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Managing *Spodoptera* Species (Lepidoptera: Noctuidae) Found in Brazilian Soybean Fields with *Bt* Soybean and Insecticides

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Abstract: Genetically modified (GM) soybeans expressing Cry1A.105/Cry2Ab2/Cry1Ac (event MON 87701 × MON 89788 × MON 87751 × MON 87708) and Cry1Ac (event MON 87701 × MON 89788) from *Bacillus thuringiensis* Berliner (*Bt*) are valuable technologies for managing key lepidopteran pests of soybean in South America, but do not provide stand-alone protection against *Spodoptera* species. Here, we evaluated the use of these *Bt* soybeans and their integration with insecticides for managing *Spodoptera* species. Cry1A.105/Cry2Ab2/Cry1Ac soybean provided reasonable levels of protection against *S. cosmioides*, *S. albula,* and *S. eridania*. However, *S. frugiperda* had higher survival on this *Bt* soybean, and Cry1Ac soybean showed low lethality against all species evaluated. *Spodoptera* larvae that survived on *Bt* and non-*Bt* soybean showed comparable susceptibility to flubendiamide and thiodicarb in diet-overlay bioassays. Regardless of soybean plant type, the field doses of flubendiamide and thiodicarb were effective in controlling surviving *Spodoptera* larvae. We conclude that Cry1A.105/Cry2Ab2/Cry1Ac soybean is effective in controlling *S. cosmioides* and *S. albula*, and also has reasonable control of *S. eridania*, but not *S. frugiperda*. Cry1Ac soybean provided poor control of all *Spodoptera* species. Nonetheless, *Spodoptera* larvae surviving on both *Bt* and non-*Bt* soybean were controlled by flubendiamide and thiodicarb. Thus, integrated control tactics would provide effective management of *Spodoptera* species in *Bt* soybean fields in South America.

Keywords: integrated pest management; transgenic soybean; chemical insecticides; defoliator species

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1. Introduction

Brazil is responsible for approximately 31% of the world soybean production, planting approximately 41 million hectares during the 2021/22 soybean season [1]. In 2013, the transgenic soybean MON 87701 × MON 89788 (commercial name Intacta RR2 PRO®), which expresses the insecticidal protein Cry1Ac from *Bacillus thuringiensis* Berliner (*Bt*) and has tolerance to glyphosate, was first planted in Brazil. Currently, this genetically modified (GM) soybean is widely cultivated (>30 million hectares per season) [2]. This *Bt* soybean provides high-level protection against key defoliator pests: *Anticarsia gemmatalis* (Hübner, 1818) (Lepidoptera: Erebidae), *Chrysodeixis includens* (Walker, 1858), *Chloridea virescens* (F. 1777), and *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae) [3–6].

In 2021, the GM soybean, MON 87751 × MON 87708 × MON 87701 × MON 89788 (commercial name Intacta 2 Xtend®), which expresses the insecticidal proteins Cry1A.105, Cry2Ab2, and Cry1Ac, became available in Brazil [7]. This soybean was obtained by conventional breeding of the following events: MON 87751, MON 87708, MON 87701 and MON 89788. MON 87751 (Cry1A.105, Cry2Ab2) and MON 87701 (Cry1Ac) express *cry* genes, which are responsible for the production of the indicated *Bt* proteins. MON 89788 produces CP4 EPSPS (5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp.)

and MON 87708 produces DMO (dicamba monooxygenase, *Stenotrophomonas maltophilia* demethylase) that confer tolerance to herbicides. This *Bt* soybean presents protection against primary lepidopteran pests of soybean: *A. gemmatalis*, *C. includens,* and *H. armigera* [8].

The use of *Bt* soybean technologies enabled great advances for integrated pest management (IPM), which is currently the main control tactic used against major lepidopteran pests that attack soybean in South America [3,9]. However, the Cry1Ac soybean is not effective in controlling *Spodoptera* species, including *Spodoptera frugiperda* (J. E. Smith, 1797), *Spodoptera eridania* (Stoll, 1782), *Spodoptera albula* (Walker, 1857), and *Spodoptera cosmioides* (Walker, 1858). On the other hand, Cry1A.105/Cry2Ab2/Cry1Ac soybean causes intermediate mortality of *S. eridania* but low lethality of *S. frugiperda*, for which resistance to Cry proteins is already widespread in Brazil [5,10–13].

In recent years, infestations and damage from *Spodoptera* species increased in soybean fields in Brazil [5]. This occurrence can be explained by the low susceptibility of these insects to Cry1Ac protein and the reductions in insecticide applications against lepidopteran pests that attack soybean, due to the effectiveness of Cry1Ac soybean in controlling the most important lepidopteran insects [5,6]. In this agricultural scenario, it is important to quantify the survival of *Spodoptera* species on *Bt* soybean and characterize their susceptibility to insecticides in order to support IPM programs.

Knowledge regarding the susceptibility of insect pests to GM crops is particularly important in tropical agroecosystems, where multiple *Bt* crops are planted in adjacent mosaics or in succession, and allows more accurate inferences about insect–host interactions and population dynamics. For these reasons, we conducted a series of experiments to evaluate the toxicity of insecticides on *Spodoptera* species surviving on *Bt* soybean expressing Cry1A.105/Cry2Ab2/Cry1Ac proteins, single Cry1Ac protein, and non-*Bt* soybean. We evaluated the toxicity of two commercially available insecticides (flubendiamide and thiodicarb) applied on *Bt* and non-*Bt* soybean to control *S. albula*, *S. cosmioides*, *S. eridania*, and *S. frugiperda*.

2. Materials and Methods

2.1. Insects

Insects (*S. eridania*, *S. cosmioides*, *S. frugiperda,* and *S. albula*) were collected in the 2019 to 2022 soybean seasons (before first plantings of Cry1A.105/Cry2Ab2/Cry1Ac soybean) (Table 1). After collection, the larvae were transported to the laboratory and maintained on an artificial diet based on white beans, wheat germ, and yeast [14]. In laboratory bioassays, the *S. frugiperda* population exhibited 60% survival until the L3 stage on *Bt* maize expressing Cry1 and Cry2 *Bt* proteins, suggesting that insects from this population had some degree of resistance to *Bt* proteins.

Table 1. Pest species tested in bioassays with *Bt* and non-*Bt* soybean.

Species	Description
S. albula	Obtained from non-*Bt* soybean fields in February of 2019 in Sapezal, MT (13°29′29″ S; 58°26′52″ W)
S. cosmioides	Obtained from non-*Bt* soybean fields in October of 2020 in Rio Verde, GO (17°06′05″ S; 50°46′57″ W)
S. eridania	Obtained from non-*Bt* soybean fields in March of 2021 in Santa Maria, RS (29°43′20″ S; 53°33′34″ W)
S. frugiperda	Obtained from non-*Bt* soybean fields in October of 2021 in Cristalina, GO (16°29′59″ S; 47°36′36″ W)

2.2. Soybean

GM soybean MON 87701 × MON 89788 × MON 87751 × MON 87708 that expresses Cry1A.105, Cry2Ab2, and Cry1Ac insecticidal proteins (NEO590 I2X, GDM Genética do Brasil Ltd.a, Passo Fundo, RS, Brazil), GM soybean MON 87701 × MON 89788 expressing Cry1Ac protein (A5547 IPRO, Bayer Crop Science, São Paulo, SP, Brazil), and non-*Bt* soybean (NA 5909 RG, Nidera Sementes Ltd.a, São Paulo, SP, Brazil) were cultivated in 12-L plastic pots (6–8 plants per pot) containing soil and plant substrate (2:1) in a greenhouse. Before the bioassays, plants were tested for the presence/absence of the expected insecticidal protein(s) with detection kits (EnviroLogix, QuickStix) for Cry proteins.

2.3. Insecticides

The insecticides evaluated were flubendiamide (Belt 480 g active ingredient (a.i.)/L, Bayer S.A., São Paulo, SP, Brazil)—ryanodine receptor modulator (IRAC MoA group 28) and thiodicarb (Larvin 800 g a.i./L, Bayer S.A., São Paulo, SP, Brazil)—acetylcholinesterase inhibitor (IRAC MoA subgroup 1A).

2.4. Evaluating Survival and Development of Spodoptera Species on Bt Soybean

Trifoliate leaves of Cry1A.105/Cry2Ab2/Cry1Ac soybean, Cry1Ac soybean, and non-*Bt* soybean from earlier varieties were excised from plants at the $V_{5–8}$ growth stages [15]. In the laboratory, a single leaf was placed onto a filter paper on top of a gelled 2% agar–water mixture in 100 mL plastic cups. Afterwards, leaves were infested with 10 neonates (<24 h old) and maintained in a climatic chamber at 25 ± 2 °C, 60 ± 10% relative humidity, and 14:10 h light: dark photoperiod. Leaves were replaced every 2 d. The experimental design was completely randomized with 10 replicates of 10 neonates, totaling 100 neonates tested per species and plant type. Survivorship and developmental time until the L3 larval stage were evaluated. Survival and developmental time data were subjected to non-parametric analysis, and the mean differences (i.e., comparison of soybean plant types within insect species) were estimated by the least square means statement (LSMEANS option of PROC GLM) using a Tukey–Kramer test at $p < 0.05$ [16].

2.5. Susceptibility to Insecticides of Spodoptera Species Surviving on Bt Soybean in Diet Assays

Surviving L3 larvae of *Spodoptera* species on *Bt* and non-*Bt* soybean were exposed to insecticides in diet-overlay bioassays. In these bioassays, the artificial diet proposed by Greene et al. [16] was poured into 24-well acrylic plates (Costar, São Paulo, SP, Brazil) (1 mL per well). After plate preparation, insecticides were diluted in distilled water to prepare the range of concentrations to be tested. A surfactant, Triton X-100 (Sigma–Aldrich, São Paulo, SP, Brazil) at 0.1%, was added to obtain a uniform spread of the solution over the diet surface. The control treatment was composed of distilled water + surfactant. For each species, 5–7 concentrations per insecticide were applied to the diet surface with a replication pipette (30 µL per well). After a drying period, a single early-L3 larva (± 1 cm in length) that had survived on *Bt* or non-*Bt* soybean leaves was transferred to each well. Then, plates were sealed with a plate cover and placed in a climatic chamber at 25 ± 2 °C, 60 ± 10% relative humidity and 14:10 h light: dark photoperiod. Bioassays were performed twice per species, with each concentration being included twice per bioassay, for a total of four to sixteen replications of 24 larvae per species and concentration. Mortality was evaluated at 48 and 96 for thiodicarb and flubendiamide, respectively. To estimate the lethal concentrations (LC_{50}) and their respective 95% confidence intervals (CIs), the concentration–mortality data from the diet-overlay bioassays were subjected to probit analysis (PROC PROBIT) in SAS® 9.1. A likelihood ratio test was performed to check if the LC_{50} values do not differ. Then, significance was stated if the 95% (CIs) did not overlap in pairwise comparisons, as suggested by Robertson et al. [17].

2.6. Efficacy of Insecticides Applied on Bt Soybean against Spodoptera Species

The *Bt* and non-*Bt* soybean varieties listed above (Section 2.2) were cultivated under field conditions at a density of 10 plants/m within each row and spacing of 0.45 m between rows. At the $V_{4–5}$ growth stage, soybeans were sprayed with the recommended field dose of each insecticide (flubendiamide at 48 g a.i./ha and thiodicarb at 80 g a.i./ha), simulating a spray volume of 150 L/ha. Insecticides were applied with a pressurized-CO_2 backpack sprayer with a 2 m bar and 0.5 m nozzle spacing (XR 110.02 fan-type nozzle tips; TeeJet Technologies Co., Glendale Heights, IL, USA). Approximately 50 min. after spraying, trifoliate leaves from the upper-third part of the plants were removed and, in the laboratory, individually placed onto a filter paper on top of a 2% agar–water gelled mixture in 100 mL plastic cups. Unsprayed plants were used as a control treatment. Each leaf was infested with 5–10 L3 larvae, which were reared on leaves of the same soybean

plant type to which they were transferred after insecticide application. Then, the cups were placed in a climatic room at 25 ± 2°C, 60 ± 10% relative humidity and 14:10 h light: dark photoperiod. Leaves were replaced every 2 d. The experimental design was randomized with 4–8 replicates of 10 larvae per species, plant type, and insecticide. The mortality (control efficacy) was assessed at 10 d. The data on the mortality on *Bt* and non-*Bt*-sprayed leaves were corrected based on unsprayed non-*Bt* soybean according to Abbott's formula [18]. Corrected mortality data were subjected to three-way analysis of variance (ANOVA) using the PROC GLM procedure with *Spodoptera* species, soybean plant type, and insecticide as the three main factors. Means were compared as described above for bioassays with *Bt* and non-*Bt* soybean leaves (Section 2.4).

3. Results

3.1. Survivorship and Developmental Time

There was significantly lower survivorship of S. cosmoides, *S. eridania*, and *S. albula* until L3 stage on Cry1A.105/Cry2Ab2/Cry1Ac soybean (10.1–17.2%) than on Cry1Ac soybean and non-*Bt* soybean (77–93% survival), which did not differ from each other ($F = 113.27$; $df = 2, 27$; $p < 0.0001$ for *S. cosmioides*; $F = 201.36$; $df = 2, 27$; $p < 0.0001$ for *S.eridania*; $F = 210.44$; $df = 2, 27$; and $p < 0.0001$ for *S. albula*) (Table 2).

Table 2. Survivorship and developmental time of *Spodoptera* species on *Bt* and non-*Bt* soybean leaves in laboratory trials.

Species/Soybean Plant Type	% Survival until L3 Stage [a]	Time until L3 Stage (d) [a]
S. cosmioides		
Cry1A.105/Cry2Ab2/Cry1Ac soybean	10.1 ± 2.4 b	8.1 ± 0.2 a
Cry1Ac soybean	79.8 ± 5.2 a	6.7 ± 0.1 b
Non-*Bt* soybean	82.0 ± 2.5 a	6.2 ± 0.1 b
S. eridania		
Cry1A.105/Cry2Ab2/Cry1Ac soybean	17.2 ± 1.8 b	7.8 ± 0.1 a
Cry1Ac soybean	84.0 ± 4.2 a	6.3 ± 0.1 b
Non-*Bt* soybean	93.0 ± 2.1 a	5.7 ± 0.1 c
S. albula		
Cry1A.105/Cry2Ab2/Cry1Ac soybean	10.6 ± 2.2 b	8.4 ± 0.2 a
Cry1Ac soybean	77.0 ± 3.8 a	7.1 ± 0.1 b
Non-*Bt* soybean	86.0 ± 2.2 a	6.4 ± 0.1 c
S. frugiperda		
Cry1A.105/Cry2Ab2/Cry1Ac soybean	36.5 ± 3.4 b	7.7 ± 0.04 a
Cry1Ac soybean	85.0 ± 2.7 a	7.0 ± 0.03 b
Non-*Bt* soybean	91.0 ± 2.3 a	6.0 ± 0.04 c

[a] Mean ± SE followed by the same letter within a column for each species are not significantly different as determinded by the LSMEANS statement using Tukey–Kramer test at $p > 0.05$.

The developmental time until L3 stage was about 1–2 d longer when these three *Spodoptera* species fed on Cry1A.105/Cry2Ab2/Cry1Ac soybean than when they fed on other soybean plant types ($F = 65.85$; $df = 2, 26$; $p < 0.0001$ for *S. cosmioides*; $F = 69.46$; $df = 2, 26$; $p < 0.0001$ for *S. eridania*; $F = 53.68$; $df = 2, 27$; and $p < 0.0001$ for *S. albula*) (Table 2). Similarly, S. frugiperda also presented significantly lower survival until L3 stage on Cry1A.105/Cry2Ab2/Cry1Ac soybean (36.5%) than on Cry1Ac soybean and non-*Bt* soybean (85% and 91%, respectively) ($F = 108.30$; $df = 2, 27$; $p < 0.0001$) (Table 2), but its survival on Cry1A.105/Cry2Ab2/Cry1Ac soybean (36.5%) was higher than that of the other insect species. The developmental time of *S. frugiperda* varied significantly between soybean plant types, being greatest on Cry1A.105/Cry2Ab2/Cry1Ac soybean (7.7 d), followed by Cry1Ac soybean (7 d) and non-*Bt* soybean (6 d) ($F = 229.94$; $df = 2, 27$; $p < 0.0001$) (Table 2).

3.2. Susceptibility to Flubendiamide in Diet-Overlay Bioassays

There was low survivorship of *S. cosmioides*, *S. eridania*, and *S. albula* on Cry1A.105/Cry-2Ab2/Cry1Ac soybean leaves (Table 2), which did not allow us to estimate the dose

response to flubendiamide. However, the LC_{50} values of flubendiamide against L3 larvae of *S. cosmioides* (0.53–0.59 µg a.i./cm^2), *S. eridania* (0.54–0.96 µg a.i./cm^2), *S. albula* (0.20–0.29 µg a.i./cm^2 of diet), and *S. frugiperda* (0.74–0.85 µg a.i./cm^2 of diet) were similar for insects developing on Cry1Ac soybean and non-*Bt* soybean, as indicated by the overlap of 95% CIs (Table 3). This similarity in susceptibility to flubendiamide was also confirmed by equality (χ^2 = 0.41–13.38; *df* = 2; *p* = 0.132–0.816) and parallelism (χ^2 = 0.02–0.55; *df* = 1; *p* = 0.460–0.886) tests.

Table 3. Concentration–mortality response to flubendiamide by *Spodoptera* species developing on *Bt* and non-*Bt* soybean in laboratory trials.

Species/Soybean Plant Type	*n* [a]	Slope ± SE	LC_{50} (95% CI) [b]	χ^2 [c]	*df* [d]
S. cosmioides					
Cry1A.105/Cry2Ab2/Cry1Ac soybean	–	– [e]	– [e]	– [e]	– [e]
Cry1Ac soybean	384	1.60 ± 0.20	0.53 (0.36–0.72) a	7.71	5
Non-*Bt* soybean	384	1.72 ± 0.22	0.59 (0.43–077) a	1.07	5
S. eridania					
Cry1A.105/Cry2Ab2/Cry1Ac soybean	–	– [e]	– [e]	– [e]	– [e]
Cry1Ac soybean	384	2.12 ± 0.24	0.54 (0.37–0.79) a	4.44	5
Non-*Bt* soybean	384	2.19 ± 0.22	0.96 (0.78–1.16) a	0.51	5
S. albula					
Cry1A.105/Cry2Ab2/Cry1Ac soybean	–	– [e]	– [e]	– [e]	– [e]
Cry1Ac soybean	384	1.24 ± 0.16	0.20 (0.14–0.28) a	2.49	5
Non-*Bt* soybean	384	1.08 ± 0.14	0.29 (0.19–0.41) a	1.90	5
S. frugiperda					
Cry1A.105/Cry2Ab2/Cry1Ac soybean	96	1.51 ± 0.36	0.58 (0.16–1.20) a	3.27	5
Cry1Ac soybean	336	1.40 ± 1.17	0.74 (0.51–0.99) a	5.04	4
Non-*Bt* soybean	336	1.43 ± 0.18	0.85 (0.59–1.14) a	4.50	4

[a] Number of tested larvae; [b] LD_{50} (µg a.i./cm^2 of diet) and 95% confidence intervals. LD_{50} values (95% CI) within a column for each Spodoptera species followed by the same letter are not significantly different from each other as indicated by overlap of 95% CIs; [c] $p > 0.05$ in the goodness-of-fit test; [d] *df* = degrees of freedom; and [e] concentration–mortality response not estimated due to limited number of larvae that developed until L3 stage.

The LC_{50} value of flubendiamide was also similar for L3 larvae of *S. frugiperda* (0.58–0.85 µg a.i./cm^2 of diet) that developed on Cry1A.105/Cry2Ab2/Cry1Ac soybean, Cry1Ac soybean, and non-*Bt* soybean as indicated by the overlap of 95% CIs and equality (χ^2 = 0.41; *df* = 2; *p* = 0.816) and parallelism (χ^2 = 0.02; *df* = 1; *p* = 0.886) tests, which confirmed that mortality curves had similar slopes and intercepts (Table 3).

3.3. Susceptibility to Thiodicarb in Diet-Overlay Bioassays

Similar susceptibility to thiodicarb was detected in L3 larvae of *S. cosmioides* (0.47–0.51 µg a.i./cm^2), *S. eridania* (1.12–1.75 µg a.i./cm^2 of diet), and *S. albula* (1.06–1.35 µg a.i./cm^2 of diet) surviving on Cry1Ac soybean and non-*Bt* soybean as demonstrated by the overlap of 95% CIs and the parallelism test (χ^2 = 0.27–0.58; *df* = 2; *p* = 0.446–0.601) (Table 4), whereas mortality curves of *S. eridania* presented different intercepts according to the equality test (χ^2 = 9.27; *df* = 2; *p* = 0.009). Unlike those of the other species, the LC_{50} values of thiodicarb were significantly lower for L3 larvae of *S. frugiperda* developing on both Cry1A.105/Cry2Ab2/Cry1Ac and Cry1Ac soybean (0.65 and 0.79 µg a.i./cm^2 of diet, respectively) than on non-*Bt* soybean (1.38 µg a.i./cm^2 of diet) (Table 4). This result was also confirmed by the non-overlap of 95% CIs and the equality test (χ^2 = 25.18; *df* = 4; *p* < 0.0001), which showed that the concentration–mortality curves had distinct slopes and intercepts.

Table 4. Concentration–mortality response to thiodicarb by *Spodoptera* species developing on *Bt* and non-*Bt* soybean in laboratory bioassays.

Species/Soybean Plant Type	*n* [a]	Slope ± SE	LC_{50} (95% CI) [b]	χ^2 [c]	*df* [d]
S. cosmioides					
Cry1A.105/Cry2Ab2/Cry1Ac soybean	–	– [e]	– [e]	– [e]	– [e]
Cry1Ac soybean	336	1.52 ± 0.18	0.47 (0.31–0.66) a	6.94	4
Non-*Bt* soybean	384	1.64 ± 0.19	0.51 (0.37–0.66) a	0.73	5
S. eridania					
Cry1A.105/Cry2Ab2/Cry1Ac soybean	–	– [e]	– [e]	– [e]	– [e]
Cry1Ac soybean	336	2.33 ± 0.28	1.12 (0.90–1.42) a	1.02	4
Non-*Bt* soybean	504	2.44 ± 0.28	1.75 (1.38–2.04) a	2.30	5
S. albula					
Cry1A.105/Cry2Ab2/Cry1Ac soybean	–	– [e]	– [e]	– [e]	– [e]
Cry1Ac soybean	336	2.05 ± 0.24	1.06 (0.85–1.30) a	4.24	4
Non-*Bt* soybean	384	2.01 ± 0.22	1.35 (1.04–1.69) a	1.80	5
S. frugiperda					
Cry1A.105/Cry2Ab2/Cry1Ac soybean	144	1.88 ± 0.36	0.65 (0.35–1.00) b	1.14	5
Cry1Ac soybean	336	2.42 ± 0.35	0.79 (0.62–0.97) b	0.87	4
Non-*Bt* soybean	384	1.82 ± 0.23	1.38 (1.10–1.92) a	1.17	4

[a] Number tested; [b] LD_{50} (μg a.i./cm^2 of diet) and 95% confidence intervals. LD_{50} (95% CI) within a column for each *Spodoptera* species followed by the same letter are not significantly different from each other as indicated by overlap of 95% CIs; [c] $p > 0.05$ in the goodness-of-fit test; [d] *df* = degrees of freedom; and [e] concentration–mortality response not estimated due to limited number of larvae that developed until L3 stage.

3.4. Efficacy of Insecticides Applied on Bt Soybean against Spodoptera Species

There were no statistically significant interaction effects of *Spodoptera* species × soybean plant type × insecticide ($F = 1.02$; $df = 17, 88$; and $p = 0.3899$), *Spodoptera* species × soybean plant type ($F = 0.25$; $df = 3, 88$; and $p = 0.8632$) or soybean plant type × insecticide ($F = 1.29$; $df = 2, 88$; and $p = 0.2806$) on control efficacy. In contrast, significant effects on control efficacy were detected for *Spodoptera* species × insecticide ($F = 4.54$; $df = 3, 88$; and $p = 0.0053$), *Spodoptera* species ($F = 10.89$; $df = 3, 88$; and $p < 0.0001$), soybean plant type ($F = 3.34$; $df = 2, 88$; and $p = 0.0398$), and insecticide ($F = 12.67$; $df = 1, 88$; and $p = 0.0006$).

The field doses of flubendiamide and thiodicarb presented similar control efficacy (87.9–100.0%) against L3 larvae of *S. frugiperda* that survived on Cry1A.105/Cry2Ab2/Cry1Ac soybean (Figure 1A) and for L3 larvae of all *Spodoptera* species that survived on Cry1Ac soybean and non-*Bt* soybean (Figure 1B). On non-*Bt* soybean, both insecticides were also effective against all *Spodoptera* species, with >81% of control (Figure 1C). Due to the reduced number of *S. cosmioides*, *S. eridania*, and *S. albula* larvae that survived on Cry1A.105/Cry2Ab2/Cry1Ac soybean until L3 stage, it was not possible to estimate the control efficacy of insecticides for those insects.

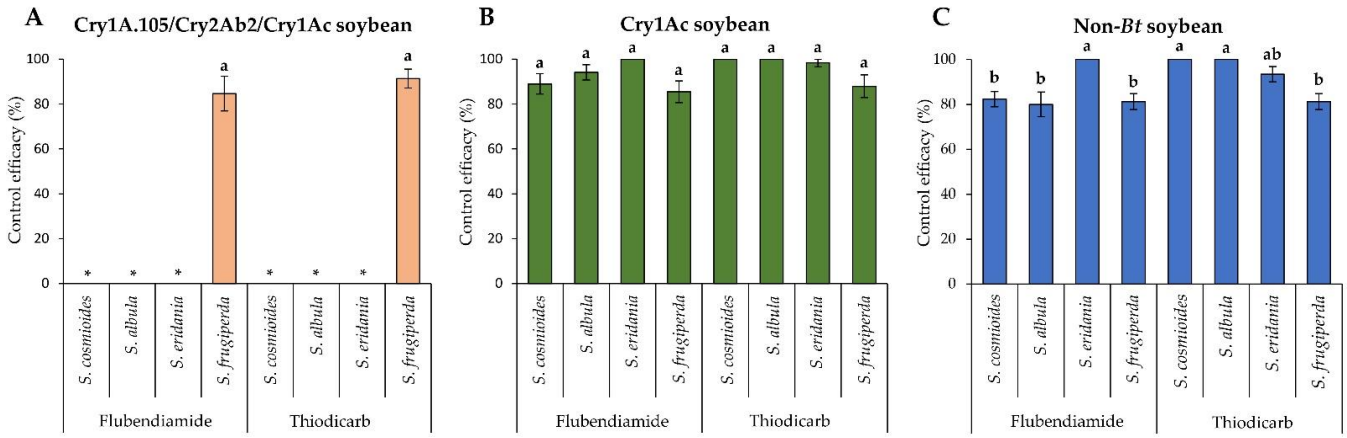

Figure 1. Control efficacy of flubendiamide and thiodicarb against *Spodoptera* species larvae that

developed to L3 stage on *Bt* and non-*Bt* soybean. Bars (±SE) with the same letter are not significantly different as determinded by the LSMEANS statement using Tukey–Kramer test at $p > 0.05$. * Not estimated due to limited number of larvae that developed until L3 stage.

4. Discussion

The GM soybean MON 87701 × MON 89788 × MON 87751 × MON 87708, which expressed Cry1A.105/Cry2Ab2/Cry1Ac insecticidal proteins, was effective at protecting soybean against *S. cosmioides* and *S. albula* (Table 2). This GM soybean also caused considerable mortality of *S. eridania* but had low lethality against *S. frugiperda*. In contrast, the GM soybean MON 87701 × MON 89788, expressing Cry1Ac protein, presented minimal effects against all *Spodoptera* species evaluated. Our findings also indicated that *Spodoptera* larvae that survived on either Cry1A.105/Cry2Ab2/Cry1Ac or Cry1Ac soybean generally had longer developmental time until L3 stage than those on non-*Bt* soybean (Table 2). Previous reports also state similar effects of Cry1A.105/Cry2Ab2/Cry1Ac against *Spodoptera* species, i.e., prolonged developmental time, reduced larval weight, and reduced population growth potential [10]. Our results are also in accordance with earlier studies that indicated low lethality of Cry1Ac protein expressed in soybean against *Spodoptera* species [5,10,11].

The reduced effectiveness of Cry1A.105/Cry2Ab2/Cry1Ac soybean in controlling *S. frugiperda* can be explained by the widespread resistance of this insect to Cry proteins expressed in multiple *Bt* plant species and the large degree of cross-resistance between Cry proteins expressed in *Bt* maize, *Bt* cotton, and *Bt* soybean in Brazil [19–21]. The low susceptibility of *Spodoptera* species to Cry1Ac protein can be attributed to the inactivation of *Bt* proteins by proteases and low specific binding sites for Cry proteins in its midgut [22,23]. Therefore, some survival of *S. eridania* and relatively higher survival of *S. frugiperda* is expected on Cry1A.105/Cry2Ab2/Cry1Ac soybean, while high-level survival of all *Spodoptera* species is expected on Cry1Ac soybean, requiring the use of other effective tactics for its control.

Spodoptera larvae surviving on *Bt* and non-*Bt* soybean showed comparable susceptibility to flubendiamide and thiodicarb in diet-overlay bioassays (Tables 3 and 4). Both insecticides also provided high control effectiveness when sprayed at the field dose over *Bt* and non-*Bt* soybean against surviving *Spodoptera* larvae (Figure 1). These findings indicate that insecticides applied over *Bt* or non-*Bt* soybean (from refuge or non-*Bt* fields) had similar effectiveness against *Spodoptera* of the same larval age or size. In contrast to our results, previous studies indicated that larvae of *S. frugiperda* surviving on Cry1Ac soybean showed more pronounced susceptibility to baculovirus-based biopesticide containing *S. frugiperda* multiple nucleopolyhedrovirus than those that developed on non-*Bt* soybean [24].

From a IPM perspective, monitoring larval and damage incidence of *S. frugiperda* and *S. eridania* on Cry1A.105/Cry2Ab2/Cry1Ac soybean and of all *Spodoptera* species on Cry1Ac soybean is important to decision making for the use of chemical control or other effective control tactics for insect management. Considering that *Spodoptera* species prolonged larval developmental time when fed *Bt* soybean, such larvae may be more exposed to biotic and abiotic mortality factors, including insecticides applied to control other soybean pests (e.g., defoliating beetles and sucking pests), which may increase mortality rates. Nevertheless, some degree of plant injury is tolerable without requiring pest control; therefore, if outbreaks of *Spodoptera* species are detected in *Bt* and non-*Bt* soybean fields, insecticide application is recommended only when action thresholds are reached (20 larvae > 1.5 cm in length per 1 m sample cloth, or 30% and 15% defoliation at the vegetative and reproductive stages, respectively) [25]. The current challenge is to preserve susceptibility of *Spodoptera* species (mainly *S. cosmioides* and *S. eridania*) to the *Bt* proteins expressed in *Bt* soybean technologies. For this, it is recommended the sowing/planting of a minimum 20% area of soybean refuge (non-*Bt*) within 800 m of the *Bt* soybean field [26].

5. Conclusions

According to the experimental results, the following conclusions were made: (i) the GM soybean MON 87701 × MON 89788 × MON 87751 × MON 87708, which expresses Cry1A.105/Cry2Ab2/Cry1Ac insecticidal proteins, was effective at protecting soybean against *S. cosmioides* and *S. albula*; (ii) this GM soybean had some lethality against *S. eridania*, but minimal control of *S. frugiperda*; (iii) the GM soybean MON 87701 × MON 89788, expressing Cry1Ac protein, showed poor control of all *Spodoptera* species evaluated; (iv) *Spodoptera* larvae that survived on *Bt* and non-*Bt* soybean had comparable susceptibility to flubendiamide and thiodicarb in diet-overlay bioassays; and (v) flubendiamide and thiodicarb were effective in controlling *Spodoptera* species that survived on *Bt* or non-*Bt* soybean. Our findings suggest that integrated control strategies are needed for controlling *Spodoptera* species in *Bt* soybean areas in South America.

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