Journal of Agricultural Science; Vol. 15, No. 12; 2023
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Damage Assessment of *Melanagromyza sojae* (Diptera: Agromyzidae) on Soybean in Brazil

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Received: February 7, 2023 Accepted: September 10, 2023 Online Published: November 15, 2023

doi:10.5539/jas.v15n12p33 URL: https://doi.org/10.5539/jas.v15n12p33

Abstract

Soybean stem fly, *Melanagromyza sojae* Zehntner (Diptera: Agromyzidae), is an important soybean (*Glycine max*) pest in Eastern Asia that has recently colonized South America. The region colonized by *M. sojae* includes Brazil and several other major soybean growing countries. Management strategies for this pest remain largely undeveloped due to lack of information regarding its potential to injury soybeans. The objective of this study was to quantify soybean yield reduction caused by *M. sojae* injury. One experiment was carried out during two summer crop seasons (2020 and 2021) at Santa Maria, RS state, Brazil. Soybean was planted during late-season to ensure that high pressure of *M. sojae* adults were present in the fields. The number of seeds, 1,000-seed weight, seed yield and number of pods were quantified for the lower, middle and upper canopy, and plant height was compared to the amount of stem injured to determine percentage of injured stem. Each 1% of injured stem in the lower, middle and upper canopy segments significantly reduced the number of seeds per plant, 1,000-seed weight, and yield. Across all canopy segments, yield reduction reached 0.9 g per plant for every 1% of injured stem. Treatments where insecticide applications started during the vegetative phase presented the lowest damage by *M. sojae.* These data suggest that *M. sojae* is an economically important herbivore of soybeans under Brazilian growing conditions and highlight the need to develop efficient and sustainable management strategies for this pest.

Keywords: *Glycine max*; soybean stem fly; invasive species, economic injury level, economic threshold

1. Introduction

Soybean (*Glycine max* (L.) Merril) is the main cash crop grown in South America. Approximately 57 million hectares in South America are currently cultivated with soybean (FAOSTAT, 2020), 38.5 million of which were located within Brazil during the 2020/2021 crop season (CONAB, 2021). Extensive cultivation and variation in planting dates make this crop highly vulnerable to pests, including several invasive species (Pozebon et al., 2020). Soybean stem fly, *Melanagromyza sojae* Zehntner (Diptera: Agromyzidae), is native to eastern Asia and was recently detected in South America in 2015 (Arnemann et al., 2016), but it is suspected to have been present in southern Brazil since the 1980s (Gassen & Schneider, 1985). Since its detection in Brazil, *M. sojae* has spread across the South American soybean belt, reaching Paraguay (Guedes et al., 2017), Bolivia (Vitorio et al., 2019) and Argentina (Trossero et al., 2020). In Southern Brazil, outbreaks of *M. sojae* have been repeatedly observed on late-season soybeans, which are planted after maize (*Zea mays* L.) harvest beginning in late December (Folmann et al., 2017; Pozebon et al., 2020).

Short lifecycle and high reproductive rate allow *M. sojae* to complete as many as five generations per soybean cycle (Pozebon et al., 2020), facilitating crop reinfestation and the occurrence of distinct life stages of the insect within the same plant. *Melanagromyza sojae* larvae damage soybean plants by boring into the main stem and impairing xylem tissues (Talekar, 1989). Adult females feed and lay their eggs on upper (*i.e.*, younger) leaves, piercing holes where one or two eggs are placed. After two to four days, the eggs hatch and the larvae bore into the leaf's main vein, reaching the main stem of the plant via the petiole. Each larva consumes around 1.4 mm of leaf tissue per hour (Lee, 1962), taking two days on average to reach the main stem after emergence and drastically reducing the possibility for control.

Melanagromyza sojae can attack soybean plants throughout the whole crop cycle, but population densities vary according to environmental factors such as rainfall, temperature, and availability of plant hosts. During periods of the year when mild temperatures predominate, *M. sojae* populations decrease drastically. The highest incidence is observed in the hottest and driest months, as intense rainfall restrains feeding and oviposition by adult females (Talekar & Chen, 1985; Yadav et al., 2015). In Asian countries, stem flies typically overwinter as pupae within dead soybean stems (Pozebon et al., 2020). In Brazil, winter survival has been facilitated by the presence of volunteer soybean plants in fields (Czepak et al., 2018) and alternative plant hosts, such as *Trifolium resupinatum* L. (Ferreira et al., 2020).

As a novel pest in South America, there is a paucity of data regarding the potential of *M. sojae* to reduce soybean yield, and integrated management programs targeting this pest remain largely undeveloped. Yield losses due to *M. sojae* have been estimated at 30% in Indonesia (Du & Hong, 1982) and 42% in India (Jadhav et al., 2013), varying according to region, crop fertilizer nutrition, soybean cultivar, planting date and management strategies adopted (Savajji, 2006). However, as South America has a completely different soybean growing environment than the endemic region of *M. sojae*, this information is still lacking for our continent. Thus, the objective of this work was to quantify *M. sojae* injury and its effect on yield under South American soybean growing conditions.

2. Material and Methods

2.1 Experimental Sites

The same experiment was carried out during two summer crop seasons (2020 and 2021), at the Federal University of Santa Maria (29º42′48″S, 53º43′59″W), in Santa Maria, RS, Brazil. Soybean varieties TMG 7063 IPRO (planted on 27 January 2020) and 6968RSF (planted on 25 January 2021) were used in crop seasons 2020 and 2021, respectively. Soybean seeds were treated with 30 g a.i. of carbendazim + 70 g a.i. of thiram per 100 kg of seeds. Weeds were controlled prior to planting with an application of 1,005 g a.i./ha of 2,4-D + 1,040 g a.i./ha of glyphosate, and at soybean growth stage V3 (Fehr & Caviness, 1977) with an application of 1,040 g a.i./ha of glyphosate. Foliar sprays of strobilurin and triazole fungicides were carried out at growth stages V3, V7, R1, R4 and R5.2 for disease control. The soybean variety used in the crop season 2020 contained an insecticidal Bt protein, Cry1Ac, to control defoliating caterpillars, but the soybean variety used in the crop season 2021 lacked such a trait. Sap-sucking pests (stink bugs and whiteflies) were monitored at the field borders and managed with an application of 60 g a.i./ha of acetamiprid + 30 g a.i./ha of pyriproxyfen and 970 g a.i./ha of acephate, before they reached the experimental plots.

2.2 Experimental Design and Treatments

The experimental design was completely randomized with one factor (spray timing) and seven treatments, plus one additional treatment in the crop season 2020 (Tables 1 and 2). Ten replicates were used for evaluation of percentage of injured stem during weekly evaluations, and four replicates were used for evaluation of soybean yield and its components at crop senescence. Each plot was 6 × 20 m (12 soybean rows spaced 0.5 m) and contained approximately 2,400 soybean plants. We used weekly foliar applications of an insecticide to develop treatments of varying *M. sojae* densities. The number of applications ranged from 0 (untreated control) to 7 (additional treatment in the crop season 2020), which represented an attempt to keep the plants free of *M. sojae* (*i.e.*, pest-free control). These applications occurred at distinct moments of the soybean growth cycle, starting at the early vegetative growth stages and ending at pod formation (*i.e.*, growth stage R5.2). The insecticide used was 26.5 g a.i./ha of lambda-cyhalothrin + 35.2 g a.i./ha of thiamethoxam. Sprays were carried out using a CO_2-pressurized backpack sprayer, with a spray volume of 150 L/ha and six spray nozzles (model XR 110 020) spaced 0.5 m from each other.

Table 1. Treatments, number of sprays and growth stages of the soybean plants at each spray. Crop season 2020, Santa Maria, RS, Brazil

Treatment	Number of sprays	Growth stage at each spray
1	7	V3-V6-V8-R2-R3-R4-R5.2
2	6	V6-V8-R2-R3-R4-R5.2
3	5	V8-R2-R3-R4-R5.2
4	4	R2-R3-R4-R5.2
5	3	R3-R4-R5.2
6	2	R4-R5.2
7	1	R5.2
8	0	-

Table 2. Treatments, number of sprays and growth stages of the soybean plants at each spray. Crop season 2021, Santa Maria, RS, Brazil

Treatment	Number of sprays	Growth stage at each spray
1	6	V5-V7-R1-R2-R3-R4-R5.3
2	5	V7-R1-R2-R3-R4-R5.3
3	4	R1-R2-R3-R4-R5.3
4	3	R2-R3-R4-R5.3
5	2	R3-R4-R5.3
6	1	R4-R5.3
7	0	-

2.3 Evaluations

Evaluations were carried out weekly by randomly sampling 10 soybean plants from each plot, prior to the insecticide application, at growth stages V3, V6, V8, R2, R3, R4, R5.2 and R5.5 for crop season 2020, and V5, V7, R1, R2, R3, R4 and R5.3 for crop season 2021. Plant height was measured from the soil line to the last node of the main stem, and the presence of *M. sojae* tunnels was assessed and tunnels measured by opening the main stem longitudinally, from bottom to top. The percentage of injured stem was determined as a ratio between plant height and tunnel length in the main stem. For quantification of soybean yield and its components, four replicates were used, each being the average of four harvested plants (*i.e.*, 16 soybean plants per treatment). Seed yield (g/plant) and yield components (number of pods, number of seeds and 1,000-seed weight) were quantified for each segment of the plant canopy (lower, middle and upper) and for the entire plant. Yield was estimated based on number of seeds and 1,000-seed weight.

2.4 Statistical Analysis

We used ANOVA and Scott-Knott test to determine if the means of the variables measured varied from each other ($P \leq 0.05$). Linear regression analysis was used to determine the relationship between the percentage of injured stem and components of soybean yield (seeds/plant, 1,000-seed weight, pods/plant and yield/plant). The percentage of injured stem was analysed using the evaluations from growth stages R1, R2, R3, R4 and R5.5 for crop season 2020 and growth stages R4 and R5.3 for crop season 2021, which presented statistical differences among treatments. Figures for the linear regression analysis are presented for crop season 2020, which showed the best correlation among variables. Statistical analyses were carried out using Microsoft Excel and SISVAR (Ferreira, 2014).

3. Results

3.1 Crop Season 2020 (Transgenic Soybean Variety)

3.1.1 Amount of *M. sojae* Injury

The number of pods, number of seeds, 1,000-seed weight and seed yield of the soybean plants were significantly affected ($P \leq 0.05$) by *M. sojae* injury in the crop season 2020. The percentage of injured stem did not differ significantly among treatments for the evaluations from V3 to V8 and R5.2 (Table 3). The other evaluation timings (R1 to R4 and R5.5) presented statistical differences for this variable, resulting in reduction in yield components. The amount of injury caused by *M. sojae* varied according to the growth stage at which soybean plants were sprayed (Table 3). The regression models presented high coefficients of determination ($R^2 > 0.60$ for number of seeds, $R^2 > 0.76$ for 1,000-seed weight and $R^2 > 0.74$ for seed yield), indicating a likely relationship between *M. sojae* injury and reduction in soybean yield components.

Table 3. Percentage of soybean stem length injured by *Melanagromyza sojae* in relation to insecticide sprays starting at different growth stages of the crop. Crop season 2020, Santa Maria, RS, Brazil

Treatment	Growth stage at first spray	Growth stage at evaluation moment								
		V3	V6	V8	R1	R2	R3	R4	R5.2	R5.5
1	V3	8.1^{ns}	14.0^{ns}	43.9^{ns}	47.1 a	29.7 a	29.5 a	20.7 a	22.6^{ns}	21.8 a
2	V6	0.9^{ns}	30.3^{ns}	55.3^{ns}	48.6 a	36.2 b	27.0 a	27.3 a	30.0^{ns}	23.0 a
3	V8	8.3^{ns}	23.0^{ns}	52.9^{ns}	57.9 b	28.7 a	25.7 a	23.4 a	28.4^{ns}	21.6 a
4	R2	5.0^{ns}	15.7^{ns}	56.1^{ns}	43.6 a	40.0 b	36.7 b	30.8 a	25.0^{ns}	31.2 b
5	R3	0.8^{ns}	33.0^{ns}	52.9^{ns}	61.2 b	52.9 c	40.2 b	37.2 a	24.3^{ns}	34.4 b
6	R4	1.7^{ns}	28.6^{ns}	59.5^{ns}	44.0 a	44.2 c	37.1 b	44.8 b	23.7^{ns}	43.2 c
7	R5.2	0.9^{ns}	18.2^{ns}	58.8^{ns}	41.3 a	47.5 c	42.6 b	44.2 b	27.8^{ns}	66.0 c
8	-	9.8^{ns}	24.5^{ns}	56.8^{ns}	53.2 b	39.3 b	42.4 b	39.6 b	32.4^{ns}	51.8 c
CV (%)[1]		324.23	102.68	40.34	30.88	30.99	35.90	33.81	44.60	42.71
SE[2]		4.56	8.64	7.40	4.84	3.97	4.071	3.58	3.90	5.23
F		0.714	1.198	0.777	2.192	4.726	3.788	6.728	1.329	9.948
P-value		0.6601	0.3152	0.6086	0.0448	0.0002	0.0015	< 0.0001	0.2491	< 0.0001

Note. [1] Coefficient of variation; [2] Standard error; [3] Means followed by the same letter do not differ among themselves by the Scott Knott test ($p \leq 0.05$); [4] Non-significant.

3.1.2 Yield Component Analysis

There was no significant difference among treatments for number of pods in the lower (P = 0.9235) and middle (P = 0.1186) segments. Treatment had a significant effect on number of pods in the upper segment (P = 0.0229) in the ANOVA but not in the Scott-Knott test. However, all remaining yield components presented significant differences according to the Scott-Knott test ($P \leq 0.05$), except for number of seeds in the lower segment (Table 4). The highest number of seeds in the middle segment (51.0 seeds/plant) was observed in treatment 1, whereas for the upper segments the highest values were obtained in treatments 1, 2 and 3, with 43.4, 40.0 and 40.5 seeds/plant, respectively. Similarly, treatments 1, 2 and 3 presented the highest 1,000-seed weight values in all three segments, differing statistically from the remaining treatments. The values observed for 1,000-seed weight in treatments 1, 2 and 3 were: 198.2, 194.5 and 192.1 g, respectively, in the lower segment; 199.7, 203.0 and 197.5 g, respectively, in the middle segment; and 187.7, 188.4 and 174.1 g, respectively, in the upper segment (Table 4).

Table 4. Yield components following *Melanagromyza sojae* injury in relation to insecticide sprays starting at different growth stages of the crop, in lower, middle and upper canopy segments of the soybean plants. Crop season 2020, Santa Maria, RS, Brazil

Treatment	Growth stage at first spray	Number of pods			Number of seeds			1000-seed weight		
		Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper
1	V3	39.3^{ns}	20.2^{ns}	16.7 a	96.9^{ns}	51.0 a	43.4 a	198.2 a	199.7 a	187.7 a
2	V6	33.3^{ns}	16.9^{ns}	15.1 a	79.2^{ns}	43.2 b	40.0 a	194.5 a	203.0 a	188.4 a
3	V8	34.3^{ns}	16.6^{ns}	15.0 a	84.3^{ns}	41.5 b	40.5 a	192.1 a	197.5 a	174.1 a
4	R2	35.5^{ns}	18.3^{ns}	13.7 b	79.8^{ns}	42.9 b	33.2 b	164.0 b	179.9 b	156.2 b
5	R3	35.3^{ns}	18.8^{ns}	13.3 b	77.4^{ns}	42.6 b	31.4 b	158.6 b	168.3 b	137.9 c
6	R4	36.9^{ns}	20.4^{ns}	14.4 b	68.2^{ns}	30.6 c	30.0 c	155.2 b	145.6 c	122.9 c
7	R5.2	37.0^{ns}	20.5^{ns}	13.8 b	64.4^{ns}	34.2 c	27.6 c	149.7 b	147.0 c	137.9 c
8	-	29.4^{ns}	19.3^{ns}	13.9 b	49.6^{ns}	28.9 c	25.7 c	145.5 b	126.7 d	109.0 d
CV (%)[1]		28.36	11.85	8.89	31.32	12.51	9.02	5.06	5.63	7.17
SE[2]		4.99	1.11	0.64	11.74	2.46	1.53	4.29	4.81	5.39
F		0.347	1.875	2.931	1.469	9.171	18.214	25.387	35.951	32.595
P-value		0.9235	0.1186	0.0229	0.2255	< 0.00001	< 0.00001	< 0.0001	< 0.00001	< 0.00001

Note. [1] Coefficient of variation; [2] Standard error; [3] Means followed by the same letter do not differ among themselves by the Scott Knott test ($p \leq 0.05$); [4] Non-significant.

No effect of treatment was observed for number of pods per plant in any canopy segment. The regression for number of seeds per plant for all canopy segments is shown on Figure 1, indicating a reduction of 0.71 seeds/plant ($R^2 = 0.6011$; $P < 0.0001$) in the middle segment and 0.75 seeds/plant ($R^2 = 0.8758$; $P < 0.0001$) in the upper segment for each 1% of stem injured by *M. sojae*. Similarly, each 1% of injured stem caused a reduction of 2.4 ($R^2 = 0.8525$; $P < 0.0001$), 3.1 ($R^2 = 0.7678$; $P < 0.0001$) and 3.3 g ($R^2 = 0.7903$; $P < 0.0001$) of 1,000-seed weight, for the lower, middle and upper segments, respectively (Figure 2).

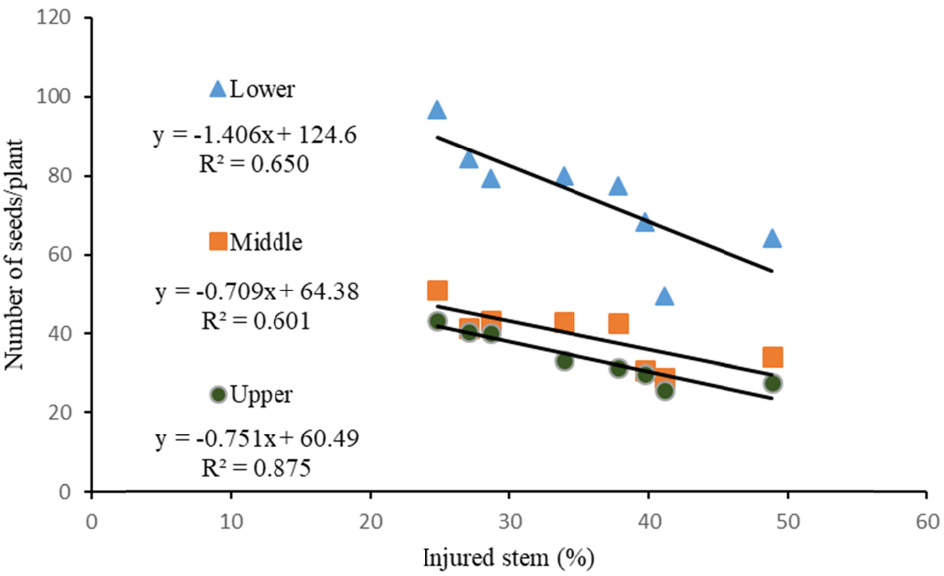

Figure 1. Relationship between percentage of stem injured by *Melanagromyza sojae* and number of seeds/plant in lower ($R^2 = 0.6504$; N = 8; $P < 0.0001$), middle ($R^2 = 0.6011$; N = 8; $P < 0.0001$) and upper ($R^2 = 0.8758$; N = 8; $P < 0.0001$) canopy segments of soybean plants. Data points in the chart represent mean values from crop season 2020

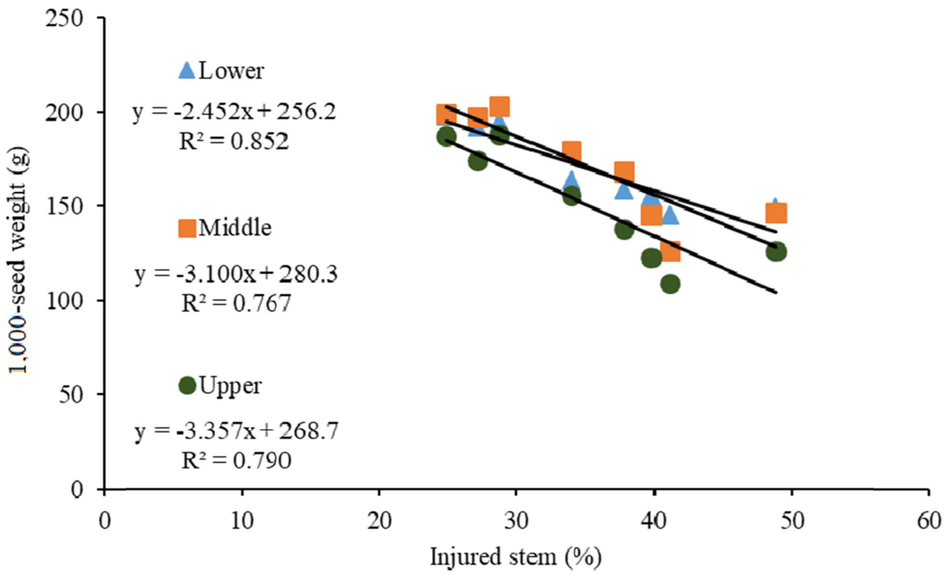

Figure 2. Relationship between percentage of stem injured by *Melanagromyza sojae* and 1,000-seed weight in lower ($R^2 = 0.8525$; N = 8; $P < 0.0001$), middle ($R^2 = 0.7678$; N = 8; $P < 0.0001$) and upper ($R^2 = 0.7903$; N = 8; $P < 0.0001$) canopy segments of soybean plants. Data points in the chart represent mean values from crop season 2020

The highest yield values were observed in treatments 1, 2, 3 and 4, which differed statistically from the remaining treatments (Scott-Knott test: $P \leq 0.05$) and yielded 37.5, 31.9, 31.4 and 26.1 g/plant, respectively (Appendix A). Lower, middle and upper segments presented yield reduction of 0.4 ($R^2 = 0.8032$; $P < 0.0001$), 0.2 ($R^2 = 0.7448$; $P < 0.0001$) and 0.2 ($R^2 = 0.8473$; $P < 0.0001$) g/plant, respectively (Figure 3). The regression model for seed yield indicated that each 1% of soybean stem injured by *M. sojae* caused a reduction of 0.9 g/plant across all canopy segments ($P < 0.0001$).

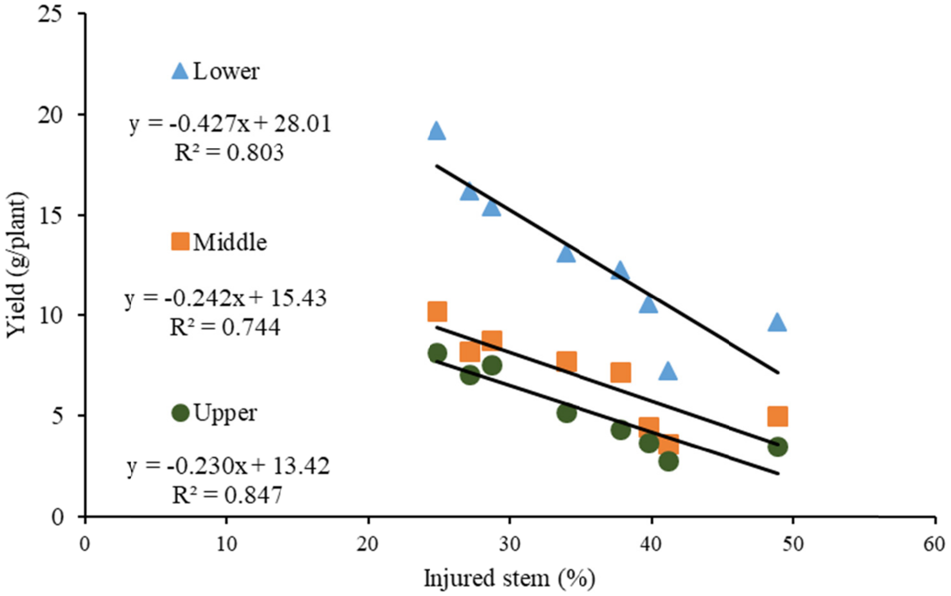

Figure 3. Relationship between percentage of stem injured by *Melanagromyza sojae* and seed yield/plant in lower ($R^2 = 0.8032$; N = 8; $P < 0.0001$), middle ($R^2 = 0.7448$; N = 8; $P < 0.0001$) and upper ($R^2 = 0.8473$; N = 8; $P < 0.0001$) canopy segments of soybean plants. Data points in the chart represent mean values from crop season 2020

3.2 Crop Season 2021 (Conventional Soybean Variety)

3.2.1 Amount of *M. sojae* Injury

Treatments 1, 2 and 3 showed the lowest percentage of stem injured by *M. sojae*, not differing among themselves by Scott-Knott test (Table 5). The remaining treatments presented higher percentage of injured stem and did not differ among themselves, ranging from 46.4 to 52.6% at growth stage R4, and from 56.3 to 65.5% at R5.3.

Table 5. Percentage of soybean stem length injured by *Melanagromyza sojae* in relation to insecticide sprays starting at different growth stages of the crop. Crop season 2021, Santa Maria, RS, Brazil

Treatment	Growth stage at first spray	Growth stage at evaluation moment						
		V5	V7	R1	R2	R3	R4	R5.3
1	V5	25.1^{ns}	50.5^{ns}	73.4^{ns}	59.0^{ns}	54.0^{ns}	39.0 a	45.9 a
2	V7	37.2^{ns}	29.1^{ns}	66.4^{ns}	50.0^{ns}	68.4^{ns}	35.6 a	44.9 a
3	R1	46.2^{ns}	58.4^{ns}	49.4^{ns}	48.6^{ns}	51.5^{ns}	32.5 a	33.6 a
4	R2	19.8^{ns}	67.5^{ns}	55.5^{ns}	46.3^{ns}	55.8^{ns}	52.6 b	56.3 b
5	R3	49.1^{ns}	53.4^{ns}	53.2^{ns}	51.2^{ns}	53.6^{ns}	46.4 b	58.0 b
6	R4	35.4^{ns}	51.6^{ns}	64.1^{ns}	55.9^{ns}	51.6^{ns}	47.4 b	59.9 b
7	-	33.1^{ns}	53.6^{ns}	67.2^{ns}	58.7^{ns}	55.3^{ns}	48.8 b	65.5 b
CV (%)[1]		102.37	60.86	38.23	41.75	35.84	34.68	37.25
SE[2]		11.37	10.01	7.41	6.97	6.31	4.73	6.12
F		0.853	1.353	1.386	0.527	0.851	2.491	3.237
P-value		0.5347	0.2474	0.2340	0.7859	0.5358	0.0316	0.0078

Note. [1] Coefficient of variation; [2] Standard error; [3] Means followed by the same letter do not differ among themselves by the Scott Knott test ($p \leq 0.05$); [4] Non-significant.

3.2.2 Yield Component Analysis

There was significant variation across the insecticide treatments for the following yield components: number of pods in the lower segment (P = 0.0023); number of seeds in the lower segment (P = 0.0073); 1,000-seed weight in the lower ($P < 0.0001$), middle ($P < 0.0001$) and upper (P = 0.0084) segments; and seed yield in the lower segment (P = 0.0021). The highest values for number of pods in the lower segment were observed in treatments 1, 3, 4 and 5, with 10.4, 11.1, 9.9 and 12.3 pods/plant, respectively, not differing significantly among themselves.

The highest values for number of seeds were observed in the lower segment of treatments 1, 3, 4 and 5, with 19.3, 20.0, 18.7 and 21.7 seeds/plant, respectively. As for 1,000-seed weight in the lower segment, the highest values were observed in treatments 1, 4 and 5, with 201.2, 206.1 and 204.0 g, respectively, not differing among themselves. In the middle segment, the highest values for 1,000-seed weight were observed in treatments 1 and 4, with 208.2 and 206.1 g, respectively, not differing from each other. In the upper segment, however, treatments 1, 2, 5 and 7 presented the highest values, with 179.3, 181.4, 181.4 and 183.4 g, respectively, not differing among themselves (Table 6).

Table 6. Yield components following *Melanagromyza sojae* injury in relation to insecticide sprays starting at different growth stages of the crop, in lower, middle and upper canopy segments of the soybean plants. Crop season 2021, Santa Maria, RS, Brazil

Treatment	Growth stage at first spray	Number of pods			Number of seeds			1000-seed weight		
		Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper
1	V5	10.4 a[3]	10.2[ns4]	11.4[ns]	19.3a	18.8[ns]	22.2[ns]	210.2 a	208.2 a	179.3 a
2	V7	6.5 b	12.8[ns]	11.1[ns]	11.4 b	23.8[ns]	21.8[ns]	195.8 b	197.9 b	181.4 a
3	R1	11.1 a	10.0[ns]	9.2[ns]	20.0 a	19.4[ns]	20.1[ns]	183.4 c	183.4 c	167.0 b
4	R2	9.9 a	11.6[ns]	10.7[ns]	18.7 a	20.3[ns]	20.3[ns]	206.1 a	206.1 a	175.2 b
5	R3	12.3 a	10.2[ns]	9.7[ns]	21.7 a	21.7[ns]	21.0[ns]	204,0 a	197.9 b	181.4 a
6	R4	5.1 b	13.1[ns]	11.4[ns]	8.9 b	23.9[ns]	24.3[ns]	173.2 d	179.3 c	171.1 b
7	-	9.6 a	9.5[ns]	9.5[ns]	16.3 b	16.0[ns]	17.5[ns]	193.7 b	195.8 b	183.4 a
CV (%)[1]		24.28	24.01	13.00	27.79	25.85	14.55	2.31	3.32	3.47
SE[2]		1.11	1.33	0.66	2.28	2.69	1.53	2.25	3.24	3.07
F		5.087	1.104	1.601	4.067	0.997	1.751	34.430	10.952	3.956
P-value		0.0023	0.3930	0.1964	0.0073	0.4531	0.1585	< 0.0001	< 0.0001	0.0084

Note. [1] Coefficient of variation; [2] Standard error; [3] Means followed by the same letter do not differ among themselves by the Scott Knott test ($p \leq 0.05$); [4] Non-significant.

Finally, the highest values for seed yield in the lower segment were observed in treatments 1, 3, 4, 5 and 7, with 4.0, 3.6, 3.8, 4.3 and 3.2 g/plant, respectively. Despite the differences observed among treatments, the resulting regression models between the aforementioned variables and the percentage of injured stem were not significant (except for 1,000-seed weight in the upper segment; P = 0.031), and have thus been omitted.

4. Discussion

4.1 Number of Seeds and 1,000-Seed Weight

Soybean yield decreased as *M. sojae* injury increased, mainly as a result of reduced number of seeds and lower 1,000-seed weight. Despite receiving weekly insecticide sprays since growth stage V3 (crop season 2020) and V5 (crop season 2021), treatment 1 still resulted in plant injury by *M. sojae* and yield reduction (although lower than all other treatments), indicating that the potential for yield reduction can be even higher than observed in this study.

The results obtained in both crop seasons indicate that suppressing *M. sojae* contributed to an increase of seed yield, as observed for the middle and upper segments in the crop season 2020 and lower segment in the crop season 2021. The treatment where a spray occurred at the onset of *M. sojae* infestation presented the highest number of seeds in all canopy segments. The regression models for the middle and upper segments in the crop season 2020 indicated a reduction of 0.71 and 0.75 seeds/plant, respectively, for each 1% of injured stem, illustrating the potential of this pest to negatively affect one of the main yield components in soybean plants.

In the crop season 2020, 1,000-seed weight also decreased as injury by *M. sojae* increased. Means were grouped together according to a clear-cut pattern in the three canopy segments: treatments 1 to 3 did not differ significantly among themselves, and the lowest seed weight values were observed in the unsprayed control plot. In the crop season 2021, treatment 1 presented the highest 1,000-seed weight values in the lower and middle segments, not differing significantly from treatment 4 in both segments and treatment 5 in the lower segment. All remaining treatments presented significant reductions in seed weight, which was expected as stem tunnelling by *M. sojae* larvae hinders xylem transport within soybean plants (Talekar, 1989), consequently affecting seed filling.

4.2 Seed Yield

Each 1% of soybean stem injured by *M. sojae* reduced seed yield by 0.9 g/plant in the crop season 2020. In comparison, studies carried out in East Asia during the 1980s estimated a yield reduction of 0.11 g/plant for each 1% of injured stem (Talekar & Chen, 1985). This value was estimated at 1.1 g/plant for the species *Melanagromyza obtuse* Malloch (Diptera: Agromyzidae) (Gangrade & Sing, 1976). Considering that modern soybean cultivars present lower leaf area index, lower height and less secondary stems than cultivars grown 40 years ago, each unit of injury was expected to produce a greater impact on yield components than previously reported.

Yield reductions as high as 63.7% were observed in treatment 8 (untreated) (Appendix B). Similarly, Jadhav et al. (2013) observed soybean yield losses ranging from 33 to 41% in India. This high potential for yield reduction is explained by the feeding behaviour of *M. sojae* larvae within the soybean stem, which damages vascular tissues and impairs xylem transport (Chiang & Norris, 1983). As a consequence, transport of water and nutrients from roots to stems and leaves is hindered (Talekar, 1989). Furthermore, soybean stems store most of the photoassimilates that are translocated to seeds during seed filling (Streeter & Jeffers, 1979). Thus, seed yield is directly affected by insect herbivory at this site within the plant.

4.3 Spray Timing and Management Strategies

Treatments where insecticide applications started during the vegetative phase (1, 2 and 3) presented the lowest damage by *M. sojae* in all evaluations of crop season 2020, not differing among themselves or treatments 8, 4 and 5 in the evaluations at R2, R4 and R5.2, respectively. Similarly, the lowest injury by *M. sojae* in the crop season 2021 was observed in treatments 1, 2 and 3, not differing among themselves according to the Scott-Knott test. Throughout the experiments, treatments with more sprays prior to evaluation presented lower percentage of stem injured by *M. sojae.* The reduction in percentage of injured stem in these treatments is probably linked to effective control of *M. sojae* adults, leading to fewer larvae boring into the main stem of the soybean plants. After the larvae reach the main stem, the possibility for control is drastically reduced, as the insect becomes virtually unreachable by insecticide sprays. The high correlation found between *M. sojae* injury level (*i.e.*, percentage of injured stem) and reduction in yield components (number of seeds, 1,000-seed weight and seed yield) points to a high potential for damage in soybean and highlights the need to develop efficient and effective management strategies for this pest.

Population outbreaks of *M. sojae* have so far remained restricted to late-season soybeans (*i.e.*, planted from December onward) in southern Brazil. However, Pozebon et al. (2020) alerts that *M. sojae* infestations are likely to become frequent in main-season soybeans as well (planted from October onward), due to the pest's adaptability to Brazilian growing conditions. Besides reducing seed yield in main-season soybeans, the development of such a scenario would boost *M. sojae* population levels during late planting, potentially compromising late-season soybean cultivation altogether. Soybean growers in southern Brazil typically cut expenses during late-season cultivation, leading to lower use of insecticides. When Bt soybean cultivars are used, the crops are sprayed with insecticide only after growth stage R3, targeting phytophagous stinkbugs. Our study included this scenario and showed the potential for *M. sojae* to damage soybean plants and reduce seed yield. Treatment 5, where two weekly insecticide sprays were carried out after growth stage R3, presented yield loss as high as 36.53%, not differing statistically from the unsprayed control plot.

Soybean pest management in Brazil relies heavily on the use of chemical insecticides and Bt cultivars. The transgenic cultivar MON 87701 × MON 89788, expressing insecticidal Bt protein Cry1Ac, was commercially released in Brazil in 2010 (Bernardi, 2012). Yu et al. (2014) studied the exposure of non-target arthropods to Cry1Ac in soybean fields and reported the presence of detectable Bt protein levels in *M. sojae* adults, with no apparent effect upon the insect's development. Bt proteins Cry4Aa, Cry4Ba, Cry11Aa, Cyt1Aa, Cry10Aa and Cyt2Ba knowingly affect dipteran insects (Ben-Dov, 2014), but no transgenic cultivar made available to date expresses these proteins. Soybean cultivars in India are classified according to their susceptibility to *M. sojae* attack, based on percentage of attacked plants (Savajji, 2006), but in Brazil this information is lacking (Curioletti, 2016). Unavailability of Bt cultivars with activity against dipteran pests, combined with the lack of information regarding susceptibility to *M. sojae* attack in current soybean cultivars, has constrained management strategies to insecticide spraying. The use of insecticide treated seeds, combined with foliar sprays shortly after plant emergence, protects soybean plants during the growth stages most vulnerable to *M. sojae* attack (Pozebon et al., 2021). Chlorantraniliprole and chlorpyrifos are recommended for seed treatment and foliar sprays, respectively (Curioletti et al., 2018).

The findings from this study highlight the importance of reducing the coexistence of pest and crop by spraying soybean plants during the vegetative phase, thus preventing yield losses and avoiding lost revenue. Further studies should assess *M. sojae* injury on main-season growing conditions and use the data to determine an economic injury level for this pest in soybean.

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Appendix A

Grain yield following *Melanagromyza sojae* injury in relation to insecticide sprays starting at different growth stages of the crop, in lower, middle and upper canopy segments and in whole soybean plants. Crop Season 2020, Santa Maria, Brazil

Treatment	Growth stage at first spray	Yield			Grain yield/plant
		Lower	Middle	Upper	
1	V3	19.2 a	10.2 a	8.1 a	37.5 a
2	V6	15.5 a	9.8 b	7.5 b	31.9 a
3	V8	16.2 a	8.1 b	7.0 b	31.4 a
4	R2	13.2 b	7.7 b	5.2 c	26.1 a
5	R3	12.3 b	7.1 b	4.3 d	23.8 b
6	R4	10.6 b	4.5 c	3.7 e	18.8 b
7	R5.2	9.6 b	5.0 c	3.5 e	18.2 b
8	-	7.1 b	3.6 c	2.8 e	13.6 b
CV (%)[1]		34.26	14.98	10.53	21.33
SE[2]		2.22	0.51	0.27	2.68
F		3.119	19.660	53.653	9.194
P-value		0.0173	< 0.00001	< 0.00001	< 0.00001

Note. [1] Coefficient of variation; [2] Standard error; [3] Means followed by the same letter do not differ among themselves by the Scott Knott test ($p \leq 0.05$); [4] Non-significant.

Appendix B

Grain yield following *Melanagromyza sojae* injury in relation to insecticide sprays starting at different growth stages of the crop, in lower, middle and upper canopy segments and in whole soybean plants. Crop season 2021, Santa Maria, RS, Brazil

Treatment	Growth stage at first spray	Yield			Grain yield/plant
		Lower	Middle	Upper	
1	V5	4.0 a[3]	4.4^{ns}[4]	3.8^{ns}	12.3^{ns}
2	V7	2.2 b	4.6^{ns}	3.9^{ns}	10.8^{ns}
3	R1	3.6 a	3.4^{ns}	3.3^{ns}	10.3^{ns}
4	R2	3.8 a	4.4^{ns}	3.8^{ns}	12.0^{ns}
5	R3	4.3 a	4.2^{ns}	3.7^{ns}	12.3^{ns}
6	R4	1.5 b	4.2^{ns}	4.1^{ns}	9.8^{ns}
7	-	3.2 a	3.1^{ns}	3.2^{ns}	9.5^{ns}
CV (%)[1]		27.60	25.22	15.21	19.63
SE[2]		0.44	0.51	0.28	1.08
F		5.183	1.209	1.464	1.211
P-value		0.0021	0.3407	0.2383	0.3393

Note. [1] Coefficient of variation; [2] Standard error; [3] Means followed by the same letter do not differ among themselves by the Scott Knott test ($p \leq 0.05$); [4] Non-significant.

Appendix C

Analyses of ANOVA for the variables injured stem, number of pods, number of grains, 1000-grain weight and yield. Crop seasons 2020 and 2021, Santa Maria, RS, Brazil

Percentage of soybean stem length injured (Crop season 2020)						
Growth stage	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
V3	Treatment	7	1038.9	148.4	0.714	0.6601
	Error	72	14963.2	207.8		
	Corrected total	79	16002.1			
	CV (%)	Standard error				
	324.22	4.55				
Growth stage	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
V6	Treatment	7	6259.8	894.2	1.198	0.3152
	Error	72	53754.6	746.6		
	Corrected total	79	60014.3			
	CV (%)	Standard error				
	102.68	8.64				
Growth stage	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
V8	Treatment	7	2977.6	425.4	0.777	0.6086
	Error	72	39419.3	547.5		
	Corrected total	79	42396.9			
	CV (%)	Standard error				
	40.34	7.39				
Growth stage	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
R1	Treatment	7	5063.2	723.3	1.469	0.1920
	Error	72	35449.2	492.3		
	Corrected total	79	40512.4			
	CV (%)	Standard error				
	40.08	7.01				

Growth stage	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
R2	Treatment	7	5227.2	746.7	4.726	0.0002
	Error	72	11375.7	158.0		
	Corrected total	79	16603.0			
	CV (%)	Standard error				
	30.99	3.97				
Growth stage	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
R3	Treatment	7	4396.4	628.0	3.788	0.0015
	Error	72	11936.6	165.8		
	Corrected total	79	16332.0			
	CV (%)	Standard error				
	35.90	4.07				
Growth stage	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
R4	Treatment	7	6048.3	864.0	6.728	< 0.00001
	Error	72	9246.1	128.4		
	Corrected total	79	15294.4			
	CV (%)	Standard error				
	33.81	3.58				
Growth stage	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
R5.2	Treatment	7	19079.9	2725.7	9.948	< 0.00001
	Error	72	19728.0	274.0		
	Corrected total	79	38807.9			
	CV (%)	Standard error				
	42.71	5.23				
Percentage of soybean stem lenght injured (Crop season 2021)						
Growth stage	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
V5	Treatment	6	6616.5	1102.7	0.853	0.5347
	Error	63	81490.8	1293.5		
	Corrected total	69	88107.4			
	CV (%)	Standard error				
	102.37	11,37				
Growth stage	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
V7	Treatment	6	8134.4	1355.7	1.353	0.2474
	Error	63	63113.2	1001.8		
	Corrected total	69	71247.6			
	CV (%)	Standard error				
	60.86	10.01				
Growth stage	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
R1	Treatment	6	4570.2	761.7	1.386	0.2340
	Error	63	34614.3	549.4		
	Corrected total	69	39184.5			
	CV (%)	Standard error				
	38.23	7.41				
Growth stage	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
R2	Treatment	6	1535.7	255.9	0.527	0.7859
	Error	63	30608.3	485.8		
	Corrected total	69	32144.0			
	CV (%)	Standard error				
	41.75	6.97				

Growth stage	Source	Degrees of freedom	Sum of squares	Means quare	Fc	P > Fc
R3	Treatment	6	2039.3	339.9	0.851	0.5358
	Error	63	25161.8	399.4		
	Corrected total	69	27201.1			
	CV (%)	Standard error				
	35.84	6.32				
Growth stage	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
R4	Treatment	6	3354.3	559.0	2.491	0.0316
	Error	63	14136.8	224.4		
	Corrected total	69	17491.1			
	CV (%)	Standard error				
	34.68	4.74				
Growth stage	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
R5.3	Treatment	6	7290.3	1215.0	3.237	0.0078
	Error	63	23646.1	375.3		
	Corrected total	69	30936.4			
	CV (%)	Standard error				
	37.25	6.12				
Yield components (Crop season 2020)						
Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
Number of pods (Lower)	Treatment	7	242.0	34.6	0.347	0.9235
	Error	24	2390.7	99.6		
	Corrected total	31	2632.7			
	CV (%)	Standard error				
	28.36	4.99				
Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
Number of pods (Middle)	Treatment	7	65.8	9.4	1.875	0.1186
	Error	24	120.2	5.0		
	Corrected total	31	186.0			
	CV (%)	Standard error				
	11.85	1.11				
Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
Number of pods (Upper)	Treatment	7	34.1	4.9	2.931	0.0229
	Error	24	39.8	1.7		
	Corrected total	31	73.9			
	CV (%)	Standard error				
	8.89	0.64				
Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
Number of grains (Lower)	Treatment	7	5668.3	809.7	1.469	0.2255
	Error	24	13234.0	551.4		
	Corrected total	31	18902.4			
	CV (%)	Standard error				
	31.32	11.74				
Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
Number of grains (Middle)	Treatment	7	1558.0	222.6	9.171	< 0.00001
	Error	24	582.4	24.3		
	Corrected total	31	2140.4			
	CV (%)	Standard error				
	12.51	2.46				

Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
Number of grains (Upper)	Treatment	7	1198.2	171.2	18.214	< 0.00001
	Error	24	225.5	9.4		
	Corrected total	31	1423.8			
	CV (%)	Standard error				
	9.02	1.53				

Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
1000-grain weight (Lower)	Treatment	7	13113.5	1873.4	25.387	< 0.00001
	Error	24	1771.0	73.8		
	Corrected total	31	14884.5			
	CV (%)	Standard error				

Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
1000-grain weight (Middle)	Treatment	7	23290.8	3327.2	35.951	< 0.00001
	Error	24	2221.9	92.5		
	Corrected total	31	25511.9			
	CV (%)	Standard error				
	5.63	4.81				

Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
1000-grain weight (Upper)	Treatment	7	26528.1	3789.7	32.595	< 0.00001
	Error	24	2790.4	116.3		
	Corrected total	31	29318.5			
	CV (%)	Standard error				
	7.17	5.39				

Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
Yield (Lower)	Treatment	7	431.5	61.6	3.119	0.0173
	Error	24	474.3	19.8		
	Corrected total	31	905.8			
	CV (%)	Standard error				
	34.26	2.22				

Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
Yield (Middle)	Treatment	7	146.7	20.9	19.660	< 0.00001
	Error	24	25.6	1.1		
	Corrected total	31	172.2			
	CV (%)	Standard error				
	14.98	0.51				

Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
Yield (Upper)	Treatment	7	116.3	16.6	53.653	< 0.00001
	Error	24	7.4	0.3		
	Corrected total	31	123.7			
	CV (%)	Standard error				
	10.53	0.28				

Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
Yield (g/plant)	Treatment	7	1852.5	264.6	9.194	< 0.00001
	Error	24	690.8	28.8		
	Corrected total	31	2543.3			
	CV (%)	Standard error				
	21.33	2.68				

Yield components (Crop season 2021)

Yieldcomponent	Source	Degrees of freedom	Sum of squares	Meansquare	Fc	P > Fc
Number of pods (Lower)	Treatment	6	152.5	25.4	5.087	0.0023
	Error	21	104.9	5.0		
	Corrected total	27	257.4			
	CV (%)	Standard error				
	24.28	1.11				
Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
Number of pods (Middle)	Treatment	6	47.0	7.8	1.104	0.3930
	Error	21	148.9	7.1		
	Corrected total	27	195.9			
	CV (%)	Standard error				
	24.01	1.33				
Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
Number of pods (Upper)	Treatment	6	16.8	2.8	1.601	0.1964
	Error	21	36.9	1.7		
	Corrected total	27	53.7			
	CV (%)	Standard error				
	13.00	0.66				
Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
Number of grains (Lower)	Treatment	6	507.2	84.5	4.067	0.0073
	Error	21	436.5	20.8		
	Corrected total	27	943.7			
	CV (%)	Standard error				
	27.79	2.28				
Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
Number of grains (Middle)	Treatment	6	173.3	28.9	0.997	0.4531
	Error	21	608.4	29.0		
	Corrected total	27	781.7			
	CV (%)	Standard error				
	25.85	2.69				
Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
Number of grains (Upper)	Treatment	6	98.0	16.3	1.751	0.1585
	Error	21	196.0	9.3		
	Corrected total	27	294.0			
	CV (%)	Standard error				
	14.55	1.53				
Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
1000-grain weight (Lower)	Treatment	6	4194.9	699.1	34.430	< 0.00001
	Error	21	426.4	20.3		
	Corrected total	27	4621.3			
	CV (%)	Standard error				
	2.31	2.25				
Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
1000-grain weight (Middle)	Treatment	6	2766.3	461.0	10.952	< 0.00001
	Error	21	884.0	42.1		
	Corrected total	27	3650.3			
	CV (%)	Standard error				
	3.32	3.24				

Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
1000-grain weight (Upper)	Treatment	6	895.7	149.3	3.956	0.0084
	Error	21	792.5	37.7		
	Corrected total	27	1688.2			
	CV (%)	Standard error				
	3.47	3.07				
Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
Yield (Lower)	Treatment	6	24.8	4.1	5.183	0.0021
	Error	21	16.8	0.8		
	Corrected total	27	41.6			
	CV (%)	Standard error				
	27.60	0.45				
Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
Yield (Middle)	Treatment	6	7.6	1.3	1.209	0.3407
	Error	21	22.1	1.0		
	Corrected total	27	29.7			
	CV (%)	Standard error				
	25.22	0.51				
Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
Yield (Upper)	Treatment	6	2.8	0.5	1.464	0.2383
	Error	21	6.7	0.3		
	Corrected total	27	9.4			
	CV (%)	Standard error				
	15.21	0.28				
Yield component	Source	Degrees of freedom	Sum of squares	Mean square	Fc	P > Fc
Yield (g/plant)	Treatment	6	34.0	5.7	1.211	0.3393
	Error	21	98.1	4.7		
	Corrected total	27	132.1			
	CV (%)	Standard error				
	19.63	1.08				

Acknowledgments

We acknowledge the Department of Crop Protection of Federal University of Santa Maria for assisting with crop cultivation and management in the experimental area. We would also like to thank every team member who took the time to participate in this study.

Authors Contributions

JAA and RPM designed the study. RPM, GP, PCR, LAC, BW, PP and GRL were responsible for experimental conduction and data collection. RPM, JGB, ACF and IV carried out statistical analysis. RPM drafted the manuscript, HP transated it to English and JDJL revised it. JAA, HP, AR, RF and JP assisted with data discussion. All authors read and approved the final manuscript.

Funding

Not applicable.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Informed Consent

Obtained.

Ethics Approval

The Publication Ethics Committee of the Canadian Center of Science and Education. The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

Provenance and Peer Review

Not commissioned; externally double-blind peer reviewed.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data Sharing Statement

No additional data are available.

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