










Comparative susceptibility of *Anticarsia gemmatalis* Hübner (Lepidoptera: *Erebidae*) and *Chrysodeixis includens* (Walker) (Lepidoptera: *Noctuidae*) to insecticides

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ABSTRACT: *Chrysodeixis includens* (Walker) and *Anticarsia gemmatalis* Hübner are important soybean defoliators. The chemical control of *A. gemmatalis* has been easier and more effective than that of *C. includens*. It is hypothesized that *C. includens* is natural tolerant to insecticides. This study quantified and compare the susceptibility of *C. includens* and *A. gemmatalis* to the insecticides flubendiamide, methomyl, and spinetoram. A susceptible population of each species, maintained under laboratory conditions without insecticides selection pressure for more than 17 generations, was used. Ingestion bioassays using five to eight concentrations of each insecticide applied on the artificial diet surface were used to estimate the LC_{50} and LC_{99} (LC = Lethal Concentration). The tolerance ratio (TR) was calculated by dividing the LC_{50} or LC_{99} of the most tolerant species by the respective value of the most susceptible species. *Chrysodeixis includens* was more tolerant to all insecticides tested than *A. gemmatalis*, with TR_{50} values of 45.9-, 10.0- and 2.6-fold for methomyl, flubendiamide, and spinetoram. These findings indicated that the risk of evolution of resistance may be higher for *C. includens* than *A. gemmatalis* due to differential survival when exposed to the same dose of insecticide applied in soybean fields. Therefore, to improve the control of both species, integrated pest management (IPM) and insect resistance management (IRM) strategies should be considered to avoid control failures in field conditions.

Key words: *Glycine max*, chemical control, integrated pest management, insect resistance management.

Suscetibilidade comparativa de *Anticarsia gemmatalis* Hübner (Lepidoptera: *Erebidae*) e *Chrysodeixis includens* (Walker) (Lepidoptera: *Noctuidae*) a inseticidas

RESUMO: *Chrysodeixis includens* (Walker) e *Anticarsia gemmatalis* Hübner são importantes desfolhadoras da cultura da soja. O controle químico de *A. gemmatalis* tem sido mais fácil e eficaz quando comparado a *C. includens*, sendo uma hipótese para isso a maior tolerância natural de *C. includens* aos inseticidas. Nesse sentido, o objetivo deste estudo foi quantificar e comparar a suscetibilidade de *C. includens* e *A. gemmatalis* aos inseticidas flubendiamida, metomil e espinetoram. Nos bioensaios foram utilizadas uma população suscetível de referência de cada espécie, mantidas em laboratório sem pressão de seleção por inseticidas a mais de 17 gerações. O método de bioensaio foi o de ingestão com aplicação de cinco a oito concentrações de cada inseticida na superfície da dieta artificial para estimativa da CL_{50} e CL_{99} (CL = Concentrações Letais). A razão de tolerância (RT) foi calculada pela divisão da CL_{50} ou CL_{99} da espécie mais tolerante pelo respectivo valor da espécie mais suscetível. *Chrysodeixis includens* foi mais tolerante aos inseticidas testados do que *A. gemmatalis*. A tolerância diferencial pode indicar o risco de evolução da resistência, nesse caso maior para *C. includens* a metomil e flubendiamida, porque apresentaram maiores valores de RT_{50} (45,9 e 10,0 vezes respectivamente) do que para espinetoram (RT_{50} 2,6 vezes). Para evitar fracassos no controle é importante adotar as premissas do Manejo Integrado de Pragas (MIP) e do Manejo da Resistência de Insetos (MRI).

Palavras-chave: *Glycine max*, controle químico, Manejo Integrado de Pragas, Manejo da Resistência de Insetos.

INTRODUCTION

Insect pests are an important biotic factor that reduces soybean [*Glycine max* L. (Merr.)] yield (OLIVEIRA et al., 2014; SILVA et al., 2020). Velvetbean caterpillar, *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: *Erebidae*), and soybean looper, *Chrysodeixis includens* (Walker, [1858]) (Lepidoptera: *Noctuidae*), are the two main soybean

defoliator pests in Brazil (BUENO et al., 2011; WILLE et al., 2017).

Chrysodeixis includens was considered a secondary pest in soybean until the end of the 90s. It rarely caused damage, mainly due to natural control by entomopathogenic fungi (MORAES et al., 1991; SPECHT et al., 2015). However, population outbreaks of this species have become more frequent since the 2000s; this could be associated with a low

incidence of natural enemies (entomopathogenic fungi) due to the increased use of fungicides (SOSA-GÓMEZ et al., 2003; BALDIN et al., 2014) and the adaptation of *C. includens* populations accompanying the expansion of soybean cultivation in different Brazilian regions (SILVA et al., 2020). However, the difference in susceptibility to insecticides between lepidopteran species is also another factor that may have contributed to the high incidence of *C. includens* in soybean (MORAES et al., 1991). *Chrysodeixis includens* has naturally been more tolerant to insecticides than other species such as *A. gemmatalis* (ROSE et al., 1988) and *Helicoverpa armigera* (HÜBNER, 1808) (Lepidoptera: Noctuidae) (SCHNEIDER & SOSA-GÓMEZ, 2016).

When exposed to a certain insecticide, the differential survival of a given species can also increase the risk of evolution of resistance (GEORGHIU, 1983; BERNARDI et al., 2012). In Brazil, intraspecific variability in susceptibility to insecticides has been reported in *C. includens*, showing that this species can adapt to insecticides (RESTELATTO et al., 2021; STACKE et al., 2019; QUEIROZ et al., 2020). The differences in insect susceptibility to insecticides affect integrated pest management (IPM) and insect resistance management (IRM) programs. Based on this, we quantified and compared the susceptibility of *A. gemmatalis* and *C. includens* to the insecticides flubendiamide, methomyl, and spinetoram.

MATERIALS AND METHODS

Rearing of *A. gemmatalis* and *C. includens*

The susceptible reference population of *A. gemmatalis* (SUSAG) was obtained from Embrapa Soybean. This population was collected in the soybean crop in May 2011 in Sertãoópolis, PR, Brazil. The susceptible reference population of *C. includens* (SUSCI-15) was provided by the company PROMIP LTDA, being collected in the soybean crop in December 2015 in Engenheiro Coelho, SP, Brazil. Both populations were maintained under laboratory conditions in the absence of insecticides selection pressure, as briefly described in the following.

Adult insects were placed in polyvinyl chloride tubes (200 mm × 200 mm) lined with bond paper (oviposition substrate). Food was supplied using Petri dishes (50 mm) with cotton soaked with 10% honey solution. Eggs were removed every two days and transferred to plastic pots (145 mL) containing a bean-based artificial diet adapted from GREENE et al. (1976). Larvae were individualized in 50-mL plastic cups containing the artificial diet when they

reached the third instar. The rearing was maintained at room temperature at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$, relative humidity of $70\% \pm 10\%$, and 14-hour photophase (PANIZZI & PARRA, 2009).

Toxicological bioassay

Artificial diet-overlay bioassays were conducted to evaluate the effects of insecticides on *C. includens* and *A. gemmatalis*. The insecticides consisted of flubendiamide (Belt[®], 480 g a.i./L, IRAC MoA group: 28), methomyl (Lannate[®], 215 g a.i./L, IRAC MoA group: 1A), and spinetoram (Exalt[®], 120 g a.i./L, IRAC MoA group: 5). An aliquot of 1.2 mL of the artificial diet was deposited in each well of 24-well acrylic plates (Costar[®], model 3526, Cambridge, Massachusetts, USA). Then, the insecticides were diluted using distilled water to prepare from five to eight concentrations. The surfactant Triton X-100[®] (Labsynth Ltda, SP, Brazil) at 0.1% was added to each concentration. An aliquot of 30 μL of the insecticide solution was applied on the diet surface of each well. After drying (~60 minutes), a single *A. gemmatalis* or *C. includens* larva at the beginning of the third instar was placed in each well.

A total of 96 to 144 larvae were tested for each concentration (four to six replicates of 24 larvae per concentration). Plates were maintained at room temperature at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$, relative humidity of $70\% \pm 10\%$, and 14-hour photophase. Mortality was evaluated 96 hours after exposure to flubendiamide (OWEN et al., 2013) and 72 hours to methomyl and spinetoram (MASCARENHAS & BOETHREL, 2000). Larvae without movement after light touch with a brush were considered dead.

Data analysis

The concentration-mortality data were submitted to Probit analysis using the SAS University Edition software version 9.4 (SAS Institute, 2020) to estimate the lethal concentrations (LC_{50} and LC_{99}) and respective confidence intervals (95% CI). The tolerance ratio (TR_{50} or TR_{99}) was calculated by dividing the LC_{50} or LC_{99} of the most tolerant species by the respective value of the most susceptible species (ROBERTSON & PREISLER, 1992).

RESULTS AND DISCUSSION

Chrysodeixis includens was more tolerant to the insecticides flubendiamide, methomyl, and spinetoram than *A. gemmatalis* (Table 1). This difference in susceptibility between species may be associated with the receptors at the insecticide site

Table 1 - Concentration-mortality response (LC; $\mu\text{g a.i./cm}^2$) of third instar *Anticarsia gemmatalis* and *Chrysodeixis includens* larvae exposed to insecticides in diet-overlay bioassays.

Species	n ^a	Slope (\pm SE)	LC ₅₀ (95% FL) ^b	LC ₉₉ (95% FL) ^a	χ^2 ^c	df ^d	TR ₅₀ ^e
-----Flubendiamide-----							
<i>A. gemmatalis</i>	912	1.23 (\pm 0.15)	0.0055 (0.0026–0.0095)	0.42 (0.17–2.06)	10.80	5	-
<i>C. includens</i>	792	2.32 (\pm 0.20)	0.055 (0.046–0.064)	0.55 (0.41–0.83)	4.68	4	10.0
-----Methomyl-----							
<i>A. gemmatalis</i>	672	0.86 (\pm 0.06)	0.083 (0.053–0.128)	41.70 (19.71–107.91)	4.15	4	-
<i>C. includens</i>	960	2.12 (\pm 0.16)	3.83 (3.13–4.55)	48.20 (36.87–68.05)	4.44	4	45.9
-----Spinetoram-----							
<i>A. gemmatalis</i>	864	1.19 (\pm 0.11)	0.0057 (0.0041–0.0075)	0.50 (0.27–1.23)	9.78	6	-
<i>C. includens</i>	600	1.78 (\pm 0.15)	0.0147 (0.0118–0.0179)	0.30 (0.19–0.53)	1.24	3	2.6

^aNumbers of larvae tested

^bLC₅₀ is the concentration of insecticide required to kill 50% of larvae. Similarly, LC₉₉ is the concentration of insecticide required to kill 99% of larvae tested.

^cChi-square values ($P > 0.05$ in the goodness-of-fit test)

^dDegrees of freedom.

^eTolerance Ratio (TR) = LC₅₀ of most tolerant species / LC₅₀ of most susceptible.

of action among insect species (QI & CASIDA, 2013), as well as in insect enzyme inhibitors, such as mixed-function oxidase, carboxylesterase, and glutathione S-transferase, that detoxify insecticides, promoting insects survival. The importance of these enzymes also varies between species, contributing to differences in tolerance (WU et al., 2007).

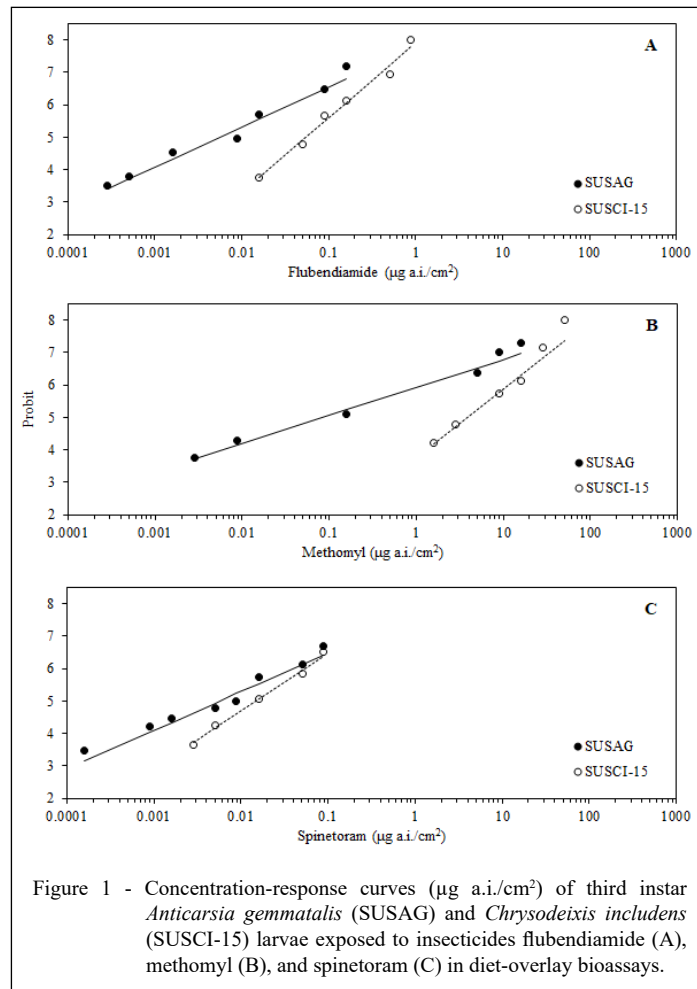
The magnitude of tolerance observed between *C. includens* and *A. gemmatalis* to the tested insecticides depended on the insecticide. The TR₅₀ was 10.0-, 45.9-, and 2.6-fold for flubendiamide, methomyl, and spinetoram, respectively (Table 1). The variation in the magnitude of tolerance of *C. includens* compared to *A. gemmatalis* has also been verified for other insecticides, such as methylparathion (341-fold) and permethrin (1.6-fold), but fenvalerate, acephate, and methomyl showed no significant differential susceptibility between the two species (ROSE et al., 1988).

The toxicity of an insecticide can be influenced by factors such as food quality, stage of insect development, and genetic variability between populations (ROSE et al., 1988; SMIRLE et al., 2013; BUENO et al., 2017; SILVA et al., 2020). Previous reports in the literature recorded that the variation in tolerance between *C. includens* and *H. armigera* populations to flubendiamide was from 11- to 2,464-fold. This variation in tolerance was related to the origin of the populations, especially for *C. includens*,

which presented a resistance ratio of up to 217-fold (SCHNEIDER & SOSA-GÓMEZ, 2016). The experiments of this study used susceptible populations maintained under laboratory conditions, with the absence of insecticides selection pressure, to avoid the influence of the presence of insecticide-resistant larvae.

The comparison between the LC₉₉ values showed no difference in tolerance between *C. includens* and *A. gemmatalis*, with TR₉₉ lower than 1.3-fold (Table 1 and Figure 1) suggesting that the difference in the tolerance depends on the insecticide concentration. Difficulties in pest control using insecticides due to differences in susceptibility usually occur in cases where this magnitude is higher than 10-fold (FFRENCH-CONSTANT & ROUSH, 1990). Thus, the decrease in insecticides residue in the field may lead to *C. includens* survival. At the same time, the mortality of *A. gemmatalis* is still observed under the same conditions. This hypothesis should be more evident for flubendiamide and methomyl because they demonstrated a higher difference of tolerance (TR₅₀ > 10.0-fold) than spinetoram (TR₅₀ = 2.6-fold) (Table 1 and Figure 1).

Based on the natural tolerance of *C. includens* compared to *A. gemmatalis* to insecticides, the importance of their correct identification in pest sampling is reinforced for the proper choice of the insecticide dose. Otherwise, this differential survival with the use of an inadequate or excessive dose of insecticides could also explain the population



outbreaks of *C. includens* observed in recent years in the soybean crop (MORAES et al., 1991). From an IRM perspective, an adequate dose of insecticide should be sufficient to cause the mortality of heterozygous resistant insects; otherwise, it may accelerate the evolution of resistance (GEORGHIU, 1983; BERNARDI et al., 2012). Thus, the differential tolerance of *C. includens* could favor the evolution of resistance of this species.

The use of genetically modified soybean by inserting the insecticidal protein Cry1Ac from *Bacillus thuringiensis* Berliner, 1915 is another important method of controlling *C. includens* and *A. gemmatilis* (BUENO et al., 2020). The natural tolerance of *C. includens* to this insecticidal protein is also identified as a risk factor for accelerating the evolution of resistance (BERNARDI et al., 2012; YANO et al., 2015).

Resistant *A. gemmatilis* populations were detected between 1987 and 1991 in the USA and Brazil for *Baculovirus anticarsia* (ABOT et al., 1996; MOTA-SANCHEZ & WISE, 2020). Moreover, resistant *C. includens* populations have been documented since the '70s in the USA for various insecticides, such as chlorinated, phosphorous, carbamates, and pyrethroids (MASCARENHAS & BOETHREL, 2000). In Brazil, this record has been recent, but populations with a resistance ratio higher than 10-fold have already been detected for flubendiamide, chlorantraniliprole, methomyl, lambda-cyhalothrin, methoxyfenozide, novaluron, teflubenzuron, and spinosad (RESTELATTO et al., 2021; STACKE et al., 2019; QUEIROZ et al., 2020). In addition, Spinetoram showed low variations in susceptibility from 2.5- to 8.6-fold (STACKE et al., 2019). This low variation in susceptibility

corroborates the hypothesis of a low risk of evolution of resistance for this active ingredient.

Thus, differential tolerance is essential to preserve the effectiveness of synthetic insecticides and *B. thuringiensis* proteins expressed in soybean for the management of *C. includens* and *A. gemmatilis*. The risk of evolution of resistance can be evaluated and changes in the frequency of resistant insects monitored (BERNARDI et al., 2012; YANO et al., 2015; STACKE et al., 2019).

Adopting IPM premises is important to delay the evolution of resistance, using adequate sampling for decision-making, rotation of insecticides with different mechanisms of action, and other control methods to reduce selection pressure. These recommendations are mainly important in the *C. includens* management in refuge areas (soybean cultivation without the expression of the insecticidal protein Cry1Ac) and other crops since this is a polyphagous species and has a high gene flow between populations and crops (SPECHT et al., 2015; BUENO et al., 2020; SILVA et al., 2020).

CONCLUSION

Chrysodeixis includens is more tolerant to the insecticides flubendiamide (10.0-fold), methomyl (45.9-fold), and spinetoram (2.6-fold) than *A. gemmatilis*.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors N.B., S.S.R., and R.E.C. contributed to the execution of experiments and writing and revising the manuscript. A.F.B. and O.B. contributed to the conception and revision of the

manuscript. M.I.C.B. contributed to the co-orientation and revision of the manuscript. C.R.F. contributed to the conception, writing, revision of the manuscript, and guidance of the master's students. All authors approved the final version of the manuscript.

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