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Research Article

Field efficacy and selectivity of synthetic pheromones for monitoring *Elasmopalpus lignosellus* and non-target lepidopterans in soybean[1]

Robson Aparecido dos Santos[2], Marilza da Silva Costa[3],
Henrique Fonseca Goulart[4], Antônio Euzébio Goulart Santana[4], Mônica Josene Barbosa Pereira[3]

ABSTRACT

The collar borer (*Elasmopalpus lignosellus*) is a soil-dwelling pest that causes severe damage to several crops, being necessary the use of early detection tools. This study aimed to evaluate the efficacy and selectivity of synthetic sex pheromone blends in monitoring *Elasmopalpus lignosellus* populations in soybean, as well as to assess their attractiveness to non-target lepidopteran species. The performances of synthetic formulations were compared with those of live virgin females in the field over two growing seasons. The ternary (Z9-14:Ac, Z11-16:Ac, and Z9-16:OAc) and the quaternary (Z9-14:Ac, Z11-16:Ac, Z11-16:OH, and Z9-16:OAc) mixtures demonstrated a strong potential as field monitoring tools.

KEYWORDS: Synthetic sex pheromone, sustainable agriculture, soil pest, integrated pest management.

RESUMO

Eficácia de campo e seletividade de feromônios sintéticos para monitoramento de *Elasmopalpus lignosellus* e lepidópteros não-alvos na soja

A broca-do-colo (*Elasmopalpus lignosellus*) é uma praga de solo que causa danos severos em várias culturas, sendo necessárias ferramentas de detecção precoce. Objetivou-se avaliar a eficácia e a seletividade de *blends* de feromônio sexual sintético no monitoramento de populações de *Elasmopalpus lignosellus* em soja, bem como a sua atratividade para espécies de lepidópteros não-alvos. Compararam-se os desempenhos de formulações sintéticas com o de fêmeas virgens vivas em campo, ao longo de duas safras. A mistura ternária (Z9-14:Ac, Z11-16:Ac e Z9-16:OAc) e a quaternária (Z9-14:Ac, Z11-16:Ac, Z11-16:OH e Z9-16:OAc) demonstraram forte potencial como ferramentas de monitoramento de campo.

PALAVRAS-CHAVE: Feromônio sexual sintético, agricultura sustentável, praga de solo, manejo integrado de pragas.

INTRODUCTION

Species-specific sex-pheromone-baited traps are essential tools for early detection and population monitoring in integrated pest management programs targeting agricultural and forest pests (Ando & Yamamoto 2020). In moths, sex pheromone communication is mediated by chemical blends released by females, with male behavioral responsiveness often depending on a precise ratio of components (Jurenka 2021).

For the lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller) (Lepidoptera: Pyralidae), the sex pheromone consists of a complex blend of ten aliphatic compounds, including acetates and alcohols with 14- and 16-carbon chains. The pheromone blend composition described for the North American population comprises 91.6 µg of (Z)-11-hexadecenal (Z11-HDA), 11.2 µg of (Z)-9-tetradecen-1-ol (Z9-TDOH), 46.4 µg of (Z)-9-tetradecenal (Z9-TDA), and 86.8 µg of (Z)-7-tetradecenal (Z7-TDA) (Lynch et al. 1984). Subsequent studies have suggested that pheromone composition and the relative proportions of these compounds may vary among geographically distinct populations, such as those from South America, potentially reflecting on local adaptation to environmental or genetic factors (Pires et al. 1992, Loera et al. 1995, Jham et al. 2005, Jham et al. 2007).

Pheromone blends vary markedly among species, ranging from single active compounds to

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[2] Universidade Federal de Mato Grosso, Cuiabá, MT, Brazil. *Email/ORCID*: robson.bio2017@gmail.com/0000-0002-5949-1006.
[3] Universidade do Estado de Mato Grosso, Departamento de Agronomia, Tangará da Serra, MT, Brazil.
Email/ORCID: marilzacosta@gmail.com/0000-0002-4226-1802; monica@unemat.br/0000-0002-5310-4140.
[4] Universidade Federal de Alagoas, Instituto de Química e Biotecnologia, Maceió, AL, Brazil.
Email/ORCID: henrique.goulart@ceca.ufal.br/0000-0003-0532-6133; aegs@ceca.ufal.br/0000-0002-3750-438X.
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complex mixtures of several components, with the biological efficacy of these blends being highly dependent on the proportion of constituents, which plays a key role in species recognition and reproductive isolation (Blankers et al. 2022). Such variation is frequently attributed to evolutionary pressures, including local adaptation and genetic divergence (Roelofs & Rooney 2003).

Monitoring soil-dwelling pest species using pheromones is particularly valuable, as direct observation of immature stages is often impractical, and trapping of adults provides the necessary early warning for the potentially damaging larval stage (Witzgall et al. 2010).

The lesser cornstalk borer is a polyphagous pest that causes significant damage to crops such as maize, soybean and peanut (Vieira et al. 2020, Bragard et al. 2021). Because its larvae develop underground, pheromone traps targeting adult males offer the most reliable method for early detection and informed management decisions (Pires et al. 1994). However, differences in pheromone response have been observed among geographically distinct populations of *E. lignosellus* (Jham et al. 2005, Jham et al. 2007). Field trials conducted in the Minas Gerais state, Brazil, reported low trap catches when using the pheromone blend originally developed for the North American population (Pires et al. 1992). Similarly, field tests in Mexico showed that the same North American blend described by Lynch et al. (1984) failed to attract local populations (Loera et al. 1995). These results highlight that pheromone formulations optimized for North American populations are not necessarily effective for South American populations of *E. lignosellus*, emphasizing the need for regionally tailored pheromone blends.

Intraspecific variation in pheromone composition was confirmed by subsequent chemical analyses. This evidence was consolidated by studies by Jham et al. (2005, 2007), which revealed significant differences in the chemical profiles of pheromone extracts from *E. lignosellus* populations collected in Minas Gerais and Goiás (Brazil), and Georgia (USA).

Building on this evidence, the present study aimed to evaluate the efficacy and selectivity of synthetic sex pheromone blends in monitoring *Elasmopalpus lignosellus* populations in soybean, as well as their attractiveness to non-target lepidopteran species. The findings are intended to inform the development of an effective, regionally adapted pheromone-based monitoring system for this economically significant pest.

MATERIAL AND METHODS

The experiments were conducted in commercial soybean production areas in the municipality of Tangará da Serra, Mato Grosso state, Brazil (14º37′10″S; 57º29′09″W), during the 2021/2022 and 2023/2024 growing seasons, from October to December. Experimental plots were established on two farms, referred to as Area I (14º18′35″S; 57º45′37″W) and Area II (14º33′43″S; 58º37′18″W).

Eggs of *Elasmopalpus lignosellus* were kindly provided by the Assist Laboratórios Agronômicos. Upon hatching, larvae were individually transferred to glass tubes (8.5 cm length × 2.5 cm diameter) containing an artificial diet following the Chalfant protocol. A layer of vermiculite was added to simulate natural soil conditions, and the larvae remained in the tubes until adult emergence.

Emerging adults were transferred to mating cages (40 × 20 cm), each containing approximately 20 pairs of individuals. The cages were internally lined with paper sheets to facilitate oviposition. Two containers were placed inside each cage: one with water and the other with a 10 % honey solution as food sources for the moths. The cages were replaced every two days, and the papers containing egg masses were stored in plastic containers until larval hatching. The colony was maintained under controlled environmental conditions (25 ± 2 ºC; 50 ± 10 % relative humidity), with an average developmental cycle of 25 to 30 days.

A randomized complete block design with six replicates was used. During the first season (2021/2022), six treatments were evaluated: four synthetic pheromone formulations, one positive control, and one negative control. The acronym “EL” (from *Elasmopalpus lignosellus*) was used to identify each treatment, followed by a number corresponding to the specific pheromone blend.

The pheromone blends (Table 1) were developed based on previous studies conducted in the same region (Viana et al. 2016) and on the identification of pheromone components extracted from the sex glands of laboratory-reared females originating from the Mato Grosso state population.

Table 1. Composition and ratios of pheromone blends evaluated in field tests for monitoring *Elasmopalpus lignosellus* in Tangará da Serra (Mato Grosso state, Brazil).

Blend	Ratio (components)
EL1	80:20 (Z9-14:Ac : Z11-16:Ac)
EL2	50:50 (Z9-14:Ac : Z11-16:Ac)
EL3	33.3:33.3:33.3 (Z9-14:Ac : Z11-16:Ac : Z9-16:OAc)
EL4	30:30:10:30 (Z9-14:Ac : Z11-16:Ac : Z11-16:OH : Z9-16:OAc)

The positive control consisted of traps baited with four virgin *E. lignosellus* females (less than 48 hours old), which were replaced weekly to ensure continuous pheromone emission. The negative control consisted of rubber septa loaded with HPLC-grade hexane only, and these were replaced together with the pheromone lures after four weeks of field exposure.

The pheromone lures were prepared using gray rubber septa (Sigma-Aldrich, USA) as release devices. Each septum was loaded with 6 mg of the respective pheromone blend, dissolved in 100 µL of HPLC-grade hexane. The negative control consisted of septa loaded with hexane only, whereas the positive control used traps baited with four virgin *E. lignosellus* females (less than 48 hours old).

Traps were deployed in soybean fields and arranged in randomized blocks, with six replications of each treatment. To minimize positional effects within each block, all traps were rotated weekly along the rows, maintaining a minimum distance of 30 m among them. The lures remained in the field for up to four weeks, after which they were replaced with freshly prepared septa. Based on the results obtained during the first season (2021/2022), only the most effective formulations (those yielding higher male captures) were evaluated again during the second season (2023/2024), along with the positive and negative controls. The average size of the experimental fields was approximately 90 hectares.

Delta traps (450 × 220 mm) lined with adhesive base inserts were used. They were mounted on 0.5 m high wooden stakes and installed within the soybean fields, maintaining a minimum border distance of 50 m from the field margins. The traps were spaced at least 200 m apart between different treatments and 150 m between trap rows of the same treatment to minimize interference.

The surrounding landscape differed slightly between the experimental sites. At the Area I (14º18'35"S; 57º45'37"W), the field was bordered on one side by a teak (*Tectona grandis*) plantation, whereas the other sides were adjacent to soybean fields. At the Area II (14º33'43"S; 58º37'18"W), the experimental area was located in an open field with no surrounding vegetation or nearby natural fragments, although a patch of natural vegetation was present near the sampling plots.

Simultaneously, *E. lignosellus* infestation in soybean crops was assessed using the 1-m row sampling technique at 30 sampling points arranged in a zigzag pattern, with 150 m spacing between points. The captured insects, both target and non-target lepidopterans, were identified based on morphological comparisons using the Soybean Pest Manual (Moreira & Aragão 2009).

All statistical analyses were performed using the R software version 4.3.1 (R Core Team 2023). For the target species, the total number of captured males was analyzed using generalized linear models with Poisson error distribution and deviance analysis at a 5 % significance level. The number of insects captured per trap was used as the dependent variable, with treatment as a fixed effect. Analysis of variance was conducted to compare treatments. For non-target Lepidoptera, the analysis followed a two-step approach. Initially, generalized linear models with a Poisson error distribution were used to model the total capture counts, assessing the overall effect of the treatments. Subsequently, the non-parametric Kruskal-Wallis test was applied to compare the median capture numbers across the treatments. This two-pronged approach was necessary because non-target species data often exhibit high variability and non-normal distributions, frequently violating the assumptions of Poisson generalized linear models (e.g., equidispersion) (Crawley 2013). The Kruskal-Wallis test, being a more robust and conservative non-parametric method, ensured a reliable comparison of treatment effects on this variable and less-structured dataset.

RESULTS AND DISCUSSION

Results for the 2021/2022 season (Figure 1A) indicated that both the virgin female treatment and the EL3 blend were the most effective in attracting males, with no significant difference between them, being both superior to the other treatments ($p < 0.05$). The EL4 blend showed an intermediate level of attractiveness, suggesting some efficacy, albeit lower than that of virgin females and EL3. The negative control with hexane, along with EL2 and EL1, resulted in significantly fewer captures, with EL1 exhibiting the lowest mean catch among all treatments. These results indicate low efficacy of EL1 and EL2 under the conditions of the Area I.

In the Area II (Figure 1B), the data revealed a different pattern. The EL4 blend was the most effective treatment, followed by EL3, virgin females, and EL1, which formed a statistically similar group ($p < 0.05$). This suggests that, in this area, EL1 performed better than in the Area I, whereas virgin females did not stand out as they did in the Area I. The reduced attractiveness of virgin females in the Area II, in contrast to the Area I, may indicate a lower male *E. lignosellus* population density in the Area II during the sampling period. In low-density situations, the probability of males encountering and responding to a point source of pheromone, such as the trap with virgin females, may be reduced, thus decreasing the capture rate. The EL2 blend showed reduced attractiveness, whereas the hexane control resulted in the lowest number of captures, confirming its role as a negative control.

Based on the data obtained during the 2021/2022 season, the blends that captured the highest number of target insects in the field and did not differ statistically from the virgin female treatment were selected for further field evaluations. Accordingly, in the 2023/2024 season, in the Area I (Figure 2A), the EL3, EL4, and virgin female treatments recorded the highest mean male captures of *E. lignosellus*, with no significant statistical differences among them ($p < 0.05$). The hexane treatment (negative control) had the lowest mean capture, significantly lower than all other treatments.

In the Area II (Figure 2B), a similar capture trend was observed, with the EL4, EL3, and virgin female treatments not differing significantly from each other ($p < 0.05$). The hexane treatment again resulted in the lowest mean capture.

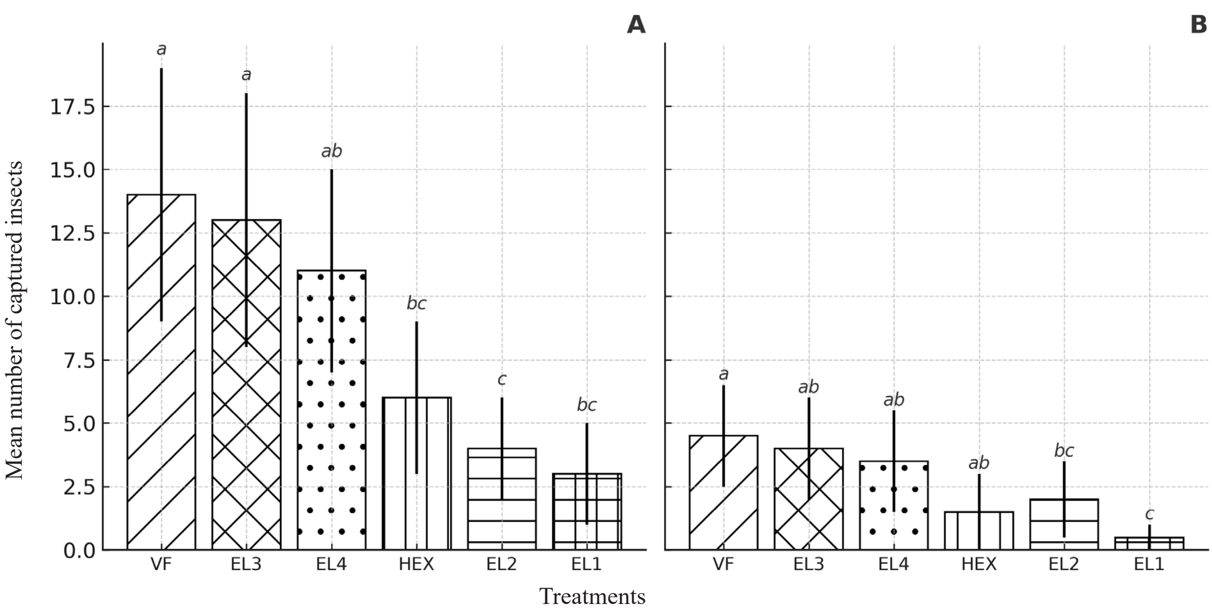

Figure 1. Mean number (± SE) of *Elasmopalpus lignosellus* (Lepidoptera: Pyralidae) males captured in soybean fields in Tangará da Serra, Mato Grosso state, Brazil, during the 2021/2022 agricultural season. Traps were deployed in the Area I (14º18’35”S; 57º45’37”W) (A) and Area II (14º33’43”S; 58º37’18”W) (B), using six treatments: four synthetic sex pheromone blends (EL1, EL2, EL3, and EL4), virgin females (VF), and hexane (HEX). Bars followed by different letters indicate significant statistical differences in male captures among the treatments (generalized linear models, deviance analysis, and analysis of variance; $p < 0.05$).

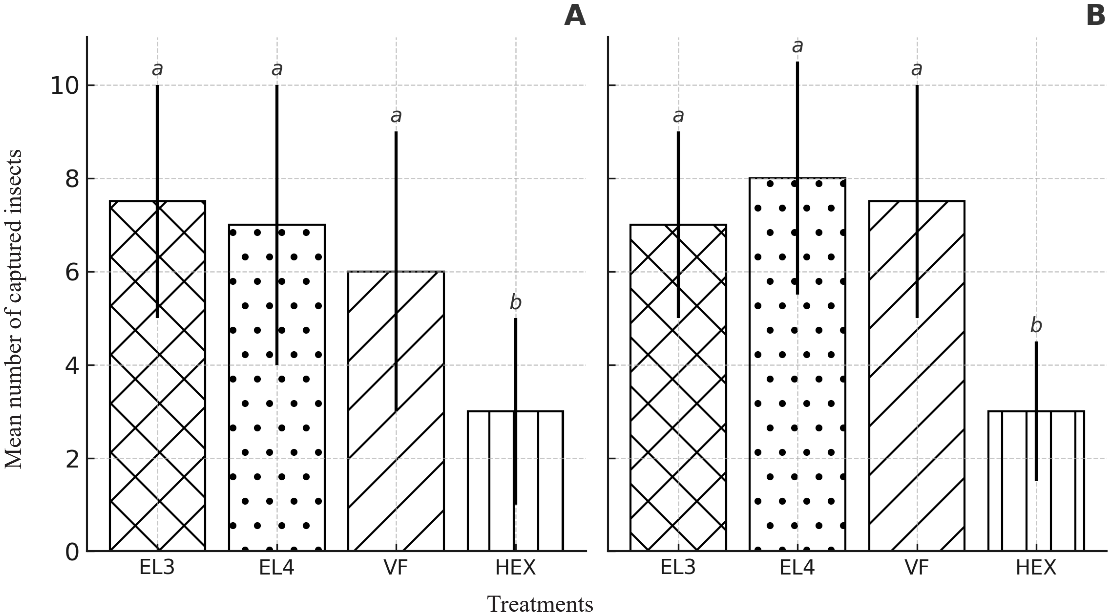

Figure 2. Mean number (± SE) of *Elasmopalpus lignosellus* (Lepidoptera: Pyralidae) males captured in soybean fields in Tangará da Serra, Mato Grosso state, Brazil, during the 2023/2024 agricultural season. Traps were deployed in the Area I (14º18'35"S; 57º45'37"W) (A) and Area II (14º33'43"S; 58º37'18"W) (B), using four treatments: two synthetic sex pheromone blends (EL3, EL4), virgin females (VF), and hexane (HEX). Bars followed by different letters indicate significant statistical differences in male captures among treatments (generalized linear models, deviance analysis, and analysis of variance; $p < 0.05$).

The EL3 and EL4 blends included among their attractive components (Z)-9-tetradecenyl acetate (Z9-14:Ac), previously identified by Attygalle et al. (1988) in another pyralid species, *Myelois cribrella* (1796), where it is part of a ternary formulation used for field monitoring. In addition, Z9-14:Ac is the sole pheromone component described for the pyralid *Dioryctria disclusa*, with proven efficacy in monitoring programs (Meyer et al. 1982). This suggests a possible conservation of pheromonal components among certain Pyralidae (Hattori et al. 2001, Huang et al. 2023).

In China, Z9-14:Ac is also an attractive component for *Spodoptera frugiperda* (Lepidoptera: Noctuidae), and when combined with (Z)-7-dodecenyl acetate (Z7-12:OAc) and (Z)-11-hexadecenyl acetate (Z11-16:OAc) at a ratio of 88:1:11, it significantly increases male attraction in both wind tunnel and field assays (Wang et al. 2022). In Brazil, this compound also attracts *S. cosmioides* and *S. frugiperda* (Blassioli-Moraes et al. 2016).

Z11-16:OAc, also present in the tested blends, is described as the sole pheromone component of *Dioryctria amatella* (Lepidoptera: Pyralidae), with field effectiveness confirmed by Meyer et al. (1986). The similarity in pheromone composition among related species supports the development of multi-component pheromone blends for simultaneous monitoring of several pest species, as exemplified by the *S. frugiperda* formulation, which includes Z11-16:OAc as a synergist (Wang et al. 2022).

Both Z9-14:Ac and Z11-16:OAc were chemically identified in pheromone gland extracts of *E. lignosellus* populations from the Brazilian states of Goiás (GO) and Minas Gerais (MG) (Jham et al. 2007). Comparative analyses between Brazilian populations and those from the United States, described by Lynch et al. (1984), also highlight the shared presence of these two main compounds.

Although Goiás and Mato Grosso are geographically close, the low capture rate observed in our field study when testing the binary blend (Z9-14:Ac and Z11-16:OAc) characteristic of the GO/MG population suggests that this blend is not optimally attractive to the *E. lignosellus* population in Mato Grosso (MT). This discrepancy between chemical identification in one region and low field efficacy in another indicates that the MT population may represent a distinct chemotype or genetic population that requires different ratios or minor components for effective attraction.

Variations in the bioactive pheromone composition across populations and regions, even when sharing core components, may indicate the existence of genetic polymorphisms or subspecies within *E. lignosellus*, as previously proposed by Jham et al. (2007).

According to Byers (2006), Z9-14:Ac is the most common compound among lepidopterans, reported in approximately 199 species, underscoring the importance of specific ratios and minor components for species and population isolation. Regarding non-target Lepidoptera captured in traps baited with different lures for monitoring *E. lignosellus* (Table 2), the analysis of gross captures of non-target Lepidoptera (Table 1) revealed notable differences in treatment selectivity. The attractiveness of the virgin female stood out due to the capture of 23 *Anticarsia gemmatalis* individuals across all seven replicates, a total significantly higher than all other treatments (e.g., 13 in EL3 and 8 in the hexane control). In contrast, the virgin female treatment demonstrated a high selectivity by capturing zero (0) individuals of the species *C. includens*, *S. cosmioides*, and *S. frugiperda*. Among the synthetic blends, EL3 was the most attractive to *S. frugiperda* (17 individuals) and *H. armigera* (15 individuals), whereas EL4 captured a total of 14 *S. frugiperda* and 11 *H. armigera*. The control treatment (hexane) recorded low captures for all species, with totals ranging from 1 (*C. virescens* and *S. cosmioides*) to 10 (*S. frugiperda*). The presence of minor, trace, or synergistic compounds not yet detected cannot be ruled out as contributors to heterospecific attraction.

According to Hansson & Stensmyr (2011), the moth olfactory system is highly sensitive and specific, allowing males to detect female pheromones at extremely low concentrations. However, this sensitivity may also cause responses to structurally similar compounds emitted by other species.

The non-target species *Chrysodeixis includens*, *Chloridea virescens*, and *Spodoptera cosmioides* showed similar mean captures across all treatments, suggesting a limited interference from the tested lures. For *Helicoverpa armigera*, the EL3 formulation resulted in a higher number of captures, indicating a possible cross-attraction to components present in this blend.

Similarly, *S. frugiperda* also responded significantly to the EL3 treatment, with higher captures, when compared to the other treatments, whereas the virgin female treatment was the least effective for this species. These results indicate that the EL4 formulation exhibited a greater selectivity, being less attractive to non-target insects than EL3 and virgin females, which is desirable in pheromone-based monitoring programs.

In the case of *C. includens*, the absence of (Z)-7-dodecenyl acetate in the tested blends was consistent with the lack of significant differences among the treatments. For *C. virescens*, although Z11-16:OH is present in the EL4 formulation, no statistically significant difference was observed, but the mean capture was higher with EL4, suggesting a potentially stronger attractive effect.

Within the *Spodoptera* complex, the observed cross-attraction suggests pheromonal interaction, which is relevant for the integrated management of these pest species. Studies conducted in China have shown that the most effective blend for *S. frugiperda*

Table 2. Mean (± SE) number of non-target moths captured per trap baited with four synthetic pheromone formulations (EL3, EL4, virgin females, and hexane) originally designed for monitoring *Elasmopalpus lignosellus*. Data were collected during field trials conducted in soybean crops.

Species	Synthetic pheromone formulations			
	EL3	EL4	Virgin females	Hexane
Anticarsia gemmatalis	1.9 ± 0.5 a*	1.7 ± 0.4 a	3.2 ± 0.6 b	1.8 ± 0.5 a
Chrysodeixis includens	0.5 ± 0.2 a	0.4 ± 0.2 a	0.0 ± 0.0 a	0.3 ± 0.2 a
Chloridea virescens	0.4 ± 0.2 a	0.6 ± 0.2 a	0.3 ± 0.2 a	0.3 ± 0.2 a
Helicoverpa armigera	2.5 ± 0.6 a	2.4 ± 0.5 ab	1.6 ± 0.4 b	2.0 ± 0.5 ab
Spodoptera cosmioides	0.5 ± 0.3 a	0.1 ± 0.1 a	0.0 ± 0.0 a	0.3 ± 0.2 a
Spodoptera frugiperda	2.2 ± 0.5 a	2.0 ± 0.4 ab	0.6 ± 0.2 c	1.6 ± 0.3 b

* Different lowercase letters within each row indicate statistically significant differences among treatments (Kruskal-Wallis test; $p < 0.05$).

includes Z9-14:OAc, Z7-12:OAc, and Z11-16:OAc (Wang et al. 2022).

The EL3 blend showed promising results in the Mato Grosso state, with significant differences observed under field conditions. This indicates its potential for monitoring *E. lignosellus* while also attracting *S. frugiperda*, thereby contributing to a more efficient integrated pest management strategy.

Therefore, the adoption of more selective pheromone blends contributes not only to the efficiency of target pest monitoring, but also to the conservation of beneficial insect fauna, reducing ecological disturbances in cropping systems (Shangguan et al. 2023).

During the field sampling process, adult insects were recorded on soybean plants and failures in the soybean stand. Based on the collected data (Figures 3A and 3B), it was possible to outline the pest's population flow pattern throughout its occurrence period over two agricultural years.

The comparative analysis between years revealed a variable dynamic of *E. lignosellus* occurrence. At the Area I (2021/2022 season), the infestation was late and of low intensity, concentrated at the end of the evaluation period, with peaks of damaged plants and adult presence, but without records of larvae and pupae. In contrast, at the Area II (2021/2022 and 2023/2024 seasons), a higher population pressure and early occurrence of the pest were observed, especially in the 2021/2022 season.

The linear meter sampling technique, although practical for assessing damage incidence, has limitations in detecting specific pest stages and may be influenced by the spatial distribution of the infestation (Southwood & Henderson 2000). The weekly sampling frequency provided a temporal overview of the infestation; however, occurrence peaks may have been underestimated between collection intervals. The absence of larvae during damage records suggests previous infestations or a lack of synchronization of larval occurrence with the sampling carried out.

Photos: Robson Aparecido dos Santos

Figure 3. Traps used for monitoring *Elasmopalpus lignosellus* in a soybean field (Tangará da Serra, Mato Grosso state, Brazil). A) Wooden stake with trap installed in the field; B) Delta trap containing a sticky insert and a rubber septum loaded with the hexane treatment.

These results underscore the need of management strategies adapted to the pest's population dynamics in each area and season, reinforcing the importance of continuous monitoring to identify critical intervention times (Fleming et al. 2021). Moreover, more selective traps help to reduce the capture of non-target species while preserving natural enemies and pollinators, which are essential for maintaining agroecological balance, thereby strengthening the sustainability of integrated pest management programs (Shangguan et al. 2023).

Future research should focus on adapting and optimizing these formulations to develop management strategies that are not only effective, but also sustainable, considering the genetic variability and adaptive capacity of *E. lignosellus*, as well as the confirmed low environmental risk.

CONCLUSIONS

The ternary blend (EL3), composed of Z9-14:Ac, Z11-16:Ac, and Z9-16:OAc, and the quaternary blend (EL4), containing Z9-14:Ac, Z11-16:Ac, Z11-16:OH, and Z9-16:OAc, demonstrated high efficacy for monitoring *Elasmopalpus lignosellus* under field conditions in the Mato Grosso state. Crucially, these formulations also proved highly selective, as they did not significantly attract non-target lepidopteran species, if compared to the control, supporting their safe integration into integrated pest management programs.

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