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POTENTIATION OF COPAÍBA OIL-RESIN WITH SYNTHETIC INSECTICIDES TO CONTROL OF FALL ARMYWORM¹

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ABSTRACT - The control of *Spodoptera frugiperda* (J. E. SMITH) (Lepidoptera: Noctuidae) has been carried out mainly with pyrethroids and organophosphates insecticides. The continuous and indiscriminate use of synthetic insecticides, for decades, has led to the selection of resistant populations and has caused concerns for human health and the environment. An alternative is the use of botanical insecticides, including through the mixtures with synthetic insecticides. This study aimed to investigate the toxicity of copaiba oil-resin mixtures (COR) with deltamethrin, beta-cyfluthrin, beta-cypermethrin, methomyl, triazophos and chlorpyrifos for *S. frugiperda*. Bioassays were performed in the laboratory using newly hatched caterpillars on a contaminated surface (filter paper). Initially, concentration-mortality curves were established for each insecticide and COR. Based on the results, experiments were conducted to verify the effect of the COR mixtures with synthetic insecticides in the proportions of 1:1 ($LC_{25} + LC_{25}$) on larvae of *S. frugiperda*. Additionally, COR concentration-response curves were established by adding 1/8 of the recommended rate of each insecticide. Notably, the use of this concentration for all insecticides was previously determined in bioassays, and no significant toxicity was found for *S. frugiperda* caterpillars. It was found that the toxicity of COR increased substantially by adding synthetic insecticides, especially with lower mixtures with beta-cypermethrin, methomyl and triazophos.

Keywords: Bioinsecticide. Additive effect. Synergism. Corn plague. Integrated pest management.

POTENCIALIZAÇÃO DO ÓLEO-RESINA DE COPAÍBA COM INSETICIDAS SINTÉTICOS PARA O CONTROLE DA LAGARTA-DO-CARTUCHO

RESUMO - O controle de *Spodoptera frugiperda* (J. E. SMITH) (Lepidoptera: Noctuidae) tem sido realizado principalmente com inseticidas piretróides e organofosforados. O uso contínuo e indiscriminado de inseticidas sintéticos, durante décadas, tem ocasionado a seleção de populações resistentes, bem como preocupações em relação à saúde humana e ao meio ambiente. Uma alternativa é o emprego de inseticidas botânicos, inclusive por meio de misturas com inseticidas sintéticos. Assim, este trabalho teve por objetivo investigar a toxicidade de misturas do óleo-resina de copaíba (ORC) com os inseticidas deltametrina, beta-ciflutrina, beta-cipermetrina, metomil, triazofós e clorpirifós para *S. frugiperda*. Foram realizados bioensaios em laboratório utilizando-se lagartas recém-eclodidas em superfície contaminada (papel filtro). Inicialmente, foram estabelecidas curvas de concentração-mortalidade para cada inseticida e para o COR. A partir dos resultados obtidos foram realizados bioensaios para verificar o efeito das misturas do ORC com os inseticidas sintéticos nas proporções de 1:1 ($CL_{25} + CL_{25}$) sobre lagartas de *S. frugiperda*. Adicionalmente, foram estabelecidas curvas de concentração-resposta do ORC adicionando-se 1/8 da dose comercial de cada inseticida. Vale destacar que o uso desta fração para todos os inseticidas foi determinada em bioensaios preliminares, não tendo apresentado toxicidade considerável para as lagartas de *S. frugiperda*. Constatou-se que a toxicidade do ORC aumentou substancialmente adicionando-se os inseticidas sintéticos, com menor destaque para as misturas com beta-cipermetrina, metomil e triazofós.

Palavras-chave: Bioinseticida. Efeito aditivo. Sinergismo. Praga de milho. Manejo integrado de pragas.

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INTRODUCTION

The control of *Spodoptera frugiperda* (J. E. SMITH) (Lepidoptera: Noctuidae) has been carried out mainly with pyrethroids and organophosphates synthetic insecticides. The continuous and indiscriminate use of these products has favored the emergence of resistant populations (OMOTO; RODRIGUEZ, 2001; RÍOS-DÍEZ; SALDAMANDO-BENJUMEA, 2011; SALEEM et al., 2016). Among the main alternatives, it is worth mentioning the use of botanical insecticides (FAZOLIN et al., 2016; SANTOS et al., 2016) and transgenic crops, although there are historical reports on *S. frugiperda*-resistant populations for the latter (SANTOS-AMAYA et al., 2015; HORIKOSHI et al., 2016).

Usually, botanical insecticides are used as powders, extracts or oils, which are easy to obtain, and generally harmless to the applicator and consumers (ISMAN, 2000; CORRÊA; SALGADO, 2011). More than 100,000 secondary metabolites with insecticidal properties have been identified from approximately 200,000 species of plants in the world, and they particularly belong to alkaloid, terpenoid, flavonoid, and quinone groups (POTENZA et al., 2004). These compounds have multiple modes of action on insects, particularly: acute toxicity, repellency, feeding inhibition, growth, development and reproduction (COITINHO et al., 2006; GERIS et al., 2008).

In this sense, the Amazon rainforest is the largest natural reservoir of the plant diversity of the planet, where each of its different forest environments has a rich and varied floristic contingent, often unique to a particular environment (OLIVEIRA; AMARAL, 2004). Despite the immense Amazonian plant diversity, the insecticide potential of species found in the forest itself is little known, since the related studies have become more frequent in the last decade (PEREIRA et al., 2008; SARRIA et al., 2011; GOUVÊA et al., 2010; LIMA-MENDONÇA et al., 2013).

Among numerous species found in the Amazon rainforest it is possible to highlight copaiba (Leguminosae-Caesalpinoideae) belongs to the family Caesalpinaceae and to the gender *Copaifera* sp. It is a native tree of Latin America and West Africa, with high rates in Brazil and widely distributed in the Amazon rainforest (FRANCISCO, 2005; ARAÚJO JÚNIOR et al., 2005). The copaiba oil comes from the flow through channels located in the plant log and it is collected through the tree trunk drilling, the volatile fraction (the distilled copaiba oil or essential oil) is obtained from oil-resin by steam distillation. This is basically composed of sesquiterpene oxygenated hydrocarbons, predominantly β -caryophyllene, β -bisabolene, β -bergamoteno and β -selinene (VEIGA JUNIOR; PINTO, 2002). Recently, Cárdenas-Ortega et al. (2015) found that the essential oil from *Salvia*

ballotiflora Benth (Lamiaceae), which also contains β -caryophyllene (12.74%) among its major components, showed insecticidal activity in *S. frugiperda*, leading to a decrease in the viability of caterpillars and pupae. However, copaiba oil-resin (COR) has been shown to be toxic to *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) (SANTOS et al., 2015).

From a toxicological point of view the recognition of the potential insecticide of occurring plants in the Amazon flora, as copaiba, is relevant because of the range of secondary metabolites often found in vegetable insecticides (SANTIAGO et al., 2005; AMBROZIN et al., 2006; GERIS et al., 2008; SARRIA et al., 2011). These compounds are needed for the immediate survival of plant cells, but they serve as an evolutionary advantage to their own survival and reproduction, may also act as a natural defense pesticides against herbivores and pathogens (GOUVÊA et al., 2010; RANI; VENKATESHWARAMMA; DEVANAND, 2011). In the case of botanical insecticides, there are different modes of action, especially the toxic potential, repellency, feeding inhibition, development and reproduction of insects (ISMAN, 2000; ALVES et al., 2012; SANTOS et al., 2016).

Most research with insecticide plants are limited to traditional toxicity tests with little research, whose purpose is the enhancement of botanical insecticides through the mixtures. However, a way of using these botanical insecticides is through the mixtures of chemical insecticides. The objective of this study was to investigate the toxicity of copaiba oil-resin (COR) mixtures with synthetic insecticides as a management alternative against *S. frugiperda*.

MATERIAL AND METHODS

Colony –stock

The colony-stock of *S. frugiperda* was established by some copies from the Agroforestry Research Center of Acre (EMBRAPA/AC). The creation of the insects was adapted from procedures adopted by Bavaresco et al. (2002). The newly hatched caterpillars were placed in separate plastic containers that had holes in the lid (5.0 × 5.5 cm) and contained artificial food, as described by Kasten Júnior, Precetti and Parra (1978), where they were kept until they reached the pupal stage. Later, the adults were sexed and after adult emergence, they were transferred to the PVC cages to obtain new oviposition and newly hatched caterpillars.

Insecticides and copaiba oil

The following insecticides were used: deltamethrin (Pyrethroid) (Decis® 25 EC),

Beta-cypermethrin (Pyrethroid) (Akito), Beta-cyfluthrin (Pyrethroid) (Turbo), triazophos (organofosforado) (Hostathion 400 BR), Chlorpyrifos (Organophosphorus) (Klorpan 480 EC) and Methomyl (Carbamate) (Lanate BR). All these insecticides were purchased in the agricultural stores. The COR was from samples of Cazumbá-Iracema and Flona Macauã/Sena Madureira reserves, provided by Agroextrativista Cooperative of Rural Producers of Vale do Rio Iaco - Sena Madureira / AC. COR extraction was performed through perforations in the trunk of the copaiba tree with an auger, about 1 to 1.5 m above the base of the plant. Then, 3–4-inch PVC pipes were inserted into the holes through which the oil drained.

Concentration-mortality curves for insecticides and COR

It has been determined, initially, the toxicity of each pesticide and COR to newly hatched caterpillars of *S. frugiperda* using concentration-mortality curves. Initially, preliminary tests to estimate the highest concentrations did not occur death of larvae were performed (lower end) and the lowest concentration that occurred with increased mortality (upper end). Based on the data obtained, were established, for each insecticide and COR, five to six concentration ranges for the definitive bioassay. The final concentrations ranged from 2.08 to 8.33, 1.25 to 20.00, 10.00 to 80.00, 53.75 to 430.00, 80.00 to 640.00, and 42.86 to 640.00 mg L⁻¹ for deltamethrin, beta-cyfluthrin, beta-cypermethrin, methomyl, triazophos, and chlorpyrifos, respectively. For copaiba oil, the final concentrations ranged from 1.56% to 25% (v/v). Acetone was used as a solvent in both bioassays.

Exposure of insects was accomplished by contact with contaminated surface, where newly hatched caterpillars were placed in separate Petri dishes (5.0 cm diameter × 1.2 cm height) containing filter papers (5.0 cm diameter) impregnated with test solutions (200 µL). Petri dishes were then covered with voile fabric to allow gas exchange. The assessment of mortality was performed at 24 hours after the start of exposure of larvae to insecticides and to COR. Larvae that showed no movement when touched lightly with a brush were considered dead. We used 5 replications, each replicate consisting of 10 larvae. The concentration-response data were subjected to probit analysis (SAS INSTITUTE, 2011).

Bioassays with mixtures of COR and insecticides

Initially, experiments were conducted to

verify the effect of COR mixtures with synthetic insecticides in the proportions of 1:1 (LC₂₅ + LC₂₅) on larvae of *S. frugiperda*. The exposure of larvae was made by using the same procedure used in bioassays of toxicity. Four replications were used, each replicate consists of 10 larvae. Mortality was assessed after 24 hours. The efficiency of the treatments was determined according to Abbott's formula. The data were analyzed by analysis of variance (ANOVA) and Tukey test ($P \leq 0.05$). Shapiro-Wilk and Cochran tests were used to check normality of residuals and homogeneity of variances, respectively.

Concentration-response curves were established when COR was combined with any one of the following insecticides: triazophos (organophosphorus), methomyl (carbamate), or beta-cypermethrin (pyrethroid). These three insecticides were chosen because of their high efficiency in bioassays when combined with COR at a ratio of 1:1 (LC₂₅ + LC₂₅), with each chemical group of insecticide. COR concentrations, previously established in bioassays as ranging from 1.56% to 25%, were combined in a solution containing 1/8th of the recommended concentration of each commercial insecticide (5.00, 53.75, and 80.00 mg L⁻¹ of beta-cypermethrin, methomyl, and triazophos, respectively). Notably, this 1/8th fraction for each insecticide was also determined through previous bioassays, and no significant toxicity was observed for newly hatched caterpillars of *S. frugiperda*. Acetone was used as a solvent in both bioassays.

Each mixture was tested on 5.0 cm diameter filter paper impregnated with five concentrations, where it was applied 200 µL pipetter of each concentration, inside Petri dishes (5.0 cm diameter × 1.2 cm height). Petri dishes containing caterpillars were covered with voile fabric to promote gas exchange. Four replications were used, each replicate being composed of ten (10) larvae. The assessment of mortality was made by counting the number of live and dead insects, 24 hours after the start of the bioassays, considering dead insects those that had not move when touched gently with a brush. The concentration-response data were subjected to probit analysis (SAS INSTITUTE, 2011).

RESULTS AND DISCUSSION

The results of the concentration-mortality curves of insecticides to the larvae are shown in Table 1. The deltamethrin showed greater toxicity to larvae and the chlorpyrifos was the least toxic. These

curves were established for testing the efficacy of commercial products and finding tests with concentrations of mixes. The probit model was suitable for concentration-mortality data obtained with insecticides, based on the low values χ^2 and the high values P , obtained for each

concentration-mortality curve ($\chi^2 < 4.1$; $P > 0.05$). With regard to COR, the following LC_{25} and LC_{50} (%) v/v were determined: 3.85 (3.20–4.45) and 5.36 (4.65–6.29), respectively ($\chi^2 = 3.951$; $P > 0.05$).

Table 1. Relative toxicity of synthetic insecticides to *S. frugiperda*.

Insecticide	Inclination \pm S.E. ¹	LC_{25} (FI 95%) mg L ⁻¹	LC_{50} (FI 95%) mg L ⁻¹	χ^2	P
Deltamethrin	5.22 \pm 0.75	2.75 (2.25-3.19)	3.71 (3.21-4.28)	1.04	0.31
Beta-Cyfluthrin	3.16 \pm 0.44	4.15 (3.32-4.97)	6.79 (5.68-8.41)	3.20	0.36
Beta-Cypermethrin	1.70 \pm 0.49	12.83 (5.69-17.88)	31.85 (23.07-65.52)	1.13	0.28
Methomyl	3.68 \pm 0.43	58.81 (47.30-69.45)	89.73 (76.58-104.75)	3.06	0.38
Triazophos	4.91 \pm 0.82	104.31 (83.40-123.31)	143.07 (120.91-172.67)	4.04	0.13
Chlorpyrifos	2.19 \pm 0.48	227.60 (172.02-326.97)	462.63 (323.10-992.15)	1.47	0.48

¹S.E. = Standard error; LC = Lethal concentration; FI 95% = Fiducial interval at 95% probability; χ^2 =Chi-square; P =Probability.

A significant variation among COR and insecticides mixtures at a ratio of 1:1 were observed ($LC_{25} + LC_{25}$) ($F_{5,18} = 283.61$; $P < 0.01$). In addition, the residues did not exhibit normal distribution ($W_c = 0.88$ and $P < 0.01$), but homogeneity of variances was detected ($C_c = 0.58$ and $P > 0.05$). It is observed in Figure 1 that the mixture of copaiba-oil and insecticides in proportions of 1:1 ($LC_{25} + LC_{25}$)

showed an additive effect, except for deltamethrin. Instead, it exhibited an antagonistic effect. The larval efficiency rate was significantly higher in COR mixtures containing one of the following insecticides: triazophos, chlorpyrifos, methomyl, and beta-cypermethrin (Figure 1). The efficiency of these mixtures was higher than that of the mixture of COR and beta-cyfluthrin.

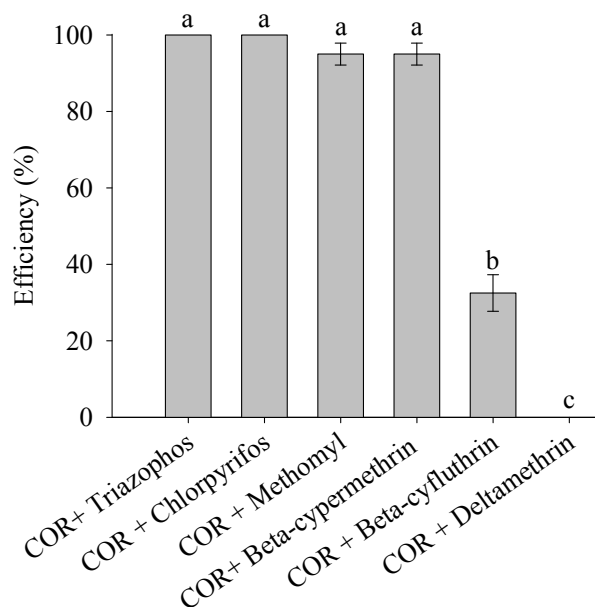


Figure 1. Efficiency (%) (\pm SE) of copaiba oil mixtures with synthetic insecticides in proportions of 1:1 ($LC_{25} + LC_{25}$) on larvae of *S. frugiperda*. Means followed by the same letter do not differ statistically with each other by Tukey Test ($P < 0.05$).

The results of the concentration-response curves of COR in admixture with insecticides (1/8 of the recommended rate of each insecticide) are shown in Table 2. In these bioassays were used only the following insecticides: triazophos (organophosphorus), methomyl (carbamate) and beta-cypermethrin (pyrethroid). They were chosen because they exhibited high efficiency when combined with COR at a ratio of 1:1, and at least one insecticide of each chemical group was represented. Chlorpyrifos was not used in the synergism tests because it exhibited a high LC₅₀ value in the toxicity bioassays (Table 1). The probit model was also suitable for the concentration-mortality data obtained with the mixtures (insecticides + COR), based on the

low value of χ^2 and high values P , obtained for each concentration-mortality curve ($\chi^2 < 7.2$; $P > 0.05$) (Table 2). The LC₅₀ of the copaiba oil separately was 5.36% v/v. Moreover, there was a substantial reduction when mixed with triazophos (1.92%), methomyl (1.82%) and beta-cypermethrin (1.71%), where synergism ratio ranged from 2.79 to 3.13 times. In general, lower amounts of COR were required to kill insects when insecticides were added to mixtures (Table 2). The results indicate that the copaiba oil toxicity was substantially increased in all mixtures when no harmful concentrations of triazophos, methomyl, and beta-cypermethrin were used.

Table 2. Toxicity of COR mixtures with insecticides (1/8 of the recommended rate of each pesticide) to *S. frugiperda*.

Mixture	Inclination \pm S.E. ¹	LC ₅₀ (FI 95%) (%) v/v	RT (LC ₅₀)	χ^2	P
COR	4.71 \pm 0.69	5.36 (4.65-6.29)	-	3.94	0.13
COR + Triazophos	3.65 \pm 0.49	1.92 (1.61-2.29)	2.79	3.99	0.13
COR + Methomyl	3.68 \pm 0.49	1.83 (1.51-2.21)	2.92	2.03	0.36
COR + Beta-cypermethrin	2.65 \pm 0.31	1.71 (0.96-1.43)	3.13	7.19	0.12

¹S.E. = Standard error of the mean; LC = Lethal concentration; FI 95% = Fiducial interval at 95% probability; RT = Reason of toxicity; χ^2 =Chi-square; P=Probability.

Although the Amazon region is one of the main centers of biodiversity, the reports of the plants with insecticidal properties are becoming more frequent from the 2000s (COITINHO et al., 2006; SILVA et al., 2007; SARRIA et al. 2011; ALVES et al., 2012; FAZOLIN et al., 2016; SANTOS et al., 2015, 2016). In general, botanical insecticides are less toxic than the synthetic ones and they degrade faster (ABBASSY; ABDELGALEIL; RABIE, 2009). Another advantage of botanical insecticides is the chemo-biodiversity of plants, which provides a variety of bioactive compounds against pests and significantly reduces the selection of resistant ones (PAVELA, 2009; PINO; SÁNCHEZ; ROJAS, 2013). The insecticidal activity of COR can be attributed to several identified chemical constituents, which belong to monoterpene, sesquiterpene, and diterpene classes (GERIS et al., 2008; LEANDRO et al., 2012).

Bioactivity studies of the chemical constituents found in COR and other botanical species revealed that they have different modes of action for insects (feeding inhibition and toxic activity repellency either by contact or by fumigation) (GERIS et al., 2008; ALVES et al., 2012; CHAUBEY, 2012; CÁRDENAS-ORTEGA et al., 2015; SANTOS et al., 2016). Recently, it was found that methanolic extracts of leaves and dried shells of *Copaifera langsdorffii* Desf. (Leguminosae-Caesalpinoideae) negatively affected the survival rate of caterpillars of *S. frugiperda*, increased protein excretion in caterpillars' feces, and inhibited the activity of trypsin in *in vitro* tests (ALVES et al., 2012).

The ratio of chemical components in these bioinsecticides as well as their synergism or antagonism effects primarily determine their effectiveness (HUMMELBRUNNER; ISMAN, 2001; MESBAH; MOURAD; ROKAIA, 2006; ABBASSY; ABDELGALEIL; RABIE, 2009; FAZOLIN et al., 2016). Increasing the efficacy of botanical insecticides by combining them with synthetic insecticides increases the chances of their use in the field. However, studies of mixtures of additive effects have been studied a little. In the present study, there was an additive effect, where COR mixtures + insecticide (triazophos, chlorpyrifos, methomyl and beta-cypermethrin) in ratios of 1:1 (LC₂₅ + LC₂₅) substantially increased the rate of mortality of larvae. According to Raffa and Priester (1985) the combined action of insecticides and oils may generate results as the sum of the effects of each compound or an overall effect higher than this sum, moreover, this mixture can minimize the environmental pollution by residues of insecticides persistent and preserve the beneficial insects.

In synergism tests, we found that the insecticidal activity of COR was enhanced when it was combined with triazophos, methomyl, or beta-cypermethrin because the toxicity of COR to caterpillars of *S. frugiperda* was substantially increased in these mixtures. According to Metcalf (1967), synergism is substantially more toxic or pharmacological action of two substances when used together. This combined action is the direct opposite of antagonism, in which the activity of the mixture is less than that of the more active constituent.

Synergism is only considered when a constituent of a mixture (synergist), which is inactive in the dosage employed, is considerably more active than the other single component in the resulting mixture (MESBAH; MOURAD; ROKAIA, 2006; FAZOLIN et al., 2016).

Thus, the increased toxicity of COR in mixtures with inactive doses of the synthetic insecticides beta-cypermethrin, methomyl, and triazophos reduce the risks of selecting resistant populations of *S. frugiperda*. This is because the increased toxicity of mixtures containing insecticides is usually associated with the activity of biological compounds based on distinct mechanisms of action (CORBEL et al., 2006; AHMAD; SALEEM; SAYYED, 2009). Furthermore, the use of insecticide mixtures may help reduce the selection speed of resistant pest populations and may directly benefit insect resistance management programs (to insecticides) if there is no cross-resistance to the insecticides used in the mixture (MCKENZIE, 1996; SAYYED; AHMAD; SALEEM, 2008). The prospects for the successful use of the mixtures depend on the ability of each component to kill a very high percentage of the exposed insects, which are genetically susceptible to it (CURTIS; HILL; KASIM, 1993), and this is also expected considering the susceptibility patterns of *S. frugiperda* populations to COR. It is noteworthy, however, that the indiscriminate use of any botanical insecticide should be avoided because of possible harmful effects on non-target organisms, such as natural enemies (SUTHISUT; FIELDS; CHANDRAPATYA, 2011).

Overall, COR exhibited high toxicity to caterpillars when combined with nonharmful concentrations of insecticides. These results are very meaningful for integrated pest management programs, considering that deleterious effects in the early stages of *S. frugiperda* caterpillar development reduce crop losses (SALEEM et al., 2016; SANTOS et al., 2016). Therefore, our investigation about the potential of additive mixtures is essential for the management of *S. frugiperda*, which may have economic implications for maize producers as well as for producers who cultivate other crops susceptible to this pest. Therefore, the impact on the net income of producers is particularly important in developing regions, where phytosanitary techniques (plant protection) are often based on conventional methods. On the other hand, production has substantially increased in some developing regions such as the Brazilian Amazon region, even with a limited storage capacity. In general, the COR mixtures with synthetic insecticides (triazifós, methomyl and beta-cypermethrin) promoted the substantial increase in the toxicity of COR.

CONCLUSIONS

The copaiba oil mixtures with insecticides in the proportions of 1:1 (LC₂₅ + LC₂₅) displayed greater additive effect in COR mixtures with insecticides triazophos, chlorpyrifos, methomyl and beta-cypermethrin. Additionally, the toxicity of COR increased substantially in admixture with insecticides (1/8 of recommended rate of each pesticide), especially with lower mixtures with beta-cypermethrin, methomyl and triazophos. COR in mixtures highlights a potential alternative to be implemented in integrated-pest management programs of *S. frugiperda*.

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