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UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Botucatu

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

Botucatu

2021

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

Dissertation presented to São Paulo State University (UNESP), School of Agriculture, Botucatu, to obtain the degree of Doctor in Agronomy: Crop Protection

Advisor: Prof. Dr. Edson Luiz Lopes Baldin
Coadvisor: Prof. Dr. Thomas Elliott Hunt

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I dedicate

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RESUMO

A produtividade da soja é frequentemente reduzida devido a injurias causadas por inúmeras pragas. *Anticarsia gemmatalis* Hübner (Lepidoptera: Erebidae) é uma dessas pragas e é considerada um dos mais importantes insetos desfolhadores da cultura. Além disso, *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae) 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 expresssão 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 expresssão de antixenose e/ou antibiose. 'TMG 7062' IPRO afetou negativamente o desenvolvimento e a viabilidade larval, indicando uma possível expresssão 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: Erebidae) is one such pest and is considered one of the most important defoliating insects of the crop. In addition, *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae) has recently become a serious phytosanitary problem for the crop, with several outbreaks occurring in isolation or associated with *A. gemmatalis*. Besides the ocurrence 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 unsucessfull 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 thoughout 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 contibute 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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SUMMARY

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 esperienced 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 gemmatalis* Hübner (Lepidoptera: Erebidae) is among the major species, requiring periodic control measures in order to avoid significant yield losses (MOSCARDI et al., 2012).

Anticarsia gemmatalis 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. gemmatalis* 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 dammage caused by *A. gemmatalis* and *C. includens* is similar, as they have the same alimentar behavior. *Anticarsia gemmatalis* 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 controled 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 apropriate 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 aproach 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 carachteristics of species behavior, avoidance, exclusion, and can be a response to many indetectable environmental factors (FOX, 1975; POLIS, 1981).

Although there are studies that describes *A. gemmatalis* 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. gemmatalis* 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. gemmatalis*; 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.

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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