation.

Lure formulations

Previous to this work, Argentine farmers and researchers reported field capture of *S. frugiperda* males using a sex pheromone lure from ChemTica (ChemTica, Heredia, Costa Rica; hereafter CH blend, see Table I) (Murúa & Virla, 2004). Thus, monitoring in Argentina is carried out using traps lured with the CH blend. In order to attempt to find a better lure for monitoring, we tested four synthetic pheromone formulations both in the laboratory and in the field. The negative control consisted on no pheromone while the positive control consisted on the CH blend. The pheromone formulations tested are described in Table I and consisted of: a) a commercial blend of three components (Z9-14:OAc, Z11-16:OAc and Z7-12:OAc), that is, the Hercon lure (Great Lakes, IPM, Vestaburg, MI; hereafter HE blend), which contains the same components than the Chemtica lure, b) two experimental blends of four components (Z9-14:OAc, Z11-16:OAc, Z7-12:OAc and Z9-12:OAc) which differ in the proportion of Z7-12:OAc (hereafter CHA and CHB blends), and c) an experimental blend of five components (hereafter CHE7 blend), which is the CHB blend plus E7-12:OAc, a component just found in the pheromone of Brazilian females (Batista-Pereira et al., 2006). CHA, CHB and CHE7 blends were developed by ChemTica and, similarly to their commercial lure, were emitted from a dispenser that consisted on a small chamber (1 cm diameter) with pheromone which was emitted through a membrane system to achieve a zero-order release (ChemTica, pers. comm.). The dispenser of the Hercon lure (HE blend) consisted of a rubber laminate strip.

Laboratory (wind-tunnel) tests

Lab tests were carried out in a glass wind tunnel (50 x 50 x 150 cm) at 27 °C, with wind speed at 30 cm/s and red light (2 lux). The air in the experimental room was constantly renewed by an external fan to avoid odor contamination. Adult males tested were 3 to 4 days old, and originated in each of five populations mentioned

	Blend CH (+ control)	Blend HE	Blend CHA	Blend CHB	Blend CHE7
(Z)-9 tetradecenyl acetate	x	100	x	x	x
(Z)-11 hexadecenyl acetate	x	23.9	x	x	x
(Z)-7 dodecenyl acetate	x	0.62	x	x	x
(Z)-9 dodecenyl acetate	-	-	x	x	x
(E)-7 dodecenyl acetate	-	-	-	-	x

CH: ChemTica® commercial blend; HE: Hercon® commercial blend; CHA: experimental blend of ChemTica; CHB: experimental blend of ChemTica; CHE7: experimental blend of ChemTica with five components

Table I. Constituents in synthetic versions of the sex pheromone blend of *Spodoptera frugiperda* used in lab and field tests. In HE blend, proportions are relative to principal compound (Net amounts *per* lure: 2 mg). The major compound in all ChemTica blends was Z9-14:OAc. The percentages of Z11-16:OAc and Z9-12:OAc were the same in all three experimental formulations.

above (section Collection of insects). Fifteen males *per* each treatment *per* each population were used. Before an experiment, males were placed individually in a plastic tube with gauze at both ends and kept in the wind tunnel room for at least 2 h to acclimatize them to the testing environment. Subsequently, a single male tube was placed at the downwind end of the tunnel and the behavior of the insect was observed and video recorded for 5 min using an IR camera (IP8330, Vivotek Inc., Taiwan). Males were scored according to the following behaviors: take off, oriented flight (Hatano et al., 2015), close approach (10 cm) to the lure and landing on the lure. Four treatments, a positive control and a negative control were tested (see the Lure formulations section). During a single experimental day, the different lures were tested in a random order until the experimental series (*i.e.*, four lures and the two controls) was completed. After each test the wind tunnel was thoroughly washed with 96% ethanol and dried. Experiments were performed during 3 to 7 hours after the beginning of the scotophase (Schofl et al., 2009). Males were used only once.

Field tests

Universal moth traps (Unitrap®, Great Lakes IPM, Vestaburg, MI) were installed in maize fields between November 2014 and end of February 2015 in two provinces of Argentina. In the selected counties, the presence of *S. frugiperda* larvae in the crops was visually corroborated. In Entre Ríos province, traps were installed in Oro Verde county (31° 49′ S; 60° 31′ W) inside Paraná Experimental Station of the Instituto Nacional de Tecnología Agropecuaria (INTA). In Tucumán province, traps were installed in San Agustín county (26° 50′ S; 64° 51′ W). At each site, traps were hung 1 m over the ground (according to the manufacturer recommendations), spaced 10 m apart and at least 10 m from the edge of the field using a complete randomized block design (Unbehend et al., 2014). The same treatments used in wind tunnel experiments were tested (see Lure formulations section). There were three replicates *per* treatment *per* county (n = 3). The insects were collected every 7 or 10 days.

Field tests were carried out for 13 weeks (November 2014 to February 2015). New lures were placed on week 0 and were replaced at beginning of week 7. In Tucumán the HE blend was replaced again at beginning of week 11.

Data analysis

R software (R Development Core Team, 2017) was used for statistical analyses and figures. For lab tests, percentages of individuals within a population were compared pairwise between treatments using pairwise comparison of proportions and p-value adjustment with the Holm correction (Holm, 1979). For field tests, weekly captures for the three replicates in each site were pooled. For analyses, data from male counts were square root transformed ($\sqrt{(x+0.5)}$) to increase the homogeneity of variance and normality. Treatments were compared using analysis of variance (ANOVA) and multi-comparison HSD Tukey Test.

RESULTS

Laboratory (wind-tunnel) tests

Fifteen repetitions *per* treatment *per* population were carried out (total N = 450). In all cases, percentages of taking off were high (at least 60%, data not shown). Figure 1 shows the percentages of oriented flight, close approach and landing for each treatment for each population. Oriented flight responses to the HE and CHB blends were relatively high and similar in Paraná, Federal and Laboratory populations (all from Entre Rios province). Thus, the percentage of males showing this behavior was higher for the HE (66% to 73%) and the CHB (33% to 40%) blends than for the other treatments (Fig. 1 (a), (b) and (e)). In the case of the Tucumán and Córdoba populations the lures that performed best regarding this behavior also tended to be HE and CHB (26% and 40% respectively, for Tucumán, and 47% and 27% respectively, for Córdoba) although no significant differences between treatments were found (Fig. 1 (c) and (d)).

In the case of the close approach behavior the lures that performed best, again tended to be HE and CHB, although a high variability in the responses was found (Fig. 1) and no significant differences between treatments were found. The percentage of males landing on the lures was 13% at most (Fig. 1 (a) and (b)), with no significant differences between treatments. While CHB evoked at least some landing in four out of five populations, CHE7 was the only lure that did not evoke any landing in any population. The HE blend evoked at least some landing in two out of five populations.

Field tests

The traps captured males using the HE blend (51 and 48 males), the CH blend (15 and 2), the CHA blend (6 and 2), the CHB blend (14 and 0), the CHE7 blend (17 and 2) and the negative control (6 and 0) in Tucumán and Paraná (respectively). The mean weekly capture indicated significant differences between treatments (df = 5, F = 3.93, p = 0.0022) and between counties (df = 1, F = 13.71, p = 0.0003). The HE blend (Fig. 2) tended to capture more males than the other blends (Entre Ríos: df = 5, F = 2.425, p = 0.0444 and Tucumán: df = 5, F = 2.415, p = 0.0442).

DISCUSSION

Wind-tunnel tests suggest that, in terms of oriented flight and close approach, the performance of HE was relatively high and only CHB approaches this performance while none of the other lures surpass it. CH, CHB and HE were able to evoke landing. However,

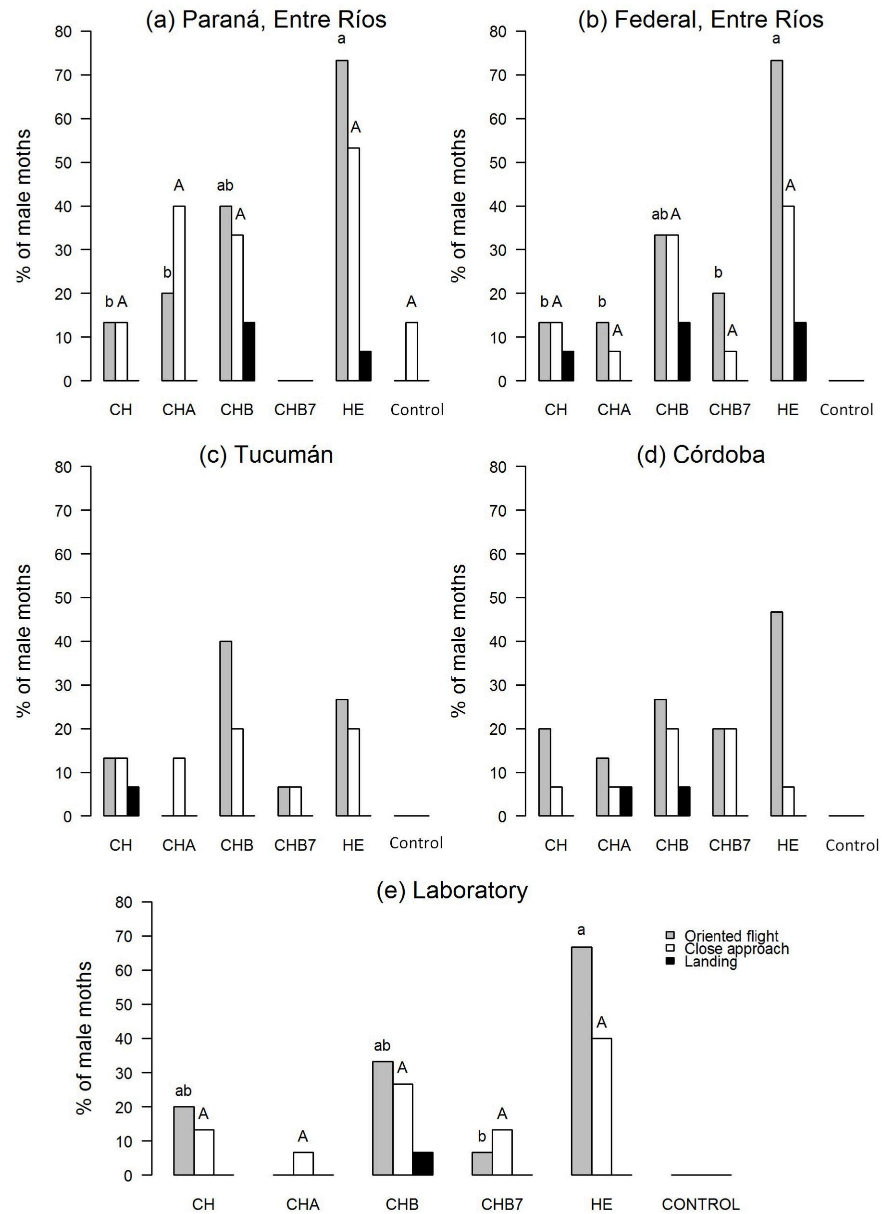

Fig. 1. Percentage of *Spodoptera frugiperda* males that responded with oriented flight, close approach and landing to different synthetic blends in laboratory wind tunnel assays. HE: Hercon® commercial blend, CH: ChemTica® commercial blend, CHA, CHB and CHE7: ChemTica® experimental blends (see text). For each behavior, bars with the same letter are not significantly different ($p<0.05$, pairwise comparison of proportions test with Holm correction).

while the CH and HE blends elicited landing in two populations, the CHB lure elicited such behavior in four out of five populations. It should be mentioned that the composition of the CHB blend is similar to that of "Blend 1" in Unbehend et al. (2014), and this blend was the most effective in capturing *S. frugiperda* in the field in Tucumán (Argentina). Responses of the three populations from Entre Ríos (Paraná, Federal and Laboratory) were similar regarding the HE and CHB lures. Thus, the fact that the laboratory population was reared for 15 generations does not seem to have affected the population preferences.

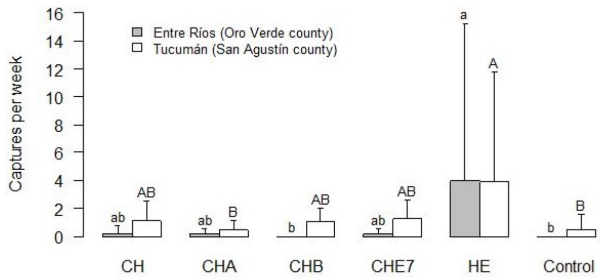

Fig. 2. Mean (± SEM) captures of *Spodoptera frugiperda* males *per* week for each formulation in Tucumán and Entre Ríos provinces. For each county, bars with the same letter are not significantly different (p<0.05, ANOVA and multi-comparison HSD Tukey Test). Non-transformed data.

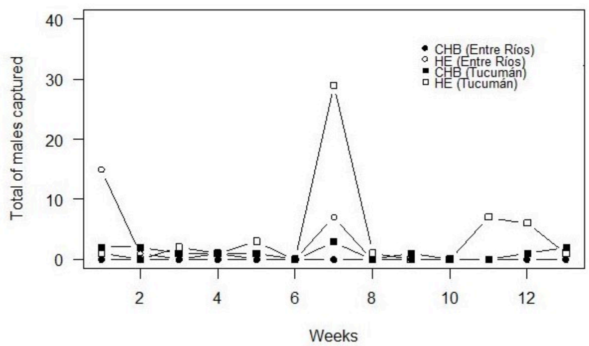

Fig. 3. Capture of *Spodoptera frugiperda* males *per* week in the field. New lures were placed of week 0 and were replaced at beginning of week 7. In Tucumán the HE lure was replaced again at beginning of week 11.

Our data indicate that the mean number of males captured in the field tests was rather low (0.10 males/trap/night) in contrast with other studies where commercial synthetic sex pheromone lures were evaluated for *S. frugiperda*. For example, Malo et al. (2001) recorded 0.37 males/trap/night in Mexico and Meagher et al. (2013) found 13 males/trap/night in the USA. In Costa Rica, capture in the field was variable when evaluating non commercial lures based on the pheromone of populations from a different geographic area (Andrade et al., 2000). Regarding the performance of the commercial lures in the field, while the ChemTica (CH) lure showed a good performance capturing male of *S. frugiperda* in México (Malo et al., 2001), Costa Rica (Andrade et al., 2000) and Brazil (Batista-Pereira et al., 2006), the Hercon (HE) lure showed a low performance in Florida, when comparing with other commercial lures (Hall et al., 2005; Bhan et al., 2013). Field work in Tucumán, previous to this work, showed that the ChemTica lure evoked relatively high capture in 2001 but not in 2002 (Murúa & Virla, 2004). This variability would be due to variations in population density between years. We thus suggest that the low capture obtained in the field in this study relates with a low population density that year as a result of the natural population dynamics. It should be mentioned that a small-scale field test carried out with the commercial ChemTica lure during summer 2017-2018 resulted in an average capture of 22 males/trap/night (Murúa, pers. obs.).

In any case, it should be taken into account that, for example, the high capture by Meagher et al. (2013) was obtained using a synthetic lure inspired by the sex pheromone of the local population. As the pheromone composition of the Argentine populations is unknown, no synthetic pheromone inspired by the blend emitted by Argentine females is available at present.

In order to relate field trapping with the results in the lab, percentage of males approaching the lure at a distance of 10 cm or less (close approach) in the wind tunnel could be compared. According to our data, the HE lure shows the highest percentages of close approach which is in agreement with field data, while the relatively good performance of CHB in the lab is not mirrored in the field. It should be noted that captures in the field depended on time elapsed after lure placement (Fig. 3). Thus, although the HE lure significantly attracted male moths in the field, that attraction was short lasted. This would mean that the odor delivery rate of the HE lure is not stable.

The fact that the CHE7 lure evoked very low attraction suggests differences between the Brazilian and the Argentine pheromone. Moreover, although in this study we found lures evoking higher attraction than the commercial ChemTica lure, the relatively low attraction to all lures tested, suggests that the pheromone formulations used here are not optimal to monitor the *S. frugiperda* populations found in Argentina. Furthermore, it suggests that the composition of the sex pheromone of the Argentine populations differ from all the blends tested here. It should be mentioned that preliminary field tests carried out using the commercial pheromone lure for *S. frugiperda* from the manufacturer Trécé® did not show consistent capture either (Casmuz, pers. comm.). Thus, in order to develop a synthetic pheromone lure to effectively monitor the Argentine populations of this moth, it would be necessary to characterize the pheromone composition emitted by females of these populations.

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