

Assessment of insecticide mortality on fall armyworm (*Spodoptera frugiperda*) between locations and modes of insecticide entry

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Abstract

Spodoptera frugiperda is a major pest of maize and plants expressing insecticide proteins from *Bacillus thuringiensis*. Control programs based on Bt maize events have increased inefficacy for this pest, often demanding insecticide sprays as a complementary measure. The aim of this work was to evaluate larval mortality in two *S. frugiperda* populations submitted to different insecticide sprays and modes of entry into the insect's body. Experimental design was completely randomized with a factorial scheme 4×2×2, plus one check. Four insecticides [azadirachtin (12 g a.i. L⁻¹), lambda-cyhalothrin (50 g a.i. L⁻¹), teflubenzuron (150 g a.i. L⁻¹), flubendiamide (480 g a.i. L⁻¹)], two modes of entry for each insecticide (topic contact and ingestion) and two *S. frugiperda* populations (from Constantina and Sertão) were tested. The mortality of individuals was assessed daily for 15 days after treatment spraying. The insecticides teflubenzuron and flubendiamide presented the highest mortality levels of *S. frugiperda*, disregarding the differences between tested populations and modes of entry. Contamination by ingestion resulted in higher mortality, especially for teflubenzuron and flubendiamide. The results suggest that *S. frugiperda* from Constantina are less susceptible to the insecticides evaluated.

Keywords: botanical insecticides; chemical control; fall armyworm; insecticides mode of entry; *Zea mays*.

Abbreviations: Bt *Bacillus thuringiensis*; IPM_Integrated Pest Management; IRM_Insect Resistance Management; NPK_nitrogen-phosphorus-potassium; V8_plant in the vegetative stage with 8 leaves; V10_plant in the vegetative stage with 10 leaves; F1_first generation of descendants.

Introduction

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae), poses a major threat to maize crops in Brazil (Ávila, 2015) and other South American countries (Pogue, 2002). *S. frugiperda* feeds on maize plants throughout its whole growing cycle, from seedling to flowering and kernel development stages, resulting in yield losses that range from 17.7 to 55.6% according to growth stage and maize genotype (Cruz, 2008). Intensive maize cultivation during two cropping seasons in Brazil (first crop from August to January, second crop from January to April), mounting up to over 17 million hectares grown with the crop (Conab, 2019), favours growth and development of *S. frugiperda* populations. While first season maize typically presents lower infestations of fall armyworm (Farias et al., 2014), farmers from southern Brazil and especially from Rio Grande do Sul state (where the majority of maize is grown in the first season) have faced severe losses due to *S. frugiperda* attack.

Control programs based on the use of transgenic maize plants expressing insecticide proteins from *Bacillus thuringiensis* Berliner have displayed increasing inefficacy for *S. frugiperda*. Since the legalization of transgenic maize cultivation in Brazil in 2007, six Bt proteins have been introduced (isolated or combined) in commercial maize events, and the management of fall armyworm in the country has relied heavily on the use of Bt maize. By disregarding Integrated Pest Management (IPM) and Insect Resistance Management (IRM) basic concepts, this inadequate approach has favoured the selection of *S. frugiperda* populations resistant to some Bt events, thus demanding insecticide sprays for damage containment (Burtet et al., 2017). Although representing a resurgence of former control strategies (prior to the advent of Bt maize), chemical insecticides can be properly combined with Bt technology in a novel approach, increasing control efficacy for the fall armyworm.

Upon eclosion, *S. frugiperda* larvae lodge itself inside the maize whorls and start to feed on the folded leaves (Busato et al., 2002), reducing the chances of direct contact with insecticide sprays, and consequently, compromising control efficacy (Gassen, 1996). Additionally, control failures can arise as a result of genetic traits in the infesting populations: successive sprayings of similar products, upon *S. frugiperda* individuals expressing alleles of resistance to certain insecticides, inevitably leads to susceptibility decrease (Onstad and Gassmann, 2014). Cases of *S. frugiperda* resistance to the insecticides lambda-cyhalothrin (Diez-Rodríguez and Omoto, 2001), chlorpyrifos (Carvalho et al., 2013), spinosad (Okuma, 2015) and lufenuron (Nascimento et al., 2016) have been reported on Brazilian populations. The mode of entry of an insecticide into the insect's organism is a key factor on raising larvae mortality, especially in management programs that combine insecticide sprays and Bt technology. Most insecticide molecules are absorbed by the insects through direct contact in the tegument, ingestion, or vapour effect (Matsumura, 1985). In the contamination by direct contact, the insecticide penetrates the tegument either from drops sprayed over the insect or spread over the leaf surface and walked upon (i.e. tarsal contact). In the contamination by ingestion, the insect acquires the product orally by feeding on plant tissues and sap. Finally, in the contamination by vapour effect, the insecticide penetrates the insect's respiratory openings (i.e. spiracles) in vaporious phase. Some insecticide products display more than one mode of entry simultaneously (Rego, 2016). Therefore, the objective of this study was to evaluate the effect of different insecticides and its modes of entry on third instar larvae from two *S. frugiperda* populations of southern Brazil.

Results and discussion

Our results show that *S. frugiperda* mortality varies according to the origin site of the population, the type of insecticide sprayed and its mode of entry into larvae body. There was significant interaction among the three factors (origin, insecticide and mode of entry), and statistical difference between them and the check (Table 1). Data regarding survival time of *S. frugiperda* according to origin, insecticide and mode of entry are shown in Figure 1, while the speed of larval mortality for each treatment is presented in Figure 2.

Entry mode of azadirachtin

The insecticide azadirachtin presented low larval mortality, regardless of mode of entry and origin site (Figures 1 and 2). The active ingredient of this product mimics the ecdysis hormone of insects, affecting the moulting process and, at high doses, interrupting it. As a consequence, azadirachtin promotes higher mortality levels at immature life phases of the insects (Martinez, 2008), and has to be ingested by the larvae to perform its growth regulator action. In addition, it presents anti-feeding and repellent effects, reducing food consumption and egg laying in the treated areas, respectively (Martinez, 2008). Viana et al. (2006), Lima et al. (2008) and Ribeiro et al. (2012) found high mortality levels among newly hatched larvae treated with azadirachtin.

Viana and Prates (2003) observed that the active ingredient caused high mortality, when ingested by the larvae through maize leaves but, presented no effect when directly sprayed over the insects. The low mortality obtained in our study is probably linked to the insects' size (third instar), since control is more efficient at early development stages. Low concentration of active ingredient can also be related, although the dose used was as prescribed by the manufacturer.

Entry mode of lambda-cyhalothrin

Lambda-cyhalothrin provided low mortality of *S. frugiperda* larvae, at both modes of entry and origin sites (Figure 1). This outcome might be a result of low susceptibility in the populations and intensive, high-dose use of the product in the region during the last years, as attested by the reports of *S. frugiperda* resistance to lambda-cyhalothrin (e.g. Diez-Rodríguez and Omoto, 2001). In addition, no significant difference was observed between the modes of entry. Since the product is recommended by the manufacturer as efficient through both contamination routes (contact and ingestion), this lack of difference can be related to the overall low mortality provided by the insecticide.

Entry mode of flubendiamide

The insecticide flubendiamide presented high mortality levels at both *S. frugiperda* populations, when acquired by the insects through ingestion (Figure 1). Similar results were found by Oliveira and Nunes (2017), who observed 100% of *S. frugiperda* mortality in soybean three days after spraying, and by Bellettini et al. (2011), who obtained control efficiencies equal or higher than 80% in cotton plants. The high efficiency of this insecticide molecule is partly explained by its novelty (released in 2007) and also by its distinct mode of action, when compared to other insecticides, consequently reducing selection pressure. Accordingly, no resistance of *S. frugiperda* to this molecule has been reported to-date (Ribeiro, 2014). Although prescribed as efficient through both modes of entry, flubendiamide provided mortality levels significantly higher, when acquired by ingestion. This is probably due to the longer time, during which the insect remained exposed to contamination, when the insecticide is sprayed rather than added into the insect's diet. The direct contact occurs only once and control chances are considerably reduced.

Entry mode of teflubenzuron

Teflubenzuron caused high mortality of *S. frugiperda* larvae, when acquired by ingestion, especially at 10 days after spraying and onwards (Figures 1 and 2). This outcome is probably related to the mode of action of this insecticide, since growth regulators display no knockdown effect and demand a longer time to exert full action. While ingestion was the mode of entry that most favoured teflubenzuron's action (as also observed for flubendiamide), contamination by contact also resulted in fairly high mortality rates, being an advantage of this product.

Table 1. Analysis of variance (ANOVA) on all factors (origin site, insecticide sprayed and mode of entry) and their respective interactions.

| | DF ¹ | SS | MS | F-value | P-value |
|--------------------------------------|-----------------|----------|---------|---------|---------|
| Origin site | 1 | 4.22177 | 4.22177 | 44.0412 | 0.0 |
| Insecticide | 3 | 20.81205 | 6.93735 | 72.3699 | 0.0 |
| Mode of entry | 1 | 5.3956 | 5.3956 | 56.2865 | 0.0 |
| Origin * Insecticide | 3 | 1.26293 | 0.42098 | 4.3916 | 0.0048 |
| Origin * Mode of entry | 1 | 1.21466 | 1.21466 | 12.6713 | 4e-04 |
| Insecticide * Mode of entry | 3 | 8.06063 | 2.68688 | 28.0293 | 0.0 |
| Origin * Insecticide * Mode of entry | 3 | 1.09132 | 0.36377 | 3.7948 | 0.0107 |
| Check vs Factorial | 1 | 2.81281 | 2.81281 | 29.343 | 0.0 |
| Statistical residual | 323 | 30.96264 | 0.09586 | | |
| Total | 339 | 75.83441 | 0.2237 | | |

¹ DG: degree of freedom; SS: sum of squares; MS: mean square.

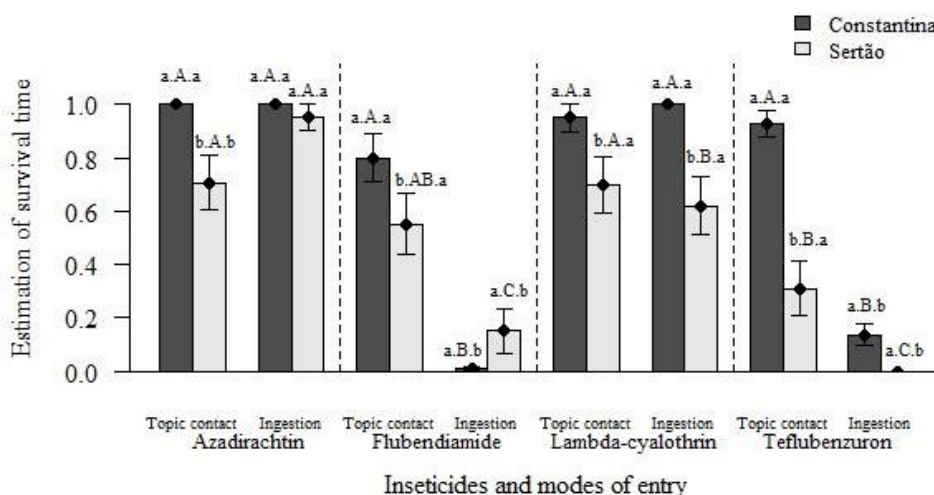


Fig 1. Estimated survival time of *S. frugiperda* larvae according to origin site, insecticide sprayed and mode of entry. Y-axis: survival time 1 = 100% of survival during the evaluated period; survival time 0 = no survival during the evaluated period. Tukey's test: a.A.a = first letter (lowercase) compares between origin sites, under the combination of factors insecticide and mode of entry; second letter (uppercase) compares between insecticides, under the combination of factors origin site and mode of entry; third letter (lowercase) compares between modes of entry, under the combination of factors origin site and insecticide.

Table 2. Trade name, active ingredient, dose and mode of action of the insecticides evaluated in relation to larval mortality, in two different *S. frugiperda* populations.

| Trade name | Active ingredient | Dose (g a.i. ha ⁻¹) | Mode of action |
|---------------|--------------------|---------------------------------|---|
| Azamax 12 EC | Azadirachtin | 4.8 | Growth regulator (feeding inhibitor and egg laying repellent) |
| Karate 50 EC | Lambda-cyhalothrin | 7.5 | Sodium channel modulator |
| Nomolt 150 SC | Teflubenzuron | 11.25 | Chitin biosynthesis inhibitor |
| Belt 480 SC | Flubendiamide | 60 | Ryanodine receptor modulator |
| Check | - | - | - |

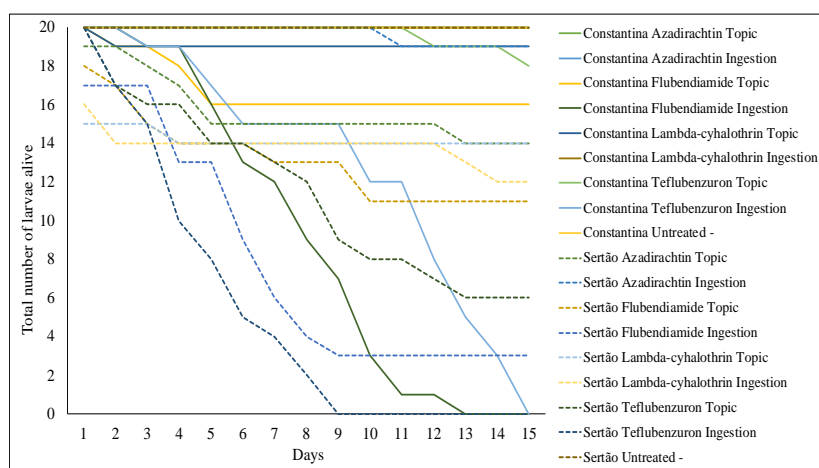


Fig 2. Mortality speed of *S. frugiperda* larvae according to origin site, insecticide sprayed and mode of entry into the insect's body.

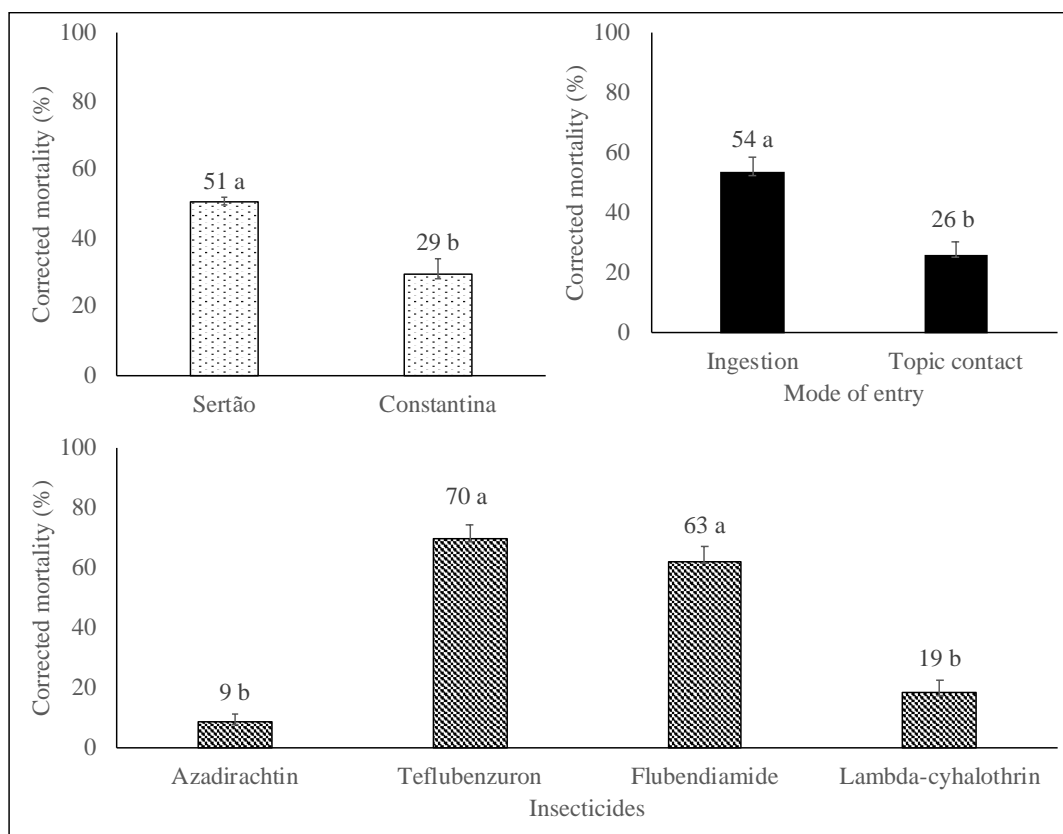


Fig 3. Means of *S. frugiperda* larval mortality corrected by Abbott (1925), for the factors: (a) origin site; (b) mode of entry; and (c) insecticide sprayed. Means followed by the same letter do not differ by Tukey's test ($P > 0.05$).

Mortality related to different origins of *S. frugiperda* populations

Overall, treatments flubendiamide + ingestion, teflubenzuron + ingestion and azadirachtin + ingestion presented the highest mortality levels and did not differ between the two armyworm populations. For the remaining treatments, larvae mortality was significantly lower in the population from Constantina, meaning that the two municipalities host the same species with different susceptibility levels to the same insecticide doses. Variations on insecticide susceptibility between populations geographically apart are frequently linked to management practices, with overdosing and inadequate use (e.g. without rotation of modes of action) increasing selection pressure and favouring the survival of more adapted, less susceptible individuals. Furthermore, many insect species are equipped with detoxification metabolic routes powered by enzymatic processes, by which certain amounts of insecticide can be converted into non-toxic compounds or readily expelled from the insect's body, preventing its action at the target site (Beckel et al., 2006).

Conclusions and implications for pest management

The means of larval mortality compared between insecticides, origin sites and modes of entry are shown on Figure 3. Flubendiamide and teflubenzuron provided the highest mortality levels, differing significantly from azadirachtin and lambda-cyhalothrin. The mode of entry by

ingestion was proved superior than contamination by contact. The population of *S. frugiperda* from Constantina presented mortality levels significantly lower than the population from Sertão. By providing better insights into the efficiency of different contamination routes for each insecticide and the variations on susceptibility between different *S. frugiperda* populations, the results presented here will help maize growers to reach more assertive decisions inside their production systems. Accordingly, further studies should explore the low susceptibility of *S. frugiperda* populations from Constantina and the causes behind this outcome to safeguard the control efficiency of the insecticides currently employed. Since genetic traits of the infesting populations are closely linked to insecticide susceptibility, deepening our knowledge on the theme will help improve current management strategies for *S. frugiperda* in maize crops and develop new ones.

Materials and methods

Collection sites and laboratory rearing of *S. frugiperda*

Larvae and eggs of *S. frugiperda* were collected in second crop maize fields at the municipalities of Sertão (28°02'41"S, 52°08'54"W), in February 2018, and Constantina (27°42'01"S, 53°00'51"W), in March 2018, both located in Rio Grande do Sul state, Brazil. The collects were supported by the project A8FA513, registered at the National System for Genetic Patrimony Management and Associated Traditional Knowledge (SISGEN). The maize field of Sertão comprised the hybrid *Agroceres* AG9000 Pro 3, sowed at January 20th, 2018, using 300 kg ha⁻¹ of the fertilizer NPK 05.20.20, 3 seeds m⁻¹ of plant density, and 0.47 m of spacing

between rows. The maize field of Constantina comprised the hybrid Agrocere AG9000 Pro 3, sowed at February 8th, 2018, using 400 kg ha⁻¹ of the fertilizer NPK 05.20.20, 3 seeds m⁻¹ of plant density, and 0.47 m of spacing between rows. The maize plants were at growth stages V8 and V10 at the moment of collection, in Sertão and Constantina, respectively. The insects sampled were sent to the Laboratory of Entomology of the Federal Institute for Education, Science and Technology of Rio Grande do Sul (IFRS – Campus Sertão) for analyses.

Newly hatched larvae were transferred to 50 mL plastic cups containing artificial diet made from beans, wheat germ and barley yeast (adapted from Greene et al., 1976). Pupae were lodged on Petri dishes containing vermiculite substrate and placed on square wooden cages, covered with “voil” fabric to shelter the adult moths. Adults were fed a honey-based solution (10%), imbued on cotton and replaced every two days to avoid contamination. Conventional maize plants were also placed inside the cages in Becker pots with water to serve as oviposition sites. The eggs were then transferred to 100 mL plastic cups containing filter paper moistened with water to maintain internal humidity. The hatched larvae (generation F1) were again submitted to artificial diet until reaching proper size for treatment spraying.

Treatments and experimental design

Treatments were arranged in a completely randomized design with 20 replicates in a factorial scheme 4×2×2, plus one check. Factor A comprised four insecticide products with distinct modes of action in the insect’s body (Table 2). Factor B comprised two modes of entry for the insecticide into the insect’s body – topic contact and ingestion. Factor C comprised of two origin sites of the populations evaluated – Constantina and Sertão.

Mortality bioassay based on the mode of entry

For the mortality bioassay on contamination by contact, third instar larvae (8-10 mm of size) were placed in Petri dishes. The insecticides were sprayed directly over the larvae (40 cm of height), using a 1.5 L manual sprayer (nozzles type “hollow cone”, model JA 0.5; Jacto, Pompéia, São Paulo, Brazil), 100 L ha⁻¹ of spray volume and 1 m s⁻¹ of flow rate. The larvae were then transferred to 50 mL plastic cups containing artificial diet.

For the mortality bioassay of contamination by ingestion, the artificial diet was treated with the respective insecticides. Each 50 mL plastic cup received 5 mL of the artificial diet and was kept on a laminar flow chamber until the mixture gelled. The insecticides were diluted with water and 0.1% of the surfactant Haiten[®] (Arysta Lifescience do Brasil Indústria Química e Agropecuária S.A, Paraíso, São Paulo, Brazil), and then applied on the respective treatments in a 50 µL dose, using a micropipette (LabTex[®] - volume range 20-200 µL). The cups were kept inside the laminar flow chamber until the insecticide solutions dried over the diets. Finally, third instar larvae (8-10 mm of size) were transferred to the cups corresponding to each treatment. The choice for third instar larvae was based on recommendations regarding the control moment for fall armyworm, as suggested by the insecticides’ manufacturers.

Evaluations and data analyses

Larval mortality was assessed by brushing the larvae on the last abdominal segments and considering them dead when no articulate movement followed. These evaluations were carried out daily for a period of 15 days. Survival time was evaluated by assigning value 1 (one) to the larvae that survived for 15 days and 0 (zero) to those that died on the first evaluation day. The speed of larval mortality was also evaluated during the whole period, considering each origin site, insecticide sprayed and mode of entry. Data normality was tested through Jarque-Bera and graphic inspection of the residuals, followed by transformation using the power function. The results obtained were submitted to variance analysis and means compared by Tukey’s test (P<0.05), using the software R (R Core Team, 2016). Overall, means of each treatment under the respective factors were also analysed by ANOVA and compared by Tukey’s test (P<0.05), using the software SISVAR (Ferreira, 2011).

Conclusions

Teflubenzuron and flubendiamide provide the highest mortality levels of *S. frugiperda*. Contamination by ingestion enhances control efficiency for insecticides especially for teflubenzuron and flubendiamide. *S. frugiperda* individuals from Constantina presented low susceptibility to the insecticides tested.

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