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First insights of soybean stem fly (SSF) *Melanagromyza sojae* control in South America

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Abstract

The occurrence, distribution, and intensity of damages caused by *Melanagromyza sojae* on soybean fields in Brazil and Paraguay indicate the need of information regarding the effect of insecticides and mode of applications to its control. The aim of this study was to evaluate insecticide applications at soybean sowing and during the early soybean stages to control *M. sojae*. Two experiments were carried. In the 1st experiment, 12 insecticides were applied at sowing as a seed treatment (ST) or in the sowing furrow (SF) and combined with presence or absence of foliar spray (FS) of insecticides at 18 and 28 days after soybean emergence (DAE) in a factorial scheme. In the 2nd experiment, 17 insecticides were sprayed foliar at 10 and 22 DAE. Control efficiency of *M. sojae* was assessed on experiment 1 at 22, 28 and 38 DAE and on experiment 2 at 22 and 38 DAE, by counting the number of larvae, pupae, and damaged plants and length of galleries. The results evidenced the need of a specific management for this pest and recommended application of insecticides such as: Chlorantraniliprole (ST), Imidacloprid + Bifenthrin (ST), Fipronil (ST), Imidacloprid (ST) and Thiamethoxam (SF) at sowing, combined with the foliar spray until 10 DAE of Chlorpyrifos, Thiametoxam + Lambda-Cyhalothrin, Thiodicarb, Bifenthrin and Imidacloprid + Beta-cyfluthrin. The foliar spray should be repeated at least once in an interval shorter than 10 days, to protect soybean plants during the most vulnerable development stages to the attack of *M. sojae.*

Keywords: Soybean stem fly; invasive pests; pest management; *Glycine max*.

Abbreviations: DAE_days after emergence; EU_experimental unit; FS_foliar spray; SF_sowing furrow; SSF_soybean stem fly; ST_seed treatment.

Introduction

The soybean stem fly (SSF), *Melanagromyza sojae* (Diptera; Agromyzidae), is endemic to many regions in the old World (Dempewolf, 2004). In soybean fields, *M. sojae* was reported occurring in parts of Russia (Strakhova et al., 2013), Asia (e.g., China (Wang and Gai, 2001)); India and Nepal (Thapa, 2012), Southeast Asia (e.g., Indonesia (Van Den Berg et al., 1995)) and in the New World in Southern Brazil (Guedes et al., 2015; Arnemann et al., 2016a, b) and Paraguay (Guedes et al., 2017).

Upon emergence, the SSF larva mines through the mesophyll leaf tissue towards the vein. During development, the larva works downward into the stem, where it feeds on the pith (Van Den Berg et al., 1995) and can reduce soybean crop yield (Van Den Berg et al., 1998). The impact of SSF on crop yield varies from 2 up to 40 %, depending on the intensity and the phenological plant stage (Jadhav et al., 2013a; Van Den Berg et al., 1998). The first four weeks after the sowing is the most susceptible period to SSF damage in soybean. The higher yield increases are achieved with plant protection programs against SSF in this period (Talekar and Chen, 1985). Wang (1979) showed that the egg stage of SSF in soybean takes between 2 and 7 days, the 3 larval instars around 7.7 days, and the pupal stage between 6 and 12 days. The adult life-span is 19 days and females lay an average of 171 eggs. Ziaee (2012) described SSF having four or five generation per year and stem pupae over-winter in the stem.

Currently, the management of *M. sojae* with insecticides is the main method adopted by soybean growers in the Old World. Insecticides are used at sowing (e.g., granules, seed treatment and liquid or granular insecticide on sowing furrow) and on foliar spray, and the combination of both methods is also reported (Talekar and Chen, 1985; Abdullah et al., 2001; Kumar et al., 2009a; Adak 2012; Jadhav et al., 2013b). Organophosphates (monocrotophos, dimethoate and omethoate) showed satisfactory SSF control (Talekar and Chen, 1985), as well seed treatment with carbamates (carbosulfan) and the combination of neonicotinoids (thiametoxam and imidacloprid) with *Beauveria bassiana* + chlorpyriphos, sprayed at flowering stage in soybean (Kumar et al., 2009b). Abdullah et al. (2001) found low SSF control efficiency (30-40%) using pyrethroids (cypermethrin).

In South America, there are a large number of insecticides permitted for pest management in soybean. Paraguay has 514 commercial formulations, composed by 46 insecticides (SENAVE, 2018). In Brazil, there are 311 commercial formulations containing 50 insecticides (AGROFIT, 2018). However, none of them are recommended to control Diptera insects or SSF on soybean. Thus, the objective of this study was to evaluate different insecticides and mode of applications, such as: seed treatment, seed furrow, and foliar applications to control *M. sojae* on soybean. This is the first insights of insecticide effects on SSF in South America, since this pest was not detected until 2015.

Results and discussion

Our study shows the first insights on SSF control considering field tests of recommended insecticides commonly used in different mode of applications such as seed treatment, seed furrow, and foliar applications to control the major soybean pests. The SSF is one of the most important soybean pests. There is no information regarding field efficacy of insecticides at different mode of applications in South America, where the largest planted area of soybean in the world is located.

Experiment 1: Soybean stem fly management using soil application (seed treatment or in-furrow) of insecticides combined with aerial spray

At 22 DAE, only the plots that received exclusively insecticides applied on sowing were evaluated. The high percentage of damaged plants (60%) on the control treatment indicated a high infestation beginning on early stages of crop development. This scenario resembles the infestation levels of soybean fields at the late growing season in Paraguay and Brazil. These results show the high potential damage of the SSF, when it occurs in the early stages of soybean, enhancing the need for specific control at planting (Table 2).

The treatment Chlorantraniliprole (1.25 g kg^{-1} seed) achieved the highest control efficiency (95.8%). The systemic insecticides Fipronil (0.5 g kg^{-1} seed), Imidacloprid + Bifenthrin (0.66 + 0.54 g kg^{-1} seed), Imidacloprid (1.2 g kg^{-1} seed), and Thiamethoxam (125 g ha^{-1}) attained intermediary control efficiency, with 70.8%, 66.7%, 62.5%, and 54.2%, respectively. This is the first result that shows the effect of Chlorantraniliprole and Fipronil applied as ST against SSF, since no previous reports were found of its effect on SSF. Thiamethoxam and Imidacloprid have proven efficiency against SSF applied as ST (Kumar et al., 2009; Adak, 2012; Jadhav et al., 2013). Our results showed that Thiamethoxam applied on seed furrow has higher efficiency than Thiamethoxam applied on seed treatment. It happened because the a.i. rate of Thiamethoxam applied on seed furrow was higher than in seed treatment, 125 g of a.i. ha^{-1} and 52.5 g of a.i. ha^{-1}, respectively.

Given intensity of injury in plants of control plots, we inferred that the performance of these insecticides are satisfactory, and could be recommended to apply as ST or SF to protect soybean plants against SSF at early stages. This intense infestation coincides with the early plant development stages, which are the first five weeks when soybeans plants are more vulnerable to SSF feeding (Van Den Berg et al., 1985).

The data of tunnel length, which measures the intensity of injury was not affected by the insecticides applied at soybean sowing. Gyawali (2002) demonstrated direct correlation of gallery length with its negative impact on soybean yield. Thus, since the insecticide treatments could not reduce the larvae injury in plant stems, the variable "number of damage plants" is the most important to discriminate the efficiency of treatments on reducing SSF damage on soybean and also to recommend packages of control.

The lowest number of pupae was found in plants treated with Thiamethoxam (1.05 g kg^{-1} seed), Fipronil (0.5 g kg^{-1} seed), Chlorantraniliprole (1.25 g kg^{-1} seed), Cyantraniliprole (0.625 g kg^{-1} seed) and Imidacloprid + Bifenthrin (0.66 + 0.54 g kg^{-1} seed). The number of larvae in all treatments differed with lower averages from the Control Treatment. These results are in accordance with the low number of damaged plants on these treatments and reflect the effect of these insecticides and their capacity to control the SSF specimens and consequently, preventing damages on plants. These systemic insecticides translocate upward from the roots towards the leaves, distributing the active ingredient in all plant tissues, especially in early developmental stages of soybean plants (Thrash et al., 2014). Thus, our study shows that the insecticide existent on the sites of larvae feeding, which are, leaf, petiole, and stem tissues of soybean plants, may efficiently suppresses the SSF larval development. These methods of application allow the insecticide to be absorbed, translocated and distributed to the sites where the insect larvae are located and feed, being able to ingest the needed dose of insecticides a.i. to reach the lethal effect. For all variables, on 28 DAE, no interaction was detected between insecticides treatment at sowing (ST or SF) compared to foliar pulverization at 18 DAE. In practice, it means that aerial spray of insecticides at 18 DAE did not result in significant SSF control improvement, compared to the ST or SF only treatments. The number of damaged plants of all insecticide treatments were different from the control treatment, except for T(8) Carbosulfan (400 g ha^{-1}) + Flubendiamide (10 g ha^{-1}) and T (10) Cadusaphos (800 g ha^{-1}) + Bifenthrin e Carbosulfan (30 + 90 g ha^{-1}). The best control efficiency was achieved using T(3) Fipronil (0.5 g kg^{-1} seed) + Clorfenapyr (240 g ha^{-1}) 52% and T(6) Cyantraniliprole (0.625 g kg^{-1} seed) + Chlorantraniliprole and Lambda-Cyhalothrin (7.5 + 3.25 g ha^{-1}) and T(12) Thiamethoxam (125 g i.a ha^{-1}) + Chlorantraniliprole and Lambda-Cyhalothrin (7.5 + 3.25 g ha^{1}) with 48.2% of control efficiency (Table 3).

The overall control efficiency decreased in relation to the previous evaluation (22 DAE). This result can be attributed first to the low efficiency of foliar spray to control SSF, attested by the absence of interaction between plots with and without foliar spray. It is known that the insecticide concentration in the plants using seed treatment (ST) or sowing furrow (SF) follows a reducing concentration gradient. This reduction is usually due to the plant metabolization and degradation of pesticide active ingredients that are triggered by climatic conditions (Stamm et al., 2015). In our study, the best control efficiency reached by Fipronil, Cyantraniliprole, and Thiamethoxan (SF) which can be attributed to a longer and efficient residual of ST and SF.

Generally, the SSF egg, larvae, and pupae phases occur inside the plant and are somehow protected against the effect of pesticides sprayed on aerial part of soybean plants. The larvae located inside the petiole and stem are not efficiently controlled by FS since the insecticides do not translocate long distances if absorbed at the leaves. Also, the specimens (eggs, larvae, and pupae) inside the leaves, petiole or stem are protected against the contact and ingestion of contaminated tissues with insecticides. This is confirmed due to the absence of dead larvae and pupae found during the evaluations.

Adult specimens are the most vulnerable to the effect of insecticides since they are active at exterior side of plant. Therefore, they are vulnerable to the contact and expose of

Table 1. Insecticide treatments applied as seed treatment to control *Melanagromyza sojae* in soybean. San Cristobal, Alto Paraná, Paraguay.

Treatments	Commercial Brand	Manufacturer	Rate (g a.i. ha^{-1})
1. Imidacloprid[st]	Punto 600 FS	Agrotec S.A.	48
2. Thiamethoxam[st]	Cruiser	Syngenta	42
3. Fipronil[st]	Standak Top	BASF	20
4. Chlorantraniliprole[st]	Dermacor	DuPont	48
5. Imidacloprid + Thiodicarb[st]	CropStar	Bayer	42 + 126
6. Cyantraniliprole[st]	Fortenza	Syngenta	25
7. Imidacloprid + Bifenthrin[st]	Rocks	FMC	21.6+ 26.4
8. Carbosulfan[lsf]	Marshal	FMC	400
9. Chlorpyrifos[lsf]	Lorsban	Dow	1200
10. Cadusafos[lsf]	Rugby 200 CS	FMC	800
11. Cadusafos[gsf]	Rugby 100 GR	FMC	800
12. Thiamethoxam[lsf]	Trigger	Glymax	125
13. Control treatment	-	-	-

[st] Insecticides applied on sowing as seed treatment. [lsf] Insecticides applied on sowing as liquid in sowing furrow. [gsf] Insecticides applied on sowing as granules in sowing furrow

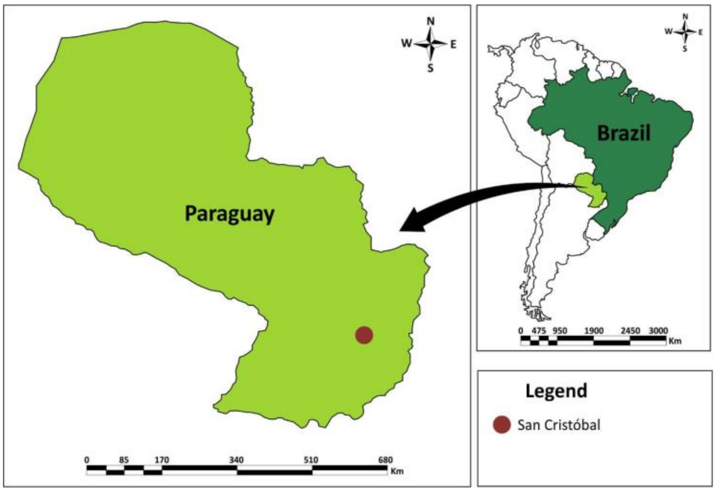

Fig 1. Experimental site location. San Cristobal, Alto Paraná, Paraguay. 2015/16 Crop season.

Table 2. Foliar spray treatments at 10 and 22 DAE to control *Melanagromyza sojae* in soybean. San Cristobal, Alto Paraná, Paraguay.

Treatments	Commercial Brand	Manufacturer	Rate(g a.i. ha^{-1})
1. Chlorantraniliprole	Premio	DuPont	10
2. Flubendiamide	Belt	Bayer	33,6
3. Chlorantraniliprole + Lambda-Cyhalothrin	Ampligo	Syngenta	7.5 + 3.75
4. Thiodicarb	Taura	Matrisoja S.A.	56
5. Methomyl	Nocaute	Tecnomyl	215
6. Chlorpyrifos	Lorsban	Dow	480
7. Acephate	Eficiente	Agrotec S.A.	750
8. Chlorfenapyr	Pirate	BASF	240
9. Indoxacarb	Avatar	DuPont	400
10. Emamectin Benzoate	Noctur	Tecnomyl	10
11. Bifenthrin	Talstar	FMC	15
12. Thiamethoxam	Trigger	Glymax	52
13. Imidacloprid	Hurano	Matrisoja	175
14. Thiamethoxam + Lambda-Cyhalothrin	Engeo Pleno	Syngenta	32.25 + 26.5
15. Imidacloprid + Beta-cyfluthrin	Connect	Bayer	100 + 12.5
16. Carbosulfan + Bifenthrin	Talisman	FMC	90 + 30
17. Control treatment	-	-	-

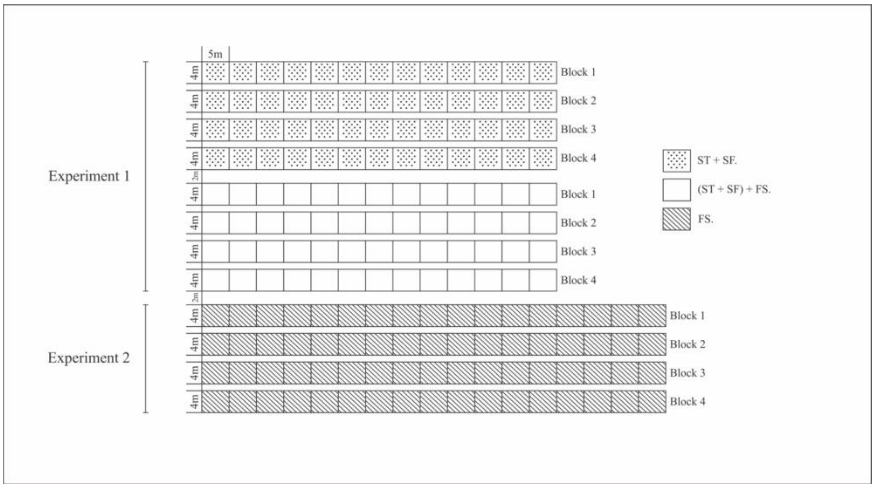

Fig 2. Experimental design of experiment 1 and 2. San Cristobal, Alto Paraná, Paraguay. 2015/16 Crop season.

Table 3. Number of damaged plants, control efficiency, length of gallery, pupae and larvae number at 22 DAE. San Cristobal, Alto Paraná, Paraguay. 2015/16 Crop season.

Treatments	Rate (g a.i. ha^{-1})	Damaged Plants[1]	Control Efficiency (%)	Length of Gallery[2] (cm)	Pupae number[1]	Larvae Number[1]
1. Imidaclopridst	48	2.25b	62.50	5.33a	0.75a	0.25b
2. Thiamethoxamst	42	3.25a	45.83	4.23a	0.50b	1.00b
3. Fipronilst	20	2.00b	66.67	4.70a	0.25b	0.75b
4. Chlorantraniliprolest	48	0.25c	95.83	1.25a	0.00b	0.00b
5. Imidacloprid + Thiodicarbst	42 + 126	3.75a	37.50	6.97a	1.00a	0.75b
6. Cyantraniliprolest	25	3.50a	41.67	5.69a	0.50b	0.75b
7. Imidacloprid + Bifenthrinst	21.6+ 26.4	1.75b	70.83	6.64a	0.00b	0.50b
8. Carbosulfan^{lsf}	400	3.75a	37.50	8.61a	1.25a	0.25b
9. Chlorpyrifos^{lsf}	1200	4.00a	33.33	5.57a	1.25a	1.00b
10. Cadusafos^{lsf}	800	3.50a	41.67	6.59a	1.25a	0.50b
11. Cadusafos^{gsf}	800	4.00a	33.33	7.03a	2.00a	0.75b
12. Thiamethoxam^{lsf}	125	2.75b	54.17	7.04a	1.25a	0.50b
13. Control treatment	-	6.00a	-	7.29a	2.25a	2.50a
CV(%)[3]		24.10	-	26.60	34.16	37.14

[1] Average number on every 10 evaluated plants. Means followed by the same letter do not differ significantly by Skott Knott test at the 5% probability. [2] Average length of the damaged plants. [3] Coefficient of Variation and the data transformed to square root of x + 0.5. st Insecticides applied on sowing as seed treatment. ^{lsf} Insecticides applied on sowing as liquid in sowing furrow. ^{gsf} Insecticides applied on sowing as granules in sowing furrow

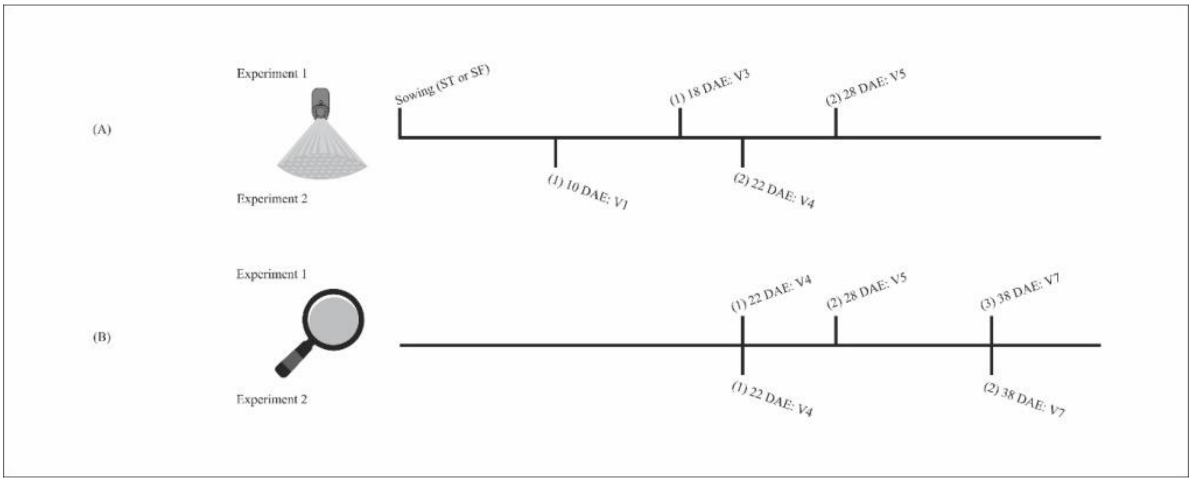

Fig 3. Description of the insecticide application and evaluation timing to the control of *Melanagromyza sojae* at different soybeans phenological stages. San Cristobal, Alto Paraná, Paraguay. 2015/16 Crop season.

Table 4. Number of damaged plants (DP), control efficiency (E), gallery length (GL), pupae and larvae number at 28 and 38 DAE. San Cristobal, Alto Paraná, Paraguay. 2015/16 Crop season.

Treatments	Rate	28 DAE					38 DAE				
	(g a.i. ha^{-1})	AP[1]	E[2]	GL[3]	Pupae	Larvae	AP	E	GL	Pupae	Larvae
1. Imidacloprid[st]	48	4.62b	28.92	7.88a	0.87a	1.00a	9.25a	7.50	15.02a	0.59a	2.00a
2. Thiamethoxam[st]	42	4.87b	25.08	8.48a	1.37a	0.92a	9.00a	10.00	14.84a	0.25a	0.50a
3. Fipronil[st]	20	3.12b	52.00	7.85a	0.65a	0.10a	9.75a	2.50	15.46a	1.32a	0.50a
4. Chlorantraniliprole[st]	48	4.12b	36.62	7.57a	0.62a	0.12a	9.75a	2.50	15.61a	0.76a	2.00a
5. Imidacloprid + Thiodicarb[st]	42 + 126	4.87b	25.08	8.08a	1.12a	0.87a	9.50a	5.00	15.76a	0.75a	3.50a
6. Cyantraniliprole[st]	25	3.37b	48.15	8.65a	1.00a	0.50a	8.75a	12.50	14.13a	1.45a	1.00a
7. Imidacloprid + Bifenthrin[st]	21.6+ 26.4	4.12b	36.62	9.24a	0.87a	0.65a	9.25a	7.50	13.69a	1.70a	1.00a
8. Carbosulfan[lsf]	400	6.62a	0.00	8.92a	1.53a	1.03a	9.50a	5.00	15.65a	0.36a	2.00a
9. Chlorpyrifos[lsf]	1200	4.25b	34.62	7.63a	1.37a	0.37a	9.50a	5.00	11.86a	1.06a	1.75a
10. Cadusafos[lsf]	800	4.25b	34.62	8.82a	0.50a	0.50a	9.75a	2.50	17.38a	1.84a	3.00a
11. Cadusafos[gsf]	800	5.87a	9.69	7.53a	0.87a	0.62a	10.00a	0.00	14.45a	0.34a	2.83a
12. Thiamethoxam[lsf]	125	3.37b	48.15	7.36a	0.12a	0.25a	10.00a	0.00	14.96a	0.94a	1.62a
13. Control treatment	-	6.50*	-	8.71	3.00*	0.50	10.00	-	18.81*	1.00	3.02
CV(%)[4]		18.66	-	26,20	29.36	24.47	4.24	-	10.17	32.06	25.61

[1]Average number on every 10 evaluated plants. Means followed by the same letter do not differ significantly by Skott Knott test at the 5% probability. [2]Control efficiency. [3]Average of gallery length of the damaged plants. [4]Coefficient of Variation and the data transformed to square root of x + 0.5. [st] Insecticides applied on sowing as seed treatment. [lsf] Insecticides applied on sowing as liquid in sowing furrow. [gsf] Insecticides applied on sowing as granules in sowing furrow. * The general insecticides averages differed from control treatment

Table 5. Number of damaged plants (DP), control efficiency (E), gallery length (GL), pupae and larvae number at 22 and 38 DAE. San Cristobal, Alto Paraná, Paraguay.

Treatments	Rate	22 DAE					38 DAE				
	(g a.i. ha^{-1})	AP[1]	E[2]	GL[3]	Pupae	Larvae	AP	E	GL	Pupae	Larvae
1. Chlorantraniliprole	10	4.75a	20.83	6.70a	2.00a	1.50a	8.25a	2.94	15.23a	1.75b	3.00a
2. Flubendiamide	33.6	5.00a	16.67	6.96a	1.25a	1.75a	9.25a	0.00	15.05a	4.00a	0.75b
3. Chlorantraniliprole + Lambda-Cyhalothrin	7.5 + 3.75	3.75a	37.50	8.68a	1.75a	1.25a	9.50a	0.00	13.06b	3.25a	3.25a
4. Thiodicarb	56	2.50b	58.33	7.43a	0.75a	0.50b	9.50a	0.00	15.89a	1.25b	2.00b
5. Methomyl	215	4.50a	25.00	6.54a	2.25a	1.50a	10.0a	0.00	17.71a	4.50a	3.75a
6. Chlorpyrifos	480	2.25b	62.50	7.20a	1.00a	0.50b	5.00c	41.18	11.66b	1.00b	0.25b
7. Acephate	750	6.25a	0.00	7.15a	1.50a	4.00a	9.25a	0.00	15.90a	2.50a	1.00b
8. Chlorfenapyr	240	3.25b	45.83	6.61a	0.75a	1.25a	9.75a	0.00	14.06a	2.75a	4.00a
9. Indoxacarb	400	4.75a	20.83	7.88a	0.50a	2.00a	9.75a	0.00	12.99b	2.50a	1.25b
10. Emamectin Benzoate	5	4.25a	29.17	5.67a	2.00a	1.00b	9.50a	0.00	11.28b	3.75a	0.75b
11. Bifenthrin	15	2.75b	54.17	6.07a	1.00a	0.50b	9.75a	0.00	15.08a	3.75a	2.75a
12. Thiamethoxam	52	3.25b	45.83	6.84a	0.50a	1.00b	6.75b	20.12	11.04b	0.75b	0.50b
13. Imidacloprid	175	3.50b	41.67	5.16a	0.75a	0.75b	7.75b	8.82	12.18b	1.00b	1.00b
14. Thiamethoxam + Lambda-Cyhalothrin	32.25 + 26.5	2.25b	62.50	5.50a	0.75a	0.25b	7.75b	8.82	10.70b	2.50a	1.50b
15. Imidacloprid + Beta-cyfluthrin	100 + 12.5	2.75b	54.17	7.26a	0.75a	0.50b	8.75a	0.00	15.02a	2.75a	2.45a
16. Carbosulfan + Bifenthrin	90 + 30	4.00a	33.33	7.11a	1.00a	1.75a	8.50a	0.00	14.36a	1.50b	1.25b
17. Control treatment	-	6.00a	-	7.29a	2.25a	2.50a	8.50a	-	13.36a	2.25a	2.50a
CV(%)[4]	-	18.78	-	14.95	30.29	35.19	8.02	-	9.02	31.05	32.37

[1]Average number on every 10 evaluated plants. Means followed by the same letter do not differ significantly by Skott Knott test at the 5% probability. [2]Control efficiency. [3]Average of gallery length of the damaged plants. [4]Coefficient of Variation and the data transformed to square root of x + 0.5.

insecticides sprayed to the surface of plants. Moreover, the SSF adults uses the ovipositor to produce punctures at the upper side of the soybean leaf blade, feeding themselves by licking the foliar cell's exudate which serves as food (Spencer, 1973). This feeding habit exposes the SSF adult to insecticide drops and its deposits on the surface of soybean leafs. Nevertheless, the quantification of the insecticide effects on SSF adults has not been done yet, given limitations imposed by the absence of tools to monitor the adult fluctuation, such as traps, pheromones and sampling methods, available for other insect species.

At 38 DAE, there was no interaction between insecticides applied at ST or SF only and with foliar application of insecticides. On control treatment, all plants were injuried by SSF . This significant raise on the mean number of injured plants indicated high infestation of SSF. In all insecticide treatments, injured plants number were superior to nine plants out of ten, except on treatment T(6), Cyantraniliprole (0.625 g kg^{-1} of seed) applied as ST and two foliar applications of Chlorantraniliprole + Lambda-Cyhalothrin (7.5 + 3.25 g ha^{-1}) and Thiamethoxam + Lambda-Cyhalothrin (100 + 12.5 g ha^{-1}), with 8.75 injured plants.

Table 6. Number of damaged plants (DP), control efficiency (E), gallery length (GL), pupae and larvae number at 28 and 38 DAE. San Cristobal, Alto Paraná, Paraguay.

Treatments	Rate	28 DAE					38 DAE				
	(g a.i. ha^{-1})	AP^{1}	E^{2}	GL^{3}	Pupae	Larvae	AP	E	GL	Pupae	Larvae
1. Imidaclopridst	48	4.62b	28.92	7.88a	0.87a	1.00a	9.25a	7.50	15.02a	0.59a	2.00a
2. Thiamethoxamst	42	4.87b	25.08	8.48a	1.37a	0.92a	9.00a	10.00	14.84a	0.25a	0.50a
3. Fipronilst	20	3.12b	52.00	7.85a	0.65a	0.10a	9.75a	2.50	15.46a	1.32a	0.50a
4. Chlorantraniliprolest	48	4.12b	36.62	7.57a	0.62a	0.12a	9.75a	2.50	15.61a	0.76a	2.00a
5. Imidacloprid + Thiodicarbst	42 + 126	4.87b	25.08	8.08a	1.12a	0.87a	9.50a	5.00	15.76a	0.75a	3.50a
6. Cyantraniliprolest	25	3.37b	48.15	8.65a	1.00a	0.50a	8.75a	12.50	14.13a	1.45a	1.00a
7. Imidacloprid + Bifenthrinst	21.6+ 26.4	4.12b	36.62	9.24a	0.87a	0.65a	9.25a	7.50	13.69a	1.70a	1.00a
8. Carbosulfan^{lsf}	400	6.62a	0.00	8.92a	1.53a	1.03a	9.50a	5.00	15.65a	0.36a	2.00a
9. Chlorpyrifos^{lsf}	1200	4.25b	34.62	7.63a	1.37a	0.37a	9.50a	5.00	11.86a	1.06a	1.75a
10. Cadusafos^{lsf}	800	4.25b	34.62	8.82a	0.50a	0.50a	9.75a	2.50	17.38a	1.84a	3.00a
11. Cadusafos^{gsf}	800	5.87a	9.69	7.53a	0.87a	0.62a	10.00a	0.00	14.45a	0.34a	2.83a
12. Thiamethoxam^{lsf}	125	3.37b	48.15	7.36a	0.12a	0.25a	10.00a	0.00	14.96a	0.94a	1.62a
13. Control treatment	-	6.50*	-	8.71	3.00*	0.50	10.00	-	18.81*	1.00	3.02
CV(%)^{4}		18.66	-	26,20	29.36	24.47	4.24	-	10.17	32.06	25.61

^{1}Average number on every 10 evaluated plants. Means followed by the same letter do not differ significantly by Skott Knott test at the 5% probability. ^{2}Control efficiency. ^{3}Average of gallery length of the damaged plants. ^{4}Coefficient of Variation and the data transformed to square root of x + 0.5. st Insecticides applied on sowing as seed treatment. ^{lsf} Insecticides applied on sowing as liquid in sowing furrow. ^{gsf} Insecticides applied on sowing as granules in sowing furrow.

Thereafter, the control efficiency was low, 12.5% for T(6) and for all other treatments below 5%. Gallery length did not show any difference among treatments, but the mean of gallery length in insecticide treatments were lower and different from control, demonstrating that the use of insecticides reduced the amount of damage to a variable that is directly related to yield reduction (Gyawali, 2002).

Experiment 2: Soybean stem fly management using aerial spray insecticides.

At 22 DAE, the higher SSF control efficiency was achieved by Chlorpyrifos (480 g ha^{-1}) and Thiamethoxam + Lambda-Cyhalothrin (32.25 + 26.5 g ha^{-1}), reaching 62.5% (Table 4). Similarly, Thiodicarb (56 g ha^{-1}) showed 58.3% and both Bifenthrin (15 g ha^{-1}) and Imidacloprid + Beta-cyfluthrin (100 + 12.5 g ha^{-1}) displayed 54.2% of SSF control. Similar results were found by Kumar et al. (2009b) with the insecticides Chlorpyrifos (76%), Thiamethoxam (55%), and Imidacloprid (44%).

Our results also showed that the gallery length was not affected by any insecticide applied in aerial mode. No difference was found on pupae number. Considering the SSF life table: egg–larvae–pupae averaged about 11-15 days (Wang, 1979), the absence of significative result is given the interval of ten days between the pulverization and the evaluation. Therefore, this interval was not sufficient to affect treatments. Furthermore, it evidenced the fact that foliar pulverization of insecticides has no effect on larvae, which already established at the time of insecticide spray. However, significantly lower larvae number of SSF in soybeans stems were found applying Thiodicarb (56 g ha^{-1}), Chlorpyrifos (480 g ha^{-1}), Emamectin Benzoate (5 g ha^{-1}), Bifenthrin (15 g ha^{-1}), Thiamethoxam (52 g ha^{-1}), Imidacloprid (175 g ha^{-1}), Thiamethoxam + Lambda-Cyhalothrin (32.25 + 26.5 g ha^{-1}) and Imidacloprid + Beta-cyfluthrin (100 + 12.5 g ha^{-1}) than the other insecticides. These results indicate that these insecticides prevented the establishment of SSF larvae on soybean stems. At the end of evaluations (38 DAE), the higher SSF control efficiency was 41.2% for Chlorpyrifos (480 g ha^{-1}) and 20.1% for Thiamethoxam (52 g ha^{-1}). The other insecticides showed SSF control efficiency less than 10%. The general average of of injured plants raised substantially, more than two times compared to the prior evaluation (1st experiment). The lower average was found in Chlorpyrifos (480 g ha^{-1}) with five injured plants, which differed from all other treatments. Thiamethoxam (52 g ha^{-1}), Imidacloprid (175 g ha^{-1}), and Thiamethoxam + Lambda-Cyhalothrin (32.25 + 26.5 g ha^{-1}) displayed average of 6.75, 7.75, and 7.75 injured plants, respectively, differing from the other treatments.

The averages of gallery length differed among treatments, with lowest means for Thiamethoxam + Lambda-Cyhalothrin (32.25 + 26.5 g ha^{-1}), Thiamethoxam (52 g ha^{-1}), Emamectin Benzoate (5 g ha^{-1}), Chlorpyrifos (480 g ha^{-1}), Imidacloprid (175 g ha^{-1}), Indoxacarb (400 g ha^{-1}), and Chlorantraniliprole + Lambda-Cyhalothrin (7.5 + 3.75 g ha^{-1}), ranging between 10.07 cm and 13.06 cm, respectively, from the lowest to the highest treatments. The difference between insecticide treatments is a result of the effect of insecticides on the delay in re-infestation of SSF.

The average number of pupae also showed difference among treatments, where the lowest means were on Thiamethoxam (52 g ha^{-1}), Chlorpyrifos (480 g ha^{-1}), Imidacloprid (175 g ha^{-1}), Thiodicarb (56 g ha^{-1}), Carbosulfan + Bifenthrin (90 + 30 g ha^{-1}), and Chlorantraniliprole (10 g ha^{-1}) with 0.75, 1.00, 1.00, 1.25, 1.50, and 1.75, respectively. The number of larvae found on Chlorpyrifos (480 g ha^{-1}), Thiamethoxam (52 g ha^{-1}), and Flubendiamide (33.6 g ha^{-1}) was the lowest, ranging with 0.25, 0.50, and 0.75, respectively.

Our results clearly show the need for a specific management to this pest, with properly timed insecticide application (in this case early in the season) of insecticides with effective control upon *M. sojae*. Van Den Berg et al. (1998) demonstrated that foliar spray of insecticide aiming at control of other insect pest population had no effect on SSF population. Combining the results of our two experiments, we suggest that the management of SSF on soybean can be done using the most efficient insecticides applied at sowing (Chlorantraniliprole, Thiamethoxam, Fipronil, Cyantraniliprole and Imidacloprid + Bifenthrin) with the most efficacious foliar sprayed insecticides (Chlorpyrifos, Thiamethoxam + Lambda-Cyhalothrin, Thiodicarb, Imidacloprid + Beta-cyfluthrin, Bifenthrin and Chlorfenapyr). In this case, the first spray should be done no later than 10 DAE and repeated at least once in an interval shorter than 10 days. These management practices are reasonable because it should provide a higher control efficiency and protection of plants on early soybean stages, when plants are more vulnerable to the SSF (Van Den Berg, 1985).

Materials and methods

Characterization of experimental area

Two experiments were performed during the 2015/16 growing season to evaluate the insecticides control efficiency on SSF on soybean plants, in San Cristobal, Alto Paraná, Paraguay, (25° 59' 56,02''S, 55° 45' 54,97''O and 336 m of altitude) (Figure 1). At the site of the experiment, the soil is classified as loamy Typic Paleustalf (USDA, 1999). Ferralsols according to WRB (2006) classification. According to Köppen classification, the predominant climate is Cfa, subtropical, humid, mesothermic, with annual precipitations between 1.600 and 1.700 mm without a defined dry season (Bade et al., 2014). The average temperature in the warmer months is higher than 22°C and in the cold months lower than 18°C.

Plant materials

The sowing was performed at 07/12/2015 and the plant emergence recorded on 13/12/2015. The soybean cultivar was TMG 7262 RR, with 15 seeds/m, the row spacing of 40cm and the fertilization rate 300 kg ha-1 of N - P_2O_5 - K_2O in the formulation 02-25-25 as base fertilization. The crop was raised following all the recommended practices for soybean crop (Rosa and Oliveira, 2014).

Experiment 1: SSF management using soil application (seed treatment or in-furrow) insecticides combined with a foliar spray

The adopted experimental arrangement was randomized blocks, with four replications, in factorial scheme (12x2), including 12 insecticides, applied in sowing only and combined with insecticide foliar spray. The experimental unity (EU) was constituted of eight soybean rows per five meters length, spaced 40 centimeters. Two lines were used as borders, and 0.5 meters of each experimental unity extremity were discarded to minimize border effects. The EU had useful area of 20 square meters (Figure 2). Insecticides were first applied in sowing as seed treatment (ST), in which the insecticides were applied to the seeds inside a plastic bag and tumbled until a consistent uniform seed coating was achieved, or directly in the sowing furrow (SF), using a CO_2 pressurized backpack sprayer and a flow rate of 50 L ha^{-1}. The insecticide foliar spray was performed at 18 and 28 days after soybean emergence (DAE; Figure 3) using a CO_2 pressurized backpack sprayer and a flow rate of 150 L ha^{-1}.

Experiment 2: SSF management using aerial spray insecticides

The experiment was carried out in a completely randomized block design with 17 treatments and four replications (Table 3). The EU size was identical to experiment one. Insecticides were sprayed at 10 and 22 DAE using a CO_2 pressurized backpack sprayer and a flow rate of 150 L ha^{-1}.

Evaluations

In the experiment one, evaluations were conducted at 22 DAE, only in the experimental units (EU) with insecticides applied in sowing, and at 28 and 38 DAE in all EUs. In the experiment two, evaluations were performed at 22 and 38 DAE. In each evaluation, 10 plants were collected from the center of the experimental units (EU). The plant evaluation was taken in three steps: (1) separation of plant shoot from the roots by cutting off the roots with a pruning shear in the plant base, (2) the plant stem was split apart lengthwise, (3) the plant stem was visually checked. In the third step, the plant was checked and the number of larvae and pupae per plant, the length of gallery (measured from the begging until the end of the tunneling) and the number of damaged plants (accounted by any presence of SSF gallery in the stem) were recorded.

Statistical analysis

All data was analyzed using ASSISTAT 7.7 (2016) and the means were grouped using the Scott-Knott test ($P \leq 0.05$). Control efficiency (E) for the treatments was calculated according to the equation of Abbott (1925) using the number of larvae on each treatment.

Conclusion

Our objectives were supported with efficient alternatives of insecticides to be applied at sowing and foliar spray to control *M. sojae* at early developmental stages of soybean. Chlorantraniliprole (ST) showed the best control efficiency of *M. sojae,* followed by Imidacloprid + Bifenthrin (ST), Fipronil (ST), Imidacloprid (ST) and Thiamethoxam (SF). Chlorpyrifos (FS) had the overall best control efficiency, followed by Thiamethoxam + Lambda-Cyhalothrin (FS), Thiodicarb (FS), Bifenthrin (FS) and Imidacloprid + Beta-cyfluthrin (FS). Furthermore, for the management of SSF in soybean we recommend the use of insecticides at sowing and combined with foliar spray until 10 DAE and repeated once in an interval shorter than 10 days, to protect soybeans plants during the most vulnerable development stages to the attack of *M. sojae*.

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