IMPROVING THE PALATABILITY OF A *Bacillus thuringiensis*-BASED INSECTICIDE TO INCREASE TOXIC ACTIVITY IN *Spodoptera frugiperda* (LEPIDOPTERA: NOCTUIDAE) LARVAE

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ABSTRACT: The colony of *Spodoptera frugiperda* was established on an artificial diet based on soy flour. The insects were maintained in controlled humidity, temperature, and photoperiod conditions. The average number of valuable larvae for bioassay was approximately 2000 individuals for each generation. The feeding stimulants were obtained by sowing, harvesting, drying, and grinding maize, sorghum, alfalfa, tomato, soybean, and pinto bean plants in fine powder and commercial safflower oil. The *Bacillus thuringiensis-based* insecticide was mixed with the corresponding feeding stimulant. The insecticide applications were carried out at the greenhouse level on maize plants with artificial infestation. The results indicated that the lowest percentage of leaf damage was observed with insecticides containing the safflower oil and pinto bean as feeding stimulants. The highest percentages of leaf damage were observed in those insecticides without feeding stimulants.

KEYWORDS: Feeding stimulant, insecticidal activity, fall armyworm, palatability, toxic activity

INTRODUCTION

Insects of the genus *Spodoptera* are native to tropical regions of the Western Hemisphere, from the United States to Argentina. In the case of *S. frugiperda*, its life cycle lasts approximately 30 days. The eggs are dome-shaped and flattened; the total egg production per female is 1500 to 2000, deposited in a monolayer on the foliage. The larva has six instars and measures up to 34 mm, forming a hard brown pupa that lasts 8 to 9 days. The adult is a moth with a wingspan of up to 40 mm; they are nocturnal and very active during the summer (Capinera, 2014). This pest is resistant to many chemical insecticides, as reported elsewhere, so one of the best options for controlling it is the application of biological insecticides.

A biological insecticide is a formulation designed explicitly against a particular pest, considering the host crop and other factors such as feeding habits and the biological cycle of the insect, making it highly effective (Rosas-García et al., 2009). The fundamental part of a biological insecticide made with the *Bacillus thuringiensis* bacteria is that the larva must ingest it to be effective. The spore-crystal complex of *B. thuringiensis*, which acts as the active ingredient, must be ingested by the larva, thus allowing these crystalline proteins to bind to the receptor molecules of the midgut through solubilization and proteolysis processes, forming pores and causing emptying of the intestinal contents, which ultimately induces the insect to death (Schnepf et al., 1998). Due to the mode of action of these insecticides, adjuvants, such as feeding stimulants, certainly play a significant role in the insecticidal activity of formulations. Therefore, their careful selection gives an essential advantage to the ingested formulations, with broad preference by the insect and, in some cases, with greater preference over its natural substrate (Rosas-García et al., 2009). Feeding stimulants are especially important in *Spodoptera* species, mainly foliage-eating pests because they can detect non-volatile compounds through their sense of taste (Kasubuchi et al., 2018; Zhou et al., 2009). The gustatory or contact chemosensory system of insects plays a crucial role in immediate responses to food (Zhou et al., 2009). In lepidopteran insects, taste neurons are located mainly on the ventral side of the labrum, maxillary palps, and galea (Schoonhoven & van Loon, 2002). The specificity of taste neurons observed as a response to certain plant compounds is genetically determined, but food selection behavior can be modified by diet (Bernays & Singer, 2005; Renwick & Huang, 1995)). For this reason, the feeding stimulants used in the development process of an insecticidal formulation must be carefully selected since they must favor the insect's taste, making it palatable and ingestible to be effective. This work aimed to improve the palatability of a biological insecticide to ensure ingestion by the insect and its toxic activity.

MATERIALS AND METHODS

Establishment of the insect colony: The insect colony was established from *Spodoptera frugiperda* pupae in a room with a controlled temperature of 26°C ± 1°C, photoperiod of 14:10 L.D, and relative humidity of 60% ± 5%. The pupae were placed in emergency chambers for adult emergence, made up of 4-liter plastic buckets lined on the inside with absorbent paper towels. Inside, a 30 ml plastic cup with cotton impregnated with a 10% sucrose solution was placed for adult feeding, and the bucket was covered with a 30 x 30 cm cheesecloth secured with a rubber band. The adults laid their eggs on the cheesecloth and absorbent paper for seven days. Cheesecloth and absorbent paper were withdrawn from the bucket, carefully folded, and placed inside a brown paper bag for the egg incubation. A 120 ml plastic cup containing an artificial diet was also placed inside the brown paper bag to allow newborn larvae to feed once the eggs hatched. Then, these larvae were taken with a camel hair brush and placed individually in 30 ml plastic cups with a fresh diet to continue their development (Fig. 1). The artificial diet was prepared according to (Rosas-García et al., 2009).

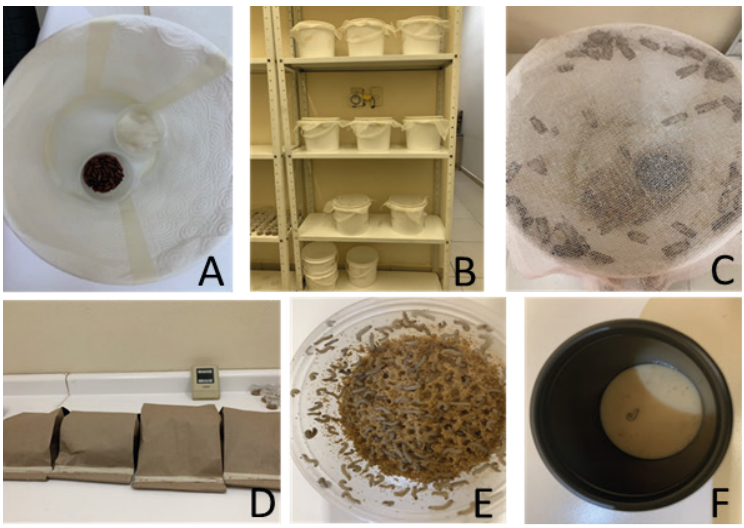

Fig. 1. Rearing method for *Spodopera frugiperda* colony. A), B) and C) emergence chambers for pupae and adult development, D) egg incubation, E) newly hatched larvae feeding on an artificial diet, F) individual larvae on an artificial diet.

Cultivation of plants for feeding stimulant production: Maize, sorghum, alfalfa, tomato, soybean, and pinto bean seeds were sown in 30 x 30 cm black plastic bags with garden soil in a greenhouse. For each type of plant, 100 bags were planted, and watering was carried out twice a week until the plants reached approximately 30 to 40 cm in height. Grown plants were cut from the root base and placed in the aeration beds covered with chicken wire netting mesh for the drying process for 7 to 10 days (Fig. 2). For complete dehydration; the dried plants were placed on trays in a forced flow drying oven at 40°C for 3 to 5 days. The fully dehydrated plants were first ground manually, wearing gloves, and then placed in a blender (Black+Decker, Crushmaster Pro). The resulting powder was weighed on a grain scale and stored in plastic containers at room temperature.

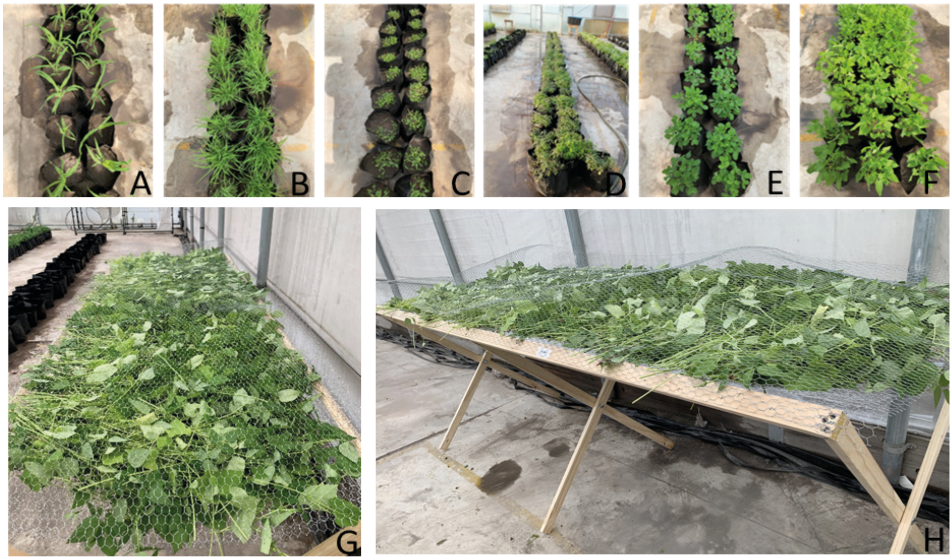

Fig. 2. Growing and drying plants. A) maize, B) sorghum, C) alfalfa, D) tomato, E) soybean, F) pinto bean, G), and H) aeration beds.

Granular feeding stimulant preparation: The feeding stimulants in the form of granules were prepared with 25 g of Capsul™, 25 g of bovine gelatin, 2 g of the feeding stimulant, and 25 ml of distilled water. The ingredients were mixed manually with a spatula until crumbs were formed. Each mixture was placed in an aluminum tray inside a forced flow drying oven at 40-45°C temperature for 48 h until dehydration. Once dried, the mixtures were ground with a mortar to obtain the granular feeding stimulants (FE): sorghum (SFE); maize (MFE), tomato (TFE), alfalfa (AFE), pinto bean (PBFE), soybean (SBFE), safflower oil (SOFE), and control (C). The granules were stored in airtight plastic containers at room temperature for later use.

Feeding preference bioassays: Two-choice bioassays were conducted in disposable 5 cm diameter Petri dishes with the bottom covered with a mixture of plaster of Paris™ and activated charcoal in a 15:1 ratio. Twenty-eight combinations of granular feeding stimulants were tested. In each dish, 0.2 g of granules to be compared were deposited as piles in opposite places. As controls, fresh leaves of the plant or Capsul™ were used. Five repetitions were performed for each confrontation. In each repetition, ten two-day-old larvae of *S. frugiperda* were placed in the center of the dish and allowed to feed for 16 h at 28ºC in complete darkness. After this, the Petri dishes were frozen at -20°C for one or two days, then the number of larvae on each pile was recorded. The data obtained were subjected to the non-parametric Mann-Whitney *U* test.

Toxic activity bioassays: The artificial infestation was conducted in 30 cm height maize plants with ten neonate larvae per plant and ten repetitions under greenhouse conditions. Each larva was placed in the plant with a camel hair brush and allowed to adapt for 24 h. Subsequently, the various mixtures of the *Bacillus thuringiensis*-based insecticide were prepared with the corresponding feeding stimulant at 4%. Immediately after, the mixture was applied with a spray pump (Fig. 3). The seven treatments tested were: control C, an insecticide with MFE, an insecticide with TFE, an insecticide with PBFE, an insecticide with SOFE, an insecticide with all FE, and insecticide without FE. The trial lasted seven days, and leaf damage was visually evaluated. The results were analyzed by ANOVA and Tukey's test for mean comparison with P≤0.05 using SPSS 18.0 (SPSS, Inc. Chicago, IL).

Fig. 3. Production and application of the *B. thuringiensis*-based insecticide with each feeding stimulant.1) and 2) insecticide production, 3) filling spray pump, 4) maize plants infested, 5) insecticide applied to maize leaves.

RESULTS

The insect colony produced an average of 2000 larvae per generation. One hundred grown plants of each different seed yielded the following yields in the dehydrated form: maize 325.94 g, sorghum 676.94 g, tomato 435.54 g, soybean 500.74 g, alfalfa 388.94 g, and pinto bean 786.94 g. The results indicated that *S. frugiperda* larvae accepted all the tested feeding stimulants. However, the most accepted feeding stimulants were maize, tomato, pinto beans, and safflower oil (F = 12,483, df = 6/243, P≤0.05) (Table 1).

Comparison	Treatment									Larvae total number*	
	SFE	MFE	TFE	AFE	PBFE	SBFE	SOFE	C	P≤ 0.05	R	NR
1	25	17							NS	42	8
2	25		21						NS	46	4
3	27			17					**	44	6
4	21				26				NS	47	3
5	23					24			NS	47	3
6	29						18		**	47	3
7	21							26	NS	47	3
8		33	14						**	47	3
9		28		16					**	44	6
10		12			34				**	46	4
11		20				29			**	49	1
12		31					17		**	48	2
13		26						20	NS	46	4
14			32	16					**	48	2
15			11		35				**	46	4
16			32			12			**	44	6
17			11				35		**	46	4
18			34					16	**	50	0
19				22	18				NS	40	10
20				13		3			**	16	34
21				14			23		NS	37	13
22				14				20	**	34	16
23					22	7			**	29	21
24					16		13		NS	29	21
25					20			15	NS	35	15
26						11	27		**	38	12
27						20		13	NS	33	17
28							28	14	**	42	8

A two-choice bioassay was conducted for each part of the treatment (granular feeding stimulants) to determine the most palatable to larvae. The total number of larvae in each comparison = 50. P values obtained from Mann-Whitney *U* test. NS, no significance, ** high significance. *Larvae total number R= responders, NR= non responders.

Table 1. Two-choice bioassays to determine feeding preference in *Spodoptera frugiperda* larvae.

These four feeding stimulants were used to improve the insecticide palatability. The insecticide was more efficient when it contained pinto beans or safflower as feeding stimulants (Table 2). Additionally, there is a significant difference between the activity of the insecticide containing all of the feeding stimulants and the insecticide without feeding stimulants. According to these results, the presence of pinto beans and safflower oil as feeding stimulants favored ingestion by larvae since less leaf damage was observed.

Treatment	N	Media percentage of damaged leaves ± SE*
Insecticide + TFE	42	16.74 ± 3.545 c
Insecticide + PBFE	31	5.00 ± 1.435 a
Insecticide + SOFE	34	3.71 ± 1.480 a
Insecticide + MFE	32	21.56 ± 3.401 d
Insecticide with all FE	39	7.23 ± 2.088 ab
Insecticide without FE	41	10.39 ± 2.438 b
Control	31	37.74 ± 6.332 e

N: number of samples. * Means with the same letter in a column are not significantly different, Tukey Test, $P < 0.01$.

Table 2. Average value of the percentage of damage caused in maize leaves by *S. frugiperda* larvae after insecticide application with feeding stimulants.

DISCUSSION

The use of feeding stimulants in insecticidal formulations has been studied for many years to increase insecticidal activity (Barry & Polavarapu, 2004; Bartelt et al., 1990; Bernklau & Bjostad, 2008; Bernklau et al., 2018; Farrar & Ridgway, 1995; Ji-Hong et al., 2000; Navon et al., 1987). In the case of insecticides that must be ingested to be effective, such as those based on *B. thuringiensis*, the use of feeding stimulants plays a fundamental role since the insecticide itself is not palatable to the insect as has been reported (Rosas-García et al., 2004; Rosas-García & de Luna-Santillana, 2006), and for the control of a pest the insecticide must be ingested even more preferably than its natural substrate. *Spodoptera frugiperda* is a generalist pest whose larvae feed on various plants. With this idea in mind, these palatable plants can be used as feeding stimulants to help increase the toxic activity of an insecticidal formulation. Results indicate interestingly that some plants can act as feeding stimulants, offering an essential effect in the insecticidal activity of insecticides that work against *S. frugiperda*. As a generalist, *S. frugiperda* feeds on any of the plants tested; however, the insect does not prefer them equally. Some of the plants used in this study are not the primary target for *S. frugiperda*. Larvae preferred to ingest insecticidal mixtures containing pinto bean and safflower oil, which is assumed by the low percentage of maize-damaged leaves observed with these treatments. Also, there is a difference in the rate of damaged leaves between insecticides containing all the feeding stimulants and insecticides without any feeding stimulant. Combining several feeding stimulants in one insecticidal formulation may favor ingestion by the larva. However, compared with the other individual feeding stimulants, the result could indicate a confusing effect of smell and taste in the larvae.

This study demonstrated that some of these plants, used as feeding stimulants, help increase insecticide ingestion, causing death to the larva and less damage to the crop. Pinto beans and soybeans were preferred over maize and other powdered plants. Pinto bean and safflower oil were more preferred than tomato. However, tomato was preferred over alfalfa, soybean, and the control. The safflower oil and pinto beans outperformed soybean preference.

Since insecticides based on the spore-crystal complex of *Bacillus thuringiensis* have an avoidance or deterrent effect on *S. frugiperda* larvae, the addition of dehydrated plant powder helps improve the insecticide's taste and smell so that the larva ingests it more easily, causing less damage to the plant. In addition, including feeding stimulants not only ensures that the insect eats the insecticide, but in some cases, this extensive ingestion has reduced the active ingredient content in these products, thus offering the same efficacy in control.

Over time, many products have been tested as feeding stimulants, some with greater success than others. However, they are still considered a fundamental element in the development of biological insecticides that must be ingested by insect pests.

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