

SABRINA ONGARATTO

***Anticarsia gemmatalis* (LEPIDOPTERA: EREBIDAE): RESISTÊNCIA DE
GENÓTIPOS DE SOJA, CARACTERIZAÇÃO DE MOVIMENTAÇÃO LARVAL E
INTERAÇÃO INTRAGUILDA COM *Chrysodeixis includens* (LEPIDOPTERA:
NOCTUIDAE)**

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SABRINA ONGARATTO

***Anticarsia gemmatalis* (LEPIDOPTERA: EREBIDAE): RESISTANCE OF
SOYBEAN GENOTYPES, CHARACTERIZATION OF LARVAL MOVEMENT AND
INTRAGUILD INTERACTION WITH *Chrysodeixis includens* (LEPIDOPTERA:
NOCTUIDAE)**

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Coadvisor: Prof. Dr. Thomas Elliott Hunt

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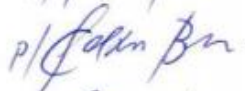
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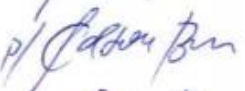
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I dedicate

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RESUMO

A produtividade da soja é frequentemente reduzida devido a injúrias causadas por inúmeras pragas. *Anticarsia gemmatalis* Hübner (Lepidoptera: Erebidæ) é uma dessas pragas e é considerada um dos mais importantes insetos desfolhadores da cultura. Além disso, *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidæ) tem se tornado um sério problema fitossanitário para a cultura, com vários surtos ocorrendo isolados ou associados a *A. gemmatalis*. Além da ocorrência no Brasil, essas espécies também ocorrem em diferentes regiões produtoras de soja dos Estados Unidos. O uso de materiais resistentes é uma ferramenta valiosa para o manejo desses lepidópteros em substituição ou em associação aos inseticidas sintéticos. Porém a rápida adoção de culturas *Bt* (soja geneticamente modificada) pelo mundo tem ocasionado uma contínua pressão de seleção das proteínas Cry sobre esses lepidópteros, o que pode resultar em populações resistentes, com consequentes falhas de controle. Assim, considerando a importância da soja, a expansão do cultivo com culturas *Bt* e o respectivo impacto na entomofauna, um entendimento mais profundo sobre o comportamento larval tem se tornado cada vez mais necessário. Os objetivos desta pesquisa foram: i) caracterizar a possível resistência de 30 genótipos de soja a *A. gemmatalis* por meio de testes em casa de vegetação e laboratório; ii) caracterizar o movimento larval de *A. gemmatalis* “na planta” e “entre plantas” de soja não *Bt* a campo e; iii) investigar as interações intra e interespecífica envolvendo *A. gemmatalis* e *C. includens* em laboratório. Os resultados dos ensaios de resistência a *A. gemmatalis* revelaram a expressão de antixenose (oviposição, atratividade e/ou consumo) nos genótipos 'TMG 133' RR, 'TMG 1179' RR, 'IAC 19', 'IAC 17', 'IAC 100', D75-10169 e IAC 78-2318. Os genótipos IAC 74-2832, 'IAC 19', 'IAC 17', 'IAC 100' e PI 274454 afetaram o comportamento e também impactaram negativamente a viabilidade larval, indicando a expressão de antixenose e/ou antibiose. 'TMG 7062' IPRO afetou negativamente o desenvolvimento e a viabilidade larval, indicando uma possível expressão de antibiose, além de baixo grau de antixenose. Os genótipos 'IAC 19', 'IAC 24', 'IAC 17' e 'IAC 100' expressaram antibiose por comprometer diferentes parâmetros biológicos, principalmente o desenvolvimento larval, viabilidade e reduzir o peso de pupas. Devido à redução da ingestão foliar por larvas em confinamento, a antixenose foi também caracterizada em 'IAC 24'. Os testes de movimentação na planta mostraram que *A. gemmatalis* tem um

comportamento bastante ativo durante o dia, especialmente em instares avançados de desenvolvimento. Verificou-se que as lagartas variam quanto à posição em que são encontradas na planta e na folha ao longo do período de observação. Quanto à movimentação entre plantas, lagartas de *A. gemmatalis* podem mover-se por longas distâncias (até 2 m) a partir da planta infestada de uma maneira não direcional. Os resultados do teste de *fitness* e de rastreamento por vídeo, mostraram que *A. gemmatalis* tem vantagem competitiva sobre *C. includens*. Nosso estudo apresenta relevantes descobertas envolvendo a interação dos dois lepidópteros em soja. Os genótipos com indicativo de resistência podem ser utilizados em programas de melhoramento visando à resistência de genótipos a *A. gemmatalis* e possivelmente a outros insetos desfolhadores. Os resultados obtidos nos testes de movimentação poderão auxiliar na escolha do momento ideal para pulverizações com inseticidas, entomopatógenos e liberação de organismos biológicos, além de auxiliar na escolha de estratégias de manejo mais assertivas. Ainda, a interação entre *A. gemmatalis* e *C. includens* poderá contribuir para um melhor entendimento do comportamento e da dinâmica de populações. Esse conjunto de informações podem auxiliar nos programas de Manejo Integrado de Pragas (MIP) e no Manejo de Resistência de Insetos (MRI) na cultura da soja.

Palavras-chave: Resistência de plantas a insetos; antixenose; antibiose; *fitness*; comportamento larval; Lepidoptera.

ABSTRACT

Soybean yield is often reduced because of the injury caused by numerous pests. *Anticarsia gemmatalis* Hübner (Lepidoptera: Erebidæ) is one such pest and is considered one of the most important defoliating insects of the crop. In addition, *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidæ) has recently become a serious phytosanitary problem for the crop, with several outbreaks occurring in isolation or associated with *A. gemmatalis*. Besides the occurrence in Brazil, these species also occur in different United States soybean production regions. The use of resistant materials is a valuable tool for the management of these lepidopterans in replacement or being used in association with synthetic insecticides. However, the rapid widespread adoption of *Bt* crops (genetically modified soybean) worldwide has caused a continuous selection pressure by Cry proteins on these lepidopteran pests, which may result in the resistant populations, with unsuccessful control. Thus, considering the importance of soybean, the expansion of the cultivation *Bt* crops and its impact on entomofauna, a deeper understanding of larval behavior has become increasingly necessary. The objectives of this research were to: i) to characterize the possible resistance of 30 soybean genotypes to *A. gemmatalis* through greenhouse and laboratory tests; ii) to characterize larval "on-plant" and "plant-to-plant" movement of *A. gemmatalis* on non-*Bt* soybean in the field and; iii) to investigate intra and interspecific interactions involving *A. gemmatalis* and *C. includens* in the laboratory. The resistance results showed antixenosis expression (oviposition, attractiveness and foliar intake) in genotypes 'TMG 133' RR, 'TMG 1179' RR, 'IAC 19', 'IAC 17', 'IAC 100', D75-10169, and IAC 78-2318. The genotypes IAC 74-2832, 'IAC 19', 'IAC 17', 'IAC 100', and PI 274454 affected behavior and also negatively impacted larval viability, indicating antixenosis and/or antibiosis expression. 'TMG 7062' IPRO negatively affected larval development and viability, indicating a possible antibiosis expression, in addition to low degree of antixenosis. Genotypes 'IAC 19', 'IAC 24', 'IAC 17', and 'IAC 100' were shown to express antibiosis resistance by compromising several biological parameters, mainly larval development and viability, and reducing pupal weight. Due to the reduction of foliar intake by confined larvae, antixenosis was also characterized in 'IAC 24'. On-plant movement tests showed that *A.*

gemmatalis has a very active behavior during the day, especially in advanced instars. It was found that larvae vary according to the position in which they are found on the plant and leaf throughout the observation period. Regarding plant-to-plant movement, *A. gemmatalis* larvae can move long distances (up to 2 m) from the infested plant in a nondirectional manner. The results of the fitness test and video tracking showed that *A. gemmatalis* has a competitive advantage over *C. includens*. Our study provides relevant findings involving the interaction of the two lepidopterans in soybean. Genotypes with indications of resistance might be used in breeding programs aimed at finding genotypes resistant to *A. gemmatalis* and possibly to other leaf-feeding insects. The results obtained in the movement tests may help in choosing the better time for insecticide, entomopathogens and releasing of biological enemy application, besides assisting in the choice of more assertive management strategies. Furthermore, the interaction between *A. gemmatalis* and *C. includens* could contribute to a better understanding of behavior and populations dynamics. This body of information can assist Integrated Pest Management (IPM) and Insect Resistance Management (IRM) programs for soybean.

Keywords: Host plant resistance; antixenosis; antibiosis; fitness; larval behavior; Lepidoptera.

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GENERAL INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is one of the most widely cultivated and used legumes worldwide due to its valuable seed composition. It can be grown in a multitude of environments using a variety of management practices, and for diverse end-user purposes. Brazil and the United States (U.S.) have been the leading producers of soybean worldwide (SOYSTATS, 2020); however, as soybean production has expanded, this crop has experienced intense phytosanitary problems, including arthropod pest attack. Among insects that feed on soybean, the leaf-eaters are considered major pests and among them, the velvetbean caterpillar, *Anticarsia gemmatilis* Hübner (Lepidoptera: Erebidæ) is among the major species, requiring periodic control measures in order to avoid significant yield losses (MOSCARDI et al., 2012).

Anticarsia gemmatilis is a common pest of non-Bt soybean in all Brazilian soybean production regions. Moreover, the insect is also considered an important species in the U.S, mainly in the south and the southeastern soybean production regions (HUSETH et al., 2021). The adults are moths with highly variable coloration, including several shades of brown (CAPINERA, 2001). Moth wingspan ranges from 30 to 38 mm, and there is a dark line that crosses the forewings and the hind wings. The 0.6 mm-diameter eggs are initially white-greenish, and therefore difficult to detect in the soybean canopy. Larval coloration is greenish during the initial instar to dark green in subsequent instars. In late instars, larvae present three white longitudinal stripes, and larval color is brownish to almost black, depending on the larval density and host plant (ANAZONWU; JOHNSON, 1986). Once the larvae completely develop, they drop to the base of the host plant and pupate under the soil surface. Pupae are initially light green and become brown as they mature. Caterpillars are very active and will spring from plants and wiggle rapidly when disturbed. Individuals less than one-half inch long "loop" when they crawl, and are often misidentified as soybean loopers (CAPINERA, 2001).

Larvae typically begin feeding in the upper canopy and move lower on the plant as the defoliation continues. Soybean is the primary host of the *A. gemmatilis* but it is polyphagous insect with several secondary hosts, including cowpea, cotton, peanut,

wheat, sunflower, rice, and also several weed species reported as winter/spring hosts (BUSCHMAN et al., 1977; SLANSKY, 1989; PANIZZI et al., 2004).

Changes in climate and agroecosystem configuration and composition may be responsible for sudden changes in ecological and evolutionary dynamics, imposing strong selective pressure and leading to a change in pest status (i.e., minor pests becoming major pests) (ALTIERI, 1999; ALVARADO-SERRANO et al., 2019; LEHMANN et al., 2020). One example is the soybean looper *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae), which has become a primary pest for soybean in Brazil (HOFFMANN-CAMPO et al., 2012; BALDIN et al., 2014; SPECHT et al., 2015; SANTOS et al., 2017) and is very common in U.S. The occurrence of *C. includens* in all Brazilian states, high polyphagia (175 species of host plants), inappropriate use of insecticides, use of fungicides after the introduction of soybean rust (consequent reduction in the occurrence of entomopathogenic fungi), and resistance to control measures in soybean are factors which have contributed to the increase of this insects' populations (SOSA-GÓMEZ et al., 2010a). In the U.S, this species is migratory, with adults migrating from Florida, Texas, Central and South America, and the Caribbean Islands into the U.S (MITCHELL et al., 1975; NEWSOM et al., 1980).

Chrysodeixis includens moths have a wingspan of approximately 35 mm, brown forewings with a small silver design in the center, and brown hindwings (SOSA-GÓMEZ et al., 2010b). Oviposition is isolated in the lower two thirds of the canopy (HERZOG; TODD, 1980). Egg color ranges from light cream to bright yellow, darkening and becoming light brown near hatching (MITCHELL, 1967; BEACH; TODD, 1985). Caterpillars are pale to dark green with two pairs of abdominal prolegs and one anal pair, with longitudinal white stripes along the side and dorsum (SULLIVAN; BOETHEL, 1994). The body tapers toward the head and when the caterpillar moves, the abdomen forms a hump or loop. Pupae are white to creamy green in color and found in silken cocoons on the underside of leaves (SHOUR; SPARKS, 1981).

The damage caused by *A. gemmatilis* and *C. includens* is similar, as they have the same alimentar behavior. *Anticarsia gemmatilis* is one of the fastest defoliating caterpillars species, able to feed their entire life and strip soybean fields in five to seven days. The larval feeding starts on the new leaves, moving to the older leaves, and also feeding on tender stems and pods, preferentially in the vegetative stage (WILKERSON et al., 1986). *Chrysodeixis includens* larvae feed on the lower leaf

surface, giving the leaf a 'window-pane' effect. Large lateral leaf veins are often not fed on, giving the plant a 'lacelike' appearance. This species prefers to feed plants in the early to mid-reproductive stage (SULLIVAN; BOETHEL, 1994).

Both species are controlled primarily by conventional synthetic insecticides. However, the inappropriate use of these compounds, without proper rotation among groups has led to serious concerns about field-evolved resistance (CATCHOT et al., 2016). Use of insecticides increases production costs, and also eliminates many natural enemies, besides having other potential negative impact to the environment. These negative consequences makes it necessary to search for new control strategies in an efficient and environmentally safe way. In this sense, the identification and use of insect resistant soybean genotypes is a promising Integrated Pest Management (IPM) strategy and tool, since this technology is persistent and compatible with other control techniques (PAINTER, 1951).

Host plant resistance to insects can be classified into three categories: antixenosis, antibiosis and tolerance (PAINTER, 1951; BALDIN et al., 2019). Plants that express antixenosis are less used as food, oviposition or shelter. In general, this happens because the plant possess allelochemicals and/or morphological characteristics that affect insect behavior during its choice for a host (PAINTER, 1951; SMITH, 2005). Antibiosis occurs when plants, based in their chemical constitution, adversely affect insect biological parameters, such as changes in development period, reduction on size and weight, lower fertility, fecundation, and high mortality rates of early insect stages (PANDA; KHUSH, 1995; SMITH, 2005). A genotype is considered tolerant to an insect when it does not affect its behavior and biological parameters, and that under the same level of insect infestation, is less damaged than others cultivars due several compensatory mechanisms (SMITH, 2005; PETERSON et al., 2017).

Among the alternative forms of host plant resistance, the use of genetically modified plants (GMOs) has been highlighted (SANTOS et al., 2009). In 2013/14 season, genetically modified soybean (MON 87701 × MON89788), which expresses the protein Cry1Ac, was launched in Brazil. This biotechnology is an important tool for integrated lepidopterous pest management and was quickly accepted by farmers (YANO et al., 2016; ISAAA, 2021). However, since this strategy has changed insect population dynamics, a deeper understanding of larval behavior becomes increasingly necessary to preserve the long-term sustainability of the technology.

Larval dispersal and survival may directly influence sampling methods used in IPM (ROSS; OSTILE, 1990; PAULA-MORAES et al., 2013; PANNUTI et al., 2015). Knowledge about the on-plant position of caterpillars throughout the day may also aid IPM, making insecticide application more effective by better directing of droplets to the insect position. Additionally, movement of early instar lepidopteran larvae on host plants largely determines where feeding sites become established (FOSTER; HOWARD, 1999; PANNUTI et al., 2019). In its turn, plant-to-plant movement is related to an adaptive mechanism by which insects seek resources and use them as an energy source, refuge and in many cases for seeking mates and mating locations (BELL, 1990; PRICE, 1997). Extensive movement among plants may also benefit insect survival in the field. Thus, it is equally necessary understand the insect behavior as they relate to these areas. Understanding movement can support the development of accurate models related to the appropriate refuge and use for Integrated Resistance Management (IRM) (PANNUTI et al., 2016; FANELA et al., 2020).

Another challenging impact on insect's behavior is the role of competition. Intraguild interactions on a given host may play a fundamental approach in the population dynamics of herbivorous insects (KAPLAN; DENNO, 2007). In this context, cannibalism and predation may intensify intraguild competition. Cannibalism may result in population self-regulation, while predation among individuals of competing species tends to "regulate" the total biomass of individuals. Both behaviors occur for different reasons, such as restrictions in food availability, starvation, intrinsic characteristics of species behavior, avoidance, exclusion, and can be a response to many undetectable environmental factors (FOX, 1975; POLIS, 1981).

Although there are studies that describes *A. gemmatilis* development in some genotypes (BEACH; TODD, 1988; HOFFMANN-CAMPO et al., 1994; FUGI et al., 2005; PIUBELLI et al., 2005; FRANCO et al., 2014; 2017), to date there are no studies that assess behavior and biological performance of this species across a wide soybean germplasm base (*Bt* and non-*Bt* genotypes). Further research is necessary in order to select new resistant genotypes. Moreover, there are no records about the characterization of larval movement for *A. gemmatilis* and its interactions with *C. includes* on soybean plants.

Thus, the specific goals of our study were: a) to characterize antixenosis and/or antibiosis in 30 soybean genotypes (*Bt* and non-*Bt*) to *A. gemmatilis*; b) to describe *A.*

gemmatalis "on-plant" and "plant-to-plant" movement in vegetative and reproductive non-*Bt* soybean plants, and c) to investigate intra and interspecific interactions involving *A. gemmatalis* and *C. includens*.

In order to reach these objectives, the dissertation was divided in three chapters, which were written according to the Journal of Economic Entomology, Journal of Pest Science and Pest Management Science's guidelines, respectively.

CHAPTER 1

RESISTANCE OF SOYBEAN GENOTYPES TO *Anticarsia gemmatalis* (LEPIDOPTERA: EREBIDAE): ANTIXENOSIS AND ANTIBIOSIS CHARACTERIZATION

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ABSTRACT

Injury by herbivores is a major biotic stress limiting soybean [*Glycine max* (L.) Merrill] crop production. Among the main soybean insect pests, *Anticarsia gemmatalis* Hübner (Lepidoptera: Erebidae) is responsible for causing significant economic damage in soybean. The primary management strategy for this insect is chemical control and use of *Bt* transgenic soybean. Alternative strategies, such as host plant resistance, can be considered an efficient and less aggressive method, especially in association with other strategies as part of an integrated pest management (IPM) approach. In this study we evaluated 30 soybean genotypes to verify antixenosis expression through oviposition, larval attractiveness, and larval foliar intake tests. From this, we selected 13 promising genotypes to verify the possible presence of antibiosis. Our results suggest that antixenosis was found in genotypes 'TMG 133' RR, 'TMG 1179' RR, 'IAC 19', 'IAC 17', 'IAC 100', D75-10169, and IAC 78-2318. By influencing on behavior and negatively impacting larval viability, antixenosis and antibiosis were indicated for the genotypes IAC 74-2832, and PI 274454. 'TMG 7062' IPRO was found to provide antibiosis resistance by negatively impacting larval development and viability. Genotypes 'IAC 19', 'IAC 24', 'IAC 17', and 'IAC 100' were shown to express antibiosis resistance by compromising several biological parameters, mainly larval development

and viability, and reducing pupal weight. Because of reduced foliar intake by larvae, antixenosis was indicated for 'IAC 24'. These genotypes should be considered in soybean breeding programs focusing on soybean resistance to *A. gemmatalis*.

Keywords: *Glycine max*; host plant resistance; velvetbean caterpillar

1.1 Introduction

Soybean [*Glycine max* (L.) Merrill] is one of the most important agricultural crops, and it has been increasingly planted worldwide (SOYSTATS 2020). Soybean grain can be used as feed, or it can be processed to produce soybean byproducts, oil, and more recently, biodiesel (USDA 2019). Soybean productivity can be severely reduced by insect pests that cause significant yield loss (Hoffmann-Campo et al. 2012, Oliveira et al. 2014, Wille et al. 2017). The velvetbean caterpillar, *Anticarsia gemmatalis* Hübner (Lepidoptera: Erebidæ) is one of the main soybean insect pests. This species causes defoliation in different soybean producing regions in the western hemisphere (Buschman et al. 1981, Sosa-Gómez et al. 2010, Bortolotto et al. 2015, Haase et al. 2015). Defoliation can be total, severely impacting yield when infestations are high (Bueno et al. 2011).

Traditionally, *A. gemmatalis* has been controlled with chemical insecticides. However, the extensive use of insecticide sprays can have significant negative effects to non-target organisms, promote pest outbreaks, and select for insecticide resistant insect populations (Vieira et al. 2011, Bel et al. 2019). In parallel, genetically modified soybean expressing a *Bacillus thuringiensis* (*Bt*) insecticidal protein has taken a prominent position in large scale management of *A. gemmatalis* (Bernardi et al. 2012). Although *Bt* technology offers good control efficiency for this pest, its extensive use without appropriate resistance management plans can compromise its durability. In addition, this technology requires the presence of specific refuge areas (with no *Bt* plants), where insecticide sprays should be avoided.

Despite the increase in the use of *Bt* technology, the size of refuge areas, the limitations for using other control methods, the high cost of seed, and many other factors have resulted in many growers preferring alternative management strategies, such as the use of resistant plants developed in classic breeding programs. Host plant

resistance can be considered an efficient and less aggressive method, especially in association with other strategies, e.g., biological control. This suggests there is a need to identify and incorporate resistant soybean genotypes as valuable pest management tools in integrated pest management (IPM) programs for soybean (Smith and Clement 2012, Canassa et al. 2017, Baldin et al. 2019).

Plant resistance can be divided into three categories: antibiosis, antixenosis and tolerance (Painter 1951). Plants expressing antibiosis negatively affect colonizing insect biology by interfering with their development, reproduction, and survival. Significant reductions in insect size, weight, longevity, and physical deformity are common effects to insects on plants presenting antibiosis. Antixenosis affects insect behavior and reduces colonization of the host by chemical, physical, and morphological factors. Tolerance is the ability of plants to resist or overcome from injury caused by pest injury, without affecting their biology or behavior (Painter 1951, Panda and Khush 1995, Smith 2005, Baldin et al. 2019). Plants exhibiting high levels of antixenosis can cause negative effects to insect development, like that of antibiosis. Thus, it is essential to differentiate antibiosis and antixenosis categories by using specific insect intake evaluation studies (Beach and Todd 1988, Smith 2005, Baldin et al. 2015, Morando et al. 2017, Coelho et al. 2020).

Although some studies have evaluated soybean resistance to *A. gemmatilis*, (Beach and Todd 1988, Hoffmann-Campo et al. 1994, Fugi et al. 2005, Piubelli et al. 2005, Franco et al. 2014, 2017), there are few studies characterizing resistance that examine a large number of soybean genotypes (with high genetic variability). Therefore, in order to identify new resistance sources, this study characterized the expression of antibiosis and antixenosis in different genotypes under laboratory and greenhouse conditions.

1.2 Materials and Methods

The initial assays were performed with 30 soybean genotypes (Table 1) that were selected to represent wide genetic variability and include sources of resistance against other pest species or commercial materials never previously tested. The plants were grown in a greenhouse and evaluated under greenhouse and laboratory conditions in 2018 and 2019 in Botucatu, São Paulo, Brazil. Polyethylene pots (2 L) containing a mixture of soil, sand, manure, and substratum (1:1:1:1) were kept in a greenhouse free

from insect infestation. All plants received recommended crop fertilization (Malavolta 2006) and other necessary cultural practices (e.g. irrigation, thinning, and cleaning). Leaves from plants at V4/V5 (Boiça Júnior et al. 2015) developmental stages (Fehr and Caviness 1977) were used in all experiments.

Rearing *A. gemmatilis*

A colony of *A. gemmatilis* was established and maintained under laboratory conditions at the Department of Plant Protection, São Paulo State University, Botucatu, São Paulo, Brazil. The methodology was based on protocols by Greene et al (1976) and Parra (2001). The insects were kept in plastic cups containing 15 mL of artificial diet in a rearing chamber (T: $25 \pm 2^\circ\text{C}$, photoperiod of 12:12 h, RH: $60 \pm 10\%$) until 48h after hatching. Insects for the antixenosis bioassays were from this colony. To provide insects for the antibiosis bioassay, eggs were commercially acquired (Pragas.com, Piracicaba, Brazil).

Antixenosis bioassays

Initially we performed three different types of bioassays (oviposition preference, attractiveness, and foliar intake) with 30 soybean genotypes. The objective was to verify the possible expression of antixenosis and select the most promising resistant genotypes for the subsequent phase of the study. At first, five plants (5 replicates) in no-choice conditions were infested with two females/plant for two days to evaluate oviposition in the entire plant in greenhouse. For attractiveness and free-choice foliar intake bioassays, two 4th-instar larvae/genotype (Boiça Júnior et al. 2015) were released in arenas (23 cm of diameter). To evaluate attractiveness, the number of larvae on leaf discs were counted after 5, 15, 30, 60, 90, and 120 minutes after release under laboratory conditions. To avoid contact among larvae and leaf tissues, discs (6.9 cm²) were covered with a plastic micro arena (50 mL), which was partially coated with organdy fabric, allowing volatile flow. Each arena with 30 genotypes represented one block, totaling ten blocks.

For the no-choice foliar intake assessment, two 4th-instar larvae/genotype were isolated in Petri dishes (8 × 2cm) with one leaf disc of each genotype, ten replicates

per genotype. Before the test, larvae were starved for 2h. In free and no-choice tests in laboratory, all replicates were observed until 90% leaf discs had experienced some feeding, at which time the study was concluded. The non-consumed food (cm²) was measured with a leaf area meter (LI-COR® LI-3100, LI-COR, Lincoln, NE). Oviposition and no-choice foliar intake tests were arranged in a completely randomized design, while attractiveness and free-choice foliar intake followed a complete randomized block design.

A. gemmatilis biology in selected genotypes

Based on antixenosis bioassays, the 11 most resistant genotypes ('IAC 19', 'IAC 100', 'IAC 17', 'IAC 24', PI 274454, IAC 74-2832, D75-10169, 'TMG 1179' RR, 'TMG 133' RR, L1-1-01, 'TMG 7062' IPRO) to *A. gemmatilis* were selected. Genotypes 'Conquista' and 'Coodetec 208' were included as susceptible commercial materials (Silva et al. 2012), totaling 13 genotypes. To reduce initial insect stress, 48 hour-old larvae were used. Fifty 48 hour-old larvae were placed into individual Petri dishes (8 × 2cm) lined with moist filter paper containing leaf disks (6.9 cm²) from a selected genotype. Each Petri dish containing one larvae corresponded to one replicate in a completely randomized design.

The following biological parameters were evaluated: duration of each larval instar, total larval period, fifth instar larvae weight (24 hour-old), larval viability, pre-pupal and pupal period duration, pupal weight (24 hour-old), pupal viability, development period (larvae - adult), pre-oviposition duration, oviposition period, and egg number. Fifth instar larvae and pupae weights were obtained with an analytical scale (AL-500, Marte Científica®, São Paulo, SP, Brasil).

For further resistance evaluation and to discriminate antixenosis and antibiosis, each individual's foliar intake (cm²) was recorded during the larval period. Leaf disks were replaced daily, and non-consumed food was measured with the leaf area meter.

Statistical analyses

Data were subjected to analysis of variance (ANOVA). Normality and homogeneity of the data were verified with Shapiro-Wilk and Levene tests (Winer et al. 1991). For the antixenosis study, data were compared using Scott-Knott test ($P < 0.05$). For the

antibiosis study, 'genotype' was considered a fixed effect and the pairwise t-test comparisons was conducted by least squares means (LS-means) ($P < 0.05$), using the SAS 'Proc Glimmix' procedure (SAS Institute 2001).

1.3 Results

Antixenosis bioassays

In the oviposition test, D75-10169, PI 229358 and 'IAC 19' genotypes (15.40, 17.80 and 18.60 eggs, respectively) were the least oviposited while 'FTS Campo Mourão' RR showed the highest eggs number (51.40) ($F = 2.64$; $df = 29$; $P = 0.0001$; Fig. 1). Considering the attractiveness mean values for all evaluations, IAC 74-2832, 'IAC 17', 'TMG 133' RR, 'KS-4202', PI 274454, 'IAC 100', 'Dowling', and 'TMG 1179' RR were the least infested (0.32 – 0.52 larvae), differing to the other genotypes, with the highest infested being 'Conquista' (1.85 larvae), 'Anta 82' (1.42), 'TMG 132' RR (1.30), L1-1-01 (1.28), 'Coodetec 208' (1.27), 'TMG 4185' (1.15), 'FTS Campo Mourão' RR (1.05), and PI 171451 (1.03) ($F = 5.12$; $df = 29$; $P < 0.0001$) (Table 2).

In the foliar intake free-choice test, the genotypes 'TMG 1179' RR, 'IAC 100', D75-10169, 'IAC 17', 'IAC 23', IAC 78-2318, 'IAC 24', 'TMG 7062' IPRO, 'IAC 19', and IAC 74-2832 were the least consumed by fourth-instar *A. gemmatilis* larvae ($< 1.12 \text{ cm}^2$) ($F = 10.01$; $df = 29$; $P < 0.0001$; Table 3). In the no-choice test, significant differences were observed among the genotypes ($F = 4.91$; $df = 29$; $P < 0.0001$; Table 3); 'IAC 100', 'TMG 7062' IPRO, IAC 78-2318, 'IAC 23', and 'TMG 1179' RR being the least consumed ($< 1.50 \text{ cm}^2$). In both tests, 'Conquista', 'Coodetec 208', and 'Anta 82' were the most consumed by *A. gemmatilis*.

A. gemmatilis biology in selected genotypes

The mean duration of first instar developmental period was significantly longer when the insects were fed with leaves of 'IAC 19' (3.66 days) and 'IAC 17' (3.34 days) compared to others 5 genotypes, including 'Conquista' (2.74 days) and 'Coodetec 208' (2.94 days) ($F = 4.09$; $df = 12$; $P < 0.0001$; Table 4). Genotypes 'IAC 19' (2.63 days), IAC 74-2832 (2.56 days) and 'IAC 17' (2.47 days) prolonged duration of the second

instars compared to 'TMG 7062' IPRO (1.13 days) and PI 274454 (2.15 days) ($F = 5.27$; $df = 12$; $P < 0.0001$). For the third instars, 'IAC 17' (3.24 days) and 'TMG 133' RR (2.88 days) resulted in the longest duration, while 'IAC 19' and 'Coodetec 208' resulted in shorter durations (< 2.40 days) ($F = 2.49$; $df = 11$; $P = 0.0048$). The longest durations of fourth instars were observed for 'IAC 17', 'IAC 100' and 'IAC 19' (2.73 – 2.97 days) compared to D75-10169, L1-1-01, and 'TMG 1179' RR with the shortest durations (< 2.30 days) ($F = 3.48$; $df = 11$; $P = 0.0001$). The longest duration of fifth instars was observed for 'Coodetec 208', 'Conquista', D75-10169, 'TMG 133' RR, and 'TMG 1179' RR (2.68 – 2.88 days), differing from most other genotypes with 'IAC 24' and PI 274454 presenting the shortest durations (2.05 and 2.09 days, respectively) ($F = 2.02$; $df = 11$; $P = 0.0256$). Sixth ($F = 1.59$; $df = 11,144$; $P = 0.1081$) and seventh ($F = 2.00$; $df = 5$; $P = 0.1222$) instars duration did not differ significantly among genotypes. The complete larval period was longest when larvae were confined to 'IAC 19', 'IAC 100', 'IAC 17', and 'IAC 24' (> 18.90 days), while 'Conquista' resulted in the shortest period (< 15.00 days) ($F = 18.64$; $df = 11$; $P < 0.0001$; Table 4).

Genotypes 'IAC 24', PI 274454, IAC 74-2832 and 'IAC 17' resulted in lower larval viability of *A. gemmatilis* ($\leq 36.00\%$) ($F = 6.08$; $df = 11$; $P < 0.0001$; Fig. 2). Insects fed with leaves of 'TMG 7062' IPRO did not complete the larval phase. 'Conquista', 'TMG 1179' RR, D75-10169 and 'TMG 133' RR were the most suitable genotypes for larval development (56.00 – 72.00%).

Second instar foliar intake was lowest for 'IAC 24' (1.14 cm²), followed by 'IAC 19' (1.22 cm²), 'IAC 100' (1.26 cm²), and 'IAC 17' (1.31 cm²) ($F = 3.18$; $df = 11$; $P = 0.0003$; Table 5). Second instar intake observed in 'TMG 7062' IPRO was negligible and could not be measured with the leaf area meter. Third instar foliar intake was lowest for 'TMG 1179' RR (6.18 cm²), 'IAC 19' (6.26 cm²), D75-10169 (6.40 cm²), and 'IAC 100' (6.49 cm²) ($F = 35.63$; $df = 11$; $P < 0.0001$). Fourth instar foliar intake was the lowest for 'IAC 100' (9.80 cm²) ($F = 25.14$; $df = 11$; $P < 0.0001$). Fifth instar foliar intake was lowest for 'IAC 19' (12.01 cm²), 'IAC 100' (12.44 cm²), 'IAC 24' (12.78 cm²), 'IAC 17' (13.59 cm²), D75-10169 (14.06 cm²), L1-1-01 (14.35 cm²), 'TMG 1179' RR (15.14 cm²), and 'TMG 133' RR (15.83 cm²) ($F = 66.78$; $df = 11$; $P < 0.0001$). Sixth instar foliar intake was lowest for D75-10169 (11.57 cm²) ($F = 4.21$; $df = 11$; $P < 0.0001$). We did not find significant differences among genotypes in seventh instar foliar intake ($F = 1.93$; $df = 5$; $P = 0.1341$). 'Conquista' was the most preferred genotype

by *A. gemmatalis* in second, third, fourth, fifth and sixth instars, with a consumption of 2.09, 14.99, 28.35, 62.97 and 24.99 cm², respectively.

Fifth instar mean weight was lowest for 'IAC 19' (0.09 g), 'IAC 24', 'IAC 100', and 'IAC 17' (0.10 g) ($F = 13.94$; $df = 11$; $P < 0.0001$; Table 6). Fifth instar weight was highest for 'Conquista' (0.18 g) and 'Coodetec 208' (0.16 g), and lowest for 'IAC 19', 'IAC 100'. Pupal weight was lowest for 'IAC 17', 'IAC 19' and 'IAC 100' (0.12 g) and 'IAC 24' (0.13 g), and highest for 'Conquista' (0.21 g) and 'Coodetec 208' (0.19 g) ($F = 16.75$; $df = 11$; $P < 0.0001$; Fig. 3). Larvae feeding on 'TMG 7062' IPRO did not become fifth instar larvae and, consequently, pupae.

Pre-pupal stage duration was longest for 'IAC 17', 'IAC 100', 'IAC 19', and 'IAC 24' (≥ 2.00 days), differing from other genotypes, and shortest for 'Conquista' (1.08 days), L1-1-01 (1.25 days), and 'TMG 1179' RR (1.28 days) ($F = 13.00$; $df = 11$; $P < 0.0001$; Table 7). Pupal stage was longest for the same genotypes as for the pre-pupal stage and 'IAC 74-2832' (> 11.20 days), differing from most of others, and shortest for 'Conquista' (9.31 days) ($F = 25.20$; $df = 11$; $P < 0.0001$; Table 7). Pupal viability did not differ significantly among genotypes ($F = 1.33$; $df = 11$; $P = 0.2087$; Fig. 4). Larvae confined to 'TMG 7062' IPRO did not reach the pupal stage. The larvae-adult duration was significantly longer when the insects were fed with 'IAC 17', 'IAC 100', 'IAC 19', and 'IAC 24' leaves (> 32.00 days) ($F = 18.24$; $df = 11$; $P < 0.0001$; Table 7). The shortest larvae-adult duration was observed for 'Conquista' (27.82 days).

Pre-oviposition period was significantly affected by genotype ($F = 8.12$; $df = 11$; $P < 0.0001$; Table 8). The longest duration was observed for 'IAC 100', 'IAC 17', 'IAC 19', and 'IAC 24' (≥ 5.00 days), differing from 'Conquista' and 'Coodetec 208' (1.85 and 2.80 days, respectively) that resulted in the shortest duration. Oviposition period was significantly affected by genotype ($F = 3.53$; $df = 11$; $P = 0.0008$). The longest duration was observed for 'Conquista' (13.54 days) and L1-1-01 (11.60 days), and shortest for 'IAC 100', 'IAC 24', 'IAC 17', and 'IAC 19' (4.75 – 5.89 days). Egg number per female was significantly affected by the genotype ($F = 5.44$; $df = 11$; $P < 0.0001$), being lowest for 'IAC 24', 'IAC 17', 'IAC 100', 'IAC 19', and 'TMG 1179' RR (110.50 – 240.43 eggs). The highest number of eggs were recovered for 'Conquista', D75-10169, L1-1-01, 'TMG 133' RR, and PI 274454 (> 400 eggs).

1.4 Discussion

Some of the soybean genotypes evaluated in this study significantly affected *A. gemmatilis* behavior and biological parameters, characterizing expression of two resistance categories, antixenosis and antibiosis (Smith 2005). Even though a previous study indicated a few promising soybean genotypes with resistance to *A. gemmatilis* (Fugi et al. 2005), in this study, for the first time, we assessed the behavior and development of *A. gemmatilis* immature and adult stages across a wide selection of soybean germplasm with high genetic variability.

In practical terms, the number of eggs laid in a field is particularly important because eggs will initiate a pest infestation. Results from the initial antixenosis bioassays in the greenhouse revealed differences among the genotypes for egg number. Several other studies also indicated differences in egg number among genotypes. In a no-choice test, PI 274453 and L1-1-01 showed the fewest egg number by *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae) (Schlick-Souza et al. 2018). In a multiple-choice oviposition test with 15 soybean genotypes, 'IAC 19' and UX-2569-159 expressed antixenosis against *Bemisia tabaci* (Gennadius) biotype B (Hemiptera: Aleyrodidae), showing the lowest number of eggs. In the same study, PI 229358 was the least attractive and least used for oviposition. The antixenosis shown by these genotypes to whitefly was related to the characteristics of their trichomes (lower density and inclined) (Valle et al. 2012, Baldin et al. 2017), which could also explain the low number of eggs observed on these genotypes in our study.

In this study, micro arenas were used to avoid the contact among larvae and leaf disks, so larvae were attracted exclusively by the volatile compounds released by each genotype. In this context, regarding the total mean of larvae attracted over the time, IAC 74-2832, 'IAC 17', 'TMG 133' RR, 'KS-4202', PI 274454, 'IAC 100', 'Dowling', and 'TMG 1179' RR had the least number of larvae (i.e. most repellent), indicating antixenosis, probably governed by a chemical factor present in the genotypes. In a similar study testing the influence of different genotypes, 'IAC 19', 'IAC 18', 'IAC 23', L1-1-01, PI 274453, PI 229358, PI 171451, 'IAC 100', 'IAC 24', 'IAC 17', and IAC 74-2832 were classified as repellents for *C. includens* (Schlick-Souza et al. 2018). The lower attractiveness or level of colonization of an insect to a genotype suggests the presence of factors that inhibit the acceptance by the specific material, and consequently feeding and/or oviposition, which usually occurs in plants exhibiting antixenosis (Smith 2005, Baldin et al. 2019).

Genotypes 'TMG 1179' RR, D75-10169, 'TMG 7062' IPRO, and the 'IAC's' genotypes presented low foliar intake in the free and no-choice tests, indicating antixenosis. Similar behavior was observed in other study where 'IAC 100' and IAC 74-2832 genotypes were rejected by larvae of *A. gemmatilis* based upon a consumption index (Hoffmann-Campo et al. 1994). Genotypes D75-10169, 'IAC 100', IAC 78-2318, and 'IAC 19' were also less attractive and less consumed by *Piezodorus guildinii* (Westwood) (Hemiptera: Pentatomidae) (Silva et al. 2014). The low consumption may indicate the presence of deterrents, or the lack of a stimulant food compound or leaf hardness, which are known to decrease feeding (Reynolds et al. 1984). However, antixenosis and antibiosis commonly overlap in resistance bioassays, which makes it difficult to interpret the two resistance categories in isolation (Panda and Khush 1995, Smith 2005). Thus, it is also important to evaluate other parameters through larval development, with the purpose to to exclude or to confirm the combined presence of antibiosis.

Larvae that fed on 'TMG 7062' IPRO were unable to transform into third instar, and this was due to the insecticidal protein (Cry1Ac) present in *Bt* plants. In general, the 'IAC's' genotypes showed a similar response, most of them resulting in larvae with a relatively short instar duration but requiring more time (days) to complete the larval phase because of an additional instar and extending the cycle. Thus, the total larval period had a difference of 5.33 days (14.53 – 19.86 days) between low and high mean, indicating a high degree of antibiosis and/or antixenosis. A previous study conducted with *P. guildinii* testing 17 soybean genotypes also indicated antibiosis resistance in some genotypes, especially 'IAC 100' and 'IAC 19', where 'IAC 100' had high impact to nymphal development, prolonging instar duration (Silva et al. 2013). Prolongation of the immature phase may result from intake of deleterious compounds, usually present in antibiotic bearing genotypes that inhibit insect development. However, genotypes with high levels of antixenosis can also cause this effect, either by hampering insect feeding, nutritional improprieties, and poor nutritional quality of a plant (Painter 1951).

All larvae died on genotype 'TMG 7062' IPRO during initial larval development, confirming its antibiotic effect. Low larval viability was observed for 'IAC 24', PI 274454, IAC 74-2832, and 'IAC 17', which suggests antibiosis, reinforcing the probable presence of toxic compounds that inhibit larvae development. For 'IAC 17' and 'IAC 24', the results confirm Fugii et al. (2005), where in addition to inducing lower larval

viability, adult deformation levels of 12.50 and 23.60% were observed for 'IAC 17' and 'IAC 24', respectively. Low larval viability was also observed when larvae of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) were fed with leaves of PI 274454 and 'IAC 17' (Coelho et al. 2020).

Genotype 'IAC 100' has significant levels of the flavonoids rutin and genistin, which suggests these compounds may be involved in resistance observed for *A. gemmatilis*, similar to that documented for *P. guildinii* (Bentivenha et al. 2018). These compounds were also related to resistance against *A. gemmatilis* in another study where leaf extracts of 'IAC 100' were offered to larvae (Piubelli et al. 2005). However, more research is needed to fully understand the role of various resistance factors through the chemical and morphological analysis for the other genotypes.

Fifth instar larvae fed 'IAC 19', 'IAC 24', 'IAC 100' and 'IAC 17' leaves weighed less, which confirms resistance. The values observed in the resistant materials (0.09 and 0.10 g) were half that observed in the susceptible genotype 'Conquista' (0.18 g). *Spodoptera cosmioides* (Walker) (Lepidoptera: Noctuidae) larvae had reduced weight when fed 'IAC 100' (Boiça Júnior et al. 2015), and fifth instar *H. armigera* had reduced weight when fed 'IAC 19' (Coelho et al. 2020).

Pupal weight was also significantly affected by the 'IAC 19', 'IAC 24', 'IAC 100', and 'IAC 17'. Larvae fed 'IAC 19', 'IAC 100', or 'IAC 17' resulted in pupal weight of 0.12 g, and 0.13 g for those fed 'IAC 24', while pupae from the susceptible genotypes 'Coodetec 208' and 'Conquista' weighed 0.19 and 0.21 g, respectively. *Anticarsia gemmatilis* fed PI 229358, 'IAC 17', 'IAC 24', and 'IAC PL-1' in a biological development study did not show significant differences in pupal weight (Fugi et al. 2005); however, in another study, pupal weight was significantly reduced from *A. gemmatilis* larvae fed 'IAC 17' (Gazzoni and Tutida 1996). The reduction in larval and pupal weight may be associated with low reserves acquired during the larval phase because of a reduction in the food's nutritional value. Together, these effects might compromise the performance of the insect in the reproductive stage by reducing the number of copulations, oviposition, and fertility (Smith and Clement 2012, Smith and Chuang 2014), which are generally associated with the expression of resistance.

In our study, the 'IAC' genotypes caused significant changes in *A. gemmatilis* life cycle (i.e., larval through pupal developmental time). Similarly, 'IAC 100' was found to provide resistance by extending the life cycle of *Spodoptera eridania* (Cramer) (Lepidoptera: Noctuidae) (Souza et al. 2014). Genotype 'IAC 17' provided the higher

numerical mean of *H. armigera* life cycle duration (Coelho et al. 2020). Genotypes that resulted in the longest duration usually have low nutritional quality, which can influence insect development (Pereyra and Sánchez 2006). The increase in life cycle duration impacts directly on the population dynamics and may determine the success of the species on the host plant (Green et al. 2002, Rajapakse and Walter 2007).

As we expected, the F1 adult parameters were severely affected by the genotypes. Differences were observed in the pre-oviposition period (1.85 – 5.50 days) and oviposition period (4.75 – 13.54 days). These results could be a consequence of reduced nutrition of resistant plants during the larval stage that translate into deficiencies, quantitative, or qualitative nutrient imbalances into the adult phase (Souza et al. 2014). We also verified a high impact of the genotypes through of the low number of eggs in 'IAC 24', 'IAC 17', 'IAC 100', and 'IAC 19' (110.50 – 178.78 eggs), confirming the resistance expression. Egg production can be affected by physical or chemical differences in the food, by the amount of food ingested during insect development, and by the nutritional value that each host can offer to the insect (Umbanhowar and Hastings 2002). Negative effects on fecundity, as well as in the number of progeny, are common in genotypes expressing antibiosis (Panda and Khush 1995, Smith 2005).

Based on the bioassays performed, high levels of antixenosis were found in genotypes 'TMG 133' RR, 'TMG 1179' RR, 'IAC 19', 'IAC 17', 'IAC 100', D75-10169, IAC 78-2318, and IAC 74-2832, as evidenced by lower rates of attractiveness, foliar intake (larvae), and oviposition (adults). By influencing behavior and impacting larval viability, genotypes IAC 74-2832 and PI 274454 were shown to express antixenosis and/or antibiosis. Genotype 'TMG 7062' IPRO was confirmed to provide antibiosis resistance by impacting larval development and larval viability. Genotypes 'IAC 19', 'IAC 24', 'IAC 17', and 'IAC 100' were shown to express antibiosis resistance by compromising several biological parameters, mainly larval development and viability, and reducing pupal weight. Because of low foliar intake by larvae on 'IAC 24', antixenosis was also characterized in the genotype.

The resistance found in some of the evaluated genotypes increases their importance as sources of resistance to *A. gemmatilis* and possibly other insects. Our study provides baseline information which can be useful for developing management strategies for this species in soybean, especially in South America. In the future, further

trials must be carried out under field conditions to validate if the genotypes are effective in suppressing *A. gemmatalis* populations.

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Table 1 - Soybean genotypes assessed for resistance to *A. gemmatilis*

Genotype	Source/Origin
'IAC 17'	D72-9601 × 'IAC-8'/IAC, Campinas, Brazil
'IAC 23'	BR-6 × IAC-83-23/IAC, Campinas, Brazil
'IAC 24'	IAC 80-1177 × IAC 83-288/IAC, Campinas, Brazil
IAC 74-2832	'Hill' × PI 274454/IAC, Campinas, Brazil
'IAC 100'	'IAC 12' × IAC 78-2318/IAC, Campinas, Brazil
IAC 78-2318	D 72-9601-1 × IAC 73-227/IAC, Campinas, Brazil
'IAC 19'	D 72-9601-1 × 'IAC 8'/IAC, Campinas, Brazil
D75-10169	'Govan' × (F4 'Bragg' × PI 229358)/IAC, Campinas, Brazil
PI 229358	Japan
PI 171451	Tóquio, Japan
PI 227687	Okinawa, Japan
PI 274453	Japan
PI 274454	Okinawa, Japan
'TMG 4182'	Tropical Melhoramento & Genética/Cambé, Brazil
'TMG 1179' RR	Tropical Melhoramento & Genética/Cambé, Brazil
'TMG 4185'	Tropical Melhoramento & Genética/Cambé, Brazil
'TMG 133' RR	Tropical Melhoramento & Genética/Cambé, Brazil
'TMG 132' RR	Tropical Melhoramento & Genética/Cambé, Brazil
'TMG 7062' IPRO	Tropical Melhoramento & Genética/Cambé, Brazil
'TMG 7262' RR	Tropical Melhoramento & Genética/Cambé, Brazil
'Anta 82'	Tropical Melhoramento & Genética/Cambé, Brazil
'KS-4202'	KS4694 × C1842/University of Nebraska, Lincoln, USA
UX-2569-159	U06-607094 × UX2324-34/University of Nebraska, Lincoln, USA
'Jackson' (PI 548657)	'Volstate' (2) × 'Palmetto' (USDA/USA)
'Dowling' (PI 548663)	'Semmes' × PI 200492 (USDA/USA)
L1-1-01	BR-6 × 'IAC-100'/ ESALQ/USP, Piracicaba, Brazil
'Conquista'	Lo 76-4484 × 'Numbaíra'/EMBRAPA, Londrina, Brazil
'Coodetec 208'	OC-4 × Williams 20/Coodetec, Cascavel, Brazil
'BMX Potência' RR	Brasmax Genética/Cambé, Brazil
'FTS Campo Mourão' RR	FT Sementes, Ponta Grossa, Brazil

Table 2 - Mean ($\pm$ SE) of fourth-instar *A. gemmatalis* attracted to 30 genotypes in the laboratory

Genotype	Attracted larvae number						Mean
	5 min	15 min	30 min	60 min	90 min	120 min	
IAC 74-2832	0.20 $\pm$ 0.13b	0.60 $\pm$ 0.22b	0.50 $\pm$ 0.22b	0.10 $\pm$ 0.10b	0.20 $\pm$ 0.13b	0.30 $\pm$ 0.15b	0.32 $\pm$ 0.08d
'IAC 17'	0.60 $\pm$ 0.27b	0.20 $\pm$ 0.13b	0.40 $\pm$ 0.22b	0.50 $\pm$ 0.22b	0.30 $\pm$ 0.15b	0.50 $\pm$ 0.22b	0.42 $\pm$ 0.06d
'TMG 133' RR	0.20 $\pm$ 0.13b	0.20 $\pm$ 0.13b	0.80 $\pm$ 0.25b	0.40 $\pm$ 0.16b	0.50 $\pm$ 0.22b	0.50 $\pm$ 0.22b	0.43 $\pm$ 0.09d
'KS-4202'	0.40 $\pm$ 0.22b	0.40 $\pm$ 0.22b	0.60 $\pm$ 0.22b	0.50 $\pm$ 0.17b	0.50 $\pm$ 0.17b	0.30 $\pm$ 0.15b	0.45 $\pm$ 0.04d
PI 274454	0.40 $\pm$ 0.16b	0.50 $\pm$ 0.17b	0.60 $\pm$ 0.27b	0.20 $\pm$ 0.13b	0.60 $\pm$ 0.22b	0.40 $\pm$ 0.22b	0.45 $\pm$ 0.06d
'IAC 100'	0.30 $\pm$ 0.15b	0.70 $\pm$ 0.30b	0.60 $\pm$ 0.22b	0.50 $\pm$ 0.22b	0.70 $\pm$ 0.26b	0.30 $\pm$ 0.15b	0.52 $\pm$ 0.07d
'Dowling'	0.80 $\pm$ 0.29a	0.50 $\pm$ 0.22b	0.20 $\pm$ 0.13b	0.50 $\pm$ 0.22b	0.50 $\pm$ 0.22b	0.60 $\pm$ 0.22b	0.52 $\pm$ 0.08d
'TMG 1179' RR	0.20 $\pm$ 0.13b	0.40 $\pm$ 0.16b	0.50 $\pm$ 0.22b	0.70 $\pm$ 0.33b	0.70 $\pm$ 0.26b	0.60 $\pm$ 0.31b	0.52 $\pm$ 0.08d
'IAC 24'	0.50 $\pm$ 0.27b	0.80 $\pm$ 0.20b	0.70 $\pm$ 0.21b	0.50 $\pm$ 0.16b	0.50 $\pm$ 0.17b	0.60 $\pm$ 0.16b	0.60 $\pm$ 0.05c
IAC 78-2318	0.40 $\pm$ 0.22b	0.70 $\pm$ 0.26b	0.90 $\pm$ 0.31b	0.60 $\pm$ 0.22b	0.40 $\pm$ 0.22b	0.80 $\pm$ 0.33b	0.63 $\pm$ 0.08c
'IAC 19'	0.30 $\pm$ 0.15b	0.50 $\pm$ 0.17b	0.30 $\pm$ 0.21b	1.00 $\pm$ 0.36a	0.80 $\pm$ 0.20b	1.00 $\pm$ 0.30a	0.65 $\pm$ 0.13c
PI 229358	0.50 $\pm$ 0.22b	0.40 $\pm$ 0.16b	0.50 $\pm$ 0.17b	0.90 $\pm$ 0.23a	0.70 $\pm$ 0.15b	0.90 $\pm$ 0.31b	0.65 $\pm$ 0.09c
'IAC 23'	0.90 $\pm$ 0.38a	0.70 $\pm$ 0.15b	0.50 $\pm$ 0.17b	0.60 $\pm$ 0.16b	0.70 $\pm$ 0.15b	0.60 $\pm$ 0.22b	0.67 $\pm$ 0.06c
'BMX Potência' RR	0.40 $\pm$ 0.16b	0.70 $\pm$ 0.21b	0.70 $\pm$ 0.30b	0.50 $\pm$ 0.17b	0.70 $\pm$ 0.15b	1.40 $\pm$ 0.27a	0.73 $\pm$ 0.14c
'TMG 7262' RR	0.40 $\pm$ 0.22b	0.50 $\pm$ 0.22 a	0.50 $\pm$ 0.17b	1.00 $\pm$ 0.33a	1.30 $\pm$ 0.30a	0.80 $\pm$ 0.20b	0.75 $\pm$ 0.14c
PI 227687	1.10 $\pm$ 0.31a	0.80 $\pm$ 0.33b	1.00 $\pm$ 0.33b	0.40 $\pm$ 0.16b	0.30 $\pm$ 0.15b	0.90 $\pm$ 0.28b	0.75 $\pm$ 0.13c
PI 274453	1.10 $\pm$ 0.28a	0.90 $\pm$ 0.23a	0.80 $\pm$ 0.20b	0.70 $\pm$ 0.21b	0.60 $\pm$ 0.22b	0.40 $\pm$ 0.22b	0.75 $\pm$ 0.10c
D75-10169	0.80 $\pm$ 0.29a	0.50 $\pm$ 0.17b	0.90 $\pm$ 0.31b	0.60 $\pm$ 0.22b	0.80 $\pm$ 0.25b	1.00 $\pm$ 0.26a	0.77 $\pm$ 0.08c
'TMG 4182'	0.50 $\pm$ 0.17b	0.40 $\pm$ 0.16b	0.50 $\pm$ 0.17b	0.60 $\pm$ 0.16b	1.00 $\pm$ 0.30b	1.70 $\pm$ 0.37a	0.78 $\pm$ 0.20c
'TMG 7062' IPRO	0.40 $\pm$ 0.16b	0.70 $\pm$ 0.30b	0.60 $\pm$ 0.22b	1.00 $\pm$ 0.33a	1.10 $\pm$ 0.28a	1.10 $\pm$ 0.18a	0.82 $\pm$ 0.12c
'Jackson'	0.50 $\pm$ 0.22b	0.60 $\pm$ 0.22b	0.90 $\pm$ 0.28b	1.00 $\pm$ 0.26a	1.00 $\pm$ 0.30b	1.10 $\pm$ 0.35a	0.85 $\pm$ 0.10c

UX-2569-159	0.70 ± 0.21a	0.80 ± 0.25b	0.90 ± 0.31b	0.60 ± 0.27b	0.90 ± 0.28b	1.30 ± 0.37a	0.87 ± 0.10c
PI 171451	1.30 ± 0.21a	1.10 ± 0.23a	1.00 ± 0.21b	0.80 ± 0.20a	1.00 ± 0.21b	1.00 ± 0.26a	1.03 ± 0.07b
'FTS Campo Mourão' RR	1.20 ± 0.42a	1.10 ± 0.35a	0.90 ± 0.28b	1.20 ± 0.42a	0.80 ± 0.25b	1.10 ± 0.35a	1.05 ± 0.07b
'TMG 4185'	0.90 ± 0.28a	0.50 ± 0.31b	0.80 ± 0.29b	1.20 ± 0.29a	1.70 ± 0.21a	1.80 ± 0.25a	1.15 ± 0.21b
'Coodetec 208'	0.50 ± 0.17b	1.40 ± 0.40a	1.40 ± 0.40a	1.10 ± 0.31a	1.40 ± 0.34a	1.80 ± 0.36a	1.27 ± 0.18b
L1-1-01	0.90 ± 0.28a	1.10 ± 0.31a	1.70 ± 0.30a	1.30 ± 0.30a	1.30 ± 0.30a	1.40 ± 0.27a	1.28 ± 0.11b
'TMG 132' RR	0.60 ± 0.50b	0.80 ± 0.33b	1.30 ± 0.42a	1.40 ± 0.40a	2.00 ± 0.36a	1.70 ± 0.45a	1.30 ± 0.22b
'Anta 82'	1.40 ± 0.27a	1.50 ± 0.31a	1.50 ± 0.31a	1.20 ± 0.35a	1.50 ± 0.27a	1.40 ± 0.40a	1.42 ± 0.05b
'Conquista'	1.20 ± 0.29a	1.50 ± 0.37a	2.00 ± 0.36a	2.10 ± 0.31a	2.10 ± 0.48a	2.20 ± 0.49a	1.85 ± 0.16a
<i>P</i>	0.0025	0.0125	0.0003	0.0002	< 0.0001	< 0.0001	< 0.0001

Means followed by the same letter in the column are not significantly different by Scott-Knott test ($P > 0.05$).

Table 3 - Mean ($\pm$ SE) foliar intake (cm^2) by fourth-instar *A. gemmatilis* on 30 genotypes in free and no-choice antixenosis tests in the laboratory

Genotype	Foliar intake (cm^2)	
	Free-choice	No-choice
'TMG 1179' RR	$0.67 \pm 0.11\text{d}$	$1.47 \pm 0.40\text{c}$
'IAC 100'	$0.88 \pm 0.24\text{d}$	$1.34 \pm 0.16\text{c}$
D75-10169	$0.89 \pm 0.13\text{d}$	$1.66 \pm 0.25\text{c}$
'IAC 17'	$0.97 \pm 0.11\text{d}$	$1.51 \pm 0.40\text{c}$
'IAC 23'	$0.99 \pm 0.13\text{d}$	$1.46 \pm 0.14\text{c}$
IAC 78-2318	$1.01 \pm 0.15\text{d}$	$1.41 \pm 0.11\text{c}$
'IAC 24'	$1.05 \pm 0.11\text{d}$	$1.70 \pm 0.43\text{c}$
'TMG 7062' IPRO	$1.05 \pm 0.18\text{d}$	$1.39 \pm 0.20\text{c}$
'IAC 19'	$1.08 \pm 0.15\text{d}$	$1.89 \pm 0.36\text{c}$
IAC 74-2832	$1.11 \pm 0.17\text{d}$	$1.65 \pm 0.35\text{c}$
L1-1-01	$1.26 \pm 0.10\text{c}$	$1.57 \pm 0.34\text{c}$
'KS-4202'	$1.33 \pm 0.21\text{c}$	$2.05 \pm 0.58\text{c}$
PI 227687	$1.40 \pm 0.13\text{c}$	$2.25 \pm 0.63\text{c}$
'TMG 133' RR	$1.46 \pm 0.16\text{c}$	$1.93 \pm 0.28\text{c}$
'FTS Campo Mourão' RR	$1.61 \pm 0.18\text{c}$	$2.24 \pm 0.28\text{c}$
'TMG 4182'	$1.73 \pm 0.23\text{b}$	$2.27 \pm 0.28\text{c}$
PI 229358	$1.79 \pm 0.27\text{b}$	$2.10 \pm 0.44\text{c}$
'BMX Potência' RR	$1.86 \pm 0.18\text{b}$	$2.56 \pm 0.24\text{c}$
'Dowling'	$1.87 \pm 0.20\text{b}$	$2.29 \pm 0.20\text{c}$
PI 274453	$1.98 \pm 0.17\text{b}$	$2.40 \pm 0.35\text{c}$
PI 171451	$2.02 \pm 0.20\text{b}$	$2.45 \pm 0.42\text{c}$
'Jackson'	$1.92 \pm 0.15\text{b}$	$2.59 \pm 0.26\text{b}$
'TMG 7262' RR	$1.94 \pm 0.22\text{b}$	$2.74 \pm 0.19\text{b}$
PI 274454	$2.03 \pm 0.17\text{b}$	$2.59 \pm 0.34\text{b}$
'TMG 4185'	$2.06 \pm 0.25\text{b}$	$2.77 \pm 0.42\text{b}$
UX-2569-159	$2.33 \pm 0.23\text{a}$	$3.03 \pm 0.35\text{b}$
'TMG 132' RR	$2.37 \pm 0.30\text{a}$	$3.08 \pm 0.22\text{b}$
'Anta 82'	$2.66 \pm 0.26\text{a}$	$3.58 \pm 0.24\text{a}$
'Coodetec 208'	$2.68 \pm 0.16\text{a}$	$4.30 \pm 0.59\text{a}$
'Conquista'	$2.95 \pm 0.17\text{a}$	$4.00 \pm 0.25\text{a}$
<i>P</i>	< 0.0001	< 0.0001

Means followed by the same letter in the column are not significantly different by Scott-Knott test ($P > 0.05$).

Table 4 - Mean ($\pm$ SE) duration of each larval instar and larval period of *A. gemmatilis* on 13 soybean genotypes in the laboratory

Genotype	Days							
	First instar	Second instar	Third instar	Fourth instar	Fifth instar	Sixth instar	Seventh instar	Larval period
'IAC 19'	3.66 $\pm$ 0.15a (n = 50)	2.63 $\pm$ 0.21a (n = 38)	2.39 $\pm$ 0.24cd (n = 31)	2.73 $\pm$ 0.29abc (n = 30)	2.19 $\pm$ 0.23bcd (n = 27)	2.61 $\pm$ 0.29a (n = 23)	2.20 $\pm$ 0.33a (n = 5)	19.86 $\pm$ 2.13a (n = 21)
'IAC 100'	3.08 $\pm$ 0.10bc (n = 50)	2.42 $\pm$ 0.17abcd (n = 43)	2.51 $\pm$ 0.19bcd (n = 41)	2.85 $\pm$ 0.24ab (n = 39)	2.48 $\pm$ 0.24abcd (n = 31)	2.26 $\pm$ 0.25a (n = 23)	2.11 $\pm$ 0.27a (n = 9)	19.24 $\pm$ 2.08a (n = 21)
'IAC 17'	3.34 $\pm$ 0.14ab (n = 50)	2.47 $\pm$ 0.16abc (n = 43)	3.24 $\pm$ 0.30a (n = 41)	2.97 $\pm$ 0.27a (n = 35)	2.64 $\pm$ 0.29abc (n = 25)	2.30 $\pm$ 0.26a (n = 20)	2.00 $\pm$ 0.27a (n = 4)	19.22 $\pm$ 2.18a (n = 18)
'IAC 24'	3.28 $\pm$ 0.13b (n = 50)	2.43 $\pm$ 0.19abcd (n = 40)	2.76 $\pm$ 0.26bcd (n = 33)	2.72 $\pm$ 0.29abcd (n = 29)	2.05 $\pm$ 0.26d (n = 22)	2.56 $\pm$ 0.34a (n = 9)	2.00 $\pm$ 0.27a (n = 3)	18.91 $\pm$ 2.50ab (n = 10)
PI 274454	3.20 $\pm$ 0.12bc (n = 50)	2.15 $\pm$ 0.14d (n = 41)	2.62 $\pm$ 0.22bcd (n = 39)	2.53 $\pm$ 0.27bcd (n = 34)	2.09 $\pm$ 0.24cd (n = 22)	2.60 $\pm$ 0.34a (n = 10)	3.00 $\pm$ 0.42a (n = 2)	17.50 $\pm$ 2.13bc (n = 14)
IAC 74-2832	3.04 $\pm$ 0.12bcd (n = 50)	2.56 $\pm$ 0.28ab (n = 39)	2.63 $\pm$ 0.24bcd (n = 35)	2.38 $\pm$ 0.22cde (n = 34)	2.65 $\pm$ 0.30abc (n = 23)	2.93 $\pm$ 0.37a (n = 15)	3.33 $\pm$ 0.49a (n = 3)	17.35 $\pm$ 1.98c (n = 17)
D75-10169	2.92 $\pm$ 0.12cd (n = 50)	2.44 $\pm$ 0.14abcd (n = 45)	2.80 $\pm$ 0.22bc (n = 44)	2.29 $\pm$ 0.18de (n = 42)	2.69 $\pm$ 0.25a (n = 35)	2.60 $\pm$ 0.38a (n = 10)	–	17.34 $\pm$ 1.49c (n = 32)
'TMG 1179' RR	2.92 $\pm$ 0.13cd (n = 50)	2.29 $\pm$ 0.12bcd (n = 45)	2.45 $\pm$ 0.18bcd (n = 42)	2.07 $\pm$ 0.13e (n = 42)	2.68 $\pm$ 0.20ab (n = 40)	2.11 $\pm$ 0.27a (n = 9)	–	17.25 $\pm$ 1.30c (n = 36)
'TMG 133' RR	3.04 $\pm$ 0.14bcd (n = 50)	2.31 $\pm$ 0.14bcd (n = 45)	2.88 $\pm$ 0.22ab (n = 42)	2.45 $\pm$ 0.19bcde (n = 40)	2.68 $\pm$ 0.26ab (n = 34)	2.28 $\pm$ 0.27a (n = 18)	–	17.21 $\pm$ 1.63c (n = 28)
L1-1-01	2.92 $\pm$ 0.11cd (n = 50)	2.20 $\pm$ 0.15cd (n = 41)	2.58 $\pm$ 0.21bcd (n = 38)	2.08 $\pm$ 0.18e (n = 36)	2.55 $\pm$ 0.26abcd (n = 31)	2.20 $\pm$ 0.28a (n = 10)	–	17.04 $\pm$ 1.77c (n = 24)
'Coodetec 208'	2.94 $\pm$ 0.11cd	2.16 $\pm$ 0.13cd	2.33 $\pm$ 0.17d	2.63 $\pm$ 0.22abcd	2.88 $\pm$ 0.32a	2.35 $\pm$ 0.33a	–	15.52 $\pm$ 1.61d

	(<i>n</i> = 50)	(<i>n</i> = 43)	(<i>n</i> = 40)	(<i>n</i> = 40)	(<i>n</i> = 33)	(<i>n</i> = 7)		(<i>n</i> = 23)
'Conquista'	2.74 ± 0.08d	2.27 ± 0.10bcd	2.56 ± 0.15bcd	2.63 ± 0.16abcd	2.73 ± 0.19a	2.00 ± 0.28a	–	14.53 ± 1.11e
	(<i>n</i> = 50)	(<i>n</i> = 48)	(<i>n</i> = 48)	(<i>n</i> = 46)	(<i>n</i> = 44)	(<i>n</i> = 2)		(<i>n</i> = 36)
'TMG 7062' IPRO	3.32 ± 0.10b	1.13 ± 0.15e	–	–	–	–	–	–
	(<i>n</i> = 50)	(<i>n</i> = 16)						
<i>P</i>	< 0.0001	< 0.0001	0.0048	0.0001	0.0256	0.1081	0.1222	< 0.0001

(–) = insects did not turn into the instar.

LS-means with the same letter in the column are not significantly different (*P* > 0.05).

Table 5 - Mean ($\pm$ SE) foliar intake per instar by *A. gemmatilis* on 13 soybean genotypes in the laboratory

Genotype	Foliar intake (cm ²)					
	Second instar	Third instar	Fourth instar	Fifth instar	Sixth instar	Seventh instar
'IAC 24'	1.14 $\pm$ 0.16c (n = 40)	6.58 $\pm$ 0.71cd (n = 33)	10.50 $\pm$ 1.03cd (n = 29)	12.78 $\pm$ 1.44d (n = 22)	15.24 $\pm$ 2.00abcd (n = 9)	13.58 $\pm$ 1.86a (n = 3)
'IAC 19'	1.22 $\pm$ 0.17bc (n = 38)	6.26 $\pm$ 0.70d (n = 31)	10.46 $\pm$ 1.02cd (n = 30)	12.01 $\pm$ 1.24d (n = 27)	14.36 $\pm$ 1.60bcd (n = 23)	12.96 $\pm$ 1.75a (n = 5)
'IAC 100'	1.26 $\pm$ 0.17bc (n = 43)	6.49 $\pm$ 0.55d (n = 41)	9.80 $\pm$ 0.73d (n = 39)	12.44 $\pm$ 1.20d (n = 31)	14.28 $\pm$ 1.58bcd (n = 23)	12.18 $\pm$ 1.57a (n = 9)
'IAC 17'	1.31 $\pm$ 0.18bc (n = 43)	6.77 $\pm$ 0.57cd (n = 41)	11.41 $\pm$ 1.05cd (n = 35)	13.59 $\pm$ 1.47d (n = 25)	16.04 $\pm$ 1.85abcd (n = 20)	10.32 $\pm$ 1.43a (n = 4)
IAC 74-2832	1.39 $\pm$ 0.18abc (n = 39)	9.38 $\pm$ 0.91b (n = 35)	14.18 $\pm$ 1.39 bcd (n = 34)	21.12 $\pm$ 2.46cd (n = 23)	17.83 $\pm$ 2.20ab (n = 15)	12.24 $\pm$ 1.72a (n = 3)
'TMG 1179' RR	1.42 $\pm$ 0.14abc (n = 45)	6.18 $\pm$ 0.49d (n = 42)	11.59 $\pm$ 0.76cd (n = 42)	15.14 $\pm$ 1.19d (n = 40)	11.92 $\pm$ 1.58cd (n = 9)	–
'TMG 133' RR	1.42 $\pm$ 0.18abc (n = 45)	7.44 $\pm$ 0.63bcd (n = 42)	14.89 $\pm$ 1.17bc (n = 40)	15.83 $\pm$ 1.38d (n = 34)	14.49 $\pm$ 1.75bcd (n = 18)	–
D75-10169	1.48 $\pm$ 0.18abc (n = 45)	6.40 $\pm$ 0.38d (n = 44)	11.37 $\pm$ 0.76cd (n = 42)	14.06 $\pm$ 1.25d (n = 35)	11.57 $\pm$ 1.52d (n = 10)	–
PI 274454	1.56 $\pm$ 0.16abc (n = 41)	8.86 $\pm$ 0.79bc (n = 39)	14.46 $\pm$ 1.39bcd (n = 34)	29.57 $\pm$ 3.85c (n = 22)	17.64 $\pm$ 2.31ab (n = 10)	15.54 $\pm$ 2.19a (n = 2)
L1-1-01	1.57 $\pm$ 0.15abc (n = 41)	9.50 $\pm$ 0.88b (n = 38)	14.59 $\pm$ 1.28bcd (n = 36)	14.35 $\pm$ 1.37d (n = 31)	16.64 $\pm$ 2.17abcd (n = 10)	–
'Coodetec 208'	1.96 $\pm$ 0.18ab	12.38 $\pm$ 0.95a	17.23 $\pm$ 1.37b	50.20 $\pm$ 4.83b	18.66 $\pm$ 2.47ab	–

	(n = 43)	(n = 40)	(n = 40)	(n = 33)	(n = 7)	
'Conquista'	2.09 ± 0.14a	14.99 ± 0.55a	28.35 ± 2.53a	62.97 ± 5.03a	24.99 ± 3.46a	–
	(n = 48)	(n = 48)	(n = 46)	(n = 44)	(n = 2)	
'TMG 7062' IPRO	–*	–	–	–	–	–
	(n = 16)					
<i>P</i>	0.0003	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.1341

(–) = insects did not turn into the instar.

(–*) = Consumption was low, and the leaf area meter did not registered the value.

LS-means with the same letter are not significantly different ($P > 0.05$).

Table 6 - Mean ($\pm$ SE) *A. gemmatalis* weight of fifth instar and pupae on 13 soybean genotypes in the laboratory

Genotype	Weight (g)	
	Fifth instar	Pupae
'IAC 19'	0.09 $\pm$ 0.02f (n = 23)	0.12 $\pm$ 0.01d (n = 20)
'IAC 24'	0.10 $\pm$ 0.01ef (n = 13)	0.13 $\pm$ 0.02cd (n = 8)
'IAC 100'	0.10 $\pm$ 0.02ef (n = 25)	0.12 $\pm$ 0.02d (n = 15)
'IAC 17'	0.10 $\pm$ 0.02ef (n = 23)	0.12 $\pm$ 0.02d (n = 15)
IAC 74-2832	0.12 $\pm$ 0.02de (n = 22)	0.16 $\pm$ 0.02b (n = 15)
PI 274454	0.13 $\pm$ 0.02cd (n = 18)	0.16 $\pm$ 0.02bc (n = 14)
'TMG 133' RR	0.14 $\pm$ 0.01bcd (n = 30)	0.16 $\pm$ 0.02b (n = 22)
D75-10169	0.15 $\pm$ 0.02bc (n = 33)	0.16 $\pm$ 0.02bc (n = 27)
'TMG 1179' RR	0.15 $\pm$ 0.02bc (n = 38)	0.16 $\pm$ 0.01b (n = 31)
L1-1-01	0.15 $\pm$ 0.02bc (n = 27)	0.16 $\pm$ 0.02b (n = 22)
'Coodetec 208'	0.16 $\pm$ 0.02b (n = 25)	0.19 $\pm$ 0.02a (n = 19)
'Conquista'	0.18 $\pm$ 0.02a (n = 39)	0.21 $\pm$ 0.02a (n = 35)
'TMG 7062' IPRO	—	—
P	< 0.0001	< 0.0001

(—) = insects did not turn into fifth instar larvae and pupae.

LS-means with the same letter in the column are not significantly different ($P > 0.05$).

Table 7 - Mean ($\pm$ SE) duration of pre-pupal, pupal, and larvae-adult periods of *A. gemmatalis* on 13 soybean genotypes in the laboratory

Genotype	Days		
	Pre-pupal	Pupal	Larvae - adult
'IAC 19'	2.00 $\pm$ 0.22a (n = 21)	11.65 $\pm$ 1.28a (n = 20)	32.47 $\pm$ 3.54a (n = 20)
'IAC 24'	2.00 $\pm$ 0.25ab (n = 10)	11.25 $\pm$ 1.46abc (n = 8)	32.25 $\pm$ 4.22a (n = 8)
'IAC 100'	2.10 $\pm$ 0.24a (n = 21)	11.73 $\pm$ 1.39a (n = 15)	32.13 $\pm$ 3.83a (n = 15)
'IAC 17'	2.11 $\pm$ 0.26a (n = 18)	11.20 $\pm$ 1.33bc (n = 15)	32.00 $\pm$ 3.75a (n = 15)
PI 274454	1.79 $\pm$ 0.22abc (n = 14)	10.86 $\pm$ 1.31cde (n = 14)	29.29 $\pm$ 3.55b (n = 14)
IAC 74-2832	1.65 $\pm$ 0.20bcd (n = 17)	11.40 $\pm$ 1.35ab (n = 15)	29.14 $\pm$ 3.40b (n = 15)
'TMG 133' RR	1.43 $\pm$ 0.16de (n = 28)	10.55 $\pm$ 1.12def (n = 22)	28.68 $\pm$ 3.05bc (n = 22)
'TMG 1179' RR	1.28 $\pm$ 0.11ef (n = 36)	10.65 $\pm$ 0.93de (n = 31)	28.65 $\pm$ 2.50bc (n = 31)
D75-10169	1.47 $\pm$ 0.14de (n = 32)	10.26 $\pm$ 0.99f (n = 27)	28.62 $\pm$ 2.76bc (n = 27)
'Coodetec 208'	1.57 $\pm$ 0.18cd (n = 23)	10.95 $\pm$ 1.22bcd (n = 19)	28.53 $\pm$ 3.20bc (n = 19)
L1-1-01	1.25 $\pm$ 0.14ef (n = 24)	10.50 $\pm$ 1.11ef (n = 22)	28.52 $\pm$ 3.02bc (n = 22)
'Conquista'	1.08 $\pm$ 0.09f (n = 36)	9.31 $\pm$ 0.73g (n = 35)	27.82 $\pm$ 2.18c (n = 35)
'TMG 7062' IPRO	—	—	—

P	< 0.0001	< 0.0001	< 0.0001
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(–) = insects did not turn into pre-pupae, pupae, and adult.

LS-means with the same letter in the column are not significantly different ($P > 0.05$).

Table 8 - Mean ($\pm$ SE) pre-oviposition and oviposition periods, and egg number of *A. gemmatalis* on 13 soybean genotypes in the laboratory

Genotype	Pre-oviposition (days)	Oviposition (days)	Number of eggs
'IAC 24'	5.00 $\pm$ 1.28abc (n = 2)	5.00 $\pm$ 1.31cd (n = 2)	110.50 $\pm$ 28.56c (n = 2)
'IAC 17'	5.00 $\pm$ 1.15ab (n = 4)	5.25 $\pm$ 1.25cd (n = 4)	131.00 $\pm$ 38.68c (n = 4)
'IAC 100'	5.50 $\pm$ 1.29a (n = 4)	4.75 $\pm$ 1.10d (n = 4)	158.75 $\pm$ 40.92c (n = 4)
'IAC 19'	5.00 $\pm$ 0.88abc (n = 9)	5.89 $\pm$ 1.03cd (n = 9)	178.78 $\pm$ 37.28c (n = 9)
'TMG 1179' RR	3.57 $\pm$ 0.70cd (n = 7)	9.43 $\pm$ 2.16bcd (n = 7)	240.43 $\pm$ 47.93c (n = 7)
IAC 74-2832	4.00 $\pm$ 0.89bcd (n = 5)	9.00 $\pm$ 2.16bcd (n = 5)	277.20 $\pm$ 76.68bc (n = 5)
'Coodetec 208'	2.80 $\pm$ 0.64de (n = 5)	9.00 $\pm$ 2.62bcd (n = 5)	292.20 $\pm$ 65.88bc (n = 5)
PI 274454	3.67 $\pm$ 0.87cd (n = 6)	8.67 $\pm$ 1.96bcd (n = 6)	419.00 $\pm$ 100.69ab (n = 6)
'TMG 133' RR	3.50 $\pm$ 0.73cd (n = 6)	10.83 $\pm$ 2.43abc (n = 6)	426.17 $\pm$ 108.50ab (n = 6)
L1-1-01	3.80 $\pm$ 0.85bcd (n = 5)	11.60 $\pm$ 2.61ab (n = 5)	451.00 $\pm$ 11.16ab (n = 5)
D75-10169	3.60 $\pm$ 0.79 cd (n = 5)	8.60 $\pm$ 1.96bcd (n = 5)	462.60 $\pm$ 115.69ab (n = 5)
'Conquista'	1.85 $\pm$ 0.15e (n = 13)	13.54 $\pm$ 1.22a (n = 13)	696.69 $\pm$ 74.34a (n = 13)
'TMG 7062' IPRO	—	—	—
<i>P</i>	< 0.0001	0.0008	< 0.0001

(—) = insects did not turn into adult.

LS-means with the same letter in the column are not significantly different ($P > 0.05$).

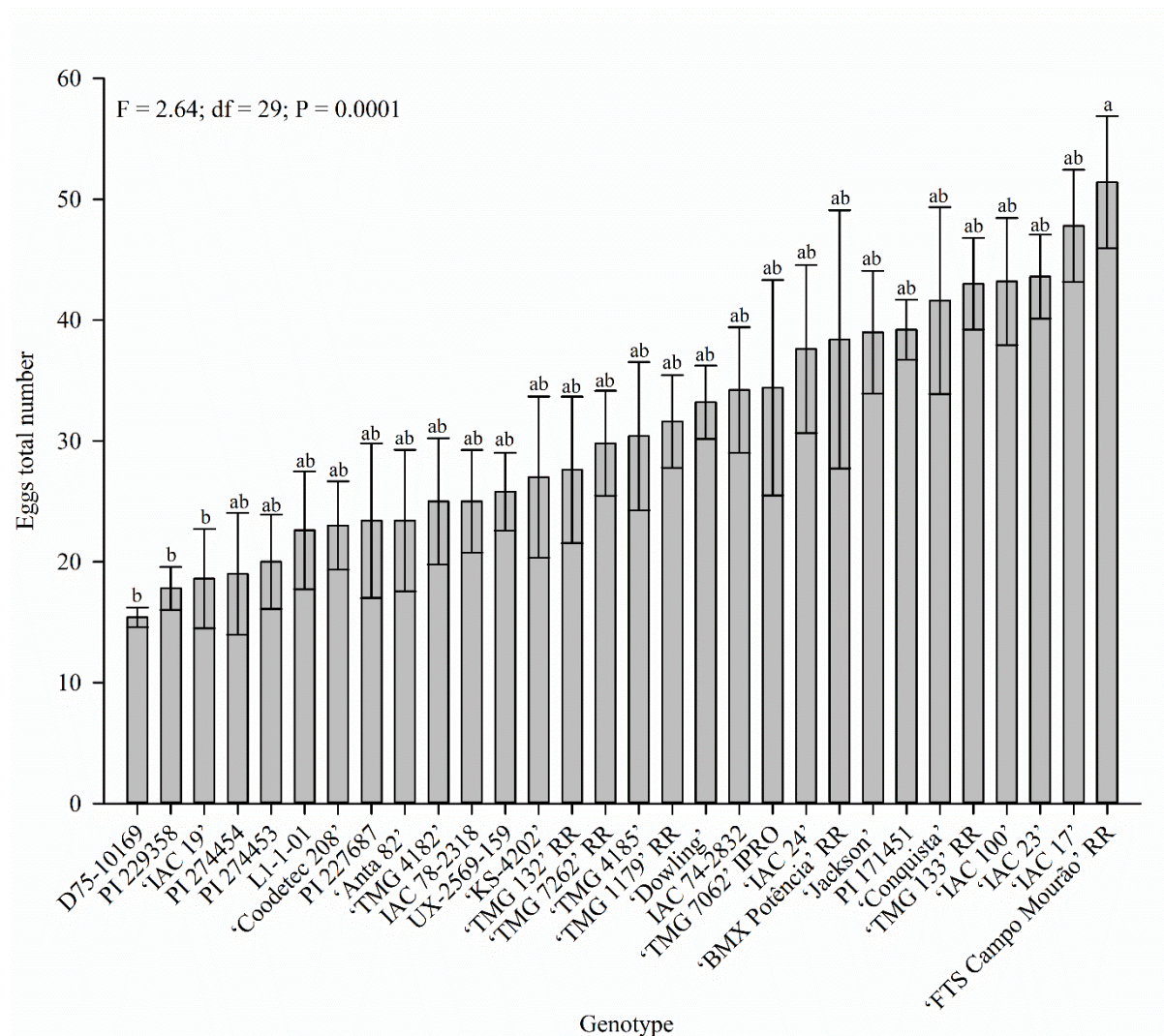


Figure 1 - Oviposition of *A. gemmatilis* on 30 soybean genotypes under greenhouse conditions.

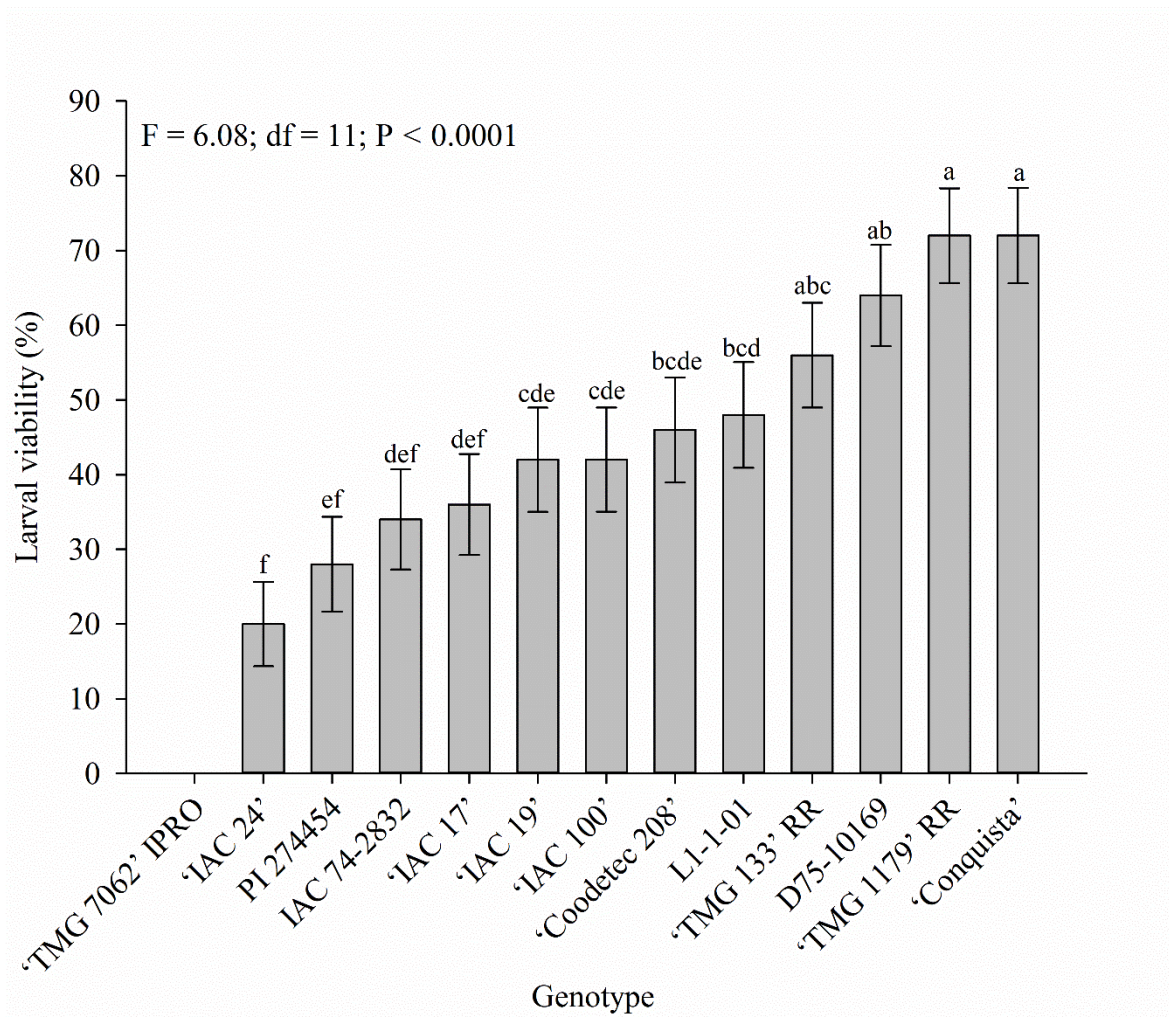


Figure 2 - Mean ($\pm$ SE) larval viability of *A. gemmatilis* on 13 soybean genotypes in the laboratory. LS-means bars with the same letter are not significantly different ($P > 0.05$).

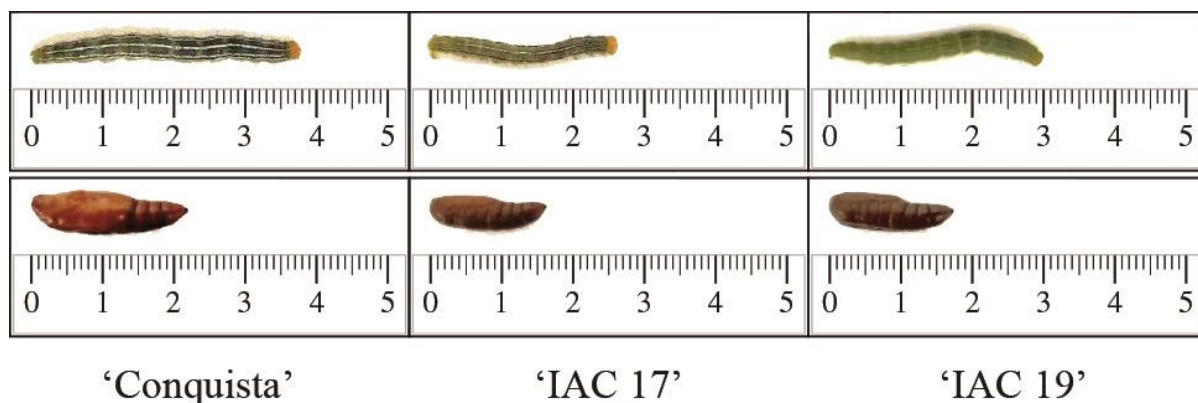


Figure 3 - Comparative development of *A. gemmatalis* fifth instar larvae and pupae on susceptible ('Conquista') and resistant ('IAC 17' and 'IAC 19') soybean genotypes.

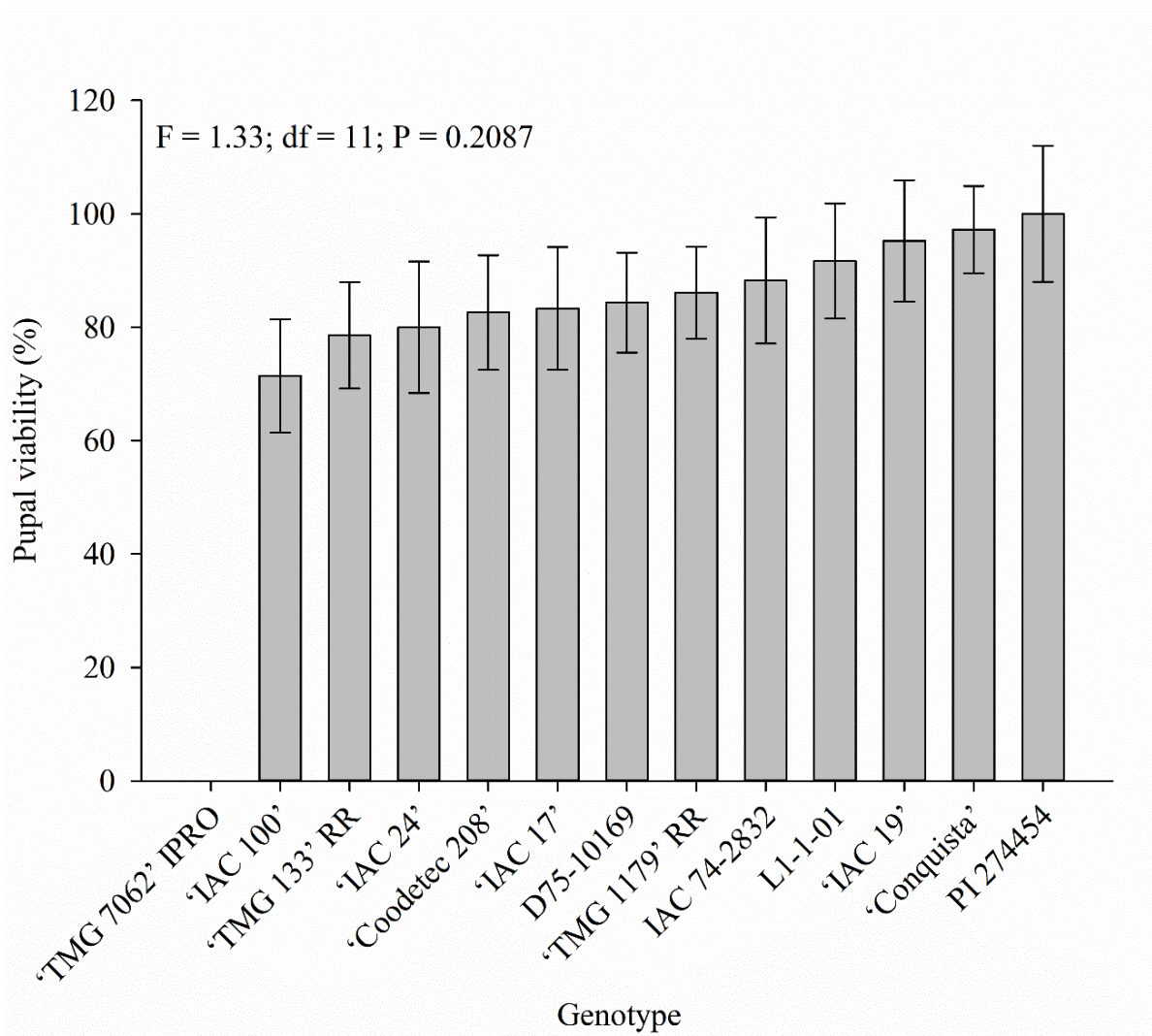


Figure 4 - Mean ($\pm$ SE) pupal viability of *A. gemmatalis* on 13 soybean genotypes in the laboratory.

CHAPTER 2

LARVAL MOVEMENT OF *Anticarsia gemmatalis* (LEPIDOPTERA: EREBIDAE) ON VEGETATIVE AND REPRODUCTIVE STAGE NON-BT SOYBEAN

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ABSTRACT

Integrated pest management (IPM) and insect resistance management (IRM) in a cropping system demands a comprehensive understanding of insect behavior, including larval movement. We evaluated the larval movement of velvetbean caterpillar, *Anticarsia gemmatalis* Hübner, in a non-Bt soybean cultivar. Experiments were performed under field conditions in the 2019 crop season. Soybean plants in vegetative and reproductive stages were infested with third to fifth instar larvae in different combinations, and on-plant movement was characterized across select time points. The dispersion among plants in different positions and orientations was assessed by releasing 24-h-old larvae on the central plant of field plots. Results indicated that *A. gemmatalis* larvae are consistent in their movement during the day, regardless of plant section or leaf position. Over the time period of assessment, larvae gradually moved down and left the plant. Although there were not significant difference

in the distance traveled by *A. gemmatalis* between orientations and positions, long distances were covered indicating that this species has nondirectional movement. This study provides relevant information regarding the movement of the velvetbean caterpillar, which can be used to improve insecticide spraying efficacy, improve probabilistic model predictive ability, and help design insect resistance management strategies.

Keywords: Velvetbean caterpillar; Larval behavior; Dispersion; Management strategies

Key Message

- The on-plant and plant-to-plant movements of *A. gemmatalis* on soybean were characterized for the first time.
- We found that *A. gemmatalis* has a highly active behavior throughout the day, changing the position where they were found on the plant and leaf. Over the period of evaluation, larvae gradually left the plant, particularly larger larvae. This is a relevant information which will help to optimize insecticide spraying and natural enemy deployment.
- Larvae have the ability to move relatively long distances from the infested plant, influencing the population dynamics in the field.
- Our findings have potential application for integrated pest management (IPM) and insecticide resistance management (IRM) for *A. gemmatalis* in non-*Bt* soybean and *Bt* soybean.

Authors' Contribution Statement

SO, ELLB and TEH conceived and designed research. SO and NL conducted experiments. KRH and EAR analyzed data. SO, ELLB and TEH wrote the manuscript. All authors read and approved the manuscript.

2.1 Introduction

Soybean [*Glycine max* (L.) Merrill] is an established crop with a high production value, being the most widely cultivated and used legumes worldwide (USDA-NASS, 2020). With the crop production expansion, several insects species adapted to this crop and became economic pests (Kogan 1981; Gagné et al. 2019). Among them, the velvetbean caterpillar, *Anticarsia gemmatilis* Hübner (Lepidoptera: Erebidae), is one important soybean pest occurring in all the western hemisphere (Puttler and Long 1980; Buschman et al. 1981; Bortolotto et al. 2015; Formentini et al. 2015; Haase et al. 2015). This species has been historically reported as a 'major' or 'serious' pest in the United States (U.S.), but are most common and problematic in the southern (Buschman et al. 1981; Huseth et al. 2021). The velvetbean caterpillar is one of the fastest defoliating caterpillars' species, able to strip soybean fields in five to seven days. The larval feeding starts on the new leaves, moving to the older ones, besides feeding on tender stems and pods. Larvae typically begin feeding in the upper canopy and move lower on the plant as the defoliation continues (Wilkerson et al. 1986).

Despite the importance of this species, a greater understanding on its larval behavior is still needed, and it includes the characterization of individual's movement, wherever on-plant and plant-to-plant, throughout their development, as already studied for other species (Paula-Moraes et al. 2012; Ramalho et al. 2014; Pannuti et al. 2015, 2016; Malaquias et al. 2017; Fanela et al. 2020; Sokame et al. 2020). These information may assist the establishment of integrated pest management (IPM) and insecticide resistance strategies (IRM) in major crops (Ross and Ostlie 1990; Onstad 2009; Tabashnik et al. 2013).

Based on previous studies, movement of early instar of lepidopteran larvae within host plants largely determines where feeding sites become established (Foster and Howard 1999; Perkins et al. 2008; Pannuti et al. 2019; Sokame et al. 2020). On-plant knowledge also helps to understand where the insects are throughout the day, where to sample and/or treat when necessary, allowing a most efficient spraying (Zalucki et al. 2002; Perkins et al. 2008; Paula-Moraes et al. 2012; Pannuti et al. 2015; Zulin et al. 2018). Control of lepidopteran pests by either biotic or abiotic measures is facilitated when we precisely know where early instars larvae are located (Vinson 1976; Zalucki et al. 2002).

The movement among plants, classified as dispersion, is the movement carried out away from a place that results in the dissemination of part of the individuals of the

original population (Price 1997). This behavior is related to an adaptive mechanism by which insects seek food resources, refuge, and mating places (Bell 1990, Price 1997). Another important reason to better understand larval behavior plants arises with the advent of soybean expressing a *Bacillus thuringiensis* (*Bt*) insecticidal protein. Brazil is the top developing country in the world planting biotech crops including soybean, while in the U.S this technology in soybean is currently being started (ISAAA 2019), and taking as example, the large scale adoption of *Bt* corn and cotton has resulted in an intense selection pressure for the development of resistance, challenging the long-term sustainability of this genetically modified organism (GMO) technology (Tabashnik et al. 2013).

The *Bt* soybean has been effective in controlling *A. gemmatalis* (Bernardi et al. 2012; Bel et al. 2019; Bacalhau et al. 2020). Nevertheless, this technology is not available in all soybean producing regions. In this sense, this pest has a capacity to cause serious loss in conventional soybean systems and in conditions of high infestation, foliar consumption can be total (Bueno et al. 2011). In addition, depending on the refuge areas settings, some early instars are able to feed in *Bt* plants and move to non-*Bt* plants, or they can move from non-*Bt* to *Bt* plants as older instars and survival in the area (Wangila et al. 2013; Head et al. 2014; Ramalho et al. 2014; Pannuti et al. 2016). Furthermore, larvae with some tolerance to *Bt* proteins, and stimulated by *Bt* plants, may move longer distances toward non-*Bt* plants (Malaquias et al. 2017).

Regarding the growing soybean market in areas such as Brazil and U.S and the constant challenges involving the management of leaf-eaters insects in the crop, this research aimed to characterize the on-plant and plant-to-plant movements of *A. gemmatalis* larvae in non-*Bt* soybean field. The results obtained in this research will serve as a baseline for future *A. gemmatalis* management studies, optimizing insecticide spraying and natural enemies releasing. Additionally, understanding larval movement capacity will help to support the choice by the best refuge strategy, concerning using *Bt* plants, and in probabilistic models to improve their predictive ability.

2.2 Materials and methods

The studies were conducted under field conditions at the University of Nebraska Haskell Agricultural Laboratory, Concord, NE, USA, during the 2019 crop season. The non-*Bt* cultivar Channel 2516R2X was used in all studies.

Insects

Anticarsia gemmatilis eggs and larvae were commercially acquired (Benzon Research Inc., Carlisle, PA, USA) and reared in plastic pots containing 15 mL of artificial diet (based on diet developed by USDA, Stoneville, MS and University of Georgia, Athens, GA, USA). The insects were kept in a rearing chamber [$25 \pm 2^\circ\text{C}$, RH $60 \pm 10\%$, 14:10 (L:D)] until the instar required for each assay (on-plant larval movement). For the plant-to-plant assay, eggs were purchased close to hatching in order to standardize infestations of the plants in the field with neonates (24-h old).

On-plant larval movement

The design was as follows: There were 4 plots of 5 non-touching soybean plants. Six velvetbean caterpillar (*A. gemmatilis*) larvae were released the middle section of each plant. Thus, each plot had 30 larvae on the plants (free of insect infestation) to start of the experiment,. The locations of the larvae were recorded in terms of both leaf and plant section position. The plant sections were divided into top, middle, lower, and off

the plant, and the leaf locations into abaxial, adaxial, and off the plant. The field infestation occurred at dusk (about 8pm) to reduce larval stress. The larval positions were measured at 10 different time points (8, 10, 12, 14, 16, 18, 20, 32, 36, 44 hours). The 8 to 20 hours are on the first day, while 32, 36, and 44 hours are the observations from the second day (i.e. 32 hours corresponds to day 2 at 8 am). The experiment was done for 4 different combinations (assays) of larval stage of development (instar), as well as soybean stage of development (vegetative and reproductive) (Fehr and Caviness 1977), which consisted in the follow combinations: 3rd instar/V7; 4th instar/R3; 5th instar/V7,R1; 5th instar/R3,R4. The assays were all analyzed separately, and time was considered a continuous scale for the analysis.

Plant-to-plant larval movement

The experiment consisted of 7 plots arranged in a randomized block design, 3.0-m long rows, totaling 400 plants per plot. Initially, soybean leaves were removed from the field (same cultivar planted to this experiment) and installed inside the plastic cages ($21.5 \times 14.5 \times 3.5$ cm). A piece of cotton moistened in water was wrapped around the base of the petioles to maintain leaf turgidity. Individual eggs were cut from organza cloth where the adults oviposited, and then placed on leaves until they hatched. After hatching, 50 larvae (24-h-old) per leaflet were kept and isolated in a Gerbox ($11 \times 11 \times 3.5$ cm) until infestation. The excess larvae were removed with a delicate paintbrush. Artificial infestation was performed by releasing the neonates at 4 locations in the middle section of the central plant of each plot, totaling 200 larvae per plant. The infestation was performed by fixing infested leaflets from the rearing cages to the upper surface of a central plant leaflet with a small piece of adhesive tape, taking care so that the neonates did not stick to the tape. This technique reduced stress to the neonates, resulting in efficient transfer of the neonates to the infested plant (Fanela et al. 2020). The field infestation occurred at dusk to reduce stress to the larvae and maximize the effectiveness of the infestation.

Destructive sampling of all plants per plot was carried out 12 d after infestation. The number/frequency of larvae were established by the position in which the insects were found in relation to the infested central plant of each plot. Larval position was demarcated in the field by small identification flags on wooden sticks placed at the plant base. Larval movement was categorized based on the presence of each insect on each plant in each plot, and the proportion of larvae present on infested plants and in rows. The rows ran north to south, and larval orientation was categorized as north (N) and south (S) in the same row with respect to the infested plant, and east (E) and west (W) when directly across rows from the infested plant. The position of the larvae was designated as zero when on the infested plant. For larvae not directly N, S, E, or W of the infested plant, the position from the release point was categorized by quadrant: northeast (NE), southeast (SE), northwest (NW), and southwest (SW). Larval mean and maximum distance from the infested plant was determined. The most distant larvae in each quadrant and orientation was considered the maximum distance (Pannuti et al. 2016).

Statistical analyses

On-plant analyses were conducted using Proc Glimmix in SAS 9.4 (SAS Institute 2001). For each leaf location and plant location, a series of Generalized Linear Mixed Models following a Binomial distribution with a logit link function were conducted to determine the proportion of larvae within each location (e.g., proportion of larvae in the top part of the plant vs not in the top part of the plant) accounting for repeated measures over time. The response variable in the binomial model was how many larvae were in each location, at a particular time point, divided by 30 (total larvae in the plot) (i.e., proportion of larvae within each location – 10/30 larvae in the top plant indicates 33% of larvae are on the top part of the plant). Our fixed effect was treating time as a factor since we have measurements for each time period. We imposed a covariance structure of spatial power to account for correlation among responses in plot and time (i.e., repeated measures). Spatial power was selected because we do not have equal distances between observations. In the analysis, leaf and plant locations were run separately.

To compare quadrants, infested rows, and orientations, a linear mixed model was conducted, using the lmer function in the lme4 package from R version 3.6.3 (R Development Core Team 2020). Direction was considered fixed effect and plot was considered as a random effect. The emmeans package was used to calculate the least squared means estimates for each direction. Tukey adjustment was used to control

type I error rates. Any analysis to compare data between rows was not possible to be conducted, since there is only 2 and 1 observation in each direction (W/E), respectively.

2.3 Results

On-plant larval movement

Considering plant location, no significant differences were observed for the estimated proportion for 3rd instar *A. gemmatilis* larvae in V7 soybean on the top ($F = 0.97$; $df =$

9; $P = 0.4815$), middle ($F = 1.04$; $df = 9$; $P = 0.4320$) and lower sections of the plant ($F = 0.47$; $df = 9$; $P = 0.8860$, Fig. 1a). However, the time effect was significant ($F = 3.01$; $df = 9$; $P = 0.0111$) on the larvae number that left the plant, since the proportion rate of larvae started with 0.35 and had an increase to 0.60. For the 4th instar larvae/R3, we observed that larvae did not congregate in the lower section of the plants ($F = 1.41$; $df = 4$; $P = 0.2794$, Fig. 1b). On the other hand, as day 1 progressed, the larvae that moved off the plant significantly increased from 0.17 at 8 h to 0.87 at 44 h ($F = 15.70$; $df = 9$; $P < 0.0001$). The larvae in the top ($F = 5.00$; $df = 9$; $P = 0.0004$) and middle ($F = 8.08$; $df = 9$; $P < 0.0001$) sections was off after 20 h point (Fig. 1b). Significant differences were observed for 5th instar larvae/V7,R1 on and before 16 h. The estimated number of larvae found in the top part of the soybean plants (proportion of 0.20 – 0.07 larvae) ($F = 4.17$; $df = 4$; $P = 0.0182$) differed from the middle section (0.02 – 0.00 larvae) ($F = 0.47$; $df = 4$; $P = 0.7583$) and larvae off the plant (0.73 – 0.90 larvae) ($F = 2.22$; $df = 4$; $P = 0.1156$). There was not enough data to obtain results for the lower portion (Fig. 1c). For the 5th instar/R3,R4 assay, we found significant differences on and before point time 18 h in the top ($F = 7.50$; $df = 5$; $P = 0.0006$), middle ($F = 8.39$; $df = 5$; $P = 0.0003$) and off plant ($F = 19.42$; $df = 5$; $P < 0.0001$). The proportion of larvae in the lower part of the plant was not significant ($F = 0.49$; $df = 5$; $P = 0.7779$). Throughout the evaluation, the estimate proportion of larvae reduced in the different plant sections, while it had an increase for larvae off plant (Fig. 1d).

Regarding leaf location, the proportion of 3rd instar larvae in V7 soybean stage significantly changes over time on the adaxial ($P < 0.0001$), abaxial part ($P < 0.0001$), and for larvae that left the plant ($P = 0.0111$, Table 1). The distribution was inversely proportional, since in the first time point, with a rate of 0.39, 0.25 and 0.35, changing to 0.19, 0.20 and 0.60, respectively, in the last evaluation time. We found a difference in the proportion of 4th instar larvae in R3 soybean in adaxial, abaxial, and off plant ($P < 0.0001$, Table 2). While in the beginning, a rate of 0.53 (adaxial) and 0.28 (abaxial) were on the plant, the proportion rate of larvae that left the plants gradually increased through time, reaching 0.87 at the point 44 (hour). For the 5th instar/V7,R1 assay, there were differences only in the time points on and before 16 hours. All insects after time point 16 were off the plant. For the adaxial part of the leaf, there was little to no data after time point 8, which is why we did not have enough data to run the model. There was no difference in the ratio of larvae counts for different time points for the abaxial

part of the leaf ($P = 0.8288$) and for larvae counts for different time points off the plant ($P = 0.1156$, Table 3). Significant differences were observed in the adaxial ($P = 0.0057$) and abaxial part of the leaf ($P = 0.0027$) with rates of 0.30 and 0.25, respectively for 5th instar larvae in R3,R4 soybean. From the first evaluation, an estimated proportion of 0.44 insects were off plant, reaching 100.00% of larvae off at the time point 20 ($P < 0.0001$, Table 4).

Plant-to-plant larval movement

There were no statistical differences ($P > 0.05$) for mean and maximum distanced moved by *A. gemmatilis* larvae among the different quadrants (NE, SE, NW, and SW), N and S orientations, within infested row (N and S axes), and across rows (E and W axes, Table 5). Numerically, the mean (104.72 cm) and maximum distance (142.52 cm) covered by *A. gemmatilis* were greater at NE direction and taking the interval distance, one larvae reaching out 202.62 cm. Following the orientation, both mean and maximum distances were observed at N (77.25 and 102.30 cm, respectively). In the infested row, the values related to the mean distance were similar [51.54 cm (N) to 56.16 cm (S)] and maximum distance were 62.25 cm (N) and 87.50 cm (S). Across rows, the distances moved were around 40.00 cm.

Regarding the quadrants, the highest larvae number was observed in the SE (13 larvae), while across row, 2 larvae were found in the W and 1 in the E. The proportion of larvae found within infested row was higher in the N position (9 larvae). However, in total, more larvae (25) were found in the S orientation, compared to the N

(24 larvae) (Table 5). The larval frequency varied from 37.14% (SE quadrant) to 17.14% (NW quadrants). With respect to N and S orientations, the larvae were distributed similarly, with frequencies of 48.98 and 51.02%, respectively. Within the infested row (N and S axes), more than 60.00% of the larvae moved North. Considering the movement of larvae across rows (W and E axes), twice the larvae were recovered on the Western axis (Table 5). A visual distribution of larvae in the plots, 12 days after release, indicating nondirectional movement is provided in Fig. 2.

2.4 Discussion

There is little information on *A. gemmatalis* larval movement on soybean plants. In the current study we assessed for the first time the movement of this species on soybean under field conditions, and observed that in all the on-plant movement scenarios, larvae had the ability to move constantly over time. For 3rd instar/V7 larvae, we observed more constancy in the distribution on the plant over the evaluation. However, for 5th instar/V7,R1 assay, for example, even in the first evaluations we did not detect larval presence in some plant sections and leaf positions. Larval size and plant growth stage affects the behavior and acceptance of a suitable host, and in turn movement patterns, stimulating larvae gradually to move off the plant. This highly active behavior was already observed for other lepidopteran species. Measuring movement of *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), 50% of the larvae had moved off the plant within 6 hours (Razze et al. 2011).

The causes of this behavior are not always clear and many of these details can influence IPM and IRM strategies. Even though we did not assess feeding behavior, it is known that timing of movement relative to feeding and the age of the larvae are important (Onstad et al. 2018). In general, it is relevant to know whether movement consists of discrete steps with each interval of feeding followed by movement or whether movement is mostly one long, continuous step from one to another section in the same plant or the plant of origin to the final host with no feeding between points. We suggest as a future research to characterize the neonate's dispersion on the plant, once the larvae will start to move and find a suitable feeding site (Perkins et al. 2008).

Larvae may be affected by environmental conditions. A noticeable characteristic during the evaluation was that we observed higher larval movement to the middle and lower plant section and to the abaxial leaf position during the warm hours of the day. Similar behavior was also reported for *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae) in dry beans (Difonzo et al. 2015). At 14 days after hatch (DAH), most 4th instars were found on the soil surface. No larvae were found on the plant or soil surface after 14 DAH. Observations by time of day clearly showed that the majority of 5th instars spent the day on or in the ground, moving up the plant in the dusk and returning to the ground by sunrise. Even in larger crops, such as corn, when *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) infestation was done above the ear zone, larvae moved downward to the ear zone over time (Pannuti et al. 2015).

This on-plant information also helps to understand the dispersion dynamic throughout the day and can improve insecticide spraying efficiency. Based in our observed larval movement, results reinforce the theory to apply insecticides and release entomopathogenic enemies early in the morning or at the dusk, when the insects will be exposed to the products. Cannibalism and predation are also causes that could impact population dynamics, and this behavior was already reported for this species and when interacting with other species (Ongaratto et al. 2021). Another important mortality factor in the agroecosystem is the presence of natural enemies, which was visually detected in our study and might also explain the number of missing larvae. The larvae position and high movement on and between plants can also in turn expose the insect to predators and parasitoids (Vinson 1976; Hermann and Thaler 2018).

Regarding the movement among plants, no significant, but notable differences in the dispersion were observed. Our results showed that long distances were covered by *A. gemmatalis* regardless of the position and orientation chosen, indicating the absence of a homogenous movement. This type of movement is a ubiquitous life-history trait of organisms, with profound consequences on the spatial and temporal dynamics of populations (Mazzi and Dorn 2012). Thus, movement of this species appears to be nondirectional and independent (Bell 1990); however, especially under field conditions, it could be influenced by biotic or abiotic factors. In a similar study with *Spodoptera eridania* (Stoll) (Lepidoptera: Noctuidae) larvae, it was also observed that the species move in all directions independent of the season or cultivar (Fanela et al. 2020). Pannuti et al. (2016) assessing *S. frugiperda* and *S. albicosta* movement observed a dispersal governed by nondirectional sensory information of both species; however, *S. frugiperda* remained nearer the release point, presented a more aggregated and symmetrical distribution.

The mean distances found here were approximately 40.00 cm in the infested row, and up to 50.00 cm for the other orientations. The mean of maximum distance varied between 38.00 to 142.52 cm, reaching 202.62 cm to the NE. Similarly, in a greenhouse and in open field trials, larvae of *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae) were observed to move from initially infested plants to at least four plants away, with the majority of larvae staying within three plants away from the release plant (Wangila et al. 2013). *Spodoptera frugiperda* larvae exhibited greater

movement to the North within the infested row; however, no significant differences were observed when considering general N and S orientations, and this northern movement may have been influenced by wind or another environmental factor (Pannuti et al. 2016).

Despite that in our studies we characterized the movement of *A. gemmatilis* in non-*Bt* soybean, we can use the behavior information to apply in a *Bt* field. In practical terms, the distance traveled by the insects may affect the evolution of resistance to *Bt* crops, in association with configuration and size of the refuge areas (Tabashnik et al. 2013; Garcia et al. 2016). Initial high-dose/refuge strategies were initially designed to delay resistance to *Bt* crops. Among them, the use of seed mixtures (refuge in the bag 'RIB') has been suggested to manage resistance to *Bt* toxins (Carroll et al. 2013). Although RIB can be an option in IRM, insects that exhibit high larval movement across plants generally favor the evolution of resistance in a seed mixture refuge, since such movement exposes insects to sublethal doses of the toxins, especially for single-gene *Bt* events (Head et al. 2014; Garcia et al. 2016).

Seed mixtures can increase the dominance of resistance by increasing survival of heterozygous larvae relative to homozygous susceptible larvae when individual larvae feed on both *Bt* and non-*Bt* plants (Mallet and Porter 1992). There are studies with other pest species and crops that demonstrate RIB is not always appropriate. In a field study conducted with seed mixes of non-*Bt* and *Bt* pyramid maize, *D. saccharalis* larvae were able to move from infested plants, as well as to adjacent rows, so larvae could feed on non-*Bt* maize until they reached a size that allowed their survival on *Bt* maize (Wangila et al. 2013). This strategy may not be ideal for resistance management of *A. gemmatilis* to the Cry1Ac toxin, and a more appropriate strategy, such the use of strip or block refuge, may be required. However, possible movement across the border between *Bt* and non-*Bt* crops must first be examined, as it may also have an important role in resistance evolution (Garcia et al. 2016).

In our open field study we recovered a lower number of larvae with high variability among plots; however, this is representative on most real conditions. We found a higher frequency of larvae recovered in the different quadrants compared to the infested or across rows, similar to observed for *S. albicosta* larvae, where about 32% of were recovered within the infested row and 75.3% within a radius of 1.7 m of the infested plant (Pannuti et al. 2016). Mortality in the early larval stages is commonly

high in Lepidoptera (Zalucki et al. 2002). The authors reported that almost 50.00% mortality occurs by first instar parasitism. Fanela et al. (2020) observed a higher mean number of recovered larvae at each distance interval for *S. eridania*. However, the study was conducted in cages, with a more controlled environmental. For instance, the crop environment will strongly influence how predation and desiccation, or the impact of rainfall, will affect survival during larval movement from plant-to-plant or to and from hiding/feeding places (Onstad et al. 2018).

Overall, our results serve as baseline for the support of *A. gemmatalis* probabilistic models and to improve their predictive ability. The present results corroborate other studies, where the findings suggest that each species must be considered independently, and one should not expect a one-size-fits-all IRM plan to be suitable for all pest species (Onstad et al. 2011). Additional *A. gemmatalis* biological and ecological studies should be conducted in association with fitness costs aiming to elucidate the relationship between larval movement and the evolution of insect resistance. In temperate areas, such as in much of the United States, selected populations of *A. gemmatalis* would not overwinter, but the selection pressure can be high in the southern United States and in tropical areas such as Brazil. As new transgenic events and/or pyramids become available worldwide, the importance of understanding *A. gemmatalis* and other target pest species' behaviors will increase.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human or animals performed by any of the authors.

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Table 1 - Estimates for the proportion of *Anticarsia gemmatalis* larvae (3rd instar/V7) in each leaf location by hour. Concord, NE, U.S, 2019 crop season

Hour	Leaf location		
	Adaxial	Abaxial	Off plant
8	0.39 ± 0.05	0.25 ± 0.06	0.35 ± 0.06
10	0.27 ± 0.04	0.36 ± 0.07	0.36 ± 0.06
12	0.08 ± 0.03	0.54 ± 0.07	0.37 ± 0.06
14	0.11 ± 0.03	0.45 ± 0.07	0.44 ± 0.06
16	0.17 ± 0.03	0.45 ± 0.07	0.38 ± 0.06
18	0.18 ± 0.04	0.30 ± 0.06	0.51 ± 0.07
20	0.32 ± 0.04	0.24 ± 0.06	0.43 ± 0.06
32	0.32 ± 0.04	0.19 ± 0.05	0.49 ± 0.07
38	0.05 ± 0.02	0.36 ± 0.07	0.59 ± 0.06
44	0.19 ± 0.04	0.20 ± 0.05	0.60 ± 0.06
<i>F</i>	7.64	6.61	3.01
<i>df</i>	9	9	9
<i>P</i>	< 0.0001	< 0.0001	0.0111

Table 2 - Estimates for the proportion of *Anticarsia gemmatalis* larvae (4th instar/R3) in each leaf location by hour. Concord, NE, U.S, 2019 crop season

Hour	Leaf location		
	Adaxial	Abaxial	Off plant
8	0.53 ± 0.07	0.28 ± 0.07	0.17 ± 0.06
10	0.10 ± 0.03	0.64 ± 0.08	0.24 ± 0.08
12	0.05 ± 0.02	0.62 ± 0.08	0.32 ± 0.09
14	0.08 ± 0.03	0.58 ± 0.08	0.33 ± 0.09
16	0.04 ± 0.02	0.56 ± 0.09	0.39 ± 0.10
18	0.08 ± 0.03	0.32 ± 0.08	0.60 ± 0.10
20	0.04 ± 0.02	0.26 ± 0.07	0.70 ± 0.09
32	0.08 ± 0.03	0.16 ± 0.05	0.76 ± 0.08
38	0.01 ± 0.01	0.18 ± 0.06	0.82 ± 0.06
44	0.11 ± 0.04	0.02 ± 0.01	0.87 ± 0.05
<i>F</i>	18.38	10.74	15.70
<i>df</i>	9	9	9
<i>P</i>	< 0.0001	< 0.0001	< 0.0001

Table 3 - Estimates for the proportion of *Anticarsia gemmatalis* larvae (5th instar/V7,R1) in each leaf location by hour. Concord, NE, U.S, 2019 crop season

Hour	Leaf location		
	Adaxial	Abaxial	Off plant
8	.	0.16 ± 0.07	0.73 ± 0.10
10	.	0.11 ± 0.05	0.88 ± 0.06
12	.	0.12 ± 0.06	0.88 ± 0.06
14	.	0.11 ± 0.05	0.89 ± 0.05
16	.	0.10 ± 0.05	0.90 ± 0.05
18	.	.	.
20	.	.	.
32	.	.	.
38	.	.	.
44	.	.	.
<i>F</i>	.	0.37	2.22
<i>df</i>	.	4	4
<i>P</i>	.	0.8288	0.1156

Table 4 - Estimates for the proportion of *Anticarsia gemmatalis* larvae (5th instar/R3,R4) in each leaf location by hour. Concord, NE, U.S, 2019 crop season

Hour	Leaf location		
	Adaxial	Abaxial	Off plant
8	0.30 ± 0.06	0.25 ± 0.06	0.44 ± 0.08
10	0.21 ± 0.05	0.24 ± 0.06	0.53 ± 0.08
12	0.16 ± 0.04	0.25 ± 0.06	0.58 ± 0.08
14	0.07 ± 0.03	0.20 ± 0.05	0.72 ± 0.07
16	0.07 ± 0.03	0.07 ± 0.03	0.86 ± 0.04
18	0.03 ± 0.02	0.03 ± 0.02	0.94 ± 0.03
20	0.02 ± 0.01	0.02 ± 0.01	0.97 ± 0.02
32	.	.	.
38	.	.	.
44	.	.	.
<i>F</i>	4.97	4.95	21.18
<i>df</i>	6	6	6
<i>P</i>	0.0057	0.0027	< 0.0001

Table 5 - Mean and maximum distance ($\pm$ SE) covered, larvae number, and frequency (%) of 24-h-old *Anticarsia gemmatalis* in different positions and orientations relative to the infested plant in non-*Bt* soybean 12 days after release. Concord, NE, U.S, 2019 crop season

Quadrants	Mean distance (cm)	Maximum distance (cm)	Larvae number	Frequency (%)
NW	90.02 $\pm$ 16.71 a (53.23 – 126.81)	124.10 $\pm$ 27.27 a (64.07 – 184.12)	6	17.14
SW	68.14 $\pm$ 14.31 a (36.58 – 99.70)	69.95 $\pm$ 23.33 a (18.51 – 121.39)	7	20.00
NE	104.72 $\pm$ 16.73 a (67.89 – 141.54)	142.52 $\pm$ 27.30 a (82.42 – 202.62)	9	25.72
SE	69.29 $\pm$ 12.67 a (41.20 – 97.38)	78.54 $\pm$ 20.65 a (32.80 – 124.29)	13	37.14
<i>F</i>	1.77	2.62		
<i>df</i>	3	3		
<i>P</i>	0.24	0.12		
Orientation				
N	77.25 $\pm$ 9.60 a (56.11 – 98.38)	102.30 $\pm$ 14.88 a (69.55 – 135.05)	24	48.98
S	66.43 $\pm$ 8.99 a (46.81 – 86.05)	78.23 $\pm$ 13.92 a (47.83 – 108.63)	25	51.02
<i>F</i>	0.76	1.56		
<i>df</i>	1	1		
<i>P</i>	0.39	0.23		
Infested Row				

N	51.54 ± 14.06 a (12.49 – 90.59)	62.25 ± 15.79 a (18.41 – 106.09)	9	64.29
S	56.16 ± 26.69 a (17.97 – 130.28)	87.50 ± 29.96 a (4.32 – 170.68)	5	35.71
<i>F</i>	0.06	1.42		
<i>df</i>	1	1		
<i>P</i>	0.82	0.3		
Across rows				
W	37.75	38.00	2	66.67
E	40.00	40.00	1	33.33
<i>F</i>	-	-	-	
<i>df</i>	-	-	-	
<i>P</i>	-	-	-	

LS-means adjusted by Tukey test, followed by the same lower case letter in each column are not significantly different ($P > 0.05$)

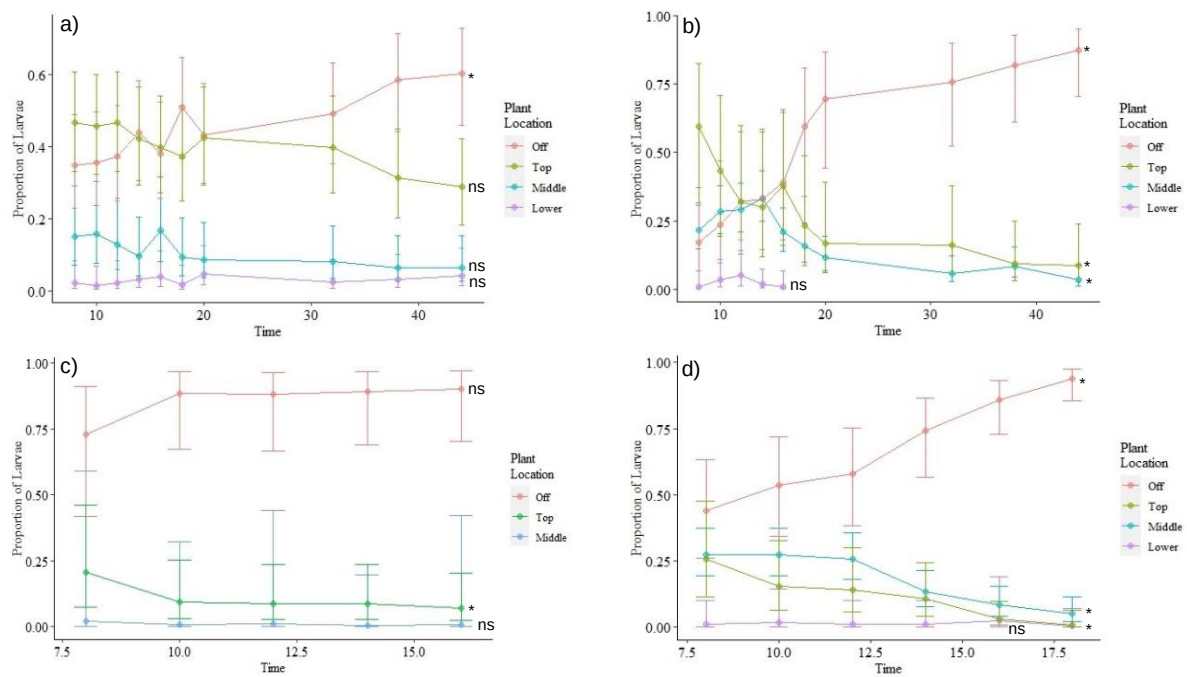


Figure 1 - Graphical distribution of estimated proportion *Anticarsia gemmatalis* larvae in different combinations involving instars (insect development) and soybean stages (vegetative and reproductive). a) 3rd instar/V7; b) 4th instar/R3; c) 5th instar/V7,R1 and d) 5th instar/R3,R4. * = significant; ns = non-significant

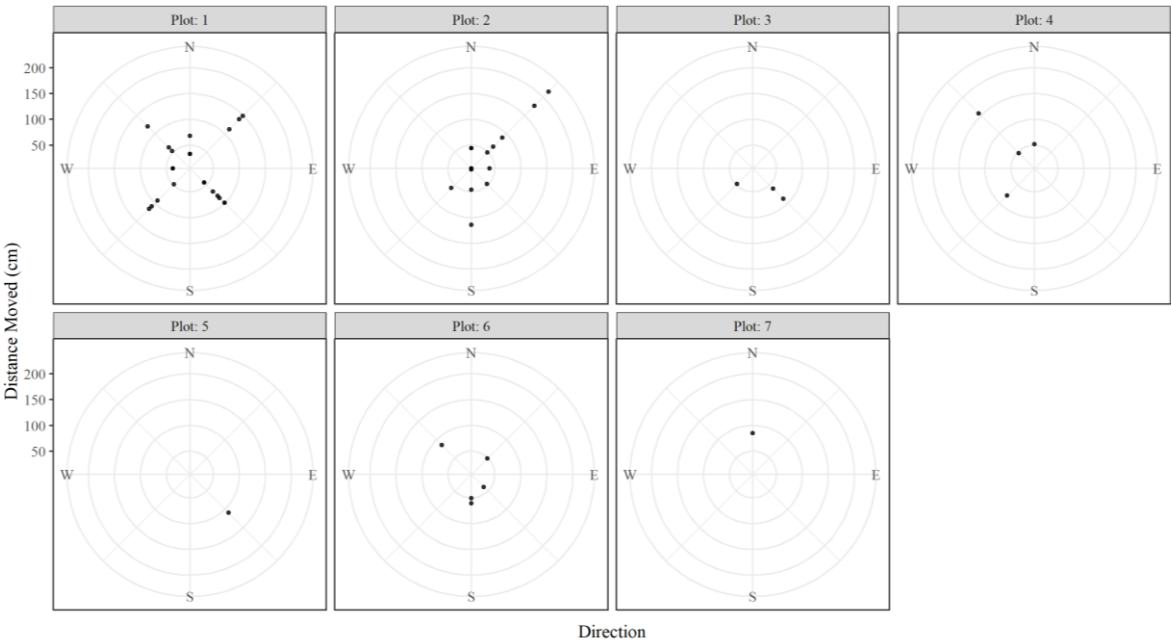


Figure 2 - Illustrative plots of distance moved by 24-h-old *Anticarsia gemmatalis* larvae relative to different positions and orientations, 12 days after release

CHAPTER 3

EFFECTS OF INTRAGUILD INTERACTIONS ON *Anticarsia gemmatalis* AND *Chrysodeixis includens* LARVAL FITNESS AND BEHAVIOR IN SOYBEAN

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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 among the major soybean 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.

3.1 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: Erebidæ), and the soybean looper, *Chrysodeixis includens* (Lepidoptera: Noctuidæ), 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 processes

which shape the patterns of these species abundance, distribution, and diversity.^{14,15} However, despite the strong evidence that herbivore competition is ubiquitous, the vast majority of investigations focus on single leaf-chewing or grain-feeding species,^{16–18} leaving the competitive interactions between leaf-eaters as a largely unexplored subject.

Several aspects should be considered when examining interaction 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 reasons.¹⁹ Among the consumptive effects, cannibalism and predation may intensify intraguild competition. Cannibalism may result in population self-regulation, while predation among individuals of competing species tends to ‘regulate’ the total biomass of individuals.^{20,21} In this sense, competitive movements are particularly 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 interactions may not have simply been due to direct effects, but also/ instead to indirect competitive effects, such as resource competition/depletion, avoidance, exclusion, and can be a response to many environmental factors. Additional factors can also increase activity and exposure to non-conspecific predation (e.g., biological control), tradeoffs of activity on non-mass/survival elements of fitness (e.g., reproduction), especially under field conditions. 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, combined 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 toxins,^{25–30} and just a few studies have been designed to understand the feeding behavior³¹ and the intraguild interactions of different lepidopteran species in corn^{32,33} 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 interaction 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.

3.2 MATERIALS AND METHODS

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 additional 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 specific colonies used in this study. To provide insects for the video tracking assay, larvae were commercially 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.

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 phenological stage V4/V5,³⁸ 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 maintained until adult emergence to verify pupal survival.

Video tracking trials

Automated video tracking software (Ethovision XT 14; Noldus Information Technology, Wageningen, The Netherlands) was used to examine potential differences in behavior between *A. gemmatilis* 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/V5,³⁸ 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 × 1.5 cm height; Fisher Scientific, Pittsburgh, PA) with or without a soybean leaf disk (2.5 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,³⁹ 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 larvae (cm) and body contact (s) was also recorded and analyzed.

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.⁴⁰ When differences occurred, Tukey's LSD's were reported at the $\alpha = 0.05$ using PROC GLIMMIX procedure in SAS 9.4.⁴¹ The same procedure was used to analyze each of the five measurements of 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 $2 \times 2 \times 3$ factorial treatment design: food – available/not available; treatment (species of larvae in which the distance moved was measured) – *A. gemmatilis*/*C. includens*; competitor – *A. gemmatilis*/*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. gemmatilis* + *A. gemmatilis*/*A. gemmatilis* + *C. includens*/*C. includens* + *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.

3.3 RESULTS

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 ($F = 0.39$; $df = 4, 26$; $P = 0.8148$). 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 ($F = 3.50$; $df = 3, 56$; $P = 0.0212$) 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.

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 $\times$ treatment and treatment $\times$ competitor at the $\alpha = 0.05$ (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 ($F = 46.77$; $df = 5, 88$; $P < 0.0001$), while for body contact, no difference occurred among the scenarios ($F = 0.40$; $df = 2,84$; $P = 0.67$) (Table 7).

3.4 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 intraspecific 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% 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, 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 larvae, 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 movement^{49,50} 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 sublethal 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 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.

CONCLUSIONS

Competition could impose a fitness cost, and the size of the competitor can impact insect development. In the conditions the study was developed, *Anticarsia gemmatalis* has a competitive advantage over *C. includens*. 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.

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CONFLICT OF INTERESTS

All authors declare no conflict of interest.

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Table 1 - Scenarios of intraguild competition involving *A. gemmatalis* and *C. includens* larvae of different sizes for fitness assay

Intraguild competition	
Treatments ^a	<i>A. gemmatalis</i> (third) versus <i>C. includens</i> (third) <i>A. gemmatalis</i> (third) versus <i>C. includens</i> (fifth) <i>A. gemmatalis</i> (fifth) versus <i>C. includens</i> (third) <i>A. gemmatalis</i> (fifth) versus <i>C. includens</i> (fifth) <i>A. gemmatalis</i> (third) versus <i>A. gemmatalis</i> (third) <i>A. gemmatalis</i> (third) versus <i>A. gemmatalis</i> (fifth) <i>A. gemmatalis</i> (fifth) versus <i>A. gemmatalis</i> (fifth) <i>C. includens</i> (third) versus <i>C. includens</i> (third) <i>C. includens</i> (third) versus <i>C. includens</i> (fifth) <i>C. includens</i> (fifth) versus <i>C. includens</i> (fifth)

^aAdapted from Dorhout and Rice and Bentivenha et al.^{29,32}

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	<i>A. gemmatalis</i> (fourth) versus <i>A. gemmatalis</i> (fourth) ^a
	<i>C. includens</i> (fourth) versus <i>C. includens</i> (fourth)
	<i>A. gemmatalis</i> (fourth) versus <i>C. includens</i> (fourth)
	<i>A. gemmatalis</i> (isolated)
	<i>C. includens</i> (isolated)
No food	<i>A. gemmatalis</i> (fourth) versus <i>A. gemmatalis</i> (fourth)
	<i>C. includens</i> (fourth) versus <i>C. includens</i> (fourth)
	<i>A. gemmatalis</i> (fourth) versus <i>C. includens</i> (fourth)
	<i>A. gemmatalis</i> (isolated)
	<i>C. includens</i> (isolated)

^a Larval development: 4-12 h after ecdysis.

Table 3 - Mean ($\pm$ SE) pupal weight (mg) of *A. gemmatalis* and *C. includens* competing in different competition scenarios under laboratory conditions

Competition scenarios		Male		Female	
Treatment	Competitor	n^a	Pupal weight (mg) ^b	n^a	Pupal weight (mg)
<i>A. gemmatalis</i> (third)	-	8	201.46 $\pm$ 4.13	7	202.64 $\pm$ 12.43 a
	<i>A. gemmatalis</i> (third)	8	178.84 $\pm$ 14.09	6	175.22 $\pm$ 15.46 ab
	<i>A. gemmatalis</i> (fifth)	1	137.50 $\pm$ 0.00	2	130.85 $\pm$ 20.65 b
	<i>C. includens</i> (third)	9	160.82 $\pm$ 11.03	4	176.73 $\pm$ 24.99 ab
	<i>C. includens</i> (fifth)	2	165.80 $\pm$ 10.00	6	145.65 $\pm$ 9.15 b
<i>P</i>			0.0744		0.0476
<i>A. gemmatalis</i> (fifth)	-	9	219.84 $\pm$ 4.92	6	224.33 $\pm$ 14.99
	<i>A. gemmatalis</i> (third)	9	203.93 $\pm$ 9.33	6	208.97 $\pm$ 13.41
	<i>A. gemmatalis</i> (fifth)	15	198.60 $\pm$ 6.27	9	201.24 $\pm$ 13.15
	<i>C. includens</i> (third)	10	203.51 $\pm$ 5.02	5	212.80 $\pm$ 13.19
	<i>C. includens</i> (fifth)	9	201.01 $\pm$ 10.20	5	214.78 $\pm$ 17.25
<i>P</i>			0.3041		0.8148
<i>C. includens</i> (third)	-	7	202.51 $\pm$ 11.46	8	193.38 $\pm$ 10.61
	<i>C. includens</i> (third)	11	174.84 $\pm$ 9.63	10	170.35 $\pm$ 14.23
	<i>C. includens</i> (fifth)	4	162.68 $\pm$ 20.84	5	162.90 $\pm$ 18.30
	<i>A. gemmatalis</i> (third)	1	159.80 $\pm$ 0.00	1	152.00 $\pm$ 0.00
	<i>A. gemmatalis</i> (fifth)	2	138.90 $\pm$ 28.40	3	131.87 $\pm$ 6.55

<i>P</i>			0.1476		0.2039
<i>C. includens</i> (fifth)	-	8	205.13 ± 9.41	7	211.33 ± 13.46
	<i>C. includens</i> (third)	5	199.76 ± 13.90	8	200.08 ± 10.61
	<i>C. includens</i> (fifth)	12	192.84 ± 12.39	13	193.96 ± 8.82
	<i>A. gemmatalis</i> (third)	4	194.25 ± 12.74	8	196.34 ± 7.92
	<i>A. gemmatalis</i> (fifth)	4	199.60 ± 6.32	6	201.18 ± 9.47
<i>P</i>			0.9472		0.7905

^a *n*, number of insects evaluated.

^b Means followed by the same letter per column do not differ by Tukey's LSD test ($P > 0.05$).

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

Competition scenarios		<i>n</i> ^a	Larval survival (%) ^b	Cannibalism/ predation (%)	Pupal survival (%)
Treatment	Competitor				
<i>A. gemmatalis</i> (third)	-	15	100.00 $\pm$ 0.00 a	-	100.00 $\pm$ 0.00 a
	<i>A. gemmatalis</i> (third)	14	46.67 $\pm$ 10.31 c	10.00 $\pm$ 5.35 b	80.00 $\pm$ 11.06 ab
	<i>A. gemmatalis</i> (fifth)	3	20.00 $\pm$ 10.69 c	40.00 $\pm$ 13.09 a	0.00 $\pm$ 0.00 c
	<i>C. includens</i> (third)	13	86.67 $\pm$ 9.09 ab	0.00 $\pm$ 0.00 b	100.00 $\pm$ 0.00 a
	<i>C. includens</i> (fifth)	8	53.33 $\pm$ 13.33 bc	0.00 $\pm$ 0.00 b	75.00 $\pm$ 16.37 b
<i>P</i>			<0.0001	0.0004	<0.0001
<i>A. gemmatalis</i> (fifth)	-	15	100.00 $\pm$ 0.00 a	-	100.00 $\pm$ 0.00
	<i>A. gemmatalis</i> (third)	15	100.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00	93.33 $\pm$ 6.67
	<i>A. gemmatalis</i> (fifth)	24	80.00 $\pm$ 8.16 b	6.67 $\pm$ 4.54	96.43 $\pm$ 3.57
	<i>C. includens</i> (third)	15	100.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00	100.00 $\pm$ 0.00
	<i>C. includens</i> (fifth)	14	93.33 $\pm$ 6.67 ab	0.00 $\pm$ 0.00	92.86 $\pm$ 7.14
<i>P</i>			0.0134	0.1037	0.6846
<i>C. includens</i> (third)	-	15	100.00 $\pm$ 0.00 a	-	100.00 $\pm$ 0.00 a
	<i>C. includens</i> (third)	21	70.00 $\pm$ 9.51 ab	0.00 $\pm$ 0.00 b	88.46 $\pm$ 6.08 a
	<i>C. includens</i> (fifth)	9	60.00 $\pm$ 13.09 b	6.67 $\pm$ 6.67 b	77.78 $\pm$ 14.70 a
	<i>A. gemmatalis</i> (third)	2	13.33 $\pm$ 9.09 c	26.67 $\pm$ 11.82 ab	100.00 $\pm$ 0.00 a
	<i>A. gemmatalis</i> (fifth)	5	33.33 $\pm$ 12.60 bc	46.67 $\pm$ 13.33 a	40.00 $\pm$ 24.49 b

<i>P</i>			<0.0001	0.0042	0.0059
<i>C. includens</i> (fifth)	-	15	100.00 ± 0.00	-	100.00 ± 0.00 a
	<i>C. includens</i> (third)	13	86.67 ± 9.09	0.00 ± 0.00 b	100.00 ± 0.00 a
	<i>C. includens</i> (fifth)	25	83.33 ± 7.97	0.00 ± 0.00 b	100.00 ± 0.00 a
	<i>A. gemmatalis</i> (third)	12	80.00 ± 10.69	0.00 ± 0.00 b	83.33 ± 11.24 ab
	<i>A. gemmatalis</i> (fifth)	10	66.67 ± 12.60	20.00 ± 10.69 a	70.00 ± 15.28 b
<i>P</i>			0.1545	0.0212	0.0166

^a*n*, number of insects evaluated.

^b 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)	-	-	1.36 (1, 44)	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 $\alpha = 0.05$ level are indicated by a bold text.

Table 6 - Mean ($\pm$ 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	Food availability				
		Distance moved (cm) ^a	Distance between larvae (cm)	Body contact (s)	Time in food (s)	Frequency in food (n)
<i>A. gemmatalis</i>	<i>A. gemmatalis</i>	256.33 $\pm$ 19.88 a	4.34 $\pm$ 0.19 a	15.84 $\pm$ 6.70 a	185.94 $\pm$ 42.12	5.30 $\pm$ 1.28 ab
	<i>C. includens</i>	245.57 $\pm$ 24.50 a	3.86 $\pm$ 0.19 ab	14.99 $\pm$ 6.34 a	144.60 $\pm$ 46.33	8.79 $\pm$ 2.73 a
	Isolated	198.78 $\pm$ 24.50 a	-	-	329.72 $\pm$ 105.63	2.27 $\pm$ 0.79 abc
<i>C. includens</i>	<i>A. gemmatalis</i>	77.75 $\pm$ 24.50 b	3.86 $\pm$ 0.19 ab	14.99 $\pm$ 6.34 a	208.01 $\pm$ 66.64	1.99 $\pm$ 0.70 bc
	<i>C. includens</i>	80.72 $\pm$ 19.88 b	3.66 $\pm$ 0.19 b	2.02 $\pm$ 0.85 b	268.97 $\pm$ 60.93	1.85 $\pm$ 0.49 bc
	Isolated	96.32 $\pm$ 24.50 b	-	-	365.49 $\pm$ 117.09	0.96 $\pm$ 0.38 c
<i>P</i>		<0.0001	0.0475	0.0009	0.25	0.0001

^a Means followed by the same letter per column do not differ by Tukey's LSD test ($P > 0.05$).

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

No Food				
Treatment	Competitor	Distance moved (cm) ^a	Distance between larvae (cm)	Body contact (s)
<i>A. gemmatalis</i>	<i>A. gemmatalis</i>	379.29 $\pm$ 19.88 a	5.01 $\pm$ 0.19 a	35.18 $\pm$ 14.89
	<i>C. includens</i>	381.37 $\pm$ 24.50 ab	4.29 $\pm$ 0.19 b	24.05 $\pm$ 10.18
	Isolated	286.05 $\pm$ 24.50 b	-	-
<i>C. includens</i>	<i>A. gemmatalis</i>	104.33 $\pm$ 24.50 c	4.29 $\pm$ 0.19 b	24.05 $\pm$ 10.18
	<i>C. includens</i>	89.06 $\pm$ 19.88 c	3.66 $\pm$ 0.19 b	21.00 $\pm$ 8.89
	Isolated	99.56 $\pm$ 24.50 c	-	-
<i>P</i>		<0.0001	<0.0001	0.6719

^a Means followed by the same letter per column do not differ by Tukey's LSD test ($P > 0.05$).

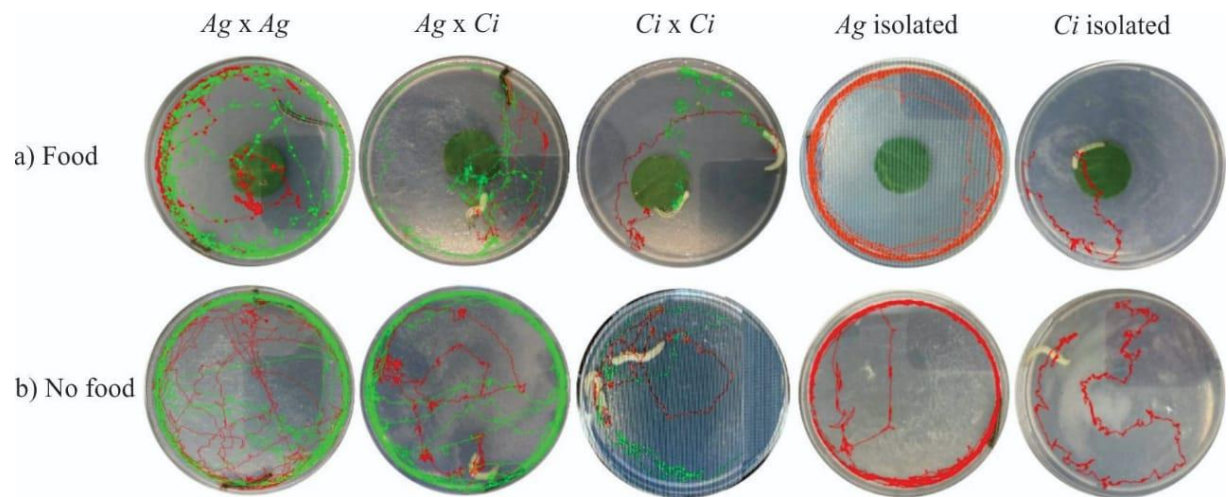


Figure 1 - Representative movement of *A. gemmatalis* (*Ag*) and *C. includens* (*Ci*) larvae recorded over 20 min in different interaction scenarios with food and no food in glass petri dish arenas. For *Ag x Ag* and *Ci x Ci* scenarios, one larva track is red and the other green. For *Ag x Ci* scenarios, *Ag* is green, and *Ci* is red. For single larva scenarios, tracks are red.

FINAL CONSIDERATIONS

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. The management of both lepidopteran species can be achieved using different control strategies such as chemical control and, in Brazil, using of *Bt* transgenic soybean. Alternative strategies, such as host plant resistance, developed in classic breeding programs, is considered an efficient and less aggressive method, especially in association with other strategies as part of an integrated pest management (IPM) approach. Also, is known that the use of *Bt* crops has been modifying Lepidoptera entomofauna in soybean. Thus, knowledge regarding behavioral aspects of pests, their movement patterns and interaction are necessary, since they share the same feeding guild in soybean.

The data obtained to *A. gemmatalis* in the chapter 1 showed that antixenosis was found in genotypes 'TMG 133' RR, 'TMG 1179' RR, 'IAC 19', 'IAC 17', 'IAC 100', D75-10169, IAC 78-2318, and IAC 74-2832, as evidenced by lower rates of attractiveness, foliar intake (larvae), and oviposition (adults). By influencing behavior and impacting larval viability, genotypes IAC 74-2832 and PI 274454 were shown to express antixenosis and/or antibiosis. Genotype 'TMG 7062' IPRO was found to provide antibiosis resistance by impacting larval development and larval viability. Genotypes 'IAC 19', 'IAC 24', 'IAC 17', and 'IAC 100' were shown to express antibiosis resistance by compromising several biological parameters, mainly larval development and viability, and reducing pupal weight. Because of low foliar intake by larvae on 'IAC 24', antixenosis was also characterized in the genotype.

The on-plant and plant-to-plant movements of *A. gemmatalis* on soybean were characterized for the first time. We found that *A. gemmatalis* has a highly active behavior throughout the day, changing the position where they were found on the plant and leaf. Over the period of evaluation, larvae gradually left the plant, particularly larger larvae. This is a relevant information which will help to optimize insecticide spraying

and natural enemy deployment. Moreover, larvae have the ability to move relatively long distances from the infested plant, influencing the population dynamics in the field.

An overall analysis of the results obtained through the fitness and video tracking indicated that if these two species, *A. gemmatalis* and *C. includens* 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.

Our findings provide significant information regarding lepidopteran development and behavior, contributing to the integrated pest management and resistance management strategies of these species.

CONCLUSIONS

Chapter 1

- Regarding the resistance of soybean genotypes to *A. gemmatalis*, the genotypes 'TMG 133' RR, 'TMG 1179' RR, 'IAC 19', 'IAC 17', 'IAC 100', D75-10169, IAC 78-2318, and IAC 74-2832 expressed antixenosis;
- IAC 74-2832 and PI 274454 were shown to express antixenosis and/or antibiosis;
- 'TMG 7062' IPRO, 'IAC 19', 'IAC 24', 'IAC 17', and 'IAC 100 were found to provide antibiosis resistance.

Chapter 2

- Field movement showed that *A. gemmatalis* larvae are consistent in their movement during the day, regardless of plant section or leaf position;
- Over the time period of assessment, larvae gradually moved down and left the plant;
- *A. gemmatalis* has a nondirectional movement in soybean field.

Chapter 3

- Intraguild competition could impose a fitness cost, and the size of the competitor can impact insect development;
- *Anticarsia gemmatalis* has a competitive advantage over *C. includens*.

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