

**Effect of resistance elicitors on the biology and feeding preference of *Spodoptera frugiperda* (J.E.Smith) (Lepidoptera: Noctuidae) in corn**

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**Abstract**

This study searches for efficient and low environmental impact alternatives to control *Spodoptera frugiperda*. Application of elicitors capable of inducing resistance in plants has already been highlighted. The elicitors trigger the plant's defense capacity against attack of herbivores and phytopathogenic organisms. Thus, the present study aimed to evaluate the effect of resistance elicitors on some biological aspects and food preference of *Spodoptera frugiperda* (J. E. Smith) in corn in laboratory conditions. The treatments consisted of: Control (distilled water); Biofertilizer (25 mL L<sup>-1</sup>); Acibenzolar-S-methyl-ASM (2 mg mL<sup>-1</sup>); Potassium silicate (10 mL L<sup>-1</sup>) and T5: Potassium silicate (10 mL L<sup>-1</sup>) + ASM (2 mg mL<sup>-1</sup>). The treatments were foliar application of elicitors with adjustable jet pressure sprayer to the whole plant with an amount of 20 mL of the solution per plant. The larval phase (weight at 7 and 14 days, duration and viability); pupal phase (weight after 24 h, duration and viability) and adult phase (number of eggs / female, number of eggs/posture/female and longevity of adults) were evaluated. Free-choice and no-choice food preference test experiments were also carried out on 1st and 3rd instar caterpillars, after which the leaves collected at 5 and 10 days after spraying. The results showed that Acibenzolar-S-methyl induces resistance in corn plants when applied either alone or together with potassium silicate. Therefore, it adversely affects development of *S. frugiperda*. The leaves treated with Potassium Silicate + ASM were less preferred and consumed by 1st and 3rd instar caterpillars in free-choice food preference test.

**Keywords:** Acibenzolar-S-Methyl; Fall Armyworm; Potassium Silicate.

**Abbreviations:** ASM\_Acibenzolar-S-Methyl; DAT\_days after treatment application; DAE\_days after emergence.

**Introduction**

Food production is constantly threatened by the insect pest attack. In the corn crop, the entomofauna is quite diverse. The caterpillar *Spodoptera frugiperda* (J.E. Smith, 1797) is a key pest in corn. It is a polyphagous insect that, besides feeding on corn, has preference for other grasses such as wheat (*Triticum aestivum* L.), sorghum (*Sorghum bicolor* L.), rice (*Oryza sativa* L.), cotton (*Gossypium hirsutum* L.) and beans (*Phaseolus vulgaris* L.) (Boregas et al., 2013).

The control of *S. frugiperda* is usually carried out with chemical insecticides. In most cases, the applications are performed late in the cropping season, when the insect population is above the control level. Thus these applications are not always efficient and can cause several problems to human and the environment. The damage magnitude caused by *S. frugiperda* has induced the development of alternative methods such as the use of

biological control (Thomazoni et al., 2014), resistant plants (Toscano et al., 2016), plants with insecticidal action (Almeida et al., 2017) and resistance elicitors (War et al., 2015).

The induction of resistance is a viable alternative, being a management practice that can be done with applications of abiotic inducers. The induced defense in plants consists in the formation of mechanical barriers and/or alteration of the biochemical responses of the plant to the attack of herbivores, increasing the synthesis of proteins, which would act as toxins or as switches of the pest metabolism (Stangarlin et al., 2011).

In the literature, there are papers that seek to investigate the use of products that promote plant resistance, which interferes with the biology of pest insects, as well as their dietary habits. Previous studies have shown that the

application of silicon to corn (*Zea mays* L.) and sunflower (*Helianthus annuus* L.) plants has reduced the number of *Chlosyne lacinia saundersii* (Doubleday and Hewitson, 1849) (Lepidoptera: Nymphalidae) in *H. annuus*. The application of silicon does not directly affect the infestation of *S. frugiperda* but favors the occurrence of the predator *Dorus* spp. (Antunes et al., 2010).

Application of silicon and acibenzolar-S-methyl (ASM) in wheat plants reduced the number of nymphs, extending the duration of the pre-reproductive period and the number of honeydew drops of the green aphid *Schizaphis graminum* (Rondani, 1958) (Pereira et al., 2010).

Considering the high expenses of controlling *S. frugiperda*, the use of elicitors capable of promoting the induction of resistance is a promising alternative, since induction of resistance allows to reduce the pest population density below the economic damage level, with minimum impact on the environment, being compatible with other control methods. In this context, the present study aimed to evaluate the effect of resistance elicitors on some biological aspects and food preference of *S. frugiperda* on corn. The results of this work can provide indication of promising resistance elicitors to be used in integrated pest management of *S. frugiperda*.

## Results and Discussion

### Biological aspects of *S. frugiperda*

Significant differences between treatments were observed throughout the whole larval phase (Table 1). Acibenzolar-S-methyl (ASM) treatment showed the lowest weight at seven days. The same result was observed at 14 days along with potassium silicate + ASM, which differed from the control. The larval viability was negatively affected by ASM (68%) and potassium silicate + ASM (64%) treatments, which were statistically similar to each other. In general, ASM-fed caterpillars had the shortest duration in the larval period (17.40 days) and an extension of this phase was observed when they were fed with potassium silicate + ASM (20.18 days) (Table 1).

The results of this research corroborate with Massey et al. (2009) who verified a reduction in the weight of *Spodoptera exempta* caterpillars (Walker, 1856), fed with different diets containing silicon.

In the present study, the lower weight and viability of *S. frugiperda* caterpillars in the treatments with ASM and its combination, with potassium silicate may be the result of increased leaf tissue stiffness due to accumulation and polymerization of silicate compounds on plant cell walls (Keeping et al., 2009; Datnoff et al., 2001), hindering penetration and chewing of these tissues by insects and decreasing the leaf digestibility (Goussain et al., 2002; Körndorfer et al., 2011), consequently interfering negatively in the development of these insects.

The larval period extension observed in the treatment of potassium silicate + ASM may be related to a food substrate

nutritional inadequacy that probably extended the larval period. This type of response is desirable in integrated pest management programs, since the increase of the larval stage may favor action of natural enemies. Therefore, the caterpillars will be exposed for a longer time. In addition, they will complete fewer generations per corn phenological cycle.

Corn leaves treated with ASM and potassium silicate + ASM provided the lowest weight gain in male and female pupae, which significantly differed from the control (Table 2). However, there was no difference between the treatments for pupae viability. On the other hand, differences were observed in the pupal period duration, where the treatment potassium silicate + ASM presented the longest duration (10.46 days) compared to the control and biofertilizer treatments (Table 2).

The efficiency of foliar applied ASM and potassium silicate + ASM in *S. frugiperda* in the present study probably occurred due to their anti-nutritive effects observed in the larval stage, which directly influenced the pupae weight. The pupae lower weight may promote changes in insect fertility resulting in small moths and non-viable eggs (Rodríguez and Vendramim, 1996). This is a positive factor, since it will possibly allow a reduction of this pest and damage to the crop, besides reducing the costs of pesticide applications.

The combination of potassium silicate + ASM provided fewer eggs / female differing from the control (Table 3). However, for the eggs / oviposition / female parameter there was no difference and the values varied between 170.45 (biofertilizer) and 133.61 (potassium silicate + ASM). The longevity of *S. frugiperda* adults was similar between treatments and varied from 12.26 (potassium silicate) to 9.21 days (potassium silicate + ASM).

The insect fertility is a biological parameter, in which the silicon effect is most observed. In the present study, the observed decrease in fertility indicates a negative influence of the potassium silicate + ASM, which can result in a lower insect population density, and consequently, lower potential for plant damage. These results corroborate with studies conducted by Nascimento et al. (2018), which showed reduced viability and numbers of eggs of *S. frugiperda* females fed with silicon treated plants applied both by leaf and soil. Silva et al. (2014) observed that the application of silicon in cotton promoted a reduction in the production of eggs / female of *S. frugiperda*.

ASM acts as an activator of the plant's natural defenses against pests and diseases (Tomquelski et al., 2007). In the present study, some biological aspects of *S. frugiperda* were affected by this compound when applied alone or in combination with potassium silicate. This may be associated to a possible induction of the synthesis of defense substances causing negative effect on this insect pest. Costa et al. (2011) assessed the effect of silicon and acibenzolar-S-methyl on the induction of green aphid (*S. graminum*) resistance in wheat and found a reduction in the number of adults and nymphs in the treatments that received the combination of silicon + ASM and ASM alone, constituting

**Table 1.** Weight at 7 and 14 days, viability and larval period (days) of *Spodoptera frugiperda* fed with corn leaves treated with different resistance elicitors.

Treatments	Weight (mg) <sup>1,3</sup>		Viability (%) (n=50) <sup>1,3</sup>	Larval <sup>2</sup> Period
	7 days (n=50)	14 days (n=50)		
Control	38.71 ± 3.36 a	308.20 ± 17.05 a	92.0 ± 3.87 a	18.33 ± 0.16 b (n=46)
Acibenzolar-S-metil - ASM	16.84 ± 2.41 c	202.84 ± 18.30 b	68.0 ± 6.66 b	17.40 ± 0.17 c (n=34)
Biofertilizer	26.11 ± 3.14 b	220.80 ± 25.0 ab	80.0 ± 5.71 ab	18.56 ± 0.24 b (n=40)
Potassium silicate	22.35 ± 2.96 bc	252.72 ± 21.38 ab	74.0 ± 6.26 ab	18.42 ± 0.21 b (n=37)
Potassium silicate + ASM	17.41 ± 2.44 bc	200.16 ± 21.05 b	64.0 ± 6.85 b	20.18 ± 0.21 a (n=32)
C.V. (%)	12.34	10.59	19.17	--

<sup>1</sup>Means (± EP) followed by the same vertical letter do not differ statistically from each other by the Tukey test at the 5% probability level; <sup>2</sup>Means (± SE) followed by the same letter vertically do not differ statistically from each other by the Kruskal-Wallis test at the 5% probability level; <sup>3</sup>Data transformed into  $\sqrt{x}$ . Values in parentheses express the number of replicates per treatment.

**Table 2.** Weight (after 24 h), viability and pupal period of *Spodoptera frugiperda* fed with corn leaves treated with different resistance elicitors.

Treatments	Pupal weight <sup>1,3</sup>		Pupal viability <sup>2</sup> (%)	Pupal Period <sup>2</sup> (Days)
	Male (X ± EP) (n)	Female (X ± EP) (n)		
Control	198.10 ± 5.74 a (n=24)	189 ± 4.75 a (n=22)	93.48 ± 3.68 a (n=46)	9.34 ± 0.20 b (n=43)
Acibenzolar-S-methyl - ASM	170.45 ± 5.23 b (n=16)	174.80 ± 5.96 ab (n=29)	100.00 ± 0.00 a (n=34)	9.64 ± 0.24 ab (n=34)
Biofertilizer	195.18 ± 8.68 ab (n=18)	166.00 ± 5.73 ab (n=22)	100.00 ± 0.00 a (n=34)	9.12 ± 0.17 b (n=24)
Potassium silicate	180.50 ± 5.55 ab (n=16)	178.70 ± 5.80 ab (n=21)	92.5 ± 4.22 a (n=37)	9.75 ± 0.20 ab (n=27)
Potassium silicate + ASM	177.31 ± 3.53 ab (n=14)	163.97 ± 8.63 b (n=18)	90.32 ± 5.40 a (n=31)	10.46 ± 0.24 a (n=28)
C.V. (%)	13.10	15.02	---	---

<sup>1</sup>Means followed by the same letter vertically do not differ statistically from each other by the Tukey test at the 5% probability level; <sup>2</sup>Means followed by the same vertical letter do not differ statistically from each other by the Kruskal-Wallis test at the 5% probability level; <sup>3</sup>Data transformed into  $\sqrt{x}$ . Values in parenthesis express the number of the treatment replications.

**Table 3.** Fertility and longevity of adults (days) of *Spodoptera frugiperda* fed with corn leaves treated with different resistance elicitors.

Treatments	Eggs/female <sup>1,2</sup>	Eggs/oviposition/ female <sup>1,2</sup>	Adult longevity <sup>3</sup>
Control	972.70 ± 76.73 a	178.70 ± 15.22 a	11.88 ± 0.72 a
Acibenzolar-S-methyl - ASM	841.47 ± 71.93 ab	141.91 ± 11.84 a	10.58 ± 0.47 a
Biofertilizer	869.09 ± 123.88 ab	170.45 ± 28.49 a	11.18 ± 1.19 a
Potassium silicate	845.21 ± 61.17 ab	165.89 ± 14.00 a	12.26 ± 0.70 a
Potassium silicate + ASM	638.28 ± 767.71 b	133.61 ± 17.71 a	9.21 ± 0.85 a
C.V. (%)	21.96	23.72	----

<sup>1</sup>Means followed by the same letter vertically do not differ statistically from each other by the Tukey test at the 5% probability level; <sup>2</sup>Data converted to  $\sqrt{x}$ ; <sup>3</sup>Means followed by the same vertical letter do not differ statistically from each other by the Kruskal-Wallis test at the 5% probability level.

**Table 4.** Average number and leaf consumption of 1st instar caterpillars of *Spodoptera frugiperda*, fed with corn leaves in free-choice feeding preference test performed at 5 and 10 days after product application.

Treatments	5 days after application					Leaf consumption <sup>2,3</sup> (cm <sup>2</sup> )
	Average number of caterpillars <sup>1</sup>					
	2h	4h	6h	12h	24h	
Control	4.10 a	4.30 a	4.80 a	4.60 a	4.70 a	0.41 a
Acibenzolar-S-methyl – ASM	2.60 a	2.20 a	2.10 a	2.10 a	1.90 b	0.24 b
Biofertilizer	2.50 a	3.00 a	3.20 a	2.70 a	2.80 b	0.27 b
Potassium silicate	3.30 a	3.20 a	2.50 a	3.70 a	3.50 b	0.23 b
Potassijum silicate +ASM	2.20 a	2.60 a	2.80 a	3.20 a	2.10 b	0.23 b
C.V. (%)	-	-	-	-	-	9.73
10 days after application						
Control	4.80 a	4.90 a	5.90 a	5.20 a	5.40 a	0.44 a
Acibenzolar-S-methyl – ASM	3.80 a	4.40 ab	3.80 b	3.70 b	3.70 b	0.26 ab
Biofertilizer	1.50 b	1.80 c	2.60 bc	2.50 bc	2.60 bc	0.30 ab
Potassium silicate	1.60 b	2.90 bc	3.00 bc	2.60 bc	2.60 bc	0.34 ab
Potassium silicate +ASM	1.60 b	1.90 c	1.60 c	2.00 c	1.90 c	0.20 b
C.V. (%)	-	-	-	-	-	10.27

<sup>1</sup>Means followed by the same letter (column) do not differ statistically from each other by the Kruskal-Wallis test at the 5% level; <sup>2</sup>Means followed by the same letter (column) do not differ statistically from each other by the Tukey test at the 5% level; <sup>3</sup>For statistical analysis the data were transformed  $\sqrt{x + 0,5}$ .

**Table 5.** Average number and leaf consumption of caterpillars of 3rd instar of *Spodoptera frugiperda*, fed with corn leaves in free-choice feeding preference test performed at 5 and 10 dias after products application.

Treatments	5 days after application <sup>1</sup>					Leaf consumption <sup>2,3</sup> (cm <sup>2</sup> )
	Number of caterpillars					
	2h	4h	6h	12h	24h	
Control	5.20 a	4.30 a	4.50 a	4.70 a	5.00 a	1.13 a
Acibenzolar-S-methyl - ASM	2.10 b	2.40 b	2.60 b	2.60 b	2.20 b	0.64 b
Biofertilizer	2.60 b	2.70 b	2.70 b	2.80 b	2.90 b	0.79 ab
Potassium silicate	2.30 b	1.80 b	1.90 b	1.90 b	1.80 b	0.58 b
Potassium silicate +ASM	2.00 b	1.80 b	1.70 b	2.20 b	2.10 b	0.63 b
C.V. (%)	-	-	-	-	-	14.80
10 days after application <sup>1</sup>						
Control	4.90 a	4.50 a	4.20 a	4.60 a	4.70 a	1.26 a
Acibenzolar-S-methyl - ASM	2.30 bc	2.50 b	2.70 ab	2.80 b	2.50 b	0.71 ab
Biofertilizante	3.40 ab	3.20 ab	3.20 ab	3.00 b	3.10 b	0.68 ab
Silicato de potássio	2.00 c	2.60 b	2.60 b	2.50 b	2.60 b	0.67 ab
Silicato de potássio +ASM	0.90 d	1.10 c	1.20 c	1.30 c	1.30 c	0.56 b
C.V. (%)	-	-	-	-	-	12.98

<sup>1</sup>Means followed by the same letter (column) do not differ statistically from each other by the Kruskal-Wallis test at the 5% level; <sup>2</sup>Means followed by the same letter (column) do not differ statistically from each other by the Tukey test at the 5% level; <sup>3</sup>For statistical analysis the data were transformed into  $\sqrt{x + 0,5}$ .

these products in an alternative management tactic for this insect.

#### Feeding preference test

In the experiment conducted five days after treatment application (5 DAT) (Table 4), no significant difference among the treatments at 2, 4, 6 and 12 h were observed on 1st instar caterpillars. The difference was only detected after 24 h in the untreated leaves (control) that were preferred and consumed by the caterpillars.

At 10 DAT (Table 4), a higher preference for feeding was observed in the leaves treated with ASM and control after 2

and 4 h. The control treatment was also preferred for feeding at 6, 12 and 24 h, differing from the other treatments, while the less preferred leaves were those treated with potassium silicate + ASM, which did not differ from biofertilizer and potassium silicate treatments. The lowest leaf consumption was verified in leaves treated with potassium silicate + ASM, differing significantly from the control (Table 4).

Biofertilizers, which are rich in microorganisms (bacteria, yeasts, filamentous fungi, actinomycetes and protozoans, among others) have great bioactive activity, triggering plant defense mechanisms, whose process occurs by stimuli or signals carried by the chemical mediators in the biofertilizer,

**Table 6.** Average number and leaf consumption of caterpillars of 3rd instar of *Spodoptera frugiperda* 24 h after release, in corn leaves in no-choice feeding preference performed at 5 and 10 days after product application (DAT).

Treatments	1st Instar			
	5 DAT		10 DAT	
	Number of caterpillars	Leaf consumption <sup>2,3</sup> (cm <sup>2</sup> )	Number of caterpillars	Leaf consumption (cm <sup>2</sup> )
Control	4.50 a	0.60 a	5.00 a	0.51 a
Acibenzolar-S-methyl - ASM	4.50 a	0.41 a	4.70 a	0.44 a
Biofertilizer	4.40 a	0.40 a	4.60 a	0.47 a
Potassium silicate	4.20 a	0.42 a	4.60 a	0.40 a
Potassium silicate +ASM	3.90 a	0.33 a	4.90 a	0.39 a
C.V. (%)		15.03		12.28
	3rd Instar			
Testemunha	1.00 a	1.21a	0.90 a	1.23 a
Acibenzolar-S-methyl - ASM	0.80 a	0.63 ab	0.80 a	0.66 b
Biofertilizante	0.90 a	0.79 ab	0.90 a	0.67 b
Silicato de potássio	0.90 a	0.57 ab	0.80 a	0.52 b
Silicato de potássio +ASM	0.70 a	0.45b	0.90 a	0.43 b
C.V. (%)		12.50		14.25

<sup>1</sup>Means followed by the same letter (column) do not differ statistically from each other by the Kruskal-Wallis test at the 5% level; <sup>2</sup>Means followed by the same letter (column) do not differ statistically from each other by the Tukey test at the 5% level; <sup>3</sup>For statistical analysis the data were transformed into  $\sqrt{x + 0,5}$ .

producing defense reactions in the most distant tissues (Barbosa, 2007). The results obtained in the present research are in accordance with Alvarenga et al. (2017), which had the lowest food and consumption preference of *S. frugiperda* first instar caterpillars for corn leaves treated with silicon and gibberellic acid. Freitas et al. (2011) also observed that silicon interferes with food preference, consumption and mortality of *Plutella xylostella* (L.) caterpillars fed with cabbage leaves.

The silicon and acibenzolar-S-methyl activities were verified by Costa et al. (2007). They evaluated the effect of these products in the resistance induction to *S. graminum* green aphid in wheat and verified that the number of aphids in the control was nine times greater than in the plants that received application of silicic acid. In the free-choice test, leaf sections of plants treated with ASM and later with silicon (calcium silicate) and only with ASM were less colonized by green aphids.

We found that untreated corn leaves (control) were preferred by the 3rd instar larvae at 5 DAT (Table 5) in all evaluation periods, differing significantly from the other treatments.

A less preference was observed for the leaves treated with potassium silicate + ASM at 10 DAT (Table 5), differing from the other treatments, while the higher food preference was verified in the control, which did not differ from the biofertilizer in the evaluations performed at 2 and 4 h. In the evaluation performed at 6 h after release of the caterpillars, biofertilizer and ASM treatments did not differ significantly from the control. In the last evaluation periods, some caterpillars migrated from these treatments to the control that was preferred for feeding, differing from other treatments. Only the plants treated with potassium silicate + ASM differed from the control, presenting lower leaf consumption.

The efficiency of the potassium silicate and ASM products observed in this study is probably due to a possible presence

of suppressive or deterrent substances to the insect that inhibited or reduced feeding. It may also be related to an increase in the production of plant compounds by activation of defense enzymes such as peroxidase, polyphenoloxidase and phenylalanine ammonia-lyase. In addition, changes in plant tissue can cause lignification, suberization and production of secondary metabolites and increased production of phytoalexins; thus, promoting plant defense against insects and pests (Gulsen et al., 2010).

Almeida et al. (2015) evaluated the effect of silicon sources application on attractiveness and preference of the *Rhopalosiphum maidis* aphid (Fitch, 1856) in two corn hybrids. They found existence of resistance elicitors has deterrence effects on corn hybrids (BM 207 C and P30K64H D) in the tests of food preference with and without a choice, decreasing the aphid attractiveness in the leaves treated with calcium and magnesium silicate.

Comparing the results obtained in the experiments with a free-choice at 5 DAT, it was observed that all tested treatments (except for the control) were less preferred and less consumed by the 1st and 3rd instar caterpillars. However, a difference in attractiveness was observed for all treatments at 10 DAT, which resulted in a higher consumption, except for potassium silicate + ASM, evidencing a possible reduction of the effect of the treatments.

In the no-choice feeding preference test at 5 and 10 DAT with 1st and 3rd instar caterpillars, no differences between treatments were detected compared to food preference (Table 6).

With respect to consumption, no significant differences were observed at 5 and 10 DAT for 1st instar caterpillars, but significant differences were observed for the 3rd instar at 10 DAT between the control and the other treatments, and at 5 DAT between the control and potassium silicate + ASM.

The treatment combining potassium silicate and ASM was more efficient than the isolated treatments. This is probably

due to the product combinations which provided some resistance to the leaves, with an increase in lignin and silicon content which was not determined during the evaluation. These results corroborate with the work conducted by Assis et al. (2015) who assessed the effects of silicon and acibenzolar-S-methyl on the resistance induction of sunflower plants to *C. lacinia saundersii*. The authors observed that the silicon application alone or combined with ASM favored the accumulation of this mineral and that ASM application provided lignin increase in sunflower plants. Different modes of silicon application in rice plants (either drench or foliar) presented greater protection against *S. frugiperda*. The treated plants were five times less preferred for feeding when compared with the control leading to a reduction in the leaf consumption (Nascimento et al., 2014).

## Materials and Methods

The research was carried out at the Entomology Laboratory of the Agronomy Biotechnology Nucleus of the Maranhão State University, Campus São Luis, Brazil, under controlled conditions of temperature ( $25 \pm 2$  ° C), relative humidity ( $70 \pm 10\%$ ) and photophase of 12 h.

### Mass rearing of *S. frugiperda*

For breeding, caterpillars were collected from corn fields in the city of São Luis, state of Maranhão, northeastern Brazil, separated and individualized in glass tubes (8.5 cm in height x 2.5 cm in diameter) containing artificial diet (Grenne et al., 1976) and packed in climatic BOD type chamber with temperature ( $25 \pm 1$  °C), relative humidity ( $70 \pm 10\%$ ) and photophase of 12 h. The caterpillars remained in the glass tubes until the pupae stage, being sexed and placed in cages made of PVC tubes (10 cm in diameter x 21 cm in height), closed at their ends by Petri dishes. The PVC tubes were coated on the inside with sulphite paper. The adults were fed a 10% honey solution. The ovipositions were placed in Petri dishes (10 cm in diameter) sealed with PVC plastic film until hatching of the caterpillars. After hatching, two caterpillars were inoculated into tubes containing the diet; thus, restarting the cycle.

### Plant material and treatments

The plants used in the experiment were grown in greenhouse using four AG1051 maize seeds per pot with 6 L capacity. The substrate was composed of the soil fertilized according to soil analysis performed by the Soil Chemistry Laboratory of the Rural Engineering Technological Nucleus from Maranhão State University, Brazil. Ten days after emergence (DAE), corn plants were thinned, leaving the two more vigorous seedlings per pot. Soil moisture was maintained by means of daily irrigation.

The treatments were foliar applied at 35 DAE (V6 stage). The treatments were: T1: Control (distilled water); T2: Biofertilizer ( $25 \text{ mL.L}^{-1}$ ); T3: Acibenzolar-S-methyl-ASM ( $2 \text{ g.L}^{-1}$ ), T4: Potassium silicate ( $10 \text{ mL.L}^{-1}$ ); T5: Potassium silicate ( $10 \text{ mL.L}^{-1}$ ) + ASM ( $2 \text{ g.L}^{-1}$ ).

The biofertilizer was produced by anaerobic fermentation in a 500 L PVC box with the following constituents: cattle manure (50 kg), cow's milk (10 L), crushed sugar cane (2 kg),

natural phosphate (1 kg) wood ash (1 kg) and boric acid (1 kg). The solution was filled with water up to 500 L and had nitrogen sources (N-NH<sub>4</sub> + and N-NO<sub>3</sub>-), N<sub>total</sub> (12.7), phosphorus ( $18 \text{ g.kg}^{-1}$ ), potassium ( $1.5 \text{ g.kg}^{-1}$ ) and pH of 6.6.

The treatment application was carried out via foliar using an adjustable spray jet pressure, applying to the whole plant with 20 mL of the solution per plant. Each plant base was protected with a polyethylene bag to avoid treatment deposition on the soil, avoiding product over-dosing. Fifty pots / treatments were used to study the biology and 10 pots / treatments for the food preference tests.

Corn leaves to be used in the bioassays were removed at five days after treatment application. This period corresponded to 40 days after emergence, at which time the crop is more susceptible to the fall armyworm attack (Cruz and Turpin, 1982). The collected leaves were taken to the laboratory, disinfected in water solution and sodium hypochlorite (10%) for 5 min, washed in running water and placed to dry on paper towel at room temperature.

### Biological aspects of *S. frugiperda*

In this bioassay, the treated leaves were collected daily and taken to the laboratory to feed *S. frugiperda*. The experiment was carried out in a completely randomized design with five treatments and 50 replications per treatment. The experimental plot was composed of a plastic cup (100 mL) coated inside with filter paper moistened with distilled water to avoid leaf dryness, a piece of corn leaf (5 cm long) and a fresh-hatched larvae.

The caterpillar transfer to the cups was carried out with a sterilized soft bristles brush. The food was changed daily, according to each treatment until the caterpillars reach the pupa stage. After 24 h, the pupae formed were collected, separated by sex, weighed on analytical balance and placed in PVC tubes (10 cm in diameter x 21 cm in height), closed at their ends by Petri dishes. Adults emerged on the same date were grouped in pairs in a ratio of 1:1, remaining in PVC cages of the same dimensions already mentioned. The inside of the cages was coated with paper sheets to serve as a substrate for the ovipositions, which were collected daily, identified and stored in air-conditioned chambers for later counting of the eggs; thus, to avoid hatching of the caterpillars inside the cages.

The biology evaluations were performed 24 h after the experiment was begun. They were: Larval phase (weight at 7 and 14 days, duration and viability); Pupal stage (weight after 24 h, duration and viability) and Adult phase (number of eggs / female, number of eggs / oviposition / female and longevity of adults).

### Food preference test

For the *S. frugiperda* free-choice and no-choice food preference tests two experiments were carried out. The first at 5 and the second at 10 days after treatment. Applications were made with 1st and 3rd instar caterpillars. In the free-choice food preference test, in trials with first instar caterpillars, leaves cut with a 3 cm<sup>2</sup> punch were used and arranged equidistantly in Petri dishes (19 cm in diameter). In the 3rd instar caterpillar test, 5 cm<sup>2</sup> leaves were used, placed in boxes 31 x 31 x 3.5 cm and arranged in

the same condition already described. In both experiments, 20 *S. frugiperda* caterpillars were released and the containers were sealed with PVC plastic film.

The experiment was laid out in randomized complete block design with five treatments and 10 replications. The food preference was evaluated by counting the number of caterpillars present in each treatment after 2, 4, 6, 12 and 24 h of the release. Foliar consumption was determined 24 h after insect release. It was calculated by the difference between the leaf area, before being supplied to the caterpillars, and leaf area remaining after the end of the experiment. The leaf sections were oven dried at 60 °C for one day and weighed on analytical balance.

The non-choice food preference test was conducted with 1st and 3rd instar larvae in two experiments both laid out in randomized complete design with five treatments and ten replications. Each experiment was composed of Petri dishes (8 cm in diameter) coated with filter paper moistened with distilled water, where a leaf disc was deposited with the dimensions already described, with the release of five first instar caterpillars. The same procedure was performed for 3rd instar caterpillars. However, only one caterpillar was released. In both experiments the plates were sealed with PVC film. After 24 h, the number of caterpillars in each treatment was assessed and foliar consumption was measured as described in the free-choice feeding preference test.

#### Statistical analysis

Data on the larval and pupal periods, pupal viability, adult longevity and food preference were compared by Kruskal-Wallis non-parametric test. The other biological parameters and leaf consumption were transformed into  $\sqrt{x}$  and  $\sqrt{x + 0,5}$ , respectively in order to perform the Analysis of Variance by the F test. The means were compared by the Tukey test at 5% significance. Statistical analysis was performed using the software Assistat, version 7.7 (Silva and Azevedo, 2016).

#### Conclusion

Acibenzolar-S-methyl (ASM) when applied alone or combined with potassium silicate induces resistance in corn plants adversely affecting *S. frugiperda* development. Leaves treated with potassium silicate + ASM were less preferred and consumed by 1st and 3rd instar caterpillars in a free-choice feeding preference test. The potassium silicate + ASM treatment showed residual effect up to 10 days after application for caterpillars of 1st instar in the free-choice feeding preference test and of the 3rd instar in the no-choice feeding preference test.

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