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CHARACTERIZATION OF THE RESISTANCE OF *Chrysodeixis includens* TO DIAMIDES[1]

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ABSTRACT – In recent decades, soybean has been one of the most widely grown crops in Brazil. The soybean looper, *Chrysodeixis includens* (Walker, [1858]; Lepidoptera: Noctuidae), is one of the main defoliating pests of soybean that cause damage and affect the cost of production. The overall objective of the present study was to characterize the resistance of *C. includens* to diamide insecticides. To this end, the baseline susceptibility of *C. includens* to flubendiamide, chlorantraniliprole, and cyantraniliprole was characterized to estimate their concentrations for diagnostic monitoring of resistance in populations collected in commercial soybean crops in southern Brazil during the 2018/19 season. Under field conditions, evaluations were made of the residual activity of three diamide insecticides: chlorantraniliprole + lambda-cyhalothrin, teflubenzuron, methoxyfenozide, and bifenthrin for discrimination between flubendiamide-susceptible and resistant *C. includens*. *Chrysodeixis includens* was more tolerant to cyantraniliprole than to flubendiamide and chlorantraniliprole. The field populations had a higher survival rate than the susceptible population. The resistant population showed a resistance ratio to flubendiamide of 70.1-fold. Under field conditions, the residue of the study insecticides, except for bifenthrin, enabled discrimination between flubendiamide-susceptible and resistant *C. includens*. The data indicate the need to study the cross-resistance relationships between insecticides to improve the rotation recommendation. They also reinforce the importance of using other good management practices, e.g., the use of insecticides based on pest sampling and the choice of other control methods to prevent *C. includens* from becoming resistant to diamide insecticides.

Keywords: *Glycine max.* Plusiinae. Chemical control. Rotation of insecticides.

CARACTERIZAÇÃO DA RESISTÊNCIA DE *Chrysodeixis includens* A DIAMIDAS

RESUMO – A soja nas últimas décadas vem sendo a cultura mais cultivada no Brasil. A lagarta falsa-medideira *Chrysodeixis includens* (Walker, [1858]) (Lepidoptera: Noctuidae) é umas das principais pragas desfolhadoras da cultura da soja que vem causado danos e comprometendo o custo de produção. O objetivo geral do trabalho foi caracterizar a resistência de *C. includens* as diamidas. Para isso foi confeccionada a linha básica de suscetibilidade de *C. includens* a flubendiamida, clorantraniliprole e ciantraniliprole para estimar as concentrações diagnósticas para monitoramento da resistência em populações coletadas em lavouras comerciais de soja na região Sul do Brasil, durante a safra 2018/19. Em condições de campo foi avaliada a atividade residual das três diamidas, clorantraniliprole+lambda-cialotrina, teflubenzurom, metoxifenozida e bifentrina na discriminação entre *C. includens* suscetível e resistente a flubendiamida. *Chrysodeixis includens* apresentou maior tolerância a ciantraniliprole do que flubendiamida e clorantraniliprole. As populações de campo apresentaram maior sobrevivência do que a população suscetível de referência. A população resistente a flubendiamida apresentou razão de resistência de 70,1 vezes. Em condições de campo, o resíduo dos inseticidas avaliados, exceto bifentrina, proporcionou discriminação entre *C. includens* suscetível e resistente a flubendiamida. Os dados indicam a necessidade de estudar as relações de resistência cruzada entre inseticidas para aprimorar a recomendação de rotação. Além, da importância do uso de outras boas práticas de manejo, como uso de inseticidas com base na amostragem de pragas e a escolha de outros métodos de controle para prevenir a evolução da resistência de *C. includens* a diamidas.

Palavras-chave: *Glycine max.* Plusiinae. Controle químico. Rotação de inseticidas.

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INTRODUCTION

Soybean has been the most largely cultivated crop and the grain with the highest production in Brazil. Southern Brazil is the second main soybean-growing region, accounting for about 40% of domestic production. Since the 2019/20 harvest, Brazil has become the world's largest producer of soybean, with a production of 124,4 million tons grown in 36,8 million hectares (CATTELAN; DALL'AGNOL, 2018; CONAB, 2020). However, there are several challenges soybean growers have to cope with that contribute to reducing crop yields, such as infestation by insect pests. Economic losses caused by insect infestations may represent 5% of production reduction (OLIVEIRA et al., 2014).

Today, in soybean fields, among the defoliating insects, the soybean looper, *Chrysodeixis includens* (Walker, [1858]) (Lepidoptera: Noctuidae), has economic importance as the main soy pest (GUEDES et al., 2015; SPECHT; PAULA-MORAES; SOSA-GÓMEZ, 2015; SILVA et al., 2020). To mitigate the damages caused by insect pests, especially larvae that feed on foliage, control measures are required based on integrated pest management (IPM). In Brazil, soybean crops are an important example that the negative impacts caused by insecticides can be minimized by adopting control levels and using other control methods integrated with chemical control (BUENO et al., 2013). However, the control methods for foliage-feeding soybean looper that have been adopted by most soybean growers are the use of chemical insecticides and genetically modified soybean with the insertion of the insecticide protein Cry1Ac of *Bacillus thuringiensis* (BT soybean) (BROOKES, 2018; OLIVEIRA et al., 2014; PERINI et al., 2019).

Due to improper use of insecticides, phytosanitary problems have been recorded more frequently, mainly those related to contamination of farmers and the environment as well as the death of natural predators. Another factor of concern has been the evolution of insecticide resistance, which diminishes the efficiency of some active ingredients in the management of insect pests (SOSA-GÓMEZ; OMOTO, 2012; PERINI et al., 2019; SPARKS et al., 2020).

After 1914, when the first case of an insecticide-resistant insect was detected, 603 species of insects or mites resistant to at least one insecticide molecule have been recorded (SPARKS et al., 2020). For *C. includens*, insecticide resistance evolution has already been recorded in the United States for acephate, BHC, DDT, cypermethrin, deltamethrin, permethrin, tefluthrin, fenvalerate, methomyl, methyl parathion, and thiodicarb (LEONARD et al., 1990). In Brazil, since the 2013/14 crop season, intraspecific variability in the susceptibility to insecticides of the diamide chemical group has been documented, such as for flubendiamide and chlorantraniliprole (RESTELATTO et al., 2021; SCHNEIDER; SOSA-GÓMES, 2016; STACKE et al., 2019) as well as for insecticides of other chemical groups like benzoylureas, pyrethroids, and diacylhydrazines (QUEIROZ et al., 2020; STACKE et al., 2019).

The chemical group of diamides is considered innovative for acting on a different site of action from other chemical groups (calcium channel modulators, IRAC MoA Group 28), being selective, and having low toxicity for mammals (SPARKS et al., 2020; TEIXEIRA; ANDALORO, 2013). Currently accounting for 12% of the world insecticide market share, diamides represent the third -bestselling chemical group of insecticides (SPARKS et al., 2020). Thus, to improve the recommended management of *C. includens* resistance to diamides, this study aimed to characterize the differential tolerance to flubendiamide, chlorantraniliprole, and cyantraniliprole, establish susceptibility baselines, monitor intraspecific variability, determine *C. includens* resistance to flubendiamide, and assess the insecticides' residual activity in discriminating flubendiamide-susceptible and resistant soybean loopers.

MATERIAL AND METHODS

Origin and creation of *C. includens* populations

The susceptible reference population of *C. includens* (SUSCI-15), collected in 2015 from soybean fields in the region of Engenheiro Coelho, SP, was provided by the company Promip Manejo Integrado de Pragas Ltda. This population has been maintained in a laboratory without suffering any kind of selection pressure from insecticides. The population of *C. includens* resistant to flubendiamide (CiResFlu) was collected in Londrina, PR (23°02'45" S 51°12'58" W) (RESTELATTO et al., 2021). This population has been kept in a laboratory for successive generations, suffering selection pressure from flubendiamide application.

During the 2018/2019 growing season, soybean loopers were collected from commercial soybean fields grown in southern Brazil (Table 1). These larvae were transported in plastic pots containing an artificial diet adapted from Greene, Leppla and Dickerson (1976) to the Entomology Laboratory of the Santa Catarina State University (UDESC), College of Agronomy and Veterinary (CAV) in the city of Lages, SC.

Table 1. Origin of *Chrysodeixis includens* (Lepidoptera: Noctuidae) populations collected from commercial soybean fields grown in southern Brazil during the 2018/2019 growing season.

City (state)	Latitude (S)	Longitude (W)	Altitude (m)	Date
Guarapuava (PR)	25°14’46”	51°35’23”	785	Mar 04, 2019
Íjui (RS)	28°16’04”	53°59’39”	328	Jan 17, 2019
Lages (SC)	24°47’27”	50°30’31”	916	Dec 10, 2018
Papanduva (SC)	26°22”04”	50°07’17”	788	Mar 05, 2019
Vargeão (SC)	26°80’99”	52° 13’67”	890	Jan 16, 2019
Vacaria (RS)	28°24’13”	50°52’49”	971	Mar 12, 2019

After obtaining adult insects, 30 moths were transferred to a 200 × 200 mm PVC tube. These brood cages were covered with a yellow bond paper to serve as oviposition overlay, and the upper face was closed with *voile* fabric. Food was provided using three Petri dishes (50 mm), one containing cotton soaked in distilled water, the other containing a solution of 10% diluted honey with the addition of sorbic acid and 1% nipagin, and the third one containing honey and beer solution at the rate of 300 mL of 10% honey and 200 mL of beer.

Every two days, the oviposition overlay paper areas with eggs were cut and placed into plastic pots (500 mL) containing an artificial diet. These plastic pots were turned upside down (lid down) to prevent contact of larva feces with the artificial diet.

Insecticides

The active ingredients (a.i.) of the insecticides used were flubendiamide (480 g a.i. L^{-1}, Belt®), chlorantraniliprole (200 a.i. L^{-1}, Premio®), cyantraniliprole (100 g a.i. L^{-1}, Benevia®) in IRAC MoA Group 28, chlorantraniliprole + lambda-cyhalothrin (100 + 50 g a.i. L^{-1}, Ampligo®) in IRAC MoA Group 28 + 3A, methoxyfenozide (240 g a.i. L^{-1}, Intrepid 240 SC®) in IRAC MoA Group 18, teflubenzuron (150 g a.i. L^{-1}, Nomolt® 150) in IRAC MoA Group 15, and bifenthrin (100 g a.i. L^{-1}, Talstar 100 EC®) in IRAC MoA Group 3A.

Characterization and monitoring of *C. includens* susceptibility to diamides in the laboratory

The bioassay method used consisted of diet ingestion with the application of insecticide to the diet overlay (MASCARENHAS; BOETHEL, 2000; RESTELATTO et al., 2021; STACKE et al., 2019). We used multiple-well culture plates (Costar®, model 3526, Cambridge, Massachusetts, US), and each well was filled with 1.5 mL of artificial diet and then sprayed with 30 µL of different insecticide concentrations. Insecticide dilutions at different concentrations were prepared with distilled water and the addition of 0.1% of Triton X-100® surfactant (Labsynth Products for Laboratory). The control treatment consisted of distilled water added with surfactant. When the insecticide was dry on the diet overlay, one early third instar larva was transferred to each well.

The plates were kept in a climate-controlled room at 25 ± 2 °C, relative humidity of 60 ± 10% and 14 h of photoperiod. The death rate was assessed after 96 hours by touching the dorsal side of the insects with a tweezer, which were considered dead when they did not show vigorous, coordinated movements (MASCARENHAS; BOETHEL, 2000; OWEN et al., 2013; RESTELATTO et al., 2021).

To determine the baseline susceptibility of the SUSCI-15 population, six to seven concentrations of each insecticide that provided a mortality rate between 5% and 99% were tested. To monitor the *C. includens* susceptibility to insecticides, we used six soybean loopers collected in the 2018/19 crop season and kept in the laboratory until F3-F4 generation. The diagnostic concentration used was determined based on lethal concentration capable of killing 99% (CL_{99}) of the SUSCI-15 population for each insecticide tested.

Procedure for selection of the flubendiamide-resistant *C. includens* population

For this stage, a population of soybean loopers was collected from a soybean crop cultivated in Londrina, PR in 2017/18, which had 75.2% of insects alive for a diagnostic concentration of 0.5053 µg flubendiamide cm^{-2} (RESTELATTO et al., 2021). Based on this result, soybean loopers with vigorous movements were selected, giving origin to a new population.

Subsequently, the descendants of this new population underwent toxicological bioassays for eight generations, using an increasing concentration that ranged from 0.5053 to 1.579 µg flubendiamide cm^{-2}. At each toxicological bioassay, the larvae that survived were selected to constitute a new population. Therefore, after eight generations of soybean loopers under selection pressure, the selected population (CiResFlu) was tested for resistance characterization based on the use of six flubendiamide dosages that caused mortality rates between 5% and 99%.

Flubendiamide residual activity under field conditions

The experiment was installed in an aluminum Cambisol soil area located in the experimental farm of the UDESC, in Lages, SC (27°44’54”S, 50° 05’08”W, and 884 m altitude). The local climate is classified as Cfb according to Köppen Climate Classification, consisting of a temperate climate with mild summer and rainfall evenly distributed over the months of the year, with no dry season. In the experimental area, a pluviometer was installed. Planting was carried out on Dec. 22, 2019, with soybean cultivar BMX Alvo RR with 50-cm spacing between rows and a sowing density of 18 seeds per meter.

Seed treatment consisted of pyraclostrobin, 25 g a.i. L^{-1} + thiophanate methyl, 225 g a.i. L^{-1} + fipronil, 250 g a.i. L^{-1} (Standak® Top) in the proportion of 200 mL of the commercial product for 100 kg^{-1} of seeds. Basal fertilization at planting consisted of 400 kg ha^{-1} (02-30-15 fertilizer), and top -dressing fertilization with 150 kg ha^{-1} of potassium chloride and 80 kg ha^{-1} of urea broadcast 38 days after emergence of the seedlings.

The experiment consisted of eight blocks with six rows of five meters in length, separated by four border lines between each treatment. The plants were marked with string placed at the third upper portion so that the leaves were removed always at the same plant segment. Spraying was carried out on Feb. 4, 2020, when the plants were at the V4 phenological stage, using a CO_2 pressurized sprayer with four fan-type nozzles at 50 cm, set to achieve 2.5 to 3 bar of pressure to obtain a spray volume of 100 L ha^{-1}. During application, air relative humidity was 67% and the temperature was 26 °C.

The insecticides applied were flubendiamide (70 mL commercial product per 100 L^{-1} water Belt®), chlorantraniliprole (33.3 mL commercial product per 100 L^{-1} water, Premio®), cyantraniliprole (125 mL commercial product per 100 L^{-1} water, Benevia®), chlorantraniliprole + lambda-cyhalothrin (50 mL commercial product per 100 L^{-1} water, Ampligo®), methoxyfenozide (150 mL commercial product per 100 L^{-1} water, Intrepid 240 SC®), teflubenzuron (100 mL commercial product per 100 L^{-1} water, Nomolt® 150) and bifenthrin (50 mL commercial product per 100 L^{-1} water, Talstar 100 EC®). To prepare the insecticide solution, 0.1% of the surfactant Assist® was added. After each insecticide application, two liters of water were sprayed to clean the sprayer boom and nozzles.

Collection of plant trefoils were carried out at 1; 3; 6; 10; and 17 days after application, and one trefoil sample was placed into a plastic pot with a lid (150 mL) and coated with Germitest paper slightly moistened with distilled water. Then, five early third instar larvae were transferred to each plastic pot, constituting one replicate. Mortality was assessed four days after transference of the larvae, using tweezers to touch the back of the insects, and those that did not exhibit vigorous movements were considered dead.

Statistical analysis

The experiments were carried out in a completely randomized design. To characterize the susceptibility baselines and resistance, the experiments were replicated four to five times with 24 soybean loopers per replicate. Susceptibility was monitored in 13 replications for each population with 24 or 48 soybean loopers per replicate. For the residual activity experiment under field conditions, each treatment consisted of ten replications with five soybean loopers per replicate.

To determine the insecticide relative toxicity for *C. includens*, the mortality data were subjected to Probit analysis to estimate the 50 and 99% lethality concentrations (LC_{50} and LC_{99}) and 95% confidence intervals (95% CI) using the SAS University Edition software program, version 9.4 (SAS INSTITUTE, 2020). To estimate the tolerance ratio (TR_{50} or TR_{99}) of *C. includens* to diamides, it was calculated by dividing the LC_{50} or LC_{99} of the SUSCI-15 population of the insecticide with the highest toxicity by the one with the lowest toxicity. To calculate the resistance ratio (RR_{50} or RR_{99}), the LC_{50} or LC_{99} of the CiResFlu population was divided by the SUSCI-15 population (ROBERTSON; PREISLER, 1992).

For the susceptibility monitoring experiment and on-field residual activity, the mortality data were subjected to analysis of variance by generalized linear mixed models. Homogeneity of variance and residuals normality was assessed by diagnostic plots (q-q plot, histogram, and residuals versus predicted values). When necessary, data were transformed using the Box-Cox method. For monitoring, the means were compared by the Tukey test ($p < 0.05$). For the insecticide residual activity experiment, data were subjected to polynomial regression analysis by adjusting the polynomial degree according to the corrected Akaike information criterion. Comparison between the populations’ intercepts and slopes was also carried out through analysis of covariance, using the SAS software University Edition, version 9.4 (SAS INSTITUTE, 2020).

RESULTS AND DISCUSSION

The diamide (IRAC MoA Group 28) toxicity for *C. includens* was associated with the active ingredient. Between the active ingredients flubendiamide and chlorantraniliprole, the confidence intervals (95% CI) of the LC_{50} values overlapped, which indicates that this population had similar susceptibility to both active ingredients. For cyantraniliprole to exhibit the same toxicity compared to other diamides, the LC_{50} needed to be about 7 and 9 times higher than the LC_{50} values of flubendiamide and chlorantraniliprole, respectively (Table 2). This difference in the tolerance of *C. includens* for the diamides was more evident when comparing the LC_{99} values. The greatest magnitude of tolerance was found between cyantraniliprole and chlorantraniliprole (TR_{99} = 37.5-fold), followed by cyantraniliprole and flubendiamide (TR_{99} = 13.9-fold) and flubendiamide and chlorantraniliprole (TR_{99} = 2.7-fold) (Table 2).

Table 2. Characterization of the susceptibility (μg cm^{-2} active ingredient) of the susceptible reference population of *Chrysodeixis includens* (Lepidoptera: Noctuidae) to diamides (IRAC MoA Group 28) in a bioassay of diet ingestion with the application of insecticide to the diet overlay.

Active ingredient	n	Slope (± SE)[a]	LC_{50} (95% CI)[b]	LC_{99} (95% CI)[b]	χ^2	df[c]	P[d]
Flubendiamide	768	1.84 (± 0.12)	0.038 (0.032–0.044)	0.70 (0.49–1.09)	8.09	5	0.1513
Chlorantraniliprole	720	2.52 (± 0.18)	0.031 (0.027–0.035)	0.26 (0.20–0.37)	2.79	5	0.7330
Cyantraniliprole	672	1.50 (± 0.16)	0.274 (0.199-0.350)	9.75 (5.82–21.00)	7.69	4	0.1037

[a]angular coefficient.
[b]LC_{50} represents the necessary insecticide concentration to kill 50% of the insects tested; LC_{99} is the insecticide concentration that is necessary to kill 99% of the insects tested.
[c]degrees of freedom.
[d]P > 0.05.

North-American populations of *C. includens* also exhibited similar susceptibility to flubendiamide and chlorantraniliprole (OWEN et al., 2013). In Brazil, other studies indicated that *C. includens* populations were less tolerant to chlorantraniliprole than to flubendiamide, but the magnitude of tolerance was dependent on the population, with a 2 to 173-fold variation (SCHNEIDER; SOSA-GÓMES, 2016) and a 3 to 24-fold variation, respectively (STACKE et al., 2019). However, in Brazilian populations, this intraspecific variation in susceptibility had already been associated with the evolution of resistance.

Based on the baseline curves, diagnostic concentrations of 0.5053 μg cm^{-2} of flubendiamide, 0.1579 μg $cm^{-2}$of chlorantraniliprole, and 5.053 μg cm^{-2} of cyantraniliprole were defined to carry out the susceptibility monitoring. Based on these diagnostic concentrations, there was a susceptibility difference for the *C. includens* populations to flubendiamide (F = 256.71; df = 6; 83; p < 0.0001), chlorantraniliprole (F = 35.09; df = 6; 84; p < 0.0001), and cyantraniliprole (F = 132.36; df = 6; 83; p < 0.0001) (Table 3).

Table 3. Monitoring susceptibility of *Chrysodeixis includens* (Lepidoptera: Noctuidae) populations to diamides (IRAC MoA Group 28) in a bioassay of diet ingestion with the application of insecticide to the diet overlay.

Population	Survival (%)		
	Flubendiamide (0.5050 μg cm^{-2})	Chlorantraniliprole (0.1579 μg cm^{-2})	Cyantraniliprole (5.053 μg cm^{-2})
SUSCI-15	0.3 ± 0.36[1] a[2]	0.0 ± 0.00 a	1.0 ± 0.57 a
Ijuí (RS)	9.3 ± 1.62 a	2.2 ± 0.86 ab	36.2 ± 2.17 d
Guarapuava (PR)	11.5 ± 5.17 a	3.8 ± 1.13 bc	5.4 ± 1.817 ab
Lages (SC)	54.8 ± 3.22 b	8.3 ± 1.42 c	31.1 ± 3.07 c
Vargeão (SC)	62.8 ± 2.34 c	2.9 ± 0.83 b	11.2 ± 2.76 b
Papanduva (SC)	71.5 ± 1.85 d	4.8 ± 1.40 bc	53.8 ± 5.44 d
Vacaria (RS)	86.7 ± 1.69 e	46.8 ± 2.58 d	58.0 ± 2.43 d

[1]Mean ± mean standard deviation.
[2]Means followed by the same letter in column do not differ statistically from each other by the Tukey test (p < 0.05).

For flubendiamide, four populations exhibited low susceptibility when compared to the SUSCI-15 population, with a variation in survival rate between 54.8 to 86.7% (Table 3). For chlorantraniliprole, only the Ijuí population exhibited a survival rate similar to SUSCI-15, with no difference to other populations (Guarapuava, Papanduva, and Vargeão). However, the magnitude of survival variation (2.9 to 8.3%) was lower when compared to flubendiamide, except for the population collected in Vacaria, where there were 46.8% of larvae alive. For cyantraniliprole, only the Guarapuava population did not differ from SUSCI-15. The survival variation for this insecticide can be considered moderate when compared to other diamides, with survival rates of 11.2 to 58%.

From the monitoring results, some inferences can be made. Firstly, there were populations collected from soybean commercial crops, which are under exposure to insecticide sprayings and have susceptibility to diamides similar to a susceptible reference population. In this case, failures in insect control with diamides would not be expected to result from resistant insects. Secondly, other populations are less susceptible to diamides, also indicating a similar susceptibility reduction for the three diamides. In soybean crops with possibly resistant soybean loopers, there may be reduced control effectiveness. However, it is important to mention that failures in the control of insects with insecticides also depend on the population density and the insect growth stage (FFRENCH-CONSTANT; ROUSH, 1990; PERINI et al., 2019).

Recently, a low genetic structure among Brazilian populations of *C. includens* (PALMA et al., 2015; SILVA et al., 2020) has been found. However, it can be seen that there is phenotypic variation in susceptibility to diamides (Table 3). In the 2013/14 and 2014/15 soybean seasons, the resistance ratio was up to 217.5 and 213.2-fold higher for flubendiamide and chlorantraniliprole, respectively, in populations collected in the midwestern region of Brazil (SCHNEIDER; SOSA-GÓMES, 2016). Other authors observed that after 2016/17, susceptibility has varied by 8.5 to 15.4-fold (STACKE et al., 2019) and by 6.2 to 24.2-fold in populations collected in southern Brazil (RESTELATTO et al., 2021).

Such variations in susceptibility may be indicative of the resistance evolution process because these molecules have been used in Brazil since 2009 (RESTELATTO et al., 2021). Cyantraniliprole has been registered more recently in the country (since 2015). Therefore, the previous uses of other diamides and the tolerance that *C. includens* has shown to this active ingredient may explain the results found in the monitoring treatment.

Based on the characterization of the concentration-response curve of a population that exhibited low susceptibility to flubendiamide, the flubendiamide-resistant population (CiResFlu) was selected with LC_{50} and LC_{99} values estimated at 2.65 µg cm^{-2} flubendiamide (2.35–2.95 95% CI) and 13.45 µg cm^{-2} flubendiamide (10.57–18.87 95% CI), respectively (χ^2 = 6.01; g.l. = 4; p = 0.1984). Compared to the susceptible reference population, there were non-overlapping confidence intervals, indicating a difference in susceptibility, with a 70.1 and 19.3-fold resistance ratio based on the LC_{50} and LC_{99}, respectively. The angular coefficient of the C.ResFlu population was 3.30 (± 0.30), indicating more homogeneity than the SUSCI-15 population [1.84 (± 0.30)] (Figure 1).

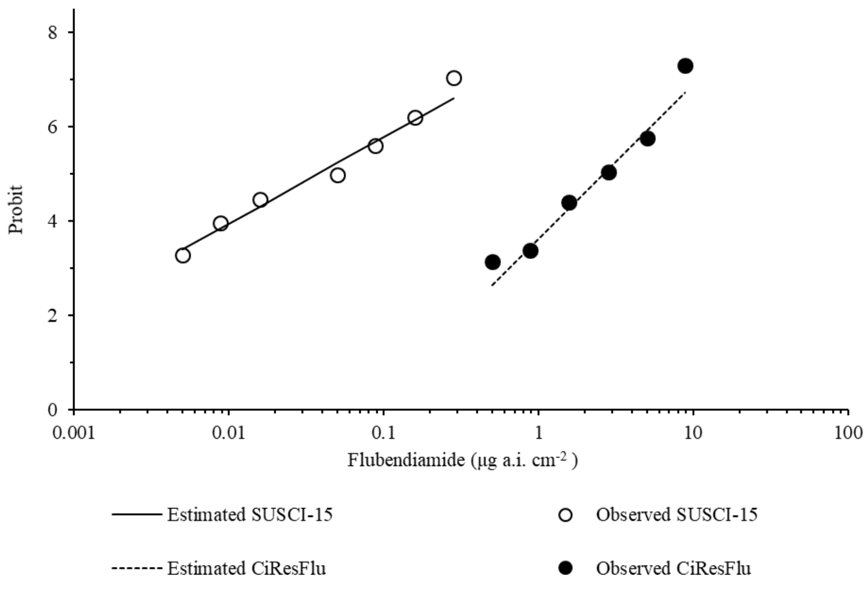

Figure 1. Concentration-response curves of the susceptible reference population (SUSCI-15) and flubendiamide-resistant population (CiResFlu) of *Chrysodeixis includens* (Lepidoptera: Noctuidae) to flubendiamide (IRAC MoA Group 28) in a bioassay of diet ingestion with application of insecticide to the diet overlay.

The residual activity curves for the insecticides assessed in the discrimination between flubendiamide-susceptible and resistant soybean loopers indicated a significant difference for the effect of time, i.e., days after application (F = 115,541.90; df = 4; 630; $p < 0.0001$), population (F = 32,185.09; df = 1; 630; $p < 0.0001$), active ingredient (F = 40,950.20; df = 6; 630; $p < 0.0001$) and in triple interaction (F = 462.83; df = 24; 630; $p < 0,0001$). Between seven and ten days after application of the insecticide, occurred precipitation of 1.7 mm in the experimental area. Arrué et al. (2014) observed that 20 mm of artificial rain 240 minutes after application of chlorantraniliprole did not affect the efficacy of control of velvetbean caterpillar *Anticarsia gemmatalis* Hübner 1818 (Lepidoptera: Erebidae) in soybean plants. Thus, the rainfall observed in the experimental area probably had little influence on the results.

The CiResFlu soybean looper population exhibited greater tolerance to flubendiamide compared to SUSCI-15 (Figure 2A). When analyzing the displacement of the residual activity curve, it can be seen that one day after application there was 100% mortality rate for the larvae of both populations. However, the flubendiamide residue after the third day of application provided a differential tolerance, with mean mortality rates of 88 ± 3.27% and 50 ± 3.33% of the soybean loopers of the SUSCI-15 and CiResFlu populations, respectively. The slope between the curves of each population was not significant, indicating a parallelism behavior, possibly due to the quantitative difference in the flubendiamide-detoxification capacity.

The relationship between the magnitude of the resistance ratio and insecticide control failures is not well defined because failure in the control with insecticides also depends on other factors such as the insect population density. However, we can assume that a ten-fold difference or over in the susceptibility to insecticides may affect insecticide effectiveness (FFRENCH-CONSTANT; ROUSH, 1990). Thus, based on the results of the residual activity, the resistance ratio found in this work may confirm this hypothesis, as there was discrimination between susceptible and resistant soybean loopers when exposed to flubendiamide residues.

These results repeated for the residual activity of chlorantraniliprole and cyantraniliprole, which also provided discrimination between flubendiamide-susceptible and resistant soybean loopers (Figure 2B-C). This result may also indicate the existence of cross-resistance between diamides for the control of *C. includens*. The possibility of occurrence of cross-resistance between diamides has favored a rapid resistance evolution in other species of Lepidoptera (ZHANG et al., 2016). Although the chlorantraniliprole + lambda-cyalothrin mixture provided greater control effectiveness than the sole diamide active ingredients, there was discrimination between soybean loopers susceptible and resistant to flubendiamide (Figure 2D).

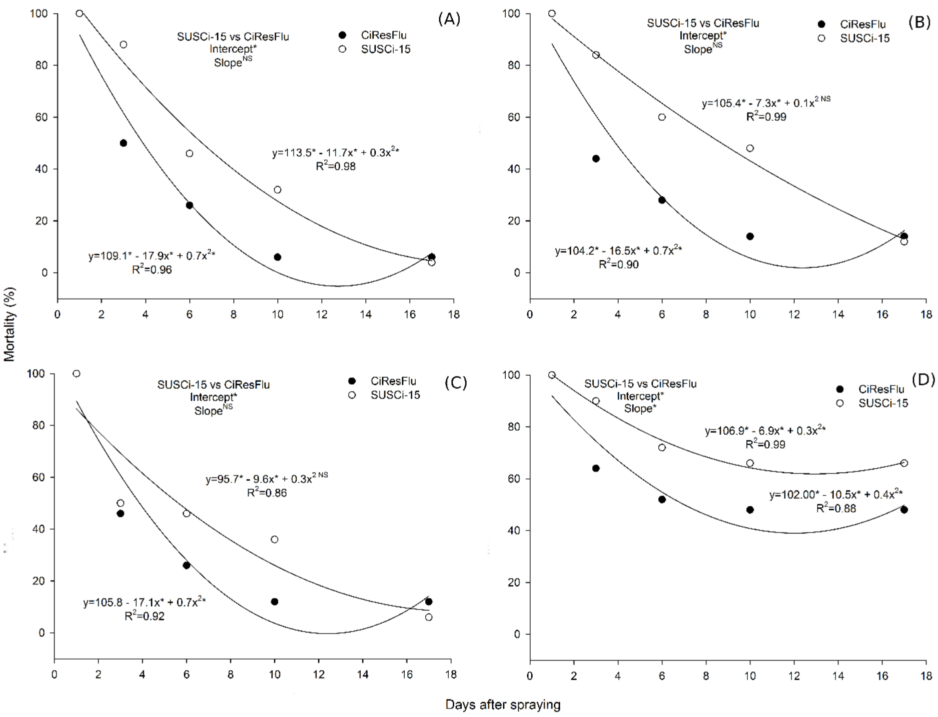

Figure 2. Residual activity curve of (A) flubendiamide, (B) chlorantraniliprole, (C) cyantraniliprole, and (D) chlorantraniliprole + lambda-cyalothrin for a susceptible reference population (SUSCI-15) and flubendiamide-resistant population (CiResFlu) of *Chrysodeixis includens* (Lepidoptera: Noctuidae) in soybean leaves under field conditions.

Insecticide rotation based on its mechanism of action has been highly recommended for resistance management (BERNARDI et al., 2012; STACKE et al., 2020a; 2020b). For example, for the management of *C. includens* resistance to teflubenzuron (IRAC Group 15), the active ingredients methoxyfenozide (IRAC Group 18), flubendiamide, and indoxacarb (IRAC Group 22A) have been considered good options for use in the rotation due to their low cross-resistance to teflubensuron (< 3.45-fold) (STACKE et al., 2020a). Although they are active ingredients with distinct mechanisms of action, the results of the residual activity of methoxyfenozide and teflubenzuron indicated that the continuous use of these molecules can affect the control effectiveness of flubendiamide because there was discrimination between susceptible and resistant soybean loopers to flubendiamide (Figure 3A-B). This situation may be aggravated because susceptibility variation in *C. includens* populations had already been detected to methoxyfenozide and teflubenzuron, with resistance ratios of up to 63 and 5,215-fold, respectively (STACKE et al., 2019).

For bifenthrin (IRAC Group 3A), there was no discrimination among the caterpillar populations, indicating the absence of cross-resistance. However, there was a low residual activity of this insecticide in the control of *C. includens.* As for other insecticides, intraspecific variability to pyrethroids, as is the case of lambda-cyalothrin, has already been found in Brazilian populations, also indicating the possible existence of pyrethroid-resistant insects (STACKE et al., 2019; 2020b). Therefore, studies addressing cross -resistance relationships are important for a proper recommendation of insecticides in rotation programs based on mechanisms of action.

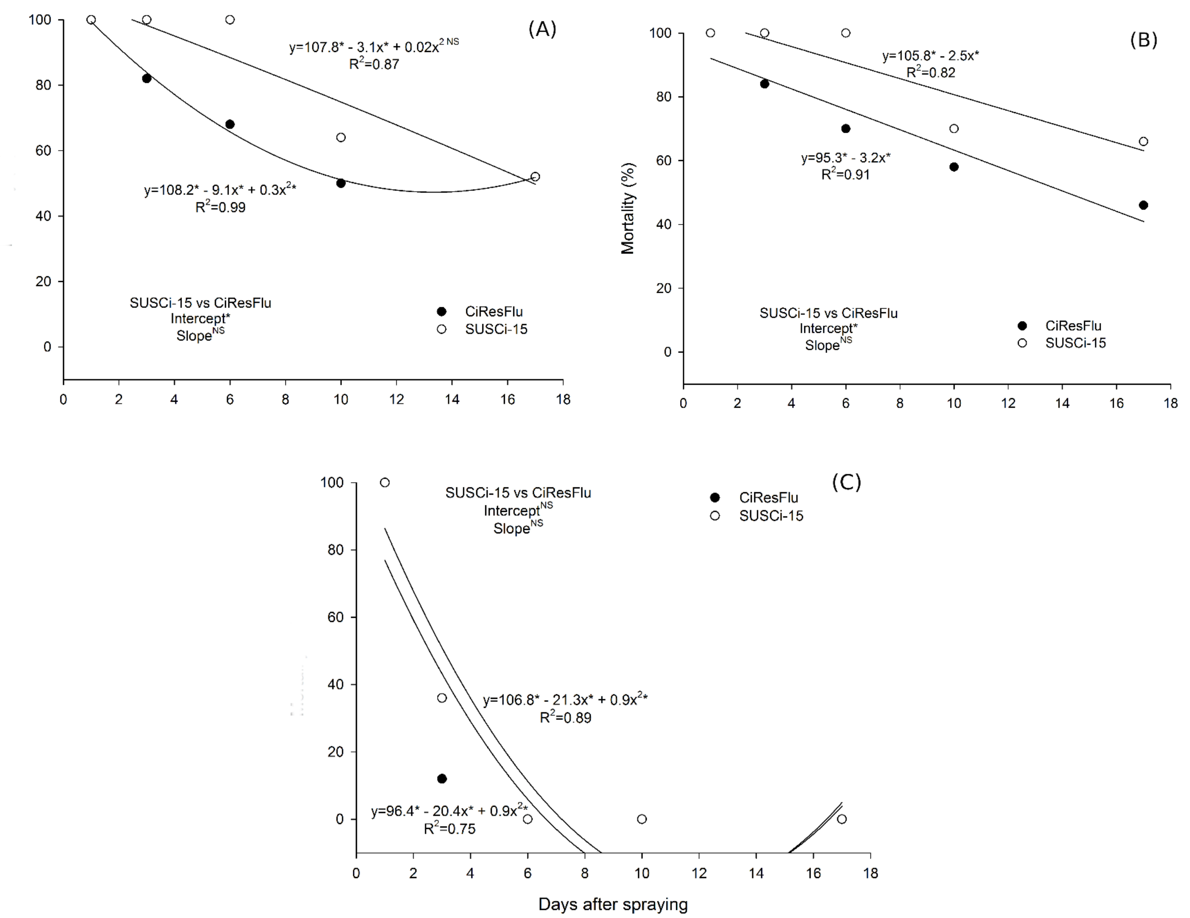

Figure 3. Residual activity curves of (A) methoxyfenozide, (B) teflubenzuron and (C) bifenthrin for a susceptible reference population (SUSCI-15) and a flubendiamide-resistant population (CiResFlu) of *Chrysodeixis includens* (Lepidoptera: Noctuidae) in soybean leaves under field conditions.

The increasing soybean cultivation has contributed to the adaptation of *C. includens*, especially with the selection of insecticide-resistant insects, in the Brazilian cropping system (SILVA et al., 2020). It is important to emphasize that in the past years the soybean culture has been the main consumer market for pesticides in the country (OLIVEIRA et al., 2014). The reduced effectiveness of control of this insect on the field with some insecticides has raised the production costs due to the need for a greater number of applications (PERINI et al., 2019).

Therefore, in addition to the rotation of insecticides based on their mechanisms of action, it is necessary to adopt other proactive resistance management practices such as the use of insecticides based on control levels. Following the control levels prescribed by the IPM, it was possible to reduce, on average, 48.8 and 53.6% of the number of insecticide applications in areas of conventional soybean and BT soybean, respectively (BUENO et al., 2021). These outcomes can be achieved due to the characteristics of current soybean cultivars regarding antibiosis effects and tolerance to defoliation by soybean loopers (WILLE et al., 2017; DURLI et al., 2020). Current soybean cultivars are capable of tolerating the defoliation levels described by IPM without affecting grain yields (BUENO et al., 2013; DURLI et al., 2020).

Recently, soybean crop areas with the insertion of the insecticidal protein Cry1Ac have increased in the country (BROOKES, 2018). This insecticidal protein is highly effective in the control of *C. includens,* contributing to the reduction of the insect population (BERNARDI et al., 2012). Thus, in addition to the benefit of reducing the number of insecticide applications in BT-soybean crop areas, the selection pressure by insecticides may diminish, allowing the reestablishment of the susceptibility of *C. includens* to chemical insecticides (BUENO et al., 2021; STACKE et al., 2020a; 2020b). However, to consolidate the IPM of soybean crops and reduce the pressure for selection of insecticides, other control management actions must be adopted, such as the use of *Baculovirus*-based insecticides (IRAC Group 31), which do not have cross-resistance with synthetic insecticides, contributing to the control of this insect in refuge areas (non-BT soybean), and even in other important agricultural cultures, since *C. includens* is a polyphagous species (GODOY et al., 2019; SPARKS et al., 2020; SPECHT; PAULA-MORAES; SOSA-GÓMEZ, 2015).

CONCLUSION

Chrysodeixis includens is more tolerant to cyantraniliprole than flubendiamide and chlorantraniliprole.

For monitoring the resistance of *C. includens* to diamides, the recommended diagnostic concentrations are 0.5053 µg cm^{-2} of flubendiamide, 0.1579 µg cm^{-2} of chlorantraniliprole, and 5.053 µg cm^{-2} of cyantraniliprole.

Chrysodeixis includens populations collected in southern Brazil exhibited varied susceptibility to flubendiamide, clorantraniliprole, and cyantraniliprole.

The resistance ratio of *C. includens* to flubendiamide is 70.1-fold, based on the LC_{50}.

Under field conditions, there is discrimination between flubendiamide-susceptible and resistant soybean loopers, *C. includens*, exposed to residues of flubendiamide, chlorantraniliprole, chlorantraniliprole + lambda-cyhalothrin, cyantraniliprole, methoxyfenozide, and teflubenzuron.

Bifenthrin residues did not result in discrimination between flubendiamide-susceptible and resistant *C. includens* caterpillars.

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