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Cut off Behavior of *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) in Soybean (*Glycine max (L.) Merrill*) Seedlings

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HIGHLIGHTS

- *Spodoptera frugiperda* has a cut off behavior on soybean seedlings.
- Soybean injury occurs as a density-dependent response.
- From the third instar there is an increasing feeding behavior of the larvae.
- Seedlings with more advanced stages present injuries only on the leaves.

Abstract: In addition to destroying the leaves, stems, pods, and grains of soybean (*Glycine max*), *Spodoptera frugiperda* larvae may also have the cut off behavior in the seedlings close to the ground, harming the establishment of the soybean crop. Therefore, this study aimed to evaluate the consequences of the fall armyworm attack by adopting the cut off behavior, which has been similarly documented for black cutworm (*Agrotis ipsilon*) in newly emerged soybean plants. The treatments were five levels of infestation with 0, 5, 10, 20 and 40 larvae per m^2. Three variables were assessed: (1) stand (relationship between attacked and initial number of plants), (2) types and amounts of injured structures, such as hypocotyl (embryonic shoot), and the cotyledons (seed leaves), and (3) level of defoliation. The variables were collected every 24 hours until the pupal stage. Additionally, the final stand of the seedlings was quantified, with the highest injury intensity observed in the plots that contained the highest number of larvae. In the second experiment, the dynamics of movement and attacks of plants by *S. frugiperda* larvae at the following times of the day: 07:00, 10:00, 13:00, 16:00, 19:00 and 21:00 were analyzed with supervised machine learning models. The injury caused by *S. frugiperda* with the behavior of the cut off and the voracity of the larvae were evident in the structures (hypocotyl and cotyledons) of the evaluated plants. The results of the present study emphasize the need to manage *S. frugiperda* during the preplanting phase of soybean.

Keywords: fall armyworm; feeding behavior; seedlings.

INTRODUCTION

The *Spodoptera* spp. complex is deeply disseminated in several parts of the world and most of these insects feed on various types of plants [1]. Among the species of this genus, *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) is known as fall armyworm, and expresses polyphagy more forcefully, mainly in relation to the number of plants registered as their hosts, of which approximately 353 specimens distributed in 76 botanical families were recently cataloged [2].

The abundance of host plants [3], added to the high movement capacity of their adults [4,5,6] and the longevity of the species [2] optimize the reproductive potential of this pest. However, some plants, such as those belonging to the Fabaceae family, such as soybean (*Glycine max*), contain allelochemicals that directly interfere with the normal development of immatures. *S. frugiperda* is no longer considered a secondary pest [7,8], starting to play a relevant role and thus limiting the of soybean yield in Brazilian agroecosystems [9], especially due to the great presence of this insect in crops [10].

Some studies that evaluated the leaf consumption of lepidopterans in soybean showed that the attack of some species of the *Spodoptera* complex, including the species *S. frugiperda*, causes simple leaf scraping to destroy the plant, consuming leaves, stems, pods, and grains [11]. In addition, larval stage of *S. frugiperda*, it can cut the seedling of soybean close to the ground. The cut off behavior of *S. frugiperda* may interfering with the crop stand, thus, characterizing an attack similar to black cutworm (*Agrotis ipsilon*), a fact reported by some researchers [12-13]. However, there is no any quantification of this cut off behavior in *S. frugiperda* published in the literature quantifying the injury caused by this behavior of the fall armyworm in soybean seedlings. In view of the above, the objective of this study was to evaluate the consequences of the attack of *S. frugiperda* assuming the behavior of the cut off on newly emerged soybean plants.

MATERIAL AND METHODS

Bioassay 1

The experiment was carried out at the Laboratory of Applied Entomology of the Federal University of Grande Dourados (UFGD), under greenhouse conditions. The soybean cultivar used was BMX Potência RR (non-Bt), which belongs to the 6.7 maturation group with an indeterminate growth habit. The material was cultivated in trays (0.55 m x 0.35 m, with approximately 0.1925 m^2), containing 9.625 liters of a compost made from 1/3 of the B horizon of a Red Distroferric Latosol, 1/3 of fine sand and 1/3 of organic substrate (Carolina Soil®) composed of 78% Sphagnum peat and 22% vermiculite.

During planting preparation, acidity and fertility correction was performed. Each tray corresponds to an experimental unit (plot). The establishment of the initial stand was 30 soybean seedlings per tray. This number was obtained by sowing 60 seeds followed by thinning to obtain the best uniformity of plants (in height, foliage, and vigor) per plot. Seed treatment was carried out with Carboxin® + Thiram® fungicides, using the dosage of 300 ml of the commercial product Vitavax-Thiram® 200 + 200 SC for 100 kg of seeds, to control a possible fungal contamination that could interfere with the germination process and stand.

The infestations by the caterpillars were carried out when the soybean cotyledons had unifoliolate leaves, and were sufficiently unrolled with their edges separated, called the cotyledonary phenological stage (VC) [14]. For the infestation of the larvae occurring at the exact moment of the defined phenological stage, a series of five visual observations of development were carried out, every 24 hours, analyzing each tray.

Spodoptera frugiperda larvae came from the stock rearing from the Laboratory of Applied Entomology at UFGD, kept in a rearing room at a temperature of 25±2 °C, relative humidity of 70±10% and a photophase of 12 h, in plastic containers of 100 ml containing artificial diet [15]. Upon reaching the 3rd instar, the larvae were infested on the trays with a soft brush, according to the following treatments: 0, 1, 2, 4 and 8 of *S. frugiperda* larvae per tray. These densities are approximately equivalent to the five levels of infestation of 0, 5, 10, 20 and 40 caterpillars per m^2, respectively. The larvae were distributed randomly. During the experiment execution, we did not observe the occurrence of cannibalism. The experimental design used was randomized blocks, with six replications. To prevent insects from escaping from the experimental plots, the trays received automotive grease on the edges.

Evaluations of three variables were performed: (1) stand (relationship between attacked and initial number of plants), (2) types and amounts of injured structures (hypocotyl and cotyledons) and (3) level of defoliation. Assessments were performed every 24 hours until pupal stage; in the event of cases of disappearance, death by cannibalism or other causes, the missing individuals were replaced immediately in the detection observed during the evaluation, corresponding to the same instar of the tray.

To measure the stand, the cut plants at its base and at the end of the study were observed; thus, the number of missing plants from the stand (initial stand minus final stand) was calculated. In the case of injured structures (hypocotyl and cotyledons), it was assumed that all parts of the plant injured by the larvae that showed consumption, cut, breakage or missing plant pieces would be included in this category, as injuries. The visual assessment of the level of defoliation, relative to the foliar consumption of the larvae, was performed using a standard grade scale proposed by Ohnesorg and Hunt [16].

Bioassay 2

The 1st instar *S. frugiperda* larvae were individually placed in transparent plastic containers (with a capacity of 2 L), filled with soil in approximately 1/3 of its volume, and covered with white voile fabric. Each cage corresponded to a plot, where 30 experimental units were established, forming a design composed of 10 treatments with 4 replications per instar.

The test was conducted in a closed room, with an average temperature of 28°C, relative humidity of 62±10% and photophase of 12 hours. Data collection was carried out at 6 different times, as follows: 7:00, 10:00, 13:00, 16:00, 19:00 and 21:00 h for 8 consecutive days. The evaluations referred to the behavior of *S. frugiperda* with the cut off behavior, total or partial consumption of the seedling, permanence in the cotyledons and stem and the arrangement of the larvae in different regions of the plant and in the cage - according to the behavioral analysis of the videos. We used open cages to make movie records easy. We provided grease on the edges of the cage to avoid scaping of the larvae. The record duration in each time interval was only 10 minutes. The evaluations of the behavioral traits were conducted by visual observations, and they were checked with movie records using the free and open-source software BORIS®.

Statistical analysis

The preference behavior of *S. frugiperda* larvae in the different structures was analyzed with a supervised multinomial regression machine learning model. Because the dependent variable offers more than two possible responses (categories), in this case more than two options, being the probability of preference of structures ((hypocotyl or cotyledons) of soybean seedlings or total consumption by *S. frugiperda*. The probabilities of occurrence were estimated for the plant structure, taking into account as a reference point the larvae found on the walls, ceiling, or floor of the cage. The multinomial model was estimated with the multinom function of the nmet package of the R program [17]. The results predicted by the model were plotted using the ggplot2 package of the R program [17].

The proportion of attacked plants and of insects with the cut off behavior was analyzed using a generalized linear model with a quasi-binomial distribution. The number of injured structures was analyzed with a model with a negative binomial distribution. The goodness of fit of the models was verified with a half-normal graph from the hnp package of R [17]. Data related to the level of defoliation were analyzed with a nonparametric regression model. All analyses were conducted with the R program [17].

RESULTS

The regression curves generated revealed that the larvae of *S. frugiperda* started the cut off behavior as their developmental phase reached the third instar and simultaneously, this intensified with the evolution of the larvae's growth (Figure 1).

More than 50% of the larvae of *S. frugiperda* remained on the stem during the first three days of infestation, only changing to the other seedling structure from the fourth day onwards. They remained in this organ close to 50% probability, at least on the first day of infestation (Figure 2).

A constant increase in the presence of larvae dispersed in the cages was observed; however, this behavior did not exceed a 25% probability (Figure 2). In terms of total or partial consumption of seedlings, shortly after the third day, there was substantial growth in the total or partial consumption of the plant by the pest (Figure 2).

Given the proportion of injured plants, an asymptotic response of this variable was observed as the density of larvae/tray increased, with an increasing response of stand loss occurring until the density of 4 caterpillars/tray (20 caterpillars/m^2), and a stabilization of the curve between this density and the highest density tested was 8 caterpillars/tray (40 caterpillars/m^2) (Figure 3A).

The highest probabilities of booth loss are estimated at approximately 0.15 (15%) with confidence intervals (95% CI) ranging from 0.10 to 0.21. Therefore, there is an overlap of confidence regions for the probability of stand loss for the highest densities tested (Figure 3A).

A linear increase in the percentage of injury was observed according to the increase in the density of larvae/tray (Figure 3B). The percentage of injury estimated by the nonparametric regression model was approximately 5.0 (CI 95%= 1 to 8%); 13 (CI 95%=9 to 17%); 21 (CI 95% =17 to 25%) and 50% (CI 95% =46 to 53%) at densities of 1, 2, 4 and 8, respectively (Figure 3B).

There was a significant increase in the number of injured structures as the density of larvae/tray increased. The number of structures predicted by the model ranged from 6 (CI 95%= 4 - 7) to 16 (CI 95%= 11 - 21), at densities from 1 to 8 caterpillars/tray (5 to 40 caterpillars/m^2), respectively (Figure 3C).

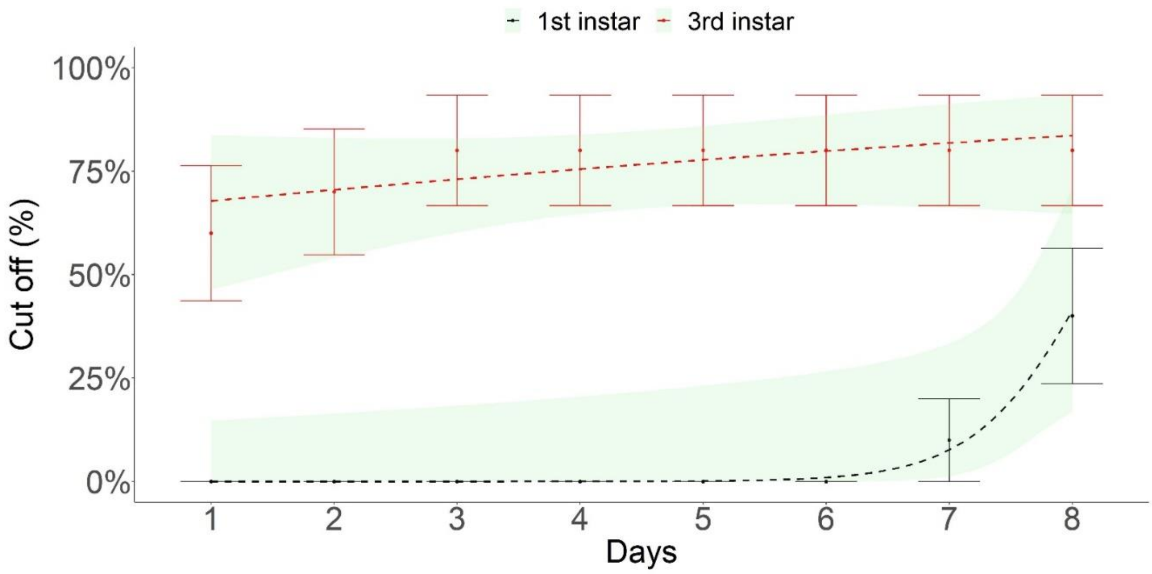

Figure 1. Percentage of first and third-instar larvae of *S. frugiperda* with cut off behavior.

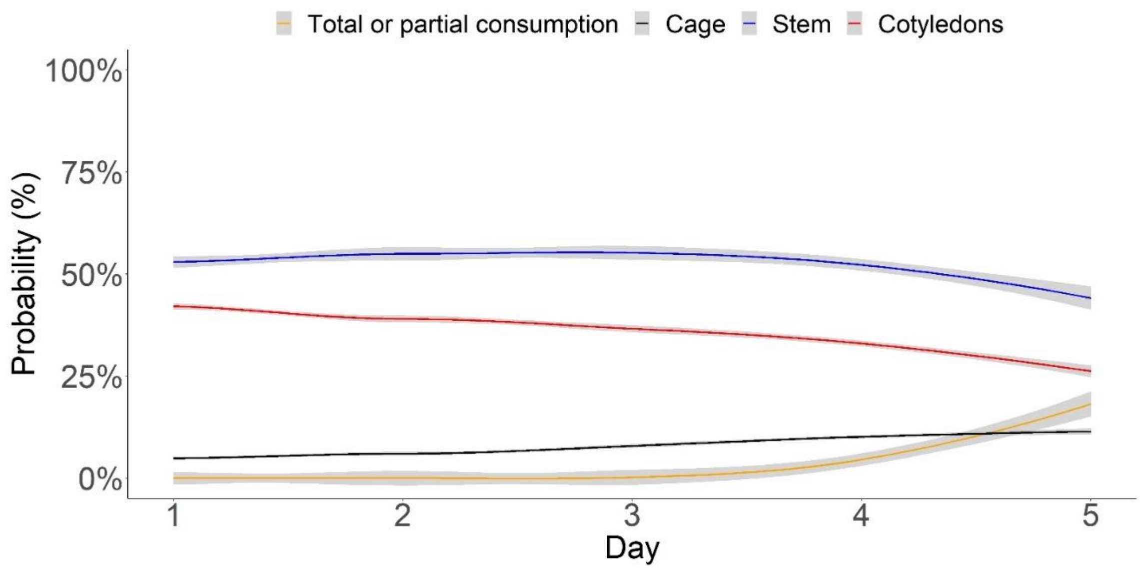

Figure 2. Probability of the preference of structures (stems or cotyledons) of soybean seedlings or total consumption by *S. frugiperda*. χ^2= 46.4235; *p-value* < 0.00001.

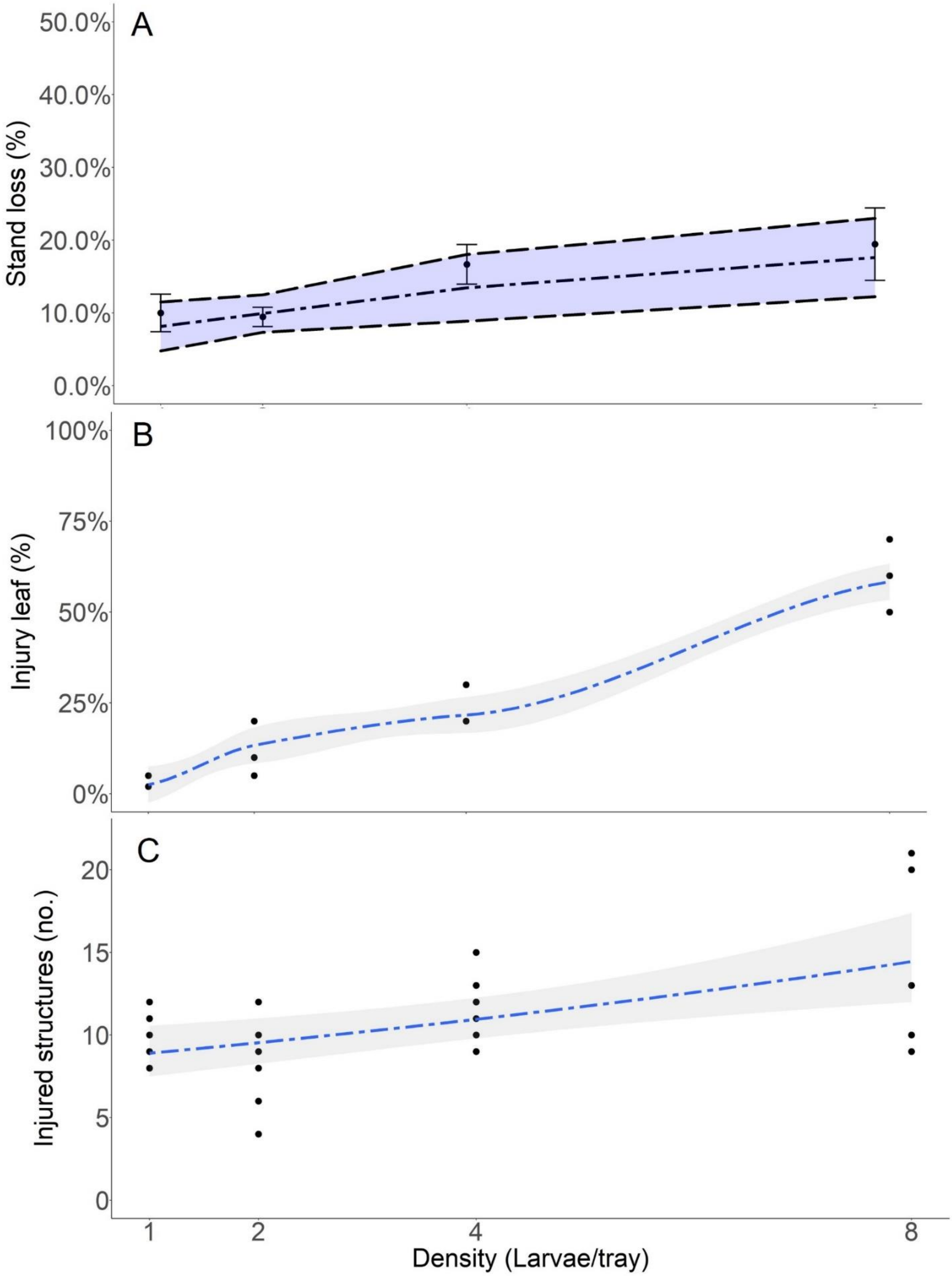

Figure 3. Proportion of stands affected by the *S. frugiperda* larvae, given by the peculiar behavior of the larvae, after 24 hours of infestation. Observed data are the dots, while the standard error shown in figure A is the bars associated with the dots. Dashed lines and regions with confidence intervals were estimated by the generalized linear model with binomial type distribution (A). Defoliation levels caused by the *S. frugiperda* larvae after 24 hours of infestation. Regions with confidence intervals were estimated by a nonparametric model (B). Number of structures injured (hypocotyl or cotyledons) by the *S. frugiperda* larvae after 24 hours of infestation. Dashed lines and regions with confidence intervals estimated by the generalized linear model with negative binomial type distribution (C).

DISCUSSION

Larvae of *S. frugiperda* can scrape the surface layer of the organs of soybean seedlings in the early stages of their development, not being commonly consumed until the third instar, with more intense and well-defined characteristics. This described feeding behavior could be observed in other plant species, mainly in corn (*Zea mays*), in which the pest consumes only the surface layer of the leaves during the developmental stage before the third instar [18].

Third instar larvae disperse and continue the feeding process, and such characteristics are linked to several factors inherent to the biochemical variability of each individual from birth, not only because of the fact related to the insect's survival instinct, but also because of multiple biotic and abiotic agents that influence the individualized dispersant behavior [19].

Among the injury caused by the larvae, the behavior of the caterpillar stands out for its severity, mainly due to the impact of this type of attack on the stand in the soybean crop. Although this is a sporadic behavior. Unusually, this behavior adopted by the larvae may also be observed in corn, where they feed on the tassel if they are wrapped in spiral leaves [20].

All densities of *S. frugiperda* larvae had a significant influence on the three variables evaluated, evidencing that the intensity of injury or death of soybean plants increased according to the density of larvae/m^2, regardless of the amounts established in the evaluation scale. When relating the density of larvae with the level of defoliation, only in the density of 40 individuals per m^2, that is, in the highest number of larvae per tray (8 larvae/tray), it exceeded the surpassing the economic damage level (NDE) reported in the literature [21], that is 30% of defoliation. For that, exceptionally, the stand variable, among the highest densities of 20 to 40 larvae per m^2, equivalent to 5 to 8 larvae per tray, showed stability of the curves. This fact could be inherent to dispute over territory, mainly because the occurrence of this behavior is related to the increase in the population density of the pest [22] or to the exposure of the larvae to a food that is not of their preference, in the case of soybean [23]. Finally, the high rate of individuals in a given space can increase competition [11]. In this case, the larvae stop dedicating itself to the most peculiar attacks, to act on what is most predictable to them and stabilize the injury caused to the plant stand. These aspects related to intraspecific interaction deserve to be studied in the future.

The injury caused by the pest was verified from the first evaluation, reinforcing the hypothesis that this insect has a cut off behavior, in addition to generalized consumption of all structures of the soybean seedling later. It is plausible to state that the behavior of the cut off occurs in this species, when the seedling is in the cotyledonary physiological stage (VC) and after that, the feeding behavior of this insect became characteristic of the category of common defoliating lepidopterans.

During the daytime the larvae drilled the soil superficially close to the base of the seedling stem, to shelter from the high temperature and solar intensity (personal observations). Direct contact with this part of the seedling supposedly increased the likelihood of larval feeding on these structures. It is possible that the fact larvae cut the base of the seedling stem instead of cotyledonary structures is linked to palatability and the low lignin concentration of this structure. At this stage, seedlings accumulate large amounts of essential nutrients for their vegetative growth [24]. Additionally, high vigor soybean seeds could mobilize high reserves of soluble proteins, starch and soluble sugars for pregerminated seedlings [25]. These compounds can act as phagostimulants, mainly sugars [26], making seedlings attractive references for insects to meet their nutritional needs.

Another important factor that we must point out is the possibility of the presence of phenolic substances in the leaves of soybean plants, which act as defense against herbivores that cause leaf injury [27], thus giving rise to another alternative, which in turn may explain the unusual behavior of this arthropod.

Given the aspect of bringing to light the optimal defense theory, which consists of the self-defense of plants through allelochemicals that protect the younger organs against the attack of phytophagous insects [28], in view of the great importance of these tissues for growth, development and consequently the perpetuation of plants [29]. Therefore, it is very likely that there was a supposed movement of larvae from the leaves to the stem, induced by the repulsion to toxic compounds, flavonoids contained in soybean leaves, characterized by the effect of antibiosis, occurring generally on cotton plants [30-31]. The cut off behavior found in our research is similar to the behavior observed in the cosmopolitan species *Agrotis ipsilon* (Hufnagel, 1766) (Lepidoptera, Noctuidae) [32]. Therefore, both species can cause injury in several seedlings early in the growing season and can open gaps in the stand, causing losses due to yield reduction and promote the need of replanting in the affected areas in soybean fields.

CONCLUSION

Spodoptera frugiperda larvae had a cut off behavior in soybean seedlings from the third instar of development. From the density of 5 caterpillars per m^2 (equivalent to 1 caterpillar per tray), injury has already occurred due to loss of stand. With the increase in density, the injury typical of a caterpillar intensified, and more intense defoliation was perceived with the gradual increase in infestation.

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