Effects of intraguild interactions on Anticarsia gemmatalis and Chrysodeixis includens larval fitness and behavior in soybean

Sabrina Ongaratto, a* Edson LL Baldin, a Thomas E Hunt, b Débora G Montezano, c Emily A Robinson d and Maria C dos Santos a

Abstract

BACKGROUND: Crop pest management requires an understanding of the complex interactions among species that potentially impact crop yield. In soybean, the velvetbean caterpillar, Anticarsia gemmatalis (Hübner), and the soybean looper, Chrysodeixis includens (Walker), are described as key pests, sharing the same feeding guild. We assessed the intraguild interactions of these species under laboratory conditions. Fitness cost study was conducted to examine the influence of competition on insect development. A video tracking system was used to evaluate behavioral parameters during larval interactions in scenarios with and without food availability.

RESULTS: In the fitness cost assay, pupal weight was not significantly affected, regardless of sex. However, larval and pupal survival were influenced by the competition, especially in third versus fifth instar scenarios. We detected 40.00% cannibalism and 46.67% predation when A. gemmatalis and C. includens third instars competed with A. gemmatalis fifth instar, respectively. Distance moved, distance between larvae, body contact (food available) and frequency in food of C. includens larvae were negatively affected by interactions. Anticarsia gemmatalis larvae showed highly active behavior, moving twice or more the distance compared to C. includens larvae, and A. gemmatalis spent more time in body contact with food.

CONCLUSION: Our results suggest that A. gemmatalis has a competitive advantage over C. includens. This study provides important information regarding lepidopteran behavior in soybean. We recommended that additional studies are necessary to understand the effects of interactions, especially in field conditions.

© 2021 The Authors. Pest Management Science published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Keywords: soybean pest; interspecific competition; intraspecific competition; integrated pest management

1 INTRODUCTION

Soybean (Glycine max (L.) Merrill) is one of the most important agricultural products and has been increasingly planted worldwide. 1,2 Despite the diverse climate conditions, important soybean producing areas in USA and South American countries are threatened by lepidopteran caterpillars. 3–5 The velvetbean caterpillar, Anticarsia gemmatalis (Lepidoptera: Erebidae), and the soybean looper, Chrysodeixis includens (Lepidoptera: Noctuidae), are among the major soybean pests, which have been causing economic damage from South to North America. 6–8

The management of both lepidopteran species can be achieved using different control strategies as part of integrated pest management (IPM) programs, but recently in Brazil, genetically modified soybean expressing a Bacillus thuringiensis (Bt) insecticidal protein has assumed a prominent position for large scale management of lepidopteran insect pests. 9,10 Although this biotechnology is an important tool for the integrated management of A. gemmatalis and C. includens, the use of Bt transgenic crops has been modifying Lepidoptera entomofauna in soybean. 9,11,12 In the US there is no Bt transgenic soybean; however, taking it as an example, the large scale adoption of Bt corn and cotton in the US has resulted in an intense selection pressure for the development of resistance and challenges the long-term sustainability of the technology. 13 Thus, how can studying interactions between insects help manage the soybean production system? In general, intra and interspecific competition dynamics among phytophagous species that are closely related are major ecological

* Correspondence to: S Ongaratto, Department of Crop Protection, School of Agriculture, São Paulo State University (FCA/UNESP), 3780 Universitária Av. - Altos do Paraíso, Botucatu, SP 18610-034, Brazil. E-mail: sabrina. ongaratto@hotmail.com

a Department of Crop Protection, School of Agriculture, São Paulo State University, Botucatu, São Paulo, Brazil

b Haskell Agricultural Laboratory, University of Nebraska-Lincoln, Concord, NE, USA
c Department of Entomology, University of Nebraska-Lincoln, Lincoln, NE, USA
d Department of Statistics, University of Nebraska-Lincoln, Lincoln, NE, USA
Among the consumptive effects, cannibalism and predation on the ef-fectors of investigations focus on single leaf-chewing or grain-feeding speci-ies,16–18 leaving the competitive interactions between leaf-eaters as a largely unexplored subject.

Several aspects should be considered when examining interact-ion of different caterpillar species. Intraguild competition has complex functions that work at any level from individual fitness to community structure pattern, being affected for many rea-sons.19 Among the consumptive effects, cannibalism and predation may intensify intraguild competition. Cannibalism may result in population self-regulation, while predation among indi-viduals of competing species tends to ‘regulate’ the total biomass of individuals.20,21 In this sense, competitive movements are par-ticularly important for the acquisition of and/or defense of limited resources, allowing insects to survive and continue development and pass their genes to the next generations. Studies indicate that for many species the genetic background of the insects is involved with competitiveness among species, including in relation to competitive behavior. Finally, the widespread occurrence of various adaptations that either inhibit or promote aggressive behavior among relatives implies the presence of selection on genes that regulate its expression.22

Mutually or alternatively, the direction of competitive interac-tions may not have simply been due to direct effects, but also/instead to indirect competitive effects, such as resource competi-tion/depletion, avoidance, exclusion, and can be a response to many environmental factors. Additional factors can also increase activity and exposure to non-conspeci succession predation (e.g., biological control), tradeoffs of activity on non-mass/survival elements of fitness (e.g., reproduction), especially under field con-ditions. Life history and physiological condition of an individual and the ecological and social conditions could also modify the likelihood of these kinds of behavior.20,21 All of these factors, com-bined or not, would lead to the consequence of larval intraguild interaction impacting aggressive behavior and fitness.

In this context, the behavior of lepidopterans exposed to insecticides has been explored in order to understand the influence on fitness23 and on the efficacy of pest management.24 In addition, some studies have investigated how pest behavioral responses to insect-resistant transgenic crops are influenced by alleles that confer resistance to plant-incorporated toxins25–30 and just a few studies have been designed to understand the feeding behavior31 and the intraguild interactions of different lepidop-teran species in corn32,33 and cotton.34 In soybean, there are only competition studies involving stinkbugs.35 Thus, further research involving other species, such as A. gemmatalis and C. includens, is highly desirable.

Considering the importance of soybean and the expansion of Bt crop cultivation, a better understanding of the behavior and inter-action between insect species has become increasingly necessary and can help in the management of A. gemmatalis and C. includens. Thus, the objective of this study was to evaluate the intraspecific and interspecific interactions of A. gemmatalis and C. includens larvae on soybean in different competition scenarios under laboratory conditions, and its effects on fitness. We also assessed the larval interactions using an automated video tracking system to describe details of larval behavior in scenarios in the presence and absence of food, under controlled conditions.

2 MATERIALS AND METHODS

2.1 Insects

For the fitness cost assay, colonies of A. gemmatalis and C. includens were kept under laboratory conditions [25 ± 2°C, RH: 60 ± 10%; 14:10 (L:D)] in the Department of Crop Protection, São Paulo State University, Botucatu, São Paulo, Brazil. The larvae were reared on artificial diet proposed by Greene et al.36 and addi-tional details on rearing methodology per Parra.37 To maintain colony vigor, insects were frequently collected from the field and from the other colonies, identified, and transferred to the spe-cific colonies used in this study.

To provide insects for the video tracking assay, larvae were com-mercially acquired (Benzon Research Inc., Carlisle, PA) and reared in plastic cups containing 15 mL of artificial diet (based on diet developed by USDA, Stoneville, MS and University of Georgia, Athens, GA). The insects were kept in a rearing chamber [25 ± 2°C, RH 60 ± 10%, 14:10 (L:D)] until the fourth instar.

2.2 Fitness cost evaluation

The intraguild interaction assay was performed in competition scenarios involving the two species under laboratory conditions at São Paulo State University, Botucatu, São Paulo, Brazil. Before testing, two larvae from each combination, based on scenarios in Table 1, were taken separately from the colony and starved for 2 h. After that, larvae were placed into transparent plastic cups (100 mL) containing soybean leaves collected from plants at phe-nological stage V4/V538 which were maintained in a greenhouse and free from insect infestation. Non-Bt soybean seeds (TMG 7262 RR) were sown in 5 L pots with sterilized soil to provide vegetative tissue for the scenarios.

Each plastic cup was considered one replicate, with 15 replicates per scenario. Larvae of the same age were held singly in plastic containers and fed with the same food source. Surviving larvae from the different competition scenarios were kept isolated in the same plastic containers and with the same food source until pupation. When the pupae were 2 days old, individuals were sexed and weighed on an analytical scale (model AY 220 0.0001G, Shimadzu Corporation, Kyoto, Japan) and main-tained until adult emergence to verify pupal survival.

2.3 Video tracking trials

Automated video tracking software (Ethovision XT 14; Noldus Information Technology, Wageningen, The Netherlands) was

| Table 1. Scenarios of intraguild competition involving A. gemmatalis and C. includens larvae of different sizes for fitness assay |
|-------------|---------------------------------|
| Intraguild competition | A. gemmatalis (third) versus C. includens (third) |
| Treatments* | A. gemmatalis (third) versus C. includens (fifth) |
| A. gemmatalis (fifth) versus C. includens (fifth) | A. gemmatalis (fifth) versus C. includens (fifth) |
| A. gemmatalis (third) versus A. gemmatalis (fifth) | C. includens (third) versus C. includens (fifth) |
| A. gemmatalis (third) versus A. gemmatalis (fifth) | C. includens (fifth) versus C. includens (fifth) |

*Adapted from Dorhout and Rice and Bentivenha et al.20,32.
used to examine potential differences in behavior between *A. gemmatalis* and *C. includens* when in competition scenarios. The experiment was conducted at the University of Nebraska, Entomology Department, Lincoln, NE, USA. Non-Bt soybean seeds (Pioneer® P25A27X) were sown in 5 L pots with sterilized soil and fertilizer (Peter’s 20-10-20 general purpose fertilizer/200 ppm Nitrogen) to provide vegetative tissue for the scenarios that offered food. The soybean leaves were collected from plants at phenological stage V4/V5, which were maintained in a greenhouse free from insect infestation. Larvae were taken separately from plastic cups with artificial diet and starved for 2 h. For each bioassay replication, a pair of larvae were confined together on opposite sides of a petri dish (8 cm diameter) as a food source, which was classified as food available or food not available. To keep the soybean tissue moist, one layer of solidified agar (2.5% wt:vol, 1.5 mm thickness) was prepared as previously described, and the leaf tissue was deposited above the layer.

Larvae interactions were assessed in five scenarios (Table 2) with and without food (10 scenarios in total). Each scenario had 15 replications, totaling 150 Petri dishes, and was recorded with a Dino-Lite AD413T-12 V camera (Big C, Torrance, CA). Ethovision software was used to collect information on the interactions of the larvae over a 20-min period. Measurements on each individual larva were taken for the distance moved (cm). When food was present, measurements on each individual larva were taken for frequency in food (n) and time in food (s). For scenarios where two larvae were present, measurements for the distance between lar-va (cm) and body contact (s) was also recorded and analyzed.

### 2.4 Statistical analyses

Fitness data (weight, survival, and cannibalism/predation) were submitted to analysis of variance, with normality and homoscedasticity using Shapiro–Wilk test and Levene’s test, respectively. When differences occurred, Tukey’s LSD’s were reported at the 0.05 using PROC GLIMMIX procedure in SAS 9.4. The same procedure was used to analyze each of the five measurements of the interest for the video-tracking assay: distance moved (cm), distance between larvae (cm), body contact (s), time in food (s), and frequency in food (n). Residual and qq-plots were used to assess normality. When normality assumptions were violated, a generalized linear mixed model (GLMM) was used to account for the underlying distribution of the data.

We analyzed the scenarios as a factorial treatment design depending on the response variable of interest and presence or absence of food. Distance moved was analyzed by a linear mixed model (LMM) with a 2 × 2 × 3 factorial treatment design: food – available/not available; treatment (species of larvae in which the distance moved was measured) – *A. gemmatalis/C. includens*; competitor – *A. gemmatalis/C. includens/isolated*. The variability due to the petri dish was considered as a random effect. Distance between larvae was analyzed using a linear model (LM) with a 2 × 3 factorial treatment design: food – available/not available; larvae combination – *A. gemmatalis + A. gemmatalis/C. includens/isolated* + *C. includens*. Body contact was analyzed as a generalized linear model (GLM) with a gamma distribution following the same 2 × 3 factorial treatment design as distance between larvae. Time in food and frequency in food were analyzed by generalized linear mixed models (GLMM) with a 2 × 3 factorial treatment design: treatment (species of larvae) – *A. gemmatalis/C. includens*; competitor – *A. gemmatalis/C. includens/isolated*. The variability due to the Petri dish was considered as a random effect. Time in food followed a gamma distribution while frequency in food followed a negative binomial distribution.

### 3 RESULTS

#### 3.1 Fitness cost evaluation

Mean pupal weights of *A. gemmatalis* did not significantly differ among noncompeting and competing larvae surviving alone or in competition scenarios as third instar males (F = 2.46; df = 4, 23; P = 0.0744; Table 3), fifth instar males (F = 1.25; df = 4, 47; P = 0.3041), and fifth instar females (F = 0.39; df = 4, 24; P = 0.8148). Mean pupal weight of *A. gemmatalis* females from third instar larvae isolated (202.64 mg) was significantly greater than the pupal weight of small larvae surviving in competition (F = 2.91; df = 4; 20; P = 0.0476). The lowest means were observed when competing against fifth instar *A. gemmatalis* and fifth instar *C. includens*, with 130.85 mg and 145.65 mg, respectively. Regarding *C. includens*, we found no significant differences in pupal weight among different competition scenarios (P > 0.05; Table 3). However, in all cases and regardless of the species (*A. gemmatalis* or *C. includens*), higher values were observed in noncompeting scenarios while third instar larvae looked to be more negatively affected.

Larval survival of third instar *A. gemmatalis* (F = 10.72; df = 4, 70; P < 0.0001) and fifth instar (F = 3.40; df = 4, 70; P = 0.0134) was significantly affected by competition. The lowest survival means were observed on intraspecific scenarios involving fifth instar *A. gemmatalis* as a competitor, with 20.00% and 80.00% survival, respectively (Table 4). Regarding third instar *C. includens* (F = 11.13; df = 4, 70; P < 0.0001), the lowest survival means were related to interspecific scenarios, when the survival was 13.33% and 33.33% competing with third and fifth instar *A. gemmatalis* larvae, respectively. No differences occurred in scenarios involving *C. includens* fifth instar larvae (F = 1.72; df = 4, 70; P = 0.1545).

Third instar *A. gemmatalis* (F = 7.17; df = 3, 56; P = 0.0004) was affected by cannibalism (40.00%) when competing with fifth instar *A. gemmatalis* (Table 4). There was no difference in percentage of cannibalism/predation of fifth instar *A. gemmatalis* (F = 2.15; df = 3, 56; P = 0.1037) against the different competitors. In scenarios involving *C. includens*, 46.67% and 20.00% predation were observed for third (F = 4.91; df = 3, 56; P = 0.0042) and fifth

---

**Table 2.** Scenarios of intraguild interaction involving *A. gemmatalis* and *C. includens* in the presence or absence of food for the video tracking assay

| Intraguild interactions | Food | No food |
|-------------------------|------|---------|
| **Food**                |      |         |
| *A. gemmatalis* (fourth) versus *A. gemmatalis* (fourth)* | A. *gemmatalis* (fourth) versus *A. gemmatalis* (fourth) |
| *C. includens* (fourth) versus *C. includens* (fourth) | C. *includens* (isolated) |
| *A. gemmatalis* (fourth) versus *C. includens* (fourth) | A. *gemmatalis* (isolated) |
| *A. gemmatalis* (isolated) | C. *includens* (isolated) |
| **No food**             |      |         |
| A. *gemmatalis* (fourth) versus *A. gemmatalis* (fourth) | A. *gemmatalis* (fourth) versus *A. gemmatalis* (fourth) |
| C. *includens* (fourth) versus *C. includens* (fourth) | A. *gemmatalis* (fourth) versus *C. includens* (fourth) |
| A. *gemmatalis* (isolated) | A. *gemmatalis* (isolated) |
| C. *includens* (isolated) | C. *includens* (isolated) |

* Larval development: 4–12 h after ecdysis.
* Adapted from Bentivenha et al.39
Table 3. Mean (± SE) pupal weight (mg) of A. gemmatalis and C. includens competing in different competition scenarios under laboratory conditions

| Competition scenarios | Male |   | Female |   |
|-----------------------|------|---|--------|---|
| Treatment             | n*  | Pupal weight (mg)† | n*  | Pupal weight (mg)† |
| A. gemmatalis (third) |      |                  |      |                  |
| -                     | 8   | 201.46 ± 4.13     | 7   | 202.64 ± 12.43 a |
| -                     | 8   | 178.84 ± 14.09    | 6   | 175.22 ± 15.46 ab|
| -                     | 1   | 137.50 ± 0.00     | 2   | 130.85 ± 20.65 b |
| C. includens (third)  | 9   | 160.82 ± 11.03    | 4   | 176.73 ± 24.99 ab|
| C. includens (fifth)  | 2   | 165.80 ± 10.00    | 6   | 145.65 ± 9.15 b  |
| P                     |      | 0.0744            |      | 0.0476            |
| A. gemmatalis (fifth) |      |                  |      |                  |
| -                     | 9   | 219.84 ± 4.92     | 6   | 224.33 ± 14.99    |
| -                     | 9   | 203.93 ± 9.33     | 6   | 208.97 ± 13.41    |
| -                     | 15  | 198.60 ± 6.27     | 9   | 201.24 ± 13.15    |
| C. includens (third)  | 10  | 203.51 ± 5.02     | 5   | 212.80 ± 13.19    |
| C. includens (fifth)  | 9   | 201.01 ± 10.20    | 5   | 214.78 ± 17.25    |
| P                     |      | 0.3041            |      | 0.8148            |
| C. includens (third)  |      |                  |      |                  |
| -                     | 7   | 202.51 ± 11.46    | 8   | 193.38 ± 10.61    |
| -                     | 11  | 174.84 ± 9.63     | 10  | 170.35 ± 14.23    |
| C. includens (fifth)  | 4   | 162.68 ± 20.84    | 5   | 162.90 ± 18.30    |
| A. gemmatalis (third) | 1   | 159.80 ± 0.00     | 1   | 152.00 ± 0.00     |
| A. gemmatalis (fifth) | 2   | 138.90 ± 28.40    | 3   | 131.87 ± 6.35     |
| P                     |      | 0.1476            |      | 0.2039            |
| C. includens (fifth)  |      |                  |      |                  |
| -                     | 8   | 205.13 ± 9.41     | 7   | 211.33 ± 13.46    |
| -                     | 5   | 199.76 ± 13.90    | 8   | 200.08 ± 10.61    |
| C. includens (fifth)  | 12  | 192.84 ± 12.39    | 13  | 193.96 ± 8.82     |
| A. gemmatalis (third) | 4   | 194.25 ± 12.74    | 8   | 196.34 ± 7.92     |
| A. gemmatalis (fifth) | 4   | 199.60 ± 6.32     | 6   | 201.18 ± 9.47     |
| P                     |      | 0.9472            |      | 0.7905            |

*n, number of insects evaluated.
† Means followed by the same letter per column do not differ by Tukey’s LSD test (P > 0.05).

(F = 3.50; df = 3, 56; P = 0.0212) instars in interspecific competition, respectively. Differences in pupal survival were detected in scenarios with third instar A. gemmatalis (F = 12.27; df = 4, 44; P < 0.0001), third (F = 4.26; df = 4, 39; P = 0.0059) and fifth instar C. includens (F = 3.30; df = 4, 59; P = 0.0166) competing larvae (Table 4). In all the scenarios, the percentage of emerging adults was lower when larvae interacted with fifth instar A. gemmatalis as a competitor, except for fifth instar A. gemmatalis (F = 0.57; df = 4, 68; P = 0.6846), where no significant difference was observed when in intra or interspecific interactions.

3.2 Video tracking trials
The model response (ANOVA) results for each of the five response variables and associated model effects are in the Table 5. In addition to the main effects, model response for the distance moved indicated significant effects of the interactions between the factor’s food × treatment and treatment × competitor at the a = 0.05 (Table 5). In conditions with food available, A. gemmatalis larvae moved a greater distance regardless of the competitor compared to C. includens larvae (F = 16.60; df = 5, 88; P < 0.0001) (Table 6; Fig. 1). Two C. includens larvae were closer together on average (3.66 cm) compared to two A. gemmatalis larvae (4.34 cm) (F = 3.16; df = 2, 84; P = 0.0475), but they had less body contact (2.02 s) compared to two A. gemmatalis larvae or differing larvae species (F = 7.69; df = 2, 84; P = 0.0009).

For time in food (Table 6), no difference was observed among the scenarios (F = 1.38; df = 5, 44; P = 0.25). Regarding frequency in food, A. gemmatalis larvae were most frequently in food (8.79) when they were interacting with C. includens larvae, while C. includens larvae were least frequently in food (0.96) when they were isolated (F = 6.50; df = 5, 44; P = 0.0001) (Table 6).

When there was no food present, A. gemmatalis larvae moved a greater distance when they were interacting with other larvae of either species compared to when A. gemmatalis were isolated and compared with larvae of C. includens, regardless of whether they were interacting with other larvae or isolated (F = 46.77; df = 5, 88; P < 0.0001). Two A. gemmatalis larvae were farther apart on average (5.01 cm) compared to any other scenario (F = 11.91; df = 2, 84; P < 0.0001), while for body contact, no difference occurred among the scenarios (F = 0.40; df = 2, 84; P = 0.67) (Table 7).

4 DISCUSSION
In this study, we assessed for the first time the effect of competitiveness on development and behavior of A. gemmatalis and C. includens, two co-occurring defoliating pests of soybean. These laboratory studies provide a clearer view of how interactions occur and specific behavior of the species under controlled conditions, which can be difficult to detect in the field where other factors such as environmental conditions, natural mortality, and disease might also influence the predominance of a species. Thus, this study provides baseline information, which can be useful for
management of these important species in soybean in North and South America.

Results from the fitness study indicated that competition could impose a fitness cost, and the size of the competitor can impact insect development. Despite the male and female pupal weight factor not being affected, in general the survival in different stages was affected. The pupal weight of *A. gemmatalis* and *C. includens* in large larvae suggest that larvae did not suffer from food competition in advanced development stages. In general, there were no statistical differences observed in pupal weight, regardless of competitor and scenario, which is an important biological parameter and can impact the adult development, capacity of oviposition, offspring generated and the life history, in general.42

The survival of *A. gemmatalis* third instars competing in an intra-specific scenario with fifth instars was affected by the competition, presenting 80.00–100.00% mortality. Similarly, *C. includens* was negatively affected when competing against *A. gemmatalis* third and fifth instar. *Anticarsia gemmatalis* fifth instar had fewer effects to its development, with more than 80.00% and 92.00% of oviposition, offspring generated and the life history, in general.42

### Table 4. Mean (± SE) larval survival (%), cannibalism/predation rate (%), and pupal survival (%) of *A. gemmatalis* and *C. includens* in different competition scenarios under laboratory conditions

| Competition scenarios | Treatment Competitor | n* | Larval survival (%)† | Cannibalism/predation (%) | Pupal survival (%) |
|------------------------|----------------------|----|----------------------|--------------------------|-------------------|
| *A. gemmatalis* (third) | -                    | 15 | 100.00 ± 0.00 a       | -                        | 100.00 ± 0.00 a   |
| A. gemmatalis (third)  | 14                   | 46.67 ± 10.31 c         | 0.00 ± 5.35 b            | 8.00 ± 11.06 ab       |
| A. gemmatalis (fifth)  | 3                    | 20.00 ± 16.69 c         | 40.00 ± 13.09 a          | 0.00 ± 0.00 c       |
| C. includens (third)   | 13                   | 86.67 ± 9.09 ab         | 0.00 ± 0.00 b            | 100.00 ± 0.00 a     |
| C. includens (fifth)   | 8                    | 53.33 ± 13.33 bc        | 0.00 ± 0.00 b            | 75.00 ± 16.37 b    |
| *P*                    |                      | <0.0001                 | 0.0004                 | <0.0001            |
| *A. gemmatalis* (fifth) | -                    | 15 | 100.00 ± 0.00 a       | -                        | 100.00 ± 0.00 a   |
| A. gemmatalis (third)  | 15                   | 100.00 ± 0.00 a         | 0.00 ± 0.00 b            | 93.33 ± 6.67       |
| A. gemmatalis (fifth)  | 24                   | 80.00 ± 8.16 b          | 6.67 ± 4.54             | 96.43 ± 3.57       |
| C. includens (third)   | 15                   | 100.00 ± 0.00 a         | 0.00 ± 0.00 b            | 100.00 ± 0.00      |
| C. includens (fifth)   | 14                   | 93.33 ± 6.67 ab         | 0.00 ± 0.00 b            | 92.86 ± 7.14       |
| *P*                    |                      | 0.0134                  | 0.1037                 | 0.6846            |
| *C. includens* (third) | -                    | 15 | 100.00 ± 0.00 a       | -                        | 100.00 ± 0.00 a   |
| C. includens (third)   | 21                   | 70.00 ± 9.51 ab         | 0.00 ± 0.00 b            | 88.46 ± 6.08 a     |
| C. includens (fifth)   | 9                    | 13.33 ± 13.09 bc        | 26.67 ± 11.82 ab        | 100.00 ± 0.00 a    |
| A. gemmatalis (fifth)  | 2                    | 33.33 ± 12.60 bc        | 46.67 ± 13.33 a          | 40.00 ± 24.49 b    |
| *C. includens* (fifth) | -                    | 15 | 100.00 ± 0.00 a       | -                        | 100.00 ± 0.00 a   |
| C. includens (third)   | 13                   | 86.67 ± 9.09 ab         | 0.00 ± 0.00 b            | 100.00 ± 0.00 a    |
| C. includens (fifth)   | 25                   | 83.33 ± 7.97            | 0.00 ± 0.00 b            | 100.00 ± 0.00 a    |
| A. gemmatalis (third)  | 12                   | 80.00 ± 10.69           | 0.00 ± 0.00 b            | 83.33 ± 11.24 ab   |
| A. gemmatalis (fifth)  | 10                   | 66.67 ± 12.60           | 20.00 ± 10.69 a          | 70.00 ± 15.28 b    |
| *P*                    |                      | 0.1545                  | 0.0212                 | 0.0166            |

n*, number of insects evaluated. † Means followed by the same letter per column do not differ by Tukey’s LSD test (*P* > 0.05).

### Table 5. Model response (ANOVA) variables and associated model effects from video tracking assay between *A. gemmatalis* and *C. includens*

| Model effect | Distance moved | Distance between larvae | Body contact | Time in food | Frequency in food |
|--------------|----------------|------------------------|--------------|--------------|-------------------|
| Food         | 20.67 (1, 88)  | 5.26 (1, 84)           | 12.15 (1, 84)| -            | -                 |
| Treatment    | 256.06 (1, 88) | -                      | -            | -            | 19.41 (1, 44)     |
| Competitor   | 2.30 (2, 88)   | -                      | -            | 2.29 (2, 44) | 4.68 (2, 44)      |
| Food × treatment | 16.86 (1, 88)   | -                      | -            | -            | -                 |
| Food × competitor | 0.45 (2, 88)   | -                      | -            | -            | -                 |
| Treatment × competitor | 4.32 (2, 88)   | -                      | -            | 0.54 (2, 44) | 0.70 (2, 44)      |
| Treatment × food × competitor | 0.22 (2, 88)   | -                      | -            | -            | -                 |
| Larvae combination | -          | 13.56 (2, 84)         | 5.31 (2, 84) | -            | -                 |
| Food × larvae combination | -         | 1.51 (2, 84)          | 2.79 (2, 84) | -            | -                 |

ANOVA results are reported as F-value (df). Significant model effects at the α = 0.05 level are indicated by a bold text.
larval and pupal survival, respectively. However, when *C. includens* fifth instar was in competition with others, larval and pupal survival were more affected, with 66.67% and 70.00% survival, respectively. Although food was available, the small surface area of the arena may have resulted in more encounters between the larvae, or not allowed escape from competitors. Furthermore,

### Table 6. Mean (± SE) distance moved, distance between larvae, body contact, time in food, and frequency in food in scenarios with *A. gemmatalis* and *C. includens* interaction with food

| Treatment | Competitor | Distance moved (cm)* | Distance between larvae (cm) | Body contact (s) | Time in food (s) | Frequency in food (n) |
|-----------|------------|----------------------|-----------------------------|----------------|----------------|----------------------|
| A. gemmatalis | A. gemmatalis | 256.33 ±19.88 a | 4.34 ±0.19 a | 15.84 ±6.70 a | 185.94 ±42.12 | 5.30 ±1.28 ab |
| C. includens | A. gemmatalis | 245.57 ±24.50 a | 3.86 ±0.19 ab | 14.99 ±6.34 a | 144.60 ±46.33 | 8.79 ±2.73 a |
| Isolated | 198.78 ±24.50 a | - | - | 329.72 ±105.63 | 2.27 ±0.79 abc |
| C. includens | A. gemmatalis | 77.75 ±24.50 b | 3.86 ±0.19 ab | 14.99 ±6.34 a | 208.01 ±66.64 | 1.99 ±0.70 abc |
| C. includens | Isolated | 80.72 ±19.88 b | 3.66 ±0.19 b | 14.99 ±6.34 a | 268.97 ±60.93 | 1.85 ±0.49 bc |
| P | <0.0001 | 0.0475 | 0.0009 | 0.25 | 0.0001 |

*Means followed by the same letter per column do not differ by Tukey’s LSD test (*P* > 0.05).

### Table 7. Mean (± SE) distance moved, distance between larvae, and body contact in scenarios with *A. gemmatalis* and *C. includens* interaction with no food

| Treatment | Competitor | Distance moved (cm)* | Distance between larvae (cm) | Body contact (s) |
|-----------|------------|----------------------|-----------------------------|----------------|
| A. gemmatalis | A. gemmatalis | 379.29 ±19.88 a | 5.01 ±0.19 a | 35.18 ±14.89 |
| C. includens | A. gemmatalis | 381.37 ±24.50 ab | 4.29 ±0.19 b | 24.05 ±10.18 |
| Isolated | 286.05 ±24.50 b | - | - | - |
| C. includens | A. gemmatalis | 104.33 ±24.50 c | 4.29 ±0.19 b | 24.05 ±10.18 |
| C. includens | Isolated | 89.06 ±19.88 c | 3.66 ±0.19 b | 21.00 ±8.89 |
| Isolated | 99.56 ±24.50 c | - | - | - |
| P | <0.0001 | <0.0001 | 0.6719 |

*Means followed by the same letter per column do not differ by Tukey’s LSD test (*P* > 0.05).

---

Figure 1. Representative movement of *A. gemmatalis* (*Ag*) and *C. includens* (*Ci*) larvae recorded over 20 min in different interaction scenarios with food and no food in glass petri dish arenas. For *Ag x Ag* and *Ci x Ci* scenarios, one larva track is red and the other green. For *Ag x Ci* scenarios, *Ag* is green, and *Ci* is red. For single larva scenarios, tracks are red.
the relative density of resources within leaves could have differed and the small laboratory arena likely created an artificial scenario where another resource pool with higher protein was readily available, the competitor larvae in this case. In a situation where the superficial area is larger than the arena used in this study, and a larva has a high velocity of movement, the larva will likely have a greater probability to escape from cannibalism or predation.

In addition, this study did not evaluate larval attack or defense behaviors, actions of which are additional factors in the system, and may result in significant injury or death of individuals without direct killing from predation/cannibalism. These behaviors may have occurred in interspecific competition involving fifth instars C. includens and A. gemmatalis, where C. includens survival was affected, but just 20.00% predation was observed. Complementary ethogram studies examining larval attack and defense behaviors could clarify these results. Some theory indicates that larvae who end up practicing aggressive movements under naturalistic conditions, such as in the field, may have lower survival by suffering injuries and death, acquiring pathogens or parasites, and having subsequent reduction in fitness, lower pupal weight, and lower rates of development.21 Further, avoiding the other competitor could have incurred relatively substantial hormonal stress, depletion of lipids, missed feeding opportunities, or other indirect costs.20

Little is known about competition behaviors in intra or interspecific interactions involving A. gemmatalis and C. includens, but is well documented for other lepidopteran species.32,33,39,43 In our interaction study under laboratory conditions, cannibalism and predation varied throughout the scenarios. Cannibalism occurred mainly in scenarios involving third versus fifth instar A. gemmatalis (40.00% observed). In addition, the highest predation rate was observed in interspecific competition between third instar C. includens versus A. gemmatalis fifth instar, and fifth versus fifth instar, with 46.67% and 20.00% respectively, suggesting that A. gemmatalis is more aggressive and could prevail over C. includens. Cannibalism and predation may be the most important mortality factors in insect populations,44 and several studies have showed that this behavior in different lepidopteran species is instar dependent, with higher rates related to delayed larval development, particularly when larvae of different instars are placed together and confined.21,33,39,45,46 However, there are other variables influencing the effect of competition resulting in aggressive behavior. Quality and quantity of food (Bt plant or not), population density, stress, and many other non-consumptive effects and environmental factors (e.g., temperature and water availability) have impact on competition and fitness, specially under field conditions.21,22

The results from the video tracking indicate that A. gemmatalis has a highly active behavior and tends to move more than C. includens. Anticarsia gemmatalis moves less when isolated than when interacting with another larva, regardless of the species, confirming that the interaction influences larval behavior. This species moved constantly and mostly in a circle, repeatedly over the evaluation period (in food or no food scenarios). Anticarsia gemmatalis is more variable in how often it visits the food, depending on its interaction with the competitor, spending more time interacting with other larvae in scenarios with food. On the other hand, C. includens moved less and in an irregular pattern, visiting the food zone less often but spending more time there.

In general, the distance moved by A. gemmatalis was greater compared with C. includens, and this behavior might be one of the characteristic responses of larvae to escape an interaction.45,47 Furthermore, due to the fact that it will be in constant movement it can result in more efficient chemical control of this species in non-Bt soybean fields. In Brazil, C. includens larvae were observed to prefer to position themselves predominantly in the lower and mid region of the soybean plants,48 less exposed to insecticide, while the capacity of A. gemmatalis to move longer distances in the canopy may expose it more readily to treatment. Moreover, larval movement behavior and the difference in distance moved between the species emphasizes the importance of understanding on-plant movement49,50 and plant-to-plant movement of lepidopterans.51–53 The movement of larvae in a seed mixture refuge strategy system, for example, might expose the insect to sub-lethal doses of Bt proteins by feeding on plant tissues of differential Bt protein content, or 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.54 Thus, the widespread release and adoption of Bt crops and the issues involving the different refuge models reinforce the necessity for further research to evaluate the effect of larval mobility and behavior on these strategies.

The distance between larvae in intra and interspecific scenarios involving A. gemmatalis was greater, which demonstrates that the species may have greater ability to escape from competitions. This species may have the habit of inciting aggressive behaviors, whether they are defensive or offensive mechanisms,55 and then, moving away from the competitor.45,47 Associated with this, regarding body contact, A. gemmatalis spent more time interacting, both in intra or interspecific scenarios, and taking advantage over C. includens. In practical terms, this characteristic may be related to competition for food, increasing cannibalism and/or predation behaviors.

Regarding the frequency at food, the two species visited the food less when isolated than when interacting with other larvae, which was expected since the species would be competing for food in intraguild scenarios. For Spodoptera frugiperda and Helicoverpa zea (Lepidoptera: Noctuidae) in corn, the same behavior was observed.39 Although not statistically significant, both species spent more 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. The decrease in larval feeding when larvae are interacting was described in a plant-to-plant movement study with S. frugiperda,52 and suggested that intraguild interactions could be related to larvae moving among plants, or may be a factor that increases the movement of larvae on alternative host plants. For insects in general, when there are restrictions in food availability and/or competitiveness, the outcome of the competition can be expressed more rapidly.56 Thus, as a secondary result, outbreaks of these species may also occur on alternative hosts, which are typically less preferred by the species, and the outcome of the competition may be more gradual or even uncertain.

Many animals will cannibalize as soon as all other food items are removed, but they may also respond simply to a reduction in the relative availability of alternatives, such as missed opportunity to feed or restrictions in food, leading to a food stress, and generally increasing food searching activity.20 Hunger triggers searching behavior, lowers the attack threshold, increases foraging time, and increases movement by stimulating locomotor activity, changing the location of foraging stations, and expanding the search area. Each of these behaviors increases the probability of intraspecific contact and predation.20 The differences in behavior in our study could be because the larvae were starved, the small
size of the arena caused stress, or simply because of the competition for the soybean leaf (food availability scenario). In practical terms, and as a secondary result of the absence of food, outbreaks of these species may also occur on alternative hosts, which are typically less preferred by the species, and the outcome of the competition may be more gradual or even uncertain.

In general, based in the results, it is assumed that if these two species occur simultaneously on the same plant and in the same phenological phase, there is a greater chance of A. gemmatalis having an advantage. In summary, interactions among the leaf-feeding Lepidoptera affect their behavior and our study showed that larvae of A. gemmatalis gain competitive advantage in intraguild interaction with C. includens. By using non-Bt soybean, this study provides the baseline of larval behavior of two economically important species in intraguild interactions. The use of Bt technology is just one more factor in a complex system, which may impact population dynamics, pest prevalence, and the competitive displacement of species that share the same ecological niche. In the future it is necessary to explore fitness and behavior under field conditions where more factors are at play.

5 CONCLUSIONS
Our findings provide significant information regarding lepidopteran development and behavior, contributing to the development of integrated pest management and resistance management strategies of these species. More research is needed to fully understand the role of larval movement, feeding behavior, intraguild interaction focusing the non-consumptive interactions, and other factors with respect to IPM and IRM.

ACKNOWLEDGEMENTS
We would thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES - Finance Code 001) for a scholarship for the senior author and Conselho Nacional de Desenvolvimento Científico e Tecnológico – Brazil (CNPq - 303892/2016-1) for the productivity scholarship in research granted to the second author. We also thank University of Nebraska-Lincoln for the financial support, for providing the Ethosview software and biological material and Dr Justin McMechan and Dr Ana M Vélez (University of Nebraska-Lincoln) for technical support.

CONFLICT OF INTERESTS
All authors declare no conflict of interest.

REFERENCES
1 CONAB, Boletim da Safra de Grãos. Available: https://www.conab.gov.br/info-agro/safras/graos/boletim-da-safra-de-graos [27 July 2020].
2 USDA, Oilseeds: World Markets and Trade. Foreign Agricultural Service, 2019. Available: https://www.fas.usda.gov/data/oilseeds-world-markets-and-trade (25 May 2020).
3 Sosa-Gómez DR, López Lastra CC and Humber RA, An overview of arthropod-associated fungi from Argentina and Brazil. Mycopathologia 170:51–76 (2010).
4 Formentini AC, Sosa-Gómez DR, Paula-Moraes SV, de Barros NM and Specht A, Lepidopteran (Insecta) associated à cultura da soja na Argentina, Brasil, Chile e Uruguai. Cienc Rural 45:2113–2120 (2015).
5 Blanco CA, Chiara Vale W, Dalla Rizza M, Farias JR, Garcia-Degano MF, Gastaminda G et al., Current situation of pests targeted by Bt crops in Latin America. Curr Opin Insect Sci 15:131–138 (2016).
6 Alford RA and Hammond AM Jr, Plusiinae (Lepidoptera: Noctuidae) populations in Louisiana soybean ecosystems as determined with loophole-baited traps. J Econ Entomol 75:647–650 (1982).
7 Haase S, McCarthy CB, Ferrelli ML, Piedre ML, Sciocco-Cap A and Romanowski V, Development of a recombination system for the generation of occlusion positive genetically modified Anticarsia gemmatalis multiple Nucleopolyhedrovirus. Viruses 7:1599–1612 (2015).
8 Bortolotto OC, Pomari-Fernandes A, Bueno RCOF, Bueno AF, da Cruz YKS, Sanzovo A et al., The use of soybean integrated pest management in Brazil: a review. Agron Sci Biotechnol 1:25–32 (2015).
9 Bernardi O, Malvestiti GS, Durando PM, Oliveira WS, Martinelli S, Berger GU et al., Assessment of the high-dose concept and level of control provided by MON 87701 × MON 89788 soybean against Anticarsia gemmatalis and Pseudoplusia includens (Lepidoptera: Noctuidae) in Brazil. Pest Manag Sci 68:1083–1091 (2012).
10 Palma L, Muñoz D, Berry C, Müllero J, Caballero P and Caballero P, Bacillus thuringiensis toxins: an overview of their biocidal activity. Toxins (Basel) 6:3296–3325 (2014).
11 Miklos JA, Aliabadi MF, Bledig SA, Connor-Ward DC, Gao A-G, Holmes BA et al., Characterization of soybean exhibiting high expression of a synthetic Bacillus thuringiensis cry1A transgene that confers a high degree of resistance to lepidopteran pests. Crop Sci 47:148–157 (2007).
12 Bel Y, Zack M, Narva K and Escribe B, Specific binding of Bacillus thuringiensis Cry1Ea toxin, and Cry1Ac and Cry1Fa competition analyses in Anticarsia gemmatalis and Chrysodeixis includens. Sci Rep 9:1–7 (2019).
13 Tabashnik BE, Brévault T and Carrière Y, Insect resistance to Bt crops: lessons from the first billion acres. Nat Biotechnol 31:510–521 (2013).
14 Denno RF, McClure MS and Ott JR, Interspecific interactions in phytophagous insects: competition reexamined and resurrected. Annu Rev Entomol 40:297–331 (1995).
15 Jones ME, Hanlon CC and Paine TD, Potential for interspecific competition between congeneric longhorned beetle species (Coleoptera: Cerambycidae) in an adventive environment. Environ Entomol 44:960–965 (2015).
16 Malling KSV, Oliveira EE and Guedes RNC, Competition between the bean weevils Acanthoscelides obtectus and Zabrottes subfasciatus in common beans. J Stored Prod Res 55:32–35 (2013).
17 Ishii Y and Shimada M, Competitive exclusion between contest and scramble strategists in Callosobruchus seed–beetle modeling. Popul Ecol 50:197–205 (2008).
18 Oliveira SOD, Rodrigues AS, Vieira JL, Rosi-Depaoli CA, Guedes NMP and Guedes RNC, Bean type modifies larval competition in Zabrottes subfasciatus (Chrysomelidae: Bruchinae). J Econ Entomol 108:2098–2106 (2015).
19 Nishimura K and Isoda Y, Evolution of cannibalism: referring to costs of cannibalism. J Theor Biol 226:293–302 (2004).
20 Fox LR, Cannibalism in natural populations. Annu Rev Ecol Syst 6:87–106 (1975).
21 Polis GA, The evolution and dynamics of intraspecific predation. Annu Rev Ecol Syst 12:225–251 (1981).
22 Raymond B, Sayed AH and Wright DJ, Genes and environment interact to determine the fitness costs of resistance to Bacillus thuringiensis. Proc R Soc B Biol Sci 272:1519–1524 (2005).
23 Stacke RF, Godoy DN, Halberstadt SA, Bronzatto ES, Giacomelli T, Hettwer BL et al., Inheritance of lambda-cyhalothrin resistance, fitness costs and cross-resistance to other pyrethroids in soybean looper, Chrysodeixis includens (Lepidoptera: Noctuidae). Crop Prot 131:105096 (2020).
24 Georghiou GP, The evolution of resistance to pesticides. Annu Rev Ecol Syst 3:133–168 (1972).
25 Gassmann AJ, Carrière Y and Tabashnik BE, Fitness costs of insect resistance to Bacillus thuringiensis. Annu Rev Entomol 54:147–163 (2009).
26 Li YX, Greenberg SM and Liu TX, Orientation behavior, development and survival of Trichopadi ni (Lepidoptera: Noctuidae) larvae on cotton expressing Cry1Ac and Cry2Ab and conventional cotton. J Insect Behav 20:473–488 (2007).
27 Harris MO, Markwick N and Sandanayake M, Is resistance to Bacillus thuringiensis endotoxin Cry1Ac associated with a change in the behavior of light brown apple moth larvae (Lepidoptera: Tortricidae)? J Econ Entomol 99:508–518 (2009).
28 Prasifka JR, Hellmich RL, Crespo ALB, Siegfried BD and Onstad DW, Video-tracking and on-plant tests show Cry1Ab resistance influences behavior and survival of neonate Ostrinia nubilalis following exposure to Bt maize. J Insect Behav 23:1–11 (2010).
Interaction effects on lepidopteran behavior

www.soci.org

29 Dorhout DL and Rice ME, Intraguild competition and enhanced survival of western bean cutworm (Lepidoptera: Noctuidae) on transgenic Cry1Ab (MON810) Bacillus thuringiensis corn. J Econ Entomol 103:54–62 (2010).

30 Wang R, Tetreau G and Wang P, Effect of crop plants on fitness costs associated with resistance to Bacillus thuringiensis toxins Cry1Ac and Cry2Ab in cabbage loopers. Sci Rep 6:20959 (2016).

31 Montezano DG, Hunt TE, Specht A, Luz PMC and Peterson JA, Survival and development of Striacosta albicosta (smith) (Lepidoptera: Noctuidae) immature stages on dry beans, non-Bt, Cry1F, and Vip3A maize. Insects 10:343 (2019).

32 Bentivenha JPF, Baldin ELL, Hunt TE, Paula-Moraes SV and Blankenship EE, Intraguild competition of three noctuid maize pests. Environ Entomol 45:999–1008 (2016).

33 Bentivenha JPF, Paula-Moraes SV, Baldin ELL, Specht A, da Silva IF and Hunt TE, Battle in the new world: Helicoverpa armigera versus Helicoverpa zea (Lepidoptera: Noctuidae). PLoS One 11:e0167182 (2016).

34 Benedict JH, Sachs ES, Altman DW, Ring DR, Stone TB and Sims SR, Impact of δ-endotoxin-producing transgenic cotton on insect-plant interactions with Heliotris virescens and Helicoverpa zeas (Lepidoptera: Noctuidae). Environ Entomol 22:1–9 (1993).

35 Tuelher ES, Silva EH, Hirose E, Guedes RNC and Oliveira EE, Competition between the phytophagous stink bugs Euschistus heros and Piezodorus guildinii in soybeans. Pest Manag Sci 72:1837–1843 (2016).

36 Greene GL, Leppla NC and Dickerson WA, Velvetbean caterpillar: a rearing procedure and artificial medium. J Econ Entomol 69:487–488 (1976).

37 Parra JRP, Técnicas de criação de insetos para programas de controle biológico. FEALQ, Piracicaba (2001).

38 Fehr W and Caviness C, Stages of soybean development, Spec Rep 2244 (1977).

39 Ferial D, Ávila CJ and Schlick-Souza EC, Population fluctuations and vertical distribution of the soybean looper (Chrysodeixis includens) in soybean culture. Am J Plant Sci 9:1544–1556 (2018).

40 Paulina Moraes SV, Hunt TE, Wright RJ, Hein GL and Blankenship EE, On-plant movement and feeding of western bean cutworm (Lepidoptera: Noctuidae) early instars on corn. Environ Entomol 41:1494–1500 (2013).

41 Raffa KF, Effect of host plant on cannibalism rates by fall armyworm (Lepidoptera: Noctuidae) larvae. Environ Entomol 16:672–675 (1987).

42 Sinner RE, Jones JW, Tuttle C and Caron RE, Population mortality and cyclicity as affected by intraspecific competition. Can Entomol 109:879–890 (1977).

43 Horner TA, Dively GP and Herbert DA, Development, survival and fitness performance of Helicoverpa zea (Lepidoptera: Noctuidae) in MON810 Bt field corn. J Econ Entomol 96:914–924 (2003).

44 Joyner K and Gould F, Developmental consequences of cannibalism in Heliothis zea (Lepidoptera: Noctuidae). Ann Entomol Soc Am 78:24–28 (1985).

45 Dial Cl and Adler PH, Larval behavior and cannibalism in Heliothis zea (Lepidoptera: Noctuidae). Ann Entomol Soc Am 83:258–263 (1990).

46 Zulin D, Montezano DG, Hunt TE, Battle in the new world: Helicoverpa armigera versus Helicoverpa zea (Lepidoptera: Noctuidae) early instars on corn. Environ Entomol 41:192–200 (2016).

47 Wangila DS, Leonard BR, Ghimire MN, Bai Y, Zhang L, Yang Y et al., Occurrence and larval movement of Diatraea saccharalis (Lepidoptera: Crambidae) in seed mixes of non-Bt and Bt pyramid corn. Pest Manag Sci 69:1163–1172 (2013).

48 Pannuti LER, Paula-Moraes SV, Hunt TE, Baldin ELL, Dana L and Malaquias JV, Plant-to-plant-to-plant movement of Striacosta albicosta (Lepidoptera: Noctuidae) and Spodoptera frugiperda (Lepidoptera: Noctuidae) in maize (Zea mays). J Econ Entomol 109:1125–1131 (2016).

49 Raffa KF, Effect of host plant on cannibalism rates by fall armyworm (Lepidoptera: Noctuidae) larvae. Environ Entomol 16:672–675 (1987).