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Intraguild interactions and behavior of *Spodoptera frugiperda* and *Helicoverpa* spp. on maize

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Running title: Intraguild interactions of *Spodoptera frugiperda* and *Helicoverpa* spp.

Abstract

**BACKGROUND:** *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is one of the major pests of maize, and is in the same feeding guild of the noctuid pests, *Helicoverpa zeae* (Boddie), and *Helicoverpa armigera* (Hübner), recently reported in South and North America. The intraguild interactions of these species were assessed in laboratory and field conditions by determining the survival of larvae in interactions scenarios with non-Bt maize.
silks and ears. Moreover, a video-tracking system was utilized to evaluate behavioral parameters during larval interactions in scenarios with or without food.

**RESULTS:** In intraguild interactions, *S. frugiperda* had greater survival (55 to 100%) when competing with *Helicoverpa* spp. in scenarios where larvae were the same instar or when they were larger (4th vs. 2nd) than their competitor. Frequency and time in food of *S. frugiperda* larvae were negatively influenced by interactions. Larvae of *S. frugiperda* moved shorter distances (less than 183.03 cm) compared to *H. zea*.

**CONCLUSION:** Overall, *S. frugiperda* had a competitive advantage over *Helicoverpa* spp. This study provides significant information regarding noctuid behavior and larval survival during intraguild interactions, which may impact pest prevalence and population dynamics, thereby affecting integrated pest management and insect resistance management of these species in maize.

**Key words:** insect behavior, Ethovision, fall armyworm, corn earworm, old world bollworm
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The fall armyworm, *Spodoptera frugiperda* (J. E. Smith, 1797), and the corn earworm, *Helicoverpa zea* (Boddie, 1850), (Lepidoptera: Noctuidae) are among the major pests of maize in the Western Hemisphere, causing economic damage due to their feeding behavior. 1,2,3,4 Moths of *H. zea* usually oviposit on maize silks, and as soon as the larvae hatch they move to and feed on kernels. 5,6,7 Damage caused by larvae of *S. frugiperda* generally occurs during the whorl stage, with foliar consumption and indirect damage to grain production due to the reduction in photosynthetic area. 1,2,8 However, *S. frugiperda* larvae may also behave similarly to *H. zea*, infesting maize ears and feeding directly on the developing kernels. 2,8,9 A better understanding of behavioral interactions between these species during the reproductive stages of maize is therefore essential. 10 Moreover, both species present difficulties in their management based on their biological characteristics and rapid adaptation to chemical (i.e. organophosphate and pyrethroid) and genetic (i.e. *Bacillus thuringiensis* transgenic hybrids) controls. 11,12,13,14

During the 2012-2013 cropping season, the occurrence of the Old World bollworm *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae) was reported for the first time in field crops in Brazil, 15,16,17 followed by reports of detection in Argentina, 18 Paraguay and Uruguay. 19 *Helicoverpa armigera* is a polyphagous pest of agricultural crops, including host plants like cotton, soybean, maize and sorghum. 17,20,21 In maize, moths of *H. armigera* prefer to oviposit on the reproductive structures (silk) and larvae tend to move to and feed on kernels. 20 On 17 June 2015, one male *H. armigera* moth was collected in the field in Brandenton, Florida, USA, causing an alert in this North America country. 22

The three aforementioned noctuids belong to the same feeding guild on maize, with the maize ear being the intraguild interaction zone. 7,9,23 Intraguild interaction among these noctuids may be significantly affected by cannibalistic behavior 5,24,25 or intraguild predation (when predation involves different species at the same food source), both considered exploitative behaviors. 26,27

These cited characteristics pose questions involving behavior, dispersion, population dynamics, and the prevalence of noctuid species in maize. Studies involving intraguild interaction of noctuids on maize are still scarce, 28, 29 as well as cannibalism, predation 30 and other larval behavior when interaction occurs, 31,32 particularly under field conditions. 33,34,35
The objective of this study was to evaluate the larval interspecific and intraspecific interactions of *S. frugiperda* when competing against *H. zeae* or *H. armigera* on maize under laboratory and field conditions using various interaction scenarios. We also evaluated the intraspecific and interspecific interactions between *S. frugiperda* and *H. zeae* under laboratory conditions using an automated video tracking system to describe details of larval behavior. A better understanding of the interactions of these noctuid pests would be relevant to their integrated pest management and insect resistance management.

### 2 MATERIALS AND METHODS

Larval interactions were assessed in three types of arenas: plastic cups with maize silks in the laboratory, plastic tubes around maize ears in the laboratory, and on maize ears in the field. Behavior was assessed for 11 distinct interaction scenarios based on species and larval stadium (Table 1). A non-Bt maize hybrid (Pioneer 30F35) was used to eliminate possible effects of Bt proteins on noctuid behavior.

#### 2.1 Insect stock colony

From 2013-2016, colonies of the three noctuid species were maintained in the laboratory (25 ± 2°C, RH: 60 ± 10%; 14:10 [L:D]) at São Paulo State University, Department of Crop Protection, Botucatu, SP, Brazil. The larvae were reared on artificial diet. In order to keep the vigor of the colony, insects were frequently collected from the field, identified and transferred to the specific colony. More details on the rearing methodology used may be found in the literature.

#### 2.2 Laboratory intraguild interaction

Two studies were conducted under laboratory conditions at LARESPI (Laboratory of Host Plant Resistance and Insecticidal Plants), São Paulo State University, Department of Crop Protection, Botucatu, SP, Brazil. In the first study, two larvae (species and stadium according to Table 1) were placed into transparent plastic cups (100 mL) with a plastic lid containing small holes in order to allow air flow. Maize silks were collected from non-Bt maize plants at growth stage R1 and cleaned with 92.8% ethanol; 100 g of maize silk was added to each cup and replaced daily to maintain quantity and quality of plant tissue.
For the second study, two larvae (species and stadium according to Table 1) were placed on a maize ear collected from non-Bt maize plants between growth stages R2 (blister) and R3 (milk) and cleaned with 92.8% ethanol. Ears were fixed to a polystyrene board base with support of two wooden dowel rods attached by rubber bands. A portion of paper towel was fixed at the base of the ear using a rubber band and moistened every two days to preserve ear turgidity. The maize ear was placed into a transparent plastic cylinder (8 cm height x 30 cm diameter), sealed on the top with organdy fabric attached to allow air flow.

Larvae used in the above studies had been removed from the artificial diet and starved individually in plastic cups one hour before initiating the scenario. Each plastic cup or tube was considered one replicate, with 20 replicates per scenario for both arenas in a completely randomized design. For the interaction study with maize silk in plastic cups, evaluations of larval survival were performed daily for 10 days. For the interaction study using maize ears, evaluations of larval survival were performed only at 10 days after infestation due to the difficulty in accessing larvae on the ear daily. These larval survival data were used to assess *S frugiperda* cannibalism and predation.

### 2.3 Field intraguild interaction

Field studies were conducted during two cropping seasons (May-August, 2015 and November, 2015-February, 2016) at São Paulo State University (22°52’48”S, 48°42’40”W, 720 m elevation). The field interaction studies were carried out in fields planted using standard agronomic practices recommended for the region, including standard irrigation management practices to ensure optimum maize growth until the reproductive stages. Natural infestations of noctuids and other maize pests were monitored by light traps and managed through the use of the insecticide chlorfluazuron (Atabron, ISK Ltd., Indaiatuba, SP, Brazil) during the vegetative stage of the plants. When the plants reached reproductive stages, insecticidal applications ceased and noctuid eggs and larvae were eliminated by hand when detected.

For each planting date, an area of approximately 1500 m² was divided into five blocks, evenly spaced, with 11 plots each (corresponding to interaction scenarios). Each plot was 4 m long and 3 rows (spaced 0.70 m apart) wide, corresponding to approximately 22 m² total. Four replicates per plot were established using the central row of each plot in a complete randomized block design, totalling 220 scenarios.

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When the plants reached the physiological stage R2-R3, a paint-brush was used to infest the maize ears with larvae (Table 1). After the infestation, each ear was carefully covered with a 25 x 30 cm bag made of organdy fabric. The upper part of the bag was held to the stalk of the maize plant with clips, leaving an internal space (≈ 10 cm) between the end of the ear and the end of the bag. The base of the bag was also fixed to the stalk and firmly fixed through two nodes and rubber brands to prevent larval escape. The survival of larvae was assessed 10 days after infestation. Once again, values of cannibalism and predation by *S. frugiperda* in each scenario were assessed.

### 2.4 Video-tracking trials

Automated video-tracking software (Ethovision XT 7.0, Noldus Information Technology, Wageningen, The Netherlands) was used to examine potential differences in behavior among *S. frugiperda* and *H. zea* when in competition scenarios (Table 2). Experiments were conducted at the Agroecosystems Entomology Laboratory, West Central Research & Extension Center, University of Nebraska-Lincoln in North Platte, NE, USA. Larvae were commercially acquired (Benzon Research Inc., Carlisle, PA, USA) and reared in plastic cups containing 15 ml of artificial diet (based on diet developed by USDA, Stoneville, MS). The insects were kept in a rearing chamber (25 ± 2°C, RH: 60 ± 10%; 14:10 [L:D]) until the 4th instar.

Non-Bt maize seeds (hybrid Channel 208-71R) were sown in 5L pots with sterilized soil and fertilizer to provide vegetative tissue for the scenarios that offered food. Each vessel held one maize plant and was maintained in a greenhouse, free from insect infestation. The maize leaves were collected from plants at phenological stage V6. Larvae were taken separately from plastic cups with artificial diet and starved for two hours. The sex of the larvae was not determined because it does not affect cannibalistic behavior. For each bioassay replication, a pair of larvae was confined together on opposite sides of a Petri dish (60 mm diameter x 15 mm height, Fisher Scientific, Pittsburgh, PA, USA) with or without a maize leaf disk (31 mm diameter) as food source, which was classified as food available or food not available. Although maize leaves are not the preferential food of *H. zea*, the larvae do feed on maize, and the objective of using leaf tissue was simply to analyze the larval interactions in scenarios with and without a food source available. To keep the maize tissue moist, two layers of solidified agar (2.5 % wt:vol, 2 and 1.5 mm thickness) were prepared, and the vegetable tissue was deposited between the two layers. A circular hole was made in the top layer (18 mm diameter), allowing the larval feeding. For treatments without food, just the first layer of agar was used.

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Each scenario was recorded with a Dino-Lite AD413T-12V camera (Big C, Torrance, CA, USA) for 15 minutes to characterize larval interactions based on movement of the larvae. The Ethovision software was used to evaluate distance moved (cm), mean distance between larvae (cm), and time (s) and frequency (number of times that the larvae visit the food zone) in the food zone for replicates with food available. For each intraguild scenario, 15 replicates were conducted. Each larva participated only once in a scenario.

2.5 Statistical Analyses

The data of larval survival in the intraguild interactions were assessed for normality with Shapiro-Wilks tests. The data were tested using Chi-square test ($\chi^2$) ($P \leq 0.05$) between the survival of scenario and its corresponding control (SAS Institute 2001). The control treatment for each interaction scenario consisted of scenarios with individuals of the same species, in the same instars as larvae for the other treatments (Table 1). For the video-tracking parameters, data were subjected to an analysis of variance, with normality assessed using the Shapiro-Wilk test and homoscedasticity evaluated using Levene’s test. When significant differences in the effects of the treatments were found, Fisher’s LSD test was used ($P \leq 0.05$) for the comparison of the means, using the statistical program PROC MIXED-SAS 9.2.
3 RESULTS

3.1 Laboratory intraguild interaction

On maize silks in cups, the survival of *S. frugiperda* for the 2nd vs. 2nd instar scenario was significantly greater when competing against *H. armigera* and *H. zea* (100% and 80% of survival, respectively) than when *S. frugiperda* competed against a conspecific (35% survival) ($\chi^2 = 21.83; df = 2; P < 0.0001$), with 100 and 65% predation, respectively (Table 3). In 2nd vs. 4th and 4th vs. 2nd instar scenarios, survival in interspecific interactions did not differ from intraspecific interactions. In 4th vs. 2nd instar scenarios, the 4th instar *S. frugiperda* had 100% survival in intraspecific interactions and with the other species, with cannibalism and predation above 90%. For the 4th vs. 4th instar scenario, the percent surviving remained higher in interaction with *H. armigera* and *H. zea* (75% and 55%) ($\chi^2 = 17.81; df = 2; P < 0.0001$) and predation varied from 75% to 45%, respectively.

On maize ears in plastic tubes in the lab, the survival of *S. frugiperda* in 2nd vs. 2nd instar scenarios was higher when interacting with *H. armigera* (90%) than with *H. zea* (65%) and *S. frugiperda* (55%) ($\chi^2 = 6.19; df = 2; P = 0.0453$) (Table 3). Cannibalism was 45%, while predation varied from 60% in *H. armigera* to 65% in *H. zea*. For 4th vs. 4th instar scenarios, *S. frugiperda* had highest survival, mainly with *H. armigera* (100%) compared to the survival in intraspecific interaction (70%) ($\chi^2 = 7.05; df = 2; P = 0.0293$). Cannibalism was low in intraspecific competition (30%), while predation levels were 55% (vs. *H. armigera*) and 50% (vs. *H. zea*). When competing on maize ears, no differences in survival of *S. frugiperda* were observed in 2nd vs. 4th instar scenarios, with survival less than 50% of 2nd instar. Cannibalism and predation rates were low in this scenario, being less than 20%. In 4th vs. 2nd instar scenarios, the survival of 4th instar remained 100% against all species of competitor. In this scenario, cannibalism was 50% and predation of *H. armigera* and *H. zea* were 60 and 70%, respectively.

3.2 Field intraguild interaction

Similar to results in the laboratory, in the 2nd vs. 2nd instar scenario the survival of *S. frugiperda* was higher in interactions with *H. armigera*, with 90% of larvae surviving, while in intraspecific interaction the survival was 45% ($\chi^2 = 9.23; df = 2; P = 0.0099$), with values of cannibalism and predation around 50%. There was no difference in survival of *S. frugiperda* in 4th vs. 4th instar scenarios, with survival above 70%. Cannibalism was 25%, while the predation was 60% of *H. armigera* and 55% of *H. zea*. In the 4th vs. 2nd scenario, larval survival was 100% in
intraspecific and interspecific interaction, and cannibalism and predation was higher than 55%. In the first field study, no difference was observed in the 2nd vs. 4th instar scenarios, and the survival of 2nd instar *S. frugiperda* was less than 40%, with low values of cannibalism and predation (less than 20%).

In the second field study, there was no difference in the survival of *S. frugiperda* against the different competitors, and the species showed high survival, above 60% in 2nd vs. 2nd instar scenarios and 75% in 4th vs. 4th instar scenarios. In 2nd vs. 2nd instar scenarios, cannibalism was 40% while predation was 50% of *H. armigera* and 70% of *H. zea*. In 4th vs. 4th instar scenarios, cannibalism was 20%, predation of *H. armigera* was 55%, and 75% of *H. zea*. Once again, 4th instar had 100% of survival in scenario 4th vs. 2nd against the conspecific and *Helicoverpa* spp. In this scenario, predation reached 100% of *H. zea* and 50% of *H. armigera*, and cannibalism reached 55%. In 2nd vs. 4th scenario, 2nd instar *S. frugiperda* showed higher survival in intraspecific interaction ($\chi^2 = 11.98; \text{df} = 2; P = 0.0025$), followed by the interaction with *H. armigera*, while against *H. zea*, larvae had 0% of survival. Cannibalism and predation did not exceed 10% in this scenario.

### 3.3 Video-tracking trials

In conditions with food available, larvae of *H. zea* moved a greater distance when they were interacting with other larvae compared to when *H. zea* were alone and compared to larvae of *S. frugiperda* regardless of whether were interacting with other larvae or alone ($F = 8.57; \text{df} = 5, 84; P < 0.0001$) (Table 5). Without food availability, larvae of *S. frugiperda* continued to move less distance than *H. zea* in intraspecific and interspecific interaction ($F = 6.77; \text{df} = 5, 84; P < 0.0001$) (Table 6) (Fig. 1). For distance between larvae, with or without food, no difference occurred among the scenarios. Larvae of *S. frugiperda* spent more time feeding (214.59 s) when the larvae were not interacting with other larvae ($F = 2.78; \text{df} = 5, 84; P = 0.0224$) (Table 5). Larvae of *S. frugiperda* and *H. zea*, when isolated, had a lower frequency in the food source (0.46 and 1.53 times, respectively), differing ($F = 6.80; \text{df} = 5, 84; P < 0.0001$) from larvae of *H. zea* in intraspecific or interspecific interaction (16.86 and 8.13 times, respectively).

### 4 DISCUSSION

Cannibalism and predation by *S. frugiperda* varied throughout the scenarios. Cannibalism was expected because this behavior has already been reported under field and laboratory conditions in previous studies. Cannibalism
values were higher when interactions occurred between larvae in different development stadium than in same larval stadium, where larger competitors had a competitive advantage over smaller conspecifics. Several studies have found that cannibalism is dependent on instar, with higher rates as larval development advances, particularly when larvae of different stages are placed together.\textsuperscript{23,26,28,33,49} In our interaction studies, cannibalism occurred mainly when larvae competed under conditions of limited food and in small arenas (e.g., maize silks in plastic cup arenas).

Previous studies reported that cannibalism rates were more frequent when larvae were confined on maize leaves than on artificial diet, indicating that cannibalistic behavior is related to a lack of food with high nutritional value.\textsuperscript{50} In another study, cannibalism rates for third instar \textit{S. frugiperda} were less than 20\% when reared on maize seedlings, and increased to 34\% when there was a shortage of food.\textsuperscript{51} Here, considering the intermediate larval survival in intraspecific competition in the maize ear, and higher cannibalism rates of other species, such as \textit{H. zea},\textsuperscript{23} it is proposed that in a maize ear it is more likely that two or more larvae of \textit{S. frugiperda} will survive compared to \textit{H. zea}, which also suggests that \textit{S. frugiperda} is less aggressive than \textit{H. zea} in an intraspecific interaction.

In interspecific interactions between \textit{S. frugiperda} and \textit{H. zea}, although both species had some mortality, \textit{S. frugiperda} prevailed over \textit{H. zea} in most of the scenarios under laboratory and field conditions. \textit{Helicoverpa zea} prevailed over \textit{S. frugiperda} only in the 4\textsuperscript{th} vs. 2\textsuperscript{nd} instar scenario, where \textit{S. frugiperda} was disadvantaged due to its smaller size. When both species occur on a maize plant, there is a higher probability that \textit{S. frugiperda} will be more developed than \textit{H. zea} and several other lepidopteran species,\textsuperscript{53} suggesting the tendency of \textit{S. frugiperda} to prevail over these other species. This mismatch in development is due to the timing of when each species colonizes the maize plant: For example, \textit{S. frugiperda} tends to infest maize crops earlier, during the vegetative stage and then migrates to the ear, while \textit{H. zea} tends to infest maize during the reproductive stages and larvae typically begin feeding on silks and kernels at the ear tip before consuming kernels down the maize ear.\textsuperscript{2,7}

For \textit{H. armigera}, its polyphagous feeding habit\textsuperscript{20,52} high voracity,\textsuperscript{53} high potential of economic loss in crops,\textsuperscript{54,55,56} and the recent introduction in Brazil\textsuperscript{15,16,17} have caused significant interest in its potential interactions with \textit{S. frugiperda}. In 2\textsuperscript{nd} vs. 2\textsuperscript{nd} instar scenarios using silks and maize ears as a food source, the higher survival of \textit{S. frugiperda} larvae relative to \textit{H. armigera} indicates a potential competitive advantage of \textit{S. frugiperda}. When larvae of \textit{S. frugiperda} were less developed than \textit{H. armigera} (2\textsuperscript{nd} vs. 4\textsuperscript{th} instar), survival of \textit{S. frugiperda} decreased, although it did not differ from survival during 2\textsuperscript{nd} vs. 4\textsuperscript{th} intraspecific interactions, which indicates the capacity of \textit{S. frugiperda} to survive in an intraguild interaction with \textit{H. armigera} even when smaller than the competitor. In this

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scenario, larvae of *S. frugiperda* might be able to survive because of its ability to escape from aggressive interactions, such as moving away from the competitor, and/or through other defensive or offensive mechanisms.\(^{57}\) In 4\(^{th}\) vs. 2\(^{nd}\) and 4\(^{th}\) vs. 4\(^{th}\) instar interactions, the high survival of *S. frugiperda* also indicates that the species is not negatively affected by *H. armigera* in interactions on non-Bt maize. Similar to *H. zea*, moths of *H. armigera* prefer to oviposit on the reproductive parts of maize plants,\(^{20}\) and again because *S. frugiperda* colonizes vegetative plant stages, the competitive advantage of *S. frugiperda* over *H. armigera* might be more accentuated under natural conditions in non-Bt maize. In addition to the infestation times of noctuids in maize, other parameters such as agricultural landscape\(^{23}\) the food quality of the host plant (including transgenic), and larval behavior\(^{31}\) may affect intraguild interactions.

Genetically modified maize hybrids expressing Bt proteins could affect intraguild interactions among noctuids and other Lepidoptera, and raises several questions involving the influence of Bt on the prevalence of species in agricultural systems. One of the hypotheses for the eastward expansion of the noctuid *Striacosta albicosta* (Smith, 1888) in the US is the pest replacement hypothesis \(^{30,58,59,60,61}\) where the wide-spread adoption of Bt maize expressing Cry1Ab may have opened a niche for *S. albicosta* as other ear-feeding lepidopterans are negatively affected by this protein, while *S. albicosta* is not.\(^{30}\) Other studies also report the influence of Bt proteins on larval cannibalism and in intraguild interactions,\(^{28,33,34,62}\) strengthening the importance of understanding intraguild insect behavior with respect to food quality.

*Spodoptera frugiperda* larvae spent longer time in the food zone when isolated than when larvae were interacting with competitors, indicating that larvae might stop feeding or feed less when intraguild interactions occur. This behavior did not differ between intraspecific and interspecific interactions, and may be a factor that increases cannibalism, predation and movement of larvae on host plants. The distance moved by *S. frugiperda* larvae was similar whether they were alone or in intraspecific or interspecific interaction, with or without food. It is possible that *S. frugiperda* larvae, hatching from relatively large egg masses and initially feeding in mass, are not stimulated to move away from potential competitors.

The time feeding results that indicated a decrease of larval feeding when interacting with other larvae and a recent study describing plant-to-plant movement of *S. frugiperda*\(^{63}\) suggest that the movement of larvae among host plants could be related to intraguild interactions. Larvae would leave the maize ear to find another food site without competing larvae. The results from the video tracking of *S. frugiperda* and *H. zea* indicates that moving and
searching for empty feeding sites might occur in interspecific interaction, even though *S. frugiperda* appears to have a competitive advantage over *Helicoverpa* spp. Future research needs to evaluate the influence of different interaction scenarios involving pairs and odd larvae combinations (i.e. 2nd vs. 2nd/2nd vs. 4th) on the movement behavior of lepidopterans

The distance moved by *H. zea* was greater compared to *S. frugiperda*, and this characteristic might be useful to escape from an interaction (i.e. move away from the competitor), which is one of the responses of larvae during an interaction. The increase in distance moved by *H. zea* raises the importance of understanding on-plant movement on plant-to-plant movement of lepidopterans.

The concerns involving larval movement among plants are increased by the hypothesis that Bt maize intensifies larval movement between plants, which may modify intraguild interactions. In addition, the adoption of the seed mixture refuge strategy (refuge-in-a-bag) and exposure to sub-lethal doses of Bt caused by the cross-pollination of maize plants raises the importance of larval behavior. The movement of larvae in a crop might expose the insect to sub-lethal doses of Bt proteins by initial feeding on a Bt plant and subsequent feeding on a non-Bt plant and vice versa, so larval mortality may not be achieved. Considering the greater mobility of *H. zea* in the observed interactions, this species may be more likely to receive sub-lethal doses of Bt proteins, in contrast to *S. frugiperda*, which moved shorter distances in this study. In previous studies, neonates have been shown to remain longer on their first host plant, although field studies showed some on-plant and in plant-to-plant movement of *S. frugiperda* larvae on non-Bt maize. For *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae), larval movement was higher on Bt plants compared to non-Bt plants, and observations with a video-tracking system demonstrated that larvae increased the distance moved, time spent moving and time away from the diet in the presence of Cry1Ab tissue. In Bt cotton, larvae of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) abandoned Bt plants with higher frequency than those developing on not-Bt plants.

Overall, interactions among the species ear-feeding Lepidoptera, in the case of this study with noctuids, affect their behavior, and larvae of *S. frugiperda* gain competitive advantage in intraguild interaction with *H. zea* and *H. armigera* on non-Bt maize. A better understanding of the intraguild interactions is essential to determine the impact they will have on species abundance in agricultural systems. It is crucial to understand these behaviors to design successful integrated pest management and resistance management strategies. By using non-Bt maize, this study provides the baseline of larval behavior of three economic important species in intraguild interactions. The extensive
use of Bt technology represents one more factor in the complex system that affects insect behavior. The widespread release and adoption of Bt crops, and the issues involving the seed mixture refuge strategy and cross-pollination among plants reinforce the necessity for further research to evaluate the effect of these practices on larval mobility, feeding behavior and intraguild interaction.
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Table 1. Scenarios of intraguild interaction involving *Spodoptera frugiperda* and *Helicoverpa* spp. in different larval stadiums.

<table>
<thead>
<tr>
<th>Scenarios</th>
<th>Intraguild interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>S. frugiperda</em> (2nd) vs. <em>H. armigera</em> (2nd)</td>
</tr>
<tr>
<td>2</td>
<td><em>S. frugiperda</em> (2nd) vs. <em>H. armigera</em> (4th)</td>
</tr>
<tr>
<td>3</td>
<td><em>S. frugiperda</em> (4th) vs. <em>H. armigera</em> (2nd)</td>
</tr>
<tr>
<td>4</td>
<td><em>S. frugiperda</em> (4th) vs. <em>H. armigera</em> (4th)</td>
</tr>
<tr>
<td>5</td>
<td><em>S. frugiperda</em> (2nd) vs. <em>H. zea</em> (2nd)</td>
</tr>
<tr>
<td>6</td>
<td><em>S. frugiperda</em> (2nd) vs. <em>H. zea</em> (4th)</td>
</tr>
<tr>
<td>7</td>
<td><em>S. frugiperda</em> (4th) vs. <em>H. zea</em> (2nd)</td>
</tr>
<tr>
<td>8</td>
<td><em>S. frugiperda</em> (4th) vs. <em>H. zea</em> (4th)</td>
</tr>
<tr>
<td>9</td>
<td><em>S. frugiperda</em> (2nd) vs. <em>S. frugiperda</em> (2nd)<em>a</em></td>
</tr>
<tr>
<td>10</td>
<td><em>S. frugiperda</em> (2nd) vs. <em>S. frugiperda</em> (4th)<em>a</em></td>
</tr>
<tr>
<td>11</td>
<td><em>S. frugiperda</em> (4th) vs. <em>S. frugiperda</em> (4th)<em>a</em></td>
</tr>
</tbody>
</table>

*a Control treatments, intraspecific interactions (Adapted from Dorhout and Rice, 2010)*

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Table 2. Scenarios of intraguild interaction involving *Spodoptera frugiperda* and *Helicoverpa zea* in presence or absence of food for the video-tracking study.

<table>
<thead>
<tr>
<th>Intraguild interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No Food</strong></td>
</tr>
<tr>
<td><em>S. frugiperda</em> (4th) vs <em>S. frugiperda</em> (4th)*</td>
</tr>
<tr>
<td><em>H. zea</em> (4th) vs <em>H. zea</em> (4th)</td>
</tr>
<tr>
<td><em>H. zea</em> (4th) vs <em>S. frugiperda</em> (4th)</td>
</tr>
<tr>
<td><em>S. frugiperda</em> (isolated)</td>
</tr>
<tr>
<td><em>H. zea</em> (isolated)</td>
</tr>
<tr>
<td><strong>Food</strong></td>
</tr>
<tr>
<td><em>S. frugiperda</em> (4th) vs <em>S. frugiperda</em> (4th)*</td>
</tr>
<tr>
<td><em>H. zea</em> (4th) vs <em>H. zea</em> (4th)</td>
</tr>
<tr>
<td><em>H. zea</em> (4th) vs <em>S. frugiperda</em> (4th)</td>
</tr>
<tr>
<td><em>S. frugiperda</em> (isolated)</td>
</tr>
<tr>
<td><em>H. zea</em> (isolated)</td>
</tr>
</tbody>
</table>

Larval development: 4-14 h after ecdysis.
Table 3. Survival and cannibalism/predation (%) of *Spodoptera frugiperda* in intraguild interaction with *Helicoverpa* spp. and in intraspecific interaction, in maize silks and maize ears in the laboratory.

<table>
<thead>
<tr>
<th>Site</th>
<th>Scenario&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Survival of <em>S. frugiperda</em> and Cannibalism/Predation by <em>S. frugiperda</em> (%)</th>
<th>vs <em>S. frugiperda</em>&lt;sup&gt;b&lt;/sup&gt;</th>
<th>vs <em>H. armigera</em>&lt;sup&gt;c&lt;/sup&gt;</th>
<th>vs <em>H. zea</em>&lt;sup&gt;c&lt;/sup&gt;</th>
<th>( \chi^2 )</th>
<th>( P^d )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. frugiperda</em> (instar)</td>
<td>Competitor (instar)</td>
<td>Survival (%)</td>
<td>Cannibalism (%)</td>
<td>Survival (%)</td>
<td>Predation (%)</td>
<td>Survival (%)</td>
</tr>
<tr>
<td>Silk</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>35</td>
<td>65</td>
<td>100</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>Silk</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Silk</td>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>100</td>
<td>90</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Silk</td>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>10</td>
<td>90</td>
<td>75</td>
<td>75</td>
<td>55</td>
</tr>
<tr>
<td>Ear</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>55</td>
<td>45</td>
<td>90</td>
<td>60</td>
<td>65</td>
</tr>
<tr>
<td>Ear</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>50</td>
<td>0</td>
<td>15</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Ear</td>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>100</td>
<td>50</td>
<td>100</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>Ear</td>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>70</td>
<td>30</td>
<td>100</td>
<td>55</td>
<td>85</td>
</tr>
</tbody>
</table>

<sup>a</sup> First size, *S. frugiperda*; second size, competitor;  
<sup>b</sup> control, intraspecific interaction;  
<sup>c</sup> n: 20 larvae;  
<sup>d</sup> \( P \) value regarding the comparison of number of survival larvae in control and vs. *Helicoverpa* spp.
Table 4. Survival and cannibalism/predation (%) of *Spodoptera frugiperda* in intraguild interaction with *Helicoverpa* spp. and in intraspecific interaction, in maize ears in two field studies.

<table>
<thead>
<tr>
<th>Site</th>
<th>Scenario&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Survival of <em>S. frugiperda</em> and Cannibalism/Predation by <em>S. frugiperda</em> (%) vs <em>S. frugiperda</em>&lt;sup&gt;b&lt;/sup&gt;</th>
<th>vs <em>H. armigera</em></th>
<th>vs <em>H. zea</em></th>
<th>( \chi^2 )</th>
<th>( p^d )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. frugiperda</em> (instar)</td>
<td>Competitor (instar)</td>
<td>Survival&lt;sup&gt;c&lt;/sup&gt; (%)</td>
<td>Cannibalism (%)</td>
<td>Survival (%)</td>
<td>Predation (%)</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; Field</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>45</td>
<td>55</td>
<td>90</td>
<td>55</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; Field</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>40</td>
<td>0</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; Field</td>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>100</td>
<td>60</td>
<td>100</td>
<td>55</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; Field</td>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>75</td>
<td>25</td>
<td>90</td>
<td>60</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; Field</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>60</td>
<td>40</td>
<td>85</td>
<td>50</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; Field</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>45</td>
<td>0</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; Field</td>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>100</td>
<td>55</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; Field</td>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>80</td>
<td>20</td>
<td>85</td>
<td>55</td>
</tr>
</tbody>
</table>

<sup>a</sup> First size, *S. frugiperda*; second size, competitor;

<sup>b</sup> control, intraspecific interaction;

<sup>c</sup> n: 20 larvae;

<sup>d</sup> P value regarding the comparison of number of survival larvae in control and vs. *Helicoverpa* spp.
Table 5. Mean (±SE) of distance moved, distance between larvae, time in food zone and frequency in food zone in scenarios with *Spodoptera frugiperda* (4th) and *Helicoverpa zea* (4th) in intraspecific and interspecific interaction with food, during 15 min.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Competitor</th>
<th>Distance moved (cm)</th>
<th>Distance between larvae (cm)</th>
<th>Time in food (s)</th>
<th>Frequency in food (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. frugiperda</td>
<td>S. frugiperda</td>
<td>146.34 ± 13.86 b</td>
<td>3.15 ± 0.15 a</td>
<td>42.68 ± 24.92 b</td>
<td>4.90 ± 1.99 cb</td>
</tr>
<tr>
<td></td>
<td>H. zea</td>
<td>183.03 ± 21.52 b</td>
<td>3.27 ± 0.10 a</td>
<td>44.59 ± 26.17 b</td>
<td>3.26 ± 1.82 cb</td>
</tr>
<tr>
<td></td>
<td>isolated</td>
<td>134.65 ± 16.99 b</td>
<td>-</td>
<td>214.59 ± 93.31 a</td>
<td>0.46 ± 0.34 c</td>
</tr>
<tr>
<td>H. zea</td>
<td>S. frugiperda</td>
<td>295.70 ± 24.81 a</td>
<td>3.00 ± 0.10 a</td>
<td>66.11 ± 24.61 b</td>
<td>16.86 ± 4.07 a</td>
</tr>
<tr>
<td></td>
<td>isolated</td>
<td>258.65 ± 23.40 a</td>
<td>3.27 ± 0.10 a</td>
<td>36.68 ± 18.94 b</td>
<td>8.13 ± 2.78 b</td>
</tr>
<tr>
<td></td>
<td>isolated</td>
<td>197.02 ± 25.45 b</td>
<td>-</td>
<td>35.18 ± 13.82 b</td>
<td>1.53 ± 0.63 c</td>
</tr>
</tbody>
</table>

*P < 0.0001 0.2793 0.0224 < 0.0001

*a* Means followed by the same letter per column do not differ by LSD test (*P* > 0.05).
Table 6. Mean (±SE) of distance moved and distance between larvae in scenarios with *Spodoptera frugiperda* (4<sup>th</sup>) and *Helicoverpa zea* (4<sup>th</sup>) in intraspecific and interspecific interaction without food, during 15 min.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Competitor</th>
<th>Distance moved (cm)</th>
<th>Distance between larvae (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. frugiperda</em></td>
<td>vs. <em>S. frugiperda</em></td>
<td>160.17 ± 6.55 dc</td>
<td>2.94 ± 0.08 a</td>
</tr>
<tr>
<td></td>
<td>vs. <em>H. zea</em></td>
<td>157.02 ± 12.85 dc</td>
<td>3.24 ± 0.16 a</td>
</tr>
<tr>
<td></td>
<td>isolated</td>
<td>145.90 ± 14.76 d</td>
<td>-</td>
</tr>
<tr>
<td><em>H. zea</em></td>
<td>vs. <em>H. zea</em></td>
<td>297.91 ± 34.57 a</td>
<td>3.12 ± 0.07 a</td>
</tr>
<tr>
<td></td>
<td>vs. <em>S. frugiperda</em></td>
<td>237.57 ± 28.51 ab</td>
<td>3.24 ± 0.16 a</td>
</tr>
<tr>
<td></td>
<td>isolated</td>
<td>216.87 ± 26.17 bc</td>
<td>-</td>
</tr>
<tr>
<td><em>P</em></td>
<td></td>
<td>&lt;0.0001</td>
<td>0.2678</td>
</tr>
</tbody>
</table>
Fig. 1. a) Distance moved by larvae of Helicoverpa zea (yellow line) and Spodoptera frugiperda (blue line) in scenario with food; b) distance moved by S. frugiperda (red line) in control scenario, without food.