Research Article

Intraspecific variation in the *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae) susceptibility to insecticides[1]

Samanta Souza Restelatto[2], Paulo Eduardo Wille[2], Nayara Buss[2],
Cleiton Luis Wille[2], Mari Inês Carissimi Boff[2], Rafael Ermenegildo Contini[2], Cláudio Roberto Franco[2]

ABSTRACT

The looper caterpillar *Chrysodeixis includens* (Walker) is considered the main defoliating pest in soybean crops, which contributes to yield losses. Delaying its resistance to insecticides is a major challenge in its management. This study aimed to characterize the susceptibility of *C. includens* to flubendiamide, cyantraniliprole, spinetoram and methomyl, as well as to establish a diagnostic concentration for monitoring resistance. The insecticide was applied to the surface of the artificial diet. For the dose-response curve, concentrations that resulted in 5 to 99 % mortality were used to estimate the lethal concentrations (LC_{50} and LC_{99}) and resistance ratios (RR_{50} and RR_{99}). The diagnostic concentrations were based on concentrations that provided 90 to 99 % mortality in the susceptible reference population. For flubendiamide and methomyl, the decrease in the susceptibility resulted in estimated RR_{50} of 6.2 to 24.2 and 4.4 to 19.6 times, respectively. For cyantraniliprole and spinetoram, there was little difference in susceptibility among the populations, with RR_{99} lower than 6.1 times. Differences in the susceptibility of *C. includens* were evident from concentrations of 0.5053, 5.053, 0.1579 and 28.42 μg cm^{-2}, respectively for flubendiamide, cyantraniliprole, spinetoram and methomyl.

KEYWORDS: *Glycine max*, Plusiinae, insecticide resistance.

RESUMO

Variabilidade intraespecífica na suscetibilidade de *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae) a inseticidas

A lagarta falsa-medideira *Chrysodeixis includens* (Walker) é considerada o principal inseto desfolhador da cultura da soja, o qual contribui para a redução da produção. Para o seu manejo, um importante desafio é retardar sua resistência a inseticidas. Objetivou-se caracterizar a suscetibilidade de *C. includens* a flubendiamida, ciantraniliprole, espinetoram e metomil, bem como definir uma concentração diagnóstica para o monitoramento da resistência. Efetuou-se aplicação de inseticida sobre a superfície da dieta artificial. Para a curva dose-resposta, foram utilizadas concentrações que proporcionaram mortalidade entre 5 e 99 %, para estimar as concentrações letais (CL_{50} e CL_{99}) e razões de resistência (RR_{50} e RR_{99}). As concentrações diagnósticas foram definidas com base em concentrações que proporcionaram mortalidade entre 90 e 99 % na população suscetível de referência. Para flubendiamida e metomil, a redução na suscetibilidade proporcionou RR_{50} estimada em 6,2 a 24,2 e 4,4 a 19,6 vezes, respectivamente. Para ciantraniliprole e espinetoram, houve pouca diferença na suscetibilidade entre as populações, com RR_{99} inferior a 6,1 vezes. A partir das concentrações de 0,5053; 5,053; 0,1579; e 28,42 μg cm^{-2}, respectivamente para flubendiamida, ciantraniliprole, espinetoram e metomil, foram evidenciadas diferenças na suscetibilidade de *C. includens*.

PALAVRAS-CHAVE: *Glycine max*, Plusiinae, resistência a inseticidas.

INTRODUCTION

The Brazilian soybean [*Glycine max* (L.) Merril] production increased by 313 % from 1990 to 2017, making it the country's main crop, in terms of grain yield and cultivated area (Cattelan & Dall'Agnol 2018).

Insect pests may reduce yield by an average of 5 % and increase production costs due to the use of insecticides (Oliveira et al. 2014). The soybean looper *Chrysodeixis includens* (Walker, [1858]) (Lepidoptera: Noctuidae) is considered the major pest among defoliating caterpillars (Specht et al. 2015, Silva et al. 2020).

The increasing use of fungicides to control Asian soybean rust is one of the hypotheses to explain *C. includens* outbreaks in soybean, due to the death of natural enemies (Sosa-Gómez et al. 2003, Specht

[1] Received: Jan. 18, 2021. Accepted: June 17, 2021. Published: Sep. 23, 2021. DOI: 10.1590/1983-40632021v5167353.
[2] Universidade do Estado de Santa Catarina, Centro de Ciências Agroveterinárias, Departamento de Agronomia, Lages, SC, Brasil. *Email/ORCID*: restelatto_sam@hotmail.com/0000-0001-6985-4512; pauloewille@gmail.com/0000-0002-5458-6102; nayara_buss@hotmail.com/0000-0001-5409-598X; cleitonwille@gmail.com/0000-0002-0097-7394; mari.boff@udesc.br/0000-0003-1700-8837; rafael-contini@hotmail.com/0000-0001-9912-4385; claudio.franco@udesc.br/0000-0001-7944-0671.

et al. 2015). The resulting indiscriminate use of insecticides to control *C. includens*, accompanied by the species' adaptation to soybean crops, has accelerated the development of resistance and compromised the use of chemical control in integrated pest management (Stacke et al. 2019, Silva et al. 2020, Bueno et al. 2021).

In Brazil, an intraspecific variation in susceptibility to insecticides such as flubendiamide and chlorantraniliprole (Schneider & Sosa-Gómez 2016, Stacke et al. 2019), lambda-cyhalothrin, methoxyfenozide, novaluron and teflubenzuron (Stacke et al. 2019), and lufenuron and spinosad (Queiroz et al. 2020) has been documented for *C. includens*. However, cases of insecticide-resistant populations have been identified in the United States since 1970 (Leonard et al. 1990, Mascarenhas & Boethrel 2000, Mota-Sanchez & Wise 2020).

The need to manage insect resistance to insecticides, in order to mitigate the damage caused by pests, is a major global challenge and justifies the importance to discover new molecules (Sparks 2013). Rotating insecticides based on their mode of action is an important recommendation in resistance management (Sparks & Nauem 2015, Stacke et al. 2019).

In addition, studies that monitor resistance are essential for detecting the presence of resistant individuals within a population (Ffrench-Constant 2006, Nunes et. 2019, Stacke et al. 2019). As such, this study aimed to assess the intraspecific variation of *C. includens* populations, as well as to determine the susceptibility baseline of the species for the insecticides flubendiamide, cyantraniliprole (diamides), spinoteram (spinosyn) and methomyl (carbamate), in order to establish diagnostic concentrations for use in insect resistance management programs in southern Brazil.

MATERIAL AND METHODS

C. includens populations were collected in soybean crops without the cry1Ac gene of *Bacilluus thuringiensis* Berliner, in the 2014/2015, 2016/2017 and 2017/2018 growing seasons, in the Brazilian states of Santa Catarina (SC), Paraná (PR) and Rio Grande do Sul (RS) (Table 1). The collected caterpillars were fed an artificial diet, adapted from Greene et al. (1976). The populations collected in Lages - SC (Lages-1) and Engenheiro Coelho - SP (SUSC-15) were considered the susceptible reference (laboratory populations), because they were kept in a laboratory without selection pressure by insecticides for more than 15 generations, before conducting the toxicological bioassays (Table 1).

The adults were kept in polyvinyl chloride (PVC) tubes (200 x 100 mm in diameter) lined with paper, used as an oviposition substrate. Food was supplied in Petri dishes (50 mm) containing cotton wool moistened with distilled water, a 10 % honey solution added with 1 % sorbic acid and nipagin (p/v), and 10 % honey with beer at a ratio of 3:2.

Table 1. Geographic and temporal origin of *Chrysodeixis includens* populations collected in soybean fields in southern Brazil.

Growing season	Location	Latitude (S)	Longitude (W)	Collection date
2013/2014	Lages - SC	27º52'01.7"	50º19'28.0"	Mar/2014
2014/2015	Lages - SC	27º52'18.0"	50º18'03.1"	Jan/2015
	Erval Velho - SC	27º13'35.8"	51º27'33.9"	Feb/2015
	Joaçaba - SC	27º11'42.5"	51º34'35.1"	Feb/2015
2015/2016	Engenheiro Coelho - SP*	-	-	Dec/2015
2016/2017	Petrolândia - SC	27º29'48.71"	49º41'5.20"	Jan/2017
	Santa Maria - RS	29º41'03"	53º48'25"	Feb/2017
2017/2018	Ituporanga - SC	27º26'56.6"	49º24'54.5"	Feb/2018
	Joaçaba - SC	27º13'10.6"	51º32'12.1"	Feb/2018
	São José do Cerrito - SC	27º42'23.5"	50º36'37.9"	Feb/2018
	Três Barras - SC	26º10'32.0"	50º14'27.4"	Feb/2018
	Vargeão - SC	26º52'57.7"	52º11'15.8"	Mar/2018
	Campo Belo - SC	27º53'56.6"	50º40'13.6"	Mar/2018
	Vacaria - RS	28º27'38.4"	51º03'53.1"	Feb/2018
	Londrina - PR	23º02'45"	51º12'58"	Mar/2018

* Population consisting of caterpillars collected from different soybean crops.

The oviposition substrates containing eggs were placed in plastic pots (145 mL) filled with the artificial diet to allow the caterpillars to develop. After the third instar, the caterpillars were transferred to plastic cups (50 mL) containing the artificial diet (3 caterpillars cup^{-1}). Breeding was carried out in an air-conditioned room (25 ± 2 ºC), with relative humidity of 60 ± 10 % and a 14-h photoperiod (Panizzi & Parra 2009).

An ingestion bioassay was conducted, applying insecticide to the surface of the artificial diet (Mascarenhas & Boethrel 1997, Mascarenhas & Boethrel 2000). The commercial insecticides used were based on the active ingredients (a.i.) flubendiamide (Belt™; 480 g L^{-1} of a.i.), cyantraniliprole (Benevia™; 100 g L^{-1} of a.i.), methomyl (Lannate™; 215 g L^{-1} of a.i.) and spinetoram (Exalt™; 120 g L^{-1} of a.i.).

The insecticides were diluted in distilled water and added with 0.1 % surfactant (Triton X-100™, Labsynth produtos para Laboratórios Ltda.). A 24-well acrylic plate (Costar™ 3526, Cambridge, Massachusetts, USA) was filled with approximately 1.2 mL of artificial diet per well. After gelation of the diet, 30 µL of the insecticide solution or distilled water + surfactant (control) were applied to the surface of the diet in each well.

Next, at the beginning of the third instar, one caterpillar was transferred to each well. The plates were kept in an air-conditioned room (25 ± 2 ºC) with relative humidity of 60 ± 10 % and 14-h photoperiod. Mortality was assessed at 72 h after the start of the bioassays for spinetoram and methomyl (Mascarenhas & Boethrel 2000) and 96 h for the diamides (flubendiamide and cyantraniliprole) (Owen et al. 2013). Caterpillars that showed no apparent movement after their last abdominal segments had been touched were considered dead.

In order to establish the susceptibility baseline, third and seventh-generation caterpillars kept in a laboratory, except for the susceptible reference populations (> 15th generation), were exposed to a logarithmic series of six to eight concentrations of each insecticide, resulting in 5 to 99 % mortality. Four to six repetitions were performed for each concentration, using 24 caterpillars per repetition. In order to estimate the LC_{50} and LC_{99} lethal concentrations and their respective confidence intervals (95 % CI), the mortality data were submitted to Probit analysis with normal distribution, using the SAS/STAT™ software (SAS Institute 2020). The difference in susceptibility was evaluated based on nonoverlapping confidence intervals (95 % CI). The resistance ratio (RR_{50} or RR_{99}) was estimated by dividing the LC_{50} or LC_{99} of the tested population by the LC_{50} or LC_{99} of the population considered susceptible (SUSCI-15) (Robertson & Preisler 1992). The mortality data of populations with RR values up to 10 were submitted to joint analysis (Proc Probit), using a binomial model and complementary log-log link (Gompertz).

In order to monitor susceptibility, diagnostic concentrations that produced 90 to 99 % mortality in susceptible reference populations were established (Mascarenhas & Boethrel 1997). To that end, an experiment was conducted to evaluate the susceptibility of field populations collected in the 2017/2018 growing season (Table 1). A completely randomized design was used, with 20 replications, each one consisting of one acrylic plate with 24 caterpillars. The data were submitted to analysis of variance and treatments compared by the Tukey test at 5 % of significance. Box-Cox transformation was performed to ensure the data met the assumptions of homoscedasticity. The model was assessed and fit to the data using diagnostic graphs (histogram, residuals *vs*. predicted values and Q-Q) and Akaike information criteria.

RESULTS AND DISCUSSION

The dose-response curves indicated intraspecific variation in *C. includens* susceptibility to the insecticides flubendiamide, cyantraniliprole, spinetoram and methomyl. The SUSCI-15 population (susceptible reference) showed a greater susceptibility to the insecticides because their LD_{50} values were lower than those of the other populations, and was therefore used to calculate the RR (Tables 2, 3, 4 and 5). In a joint analysis, the mortality data did not fit the probit model ($p < 0.05$), also indicating response heterogeneity or due to the number of populations assessed.

For flubendiamide (IRAC MoA Group 28), none of the field populations, except for Santa Maria, showed overlapping confidence intervals for lethal concentrations, when compared to the susceptible population (SUSCI-15) (Table 2). The estimated RR_{50} and RR_{99} were 6.2 to 24.2 and 61.1 to 245.7, respectively. For cyantraniliprole (IRAC MoA Group 28), based solely on LC_{99}, nonoverlapping confidence intervals indicated differences in susceptibility

(Table 3). The Londrina and Ituporanga populations exhibited the lowest susceptibility, with a maximum RR_{99} of 6.1.

Studies on the intraspecific variation of insects to insecticides are an important source of information for integrated pest and insect resistance management, particularly if baseline susceptibility is established before widespread insecticide use in a region (Ffrench-Constant 2006, Owen et al. 2013, Teixeira & Andaloro 2013, Zhang et al. 2016).

This may be the case for insecticides in the chemical group of diamides, since they were discovered fairly recently, between 2003 and 2008 (Sparks 2013, Sparks & Nauen 2015). Prior to the commercial use of diamides in the USA, the natural intraspecific variation in *C. includens* susceptibility to flubendiamide and chlorantraniliprole was 9.2 and 6.2, respectively (Owen et al. 2013). This same magnitude of natural variation (< 10) was also reported for other Lepidoptera species, including susceptibility to cyantraniliprole (Sial et al. 2010, Silva et al. 2012, Teixeira & Andaloro 2013, Zhang et al. 2016).

Flubendiamide was registered in Brazil in 2009 (Brasil 2021). As such, given the lack of information prior to this date, the variation in susceptibility to this insecticide may indicate the presence of resistant caterpillars in the phenotypic composition of populations, due to their exposure to this insecticide. The obtained data corroborate the literature, which indicates the existence of variations in susceptibility to flubendiamide among Brazilian *C. includens* populations since the 2013/2014 growing season (Schneider & Sosa-Gómez 2016, Stacke et al. 2019).

Cyantraniliprole was registered in Brazil in 2015 (Brasil 2021). Thus, the results obtained for

Table 2. Dose-response curve of third-instar *Chrysodeixis includens* in ingestion bioassays with surface treatment of the artificial diet with flubendiamide (IRAC MoA Group 28).

Population	n[a]	Slope (±SE)	μg cm^{-2} (95 % FL) LC_{50}	μg cm^{-2} (95 % FL) LC_{99}	χ2	df[b]	p	RR[c]
Lab strain								
Lages-1	840	1.63 (±0.15)	0.185 (0.125-0.256)	4.97 (2.69-12.97)	10.70	5	0.058	-
SUSCI-15	552	2.47 (±0.24)	0.055 (0.046-0.064)	0.484 (0.354-0.755)	2.31	3	0.552	-
2014/2015								
Lages-2	1,440	1.19 (±0.06)	1.33 (1.09-1.61)	118.91 (79.71-192.91)	10.40	10	0.406	24.2
Joaçaba	1,440	1.28 (±0.06)	0.728 (0.619-0.852)	48.12 (32.95-75.75)	9.50	9	0.392	13.2
Erval Velho	1,080	1.22 (±0.12)	0.431 (0.221-0.712)	34.86 (17.34-99.29)	13.50	7	0.066	7.8
2016/2017								
Santa Maria	624	2.28 (±0.26)	0.064 (0.048-0.078)	0.665 (0.481-1.089)	7.38	4	0.117	1.2
2017/2018								
Londrina	528	1.20 (±0.14)	0.341 (0.220-0.468)	29.59 (15.59-78.84)	3.01	4	0.556	6.2
Ituporanga	576	1.18 (±0.13)	0.599 (0.383-0.849)	56.50 (29.19-149.74)	6.87	5	0.231	10.8
Joint analysis	2,016	1.33 (±0.23)	0.083 (0.035-0.142)	2.21 (1.03-10.04)	250.60	16	< 0.001	-

[a] Number of tested caterpillars; [b] degrees of freedom; [c] resistance ratio obtained by dividing the LC_{50} of each population by the LC_{50} of the lab strain (SUSCI-15).

Table 3. Dose-response curve of third-instar *Chrysodeixis includens* in ingestion bioassays with surface treatment of the artificial diet with cyantraniliprole (IRAC MoA Group 28).

Population	n[a]	Slope (±SE)	μg cm^{-2} (95 % FL) LC_{50}	μg cm^{-2} (95 % FL) LC_{99}	χ2	df[b]	p	RR[c]
Lab strain								
SUSCI-15	528	1.77 (±0.23)	0.388 (0.254-0.519)	8.04 (5.07-16.77)	7.23	5	0.2038	-
2016/2017								
Santa Maria	576	1.70 (±0.18)	0.491 (0.349-0.639)	11.45 (7.27-22.38)	5.55	4	0.2355	1.3
2017/2018								
Londrina	552	0.90 (±0.08)	0.127 (0.076-0.193)	47.86 (22.72-134.28)	5.85	4	0.2108	0.3
Ituporanga	504	1.00 (±0.10)	0.230 (0.139-0.340)	49.18 (23.78-141.10)	4.37	4	0.3577	0.6
Joint analysis	2,160	1.22 (±0.08)	0.301 (0.220-0.389)	10.66 (7.96-15.37)	37.40	23	0.0290	-

[a] Number of tested caterpillars; [b] degrees of freedom; [c] resistance ratio obtained by dividing the LC_{50} of each population by the LC_{50} of the lab strain (SUSCI-15).

2015/2016 and 2016/2017 may represent natural susceptibility variations, since they were similar to the natural variation in diamide susceptibility observed for other Lepidoptera species (Owen et al. 2013, Teixeira & Andaloro 2013, Zhang et al. 2016).

This same observation may be extrapolated for spinetoram (IRAC MoA Group 5), because it was registered in the country in 2014 (Brasil 2021). For spinetoram, based on LC_{50}, only the Ituporanga population showed less susceptibility, with an RR of 3.9. However, based on the LC_{99} value, Londrina also exhibited less susceptibility, in relation to SUSCI-15 (Table 4), with an estimated RR_{99} of 10.4. The susceptibility variation of up to 3.9 may be considered similar to that reported for other Brazilian *C. includens* populations (up to 8.6) (Stacke et al. 2019) and Lepidoptera species (3.6 to 7.6) (Sial et al. 2010, Li et al. 2015). For spinosad, also from the chemical group of spinosyns, susceptibility variations of 2.2 to 7.8 have been detected in populations in the Mato Grosso state (Queiroz et al. 2020). These differences may contribute to accelerate the resistance evolution, due to the possible cross-resistance relationship among these active ingredients (Sial et al. 2010, Li et al. 2015, Sparks & Nauem 2015).

For methomyl (IRAC MoA Group 1A), the Lages-2, Joaçaba-1, Ituporanga and Londrina populations differed from SUSCI-15, based on LC_{50} (RR_{50} of 4.4 to 19.6), while Lages-2, Erval Velho, Ituporanga and Londrina differed based on LC_{99} (Table 5), with an estimated RR_{99} between 4.1 and 31.4.

Among the insecticides tested here, methomyl has been used to manage insect pests, including

Table 4. Dose-response curve of third-instar *Chrysodeixis includens* in ingestion bioassays with surface treatment of the artificial diet with spinetoram (IRAC MoA Group 5).

Population	n[a]	Slope (±SE)	µg cm^{-2} (95 % FL) LC_{50}	µg cm^{-2} (95 % FL) LC_{99}	χ2	df[b]	p	RR[c]
Lab strain								
SUSCI-15	624	1.92 (±0.14)	0.014 (0.012-0.017)	0.232 (0.165-0.359)	5.09	4	0.2784	-
2016/2017								
Santa Maria	576	1.68 (±0.19)	0.005 (0.004-0.007)	0.133 (0.085-0.265)	2.57	3	0.4625	0.4
2017/2018								
Londrina	576	1.30 (±0.12)	0.016 (0.011-0.020)	0.970 (0.560-2.121)	5.05	4	0.2821	1.1
Ituporanga	528	1.42 (±0.12)	0.055 (0.041-0.071)	2.42 (1.46-4.77)	5.47	4	0.2420	3.9
Joint analysis	2,304	1.07 (±0.24)	0.014 (0.003-0.028)	0.841 (0.301-10.27)	388.50	21	< 0.0001	-

[a] Number of tested caterpillars; [b] degrees of freedom; [c] resistance ratio obtained by dividing the LC_{50} of each population by the LC_{50} of the lab strain (SUSCI-15).

Table 5. Dose-response curve of third-instar *Chrysodeixis includens* in ingestion bioassays with surface treatment of the artificial diet with methomyl (IRAC MoA Group 1A).

Population	n[a]	Slope (± SE)	µg cm^{-2} (95 % FL) LC_{50}	µg cm^{-2} (95 % FL) LC_{99}	χ2	df[b]	p	RR[c]
Lab strain								
Lages-1	600	3.25 (±0.23)	15.51 (14.12-17.00)	80.71 (65.41-105.82)	1.49	3	0.6851	
SUSCI-15	576	2.04 (±0.16)	3.52 (2.89-4.16)	48.34 (35.28-73.50)	4.22	3	0.2383	-
2014/2015								
Lages-2	1,080	1.73 (±0.11)	68.83 (57.23-81.24)	1,520 (1,098-2,292)	8.34	7	0.3033	19.6
Joaçaba-1	600	3.71 (±0.27)	15.51 (14.15-16.93)	65.62 (54.54-83.37)	4.95	3	0.1758	4.4
Erval Velho	720	1.75 (±0.24)	9.28 (3.97-16.43)	197.06 (88.82-956.85)	14.41	4	0.0061	2.6
2016/2017								
Santa Maria	528	2.14 (±0.27)	5.82 (4.32-7.20)	71.13 (47.93-133.60)	6.13	3	0.1054	1.7
2017/2018								
Londrina	600	1.73 (±0.14)	26.15 (21.61-31.29)	578.63 (375.36-1,039)	4.41	6	0.6218	7.4
Ituporanga			36.36 (28.5-45.1)	1,468 (950.6-2,588)				10.3
Joint analysis	3,024	1.98 (±0.39)	9.87 (5.43-14.31)	88.89 (50.77-292.74)	661.60	24	< 0.0001	-

[a] Number of tested caterpillars; [b] degrees of freedom; [c] resistance ratio obtained by dividing the LC_{50} of each population by the LC_{50} of the lab strain (SUSCI-15).

C. includens, having been available on the global market since 1960-1970 (Sparks 2013, Sparks & Nauem 2015). In the USA, the evolution of methyl resistance in *C. includens* has been reported since 1978 (Leonard et al. 1990). In Brazil, its use has been recommended in soybean since at least the 1980s (Andrei 1987, Brasil 2021), what could explain the reduced susceptibility observed in *C. includens* populations.

Under experimental field conditions, differences were observed in the *C. includens* efficiency control with insecticides, for example, less effective control with diamides (flubendiamide and chlorantraniliprole), when compared to spinetoram, indoxacarb and chlorfenapyr (Perini et al. 2019). An RR greater than 10 generally compromises the insecticide effectiveness in the field (Ffrench-constant & Roush 1990). Thus, for flubendiamide and methomyl, the presence of resistant caterpillars suggests that control failures may already be occurring.

Thus, based on the SUSCI-15 dose-response curve, diagnostic concentrations of 0.5053, 5.053, 0.1579 and 28.42 μg cm^{-2} of flubendiamide, cyantraniliprole, spinetoram and methomyl, respectively, were established to monitor susceptibility (Table 6). The results demonstrated differences in survival among populations ($F = 68.33$; df = 8, 628; $p < 0.0001$) and population-insecticide interaction ($F = 13.80$; df = 24, 628; $p < 0.0001$), and a significant effect among insecticides ($F = 427.29$; df = 3, 628; $p < 0.0001$) (Table 6).

The susceptibility monitoring results demonstrate the existence of *C. includens* populations that are less susceptible to this insecticide. In addition, these populations also exhibited a greater susceptibility to cyantraniliprole (3.7-30 % of survival) than to flubendiamide (48.9-76.8 %) (Table 6). This information is important, because the possibility of cross-resistance between active ingredients of the chemical group of diamides may favor the evolution of resistance to cyantraniliprole (Wang et al. 2013, Zhang et al. 2016).

In Brazil, there are several hypotheses to explain *C. includens* outbreaks, such as the increased use of fungicides to control Asian rust, which may reduce the action of natural biological control agents; the expansion of soybean monocropping under intensive production systems, with the use of fertilizers, insecticides, smaller plant spacing and early-cycle cultivars; and, more recently, the use of insect-resistant genetically modified plants (Sosa-Gómez et al. 2003, Specht et al. 2015, Silva et al. 2020).

This adaptation of *C. includens* to soybean crops has been attributed to evidence of low genetic diversity among Brazilian *C. includens* populations (Palma et al 2015, Silva et al. 2020). Although the literature suggests a poor genetic diversity, the results indicate significant differences in susceptibility to insecticides among phenotypes, even among populations located close together, that is, collected in southern Brazil since the 2014/2015 growing season.

As such, in order to contribute to improve the integrated pest and insect resistance management, in addition to rotating insecticides based on their mode of action, a continuous monitoring of changes in the frequency of resistant insects is also important. However, given that variation in susceptibility among *C. includens* populations has been detected for

Table 6. Survival (%) (mean ± SE) of *Chrysodeixis includens* populations collected in southern Brazil in the 2017/2018 growing season, under diagnostic insecticide concentrations.

Population	Flubendiamide (0.5053 μg cm^{-2})	Cyantraniliprole (5.053 μg cm^{-2})	Spinetoram (0.1579 μg cm^{-2})	Methomyl (28.42 μg cm^{-2})
SUSCI-15	2.5 ± 1.66 Aa*	1.4 ± 0.89 Aa	0.0 ± 0.00 Aa	0.8 ± 1.33 Aa
Vacaria - RS	48.9 ± 4.52 Bb	9.3 ± 1.53 Da	18.1 ± 1.52 Cb	66.7 ± 9.17 Ac
São José do Cerrito - SC	50.2 ± 3.96 Bbc	9.1 ± 1.58 Da	17.0 ± 1.89 Cb	60.0 ± 7.67 Ac
Ituporanga - SC	54.6 ± 3.67 Abcd	3.7 ± 0.95 Da	19.7 ± 1.74 Cb	34.6 ± 9.42 Bb
Vargeão - SC	60.2 ± 3.10 Abcde	9.7 ± 1.65 Da	20.7 ± 3.06 Cb	41.9 ± 18.00 Bb
Joaçaba - SC	63.5 ± 4.10 Acdef	20.0 ± 1.23 Cb	44.5 ± 2.01 Bd	45.6 ± 9.40 Bb
Campo Belo - SC	66.3 ± 3.73 Adef	8.1 ± 0.92 Da	31.6 ± 2.38 Cc	45.8 ± 7.08 Bb
Três Barras - SC	73.4 ± 3.43 Aef	30.0 ± 1.30 Bb	21.3 ± 2.05 Cbc	70.6 ± 7.75 Ac
Londrina - PR	76.8 ± 2.89 Af	8.9 ± 2.14 Da	23.2 ± 2.01 Cbc	33.3 ± 9.58 Bb

* Means followed by the same uppercase letter in the rows and lowercase letter in the columns do not differ according to the Tukey test ($p < 0.05$).

different modes of action and active ingredients, other control methods are also important, such as using plants that express the insecticidal protein Cry1Ac and exploring micro and macrobiological agents to reduce the selection pressure from continuous insecticide use. Thus, maintaining the effectiveness of chemical control as a management option for *C. includens* is important in soybean, refuge areas (soybean without the Cry1Ac protein) and other economically important crops that host the insect.

CONCLUSIONS

1. *Chrysodeixis includens* populations collected in southern Brazil between the 2014/2015 and 2017/2018 growing seasons showed variation in susceptibility to diamides, spinosyn and carbamate. The variation magnitude depends on the insecticide. Cyantraniliprole and spinetoram showed a low variation in susceptibility, with a resistance rate of less than 3.9;
2. Diagnostic concentrations of 0.5053, 5.053, 0.1579 and 28,42 μg cm^{-2} of flubendiamide, cyantraniliprole, spinetoram and methomyl, respectively, can be used to monitor *C. includens* resistance, because they enable the detection of intraspecific variation.

ACKNOWLEDGMENTS

To the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundo de Apoio à Manutenção e ao Desenvolvimento da Educação Superior (Uniedu/Fumdes), for the scholarships awarded. We are also grateful for the financial resources received from the Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC) (FAPESC/PAP/Udesc) and Comitê de Ação à Resistência a Inseticidas (IRAC-BR) (FIEPE/CAV 001/2017).

REFERENCES

ANDREI, E. *Compêndio de defensivos agrícolas*: guia prático de produtos fitossanitários para uso agrícola. São Paulo: Organização Andrei, 1987.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. *Sistema de agrotóxicos fitossanitários*: Agrofit. 2003. Available at: https://www.gov.br/agricultura/pt-br. Access on: 13 Jan. 2021.

BUENO, A. F.; PANIZZI, A. R.; HUNT, T. E.; DOURADO, P. M.; PITTA, R. M.; GONÇALVES, J. Challenges for adoption of integrated pest management (IPM): the soybean example. *Neotropical Entomology*, v. 50, n. 1, p. 5-20, 2021.

CATTELAN, A. J.; DALL'AGNOL, A. The rapid soybean growth in Brazil. *Oilseeds and Fats, Crops and Lipids*, v. 25, n. 1, eD102, 2018.

FFRENCH-CONSTANT, R. H. Which came first: insecticide or resistance? *Trends in Genetics*, v. 23, n. 1, p. 1-4, 2006.

FFRENCH-CONSTANT, R. H.; ROUSH, R.T. Resistance detection and documentation: the relative roles of pesticidal and biochemical assays. *In*: ROUSH, R. T.; TABASHNIK, B. E. *Pesticide resistance in arthropods*. New York: Chapman & Hall, 1990. p. 4-38.

GREENE, G. L.; LEPPLA, N. C.; DICKERSON, W. A. Velvetbean caterpillar: a rearing procedure and artificial medium. *Journal of Economic Entomology*, v. 69, n. 4, p. 487-488, 1976.

LEONARD, B. R.; BOETHEL, D. J.; SPARKS, A. N.; LAYTON, M. B.; MINK, J. S.; PAVLOFF, A. M.; BURRIS, E.; GRAVES, J. B. Variations in response of soybean looper (Lepidoptera: Noctuidae) to selected insecticides in Louisiana. *Journal of Economic Entomology*, v. 83, n. 1, p. 27-34, 1990.

LI, W.; ZHANG, J.; ZHANG, P.; LIN, W.; LIN, Q.; LI, Z.; HANG, F.; ZHANG, Z.; LU, Y. Baseline susceptibility of *Plutella xylostella* (Lepidoptera: Plutellidae) to the novel insecticide spinetoram in China. *Journal of Economic Entomology*, v. 108, n. 2, p. 736-741, 2015.

MASCARENHAS, R. N.; BOETHEL, D. J. Development of diagnostic concentrations for insecticide resistance monitoring in soybean looper (Lepidoptera: Noctuidae) larvae using an artificial diet overlay bioassay. *Journal of Economic Entomology*, v. 93, n. 3, p. 897-904, 2000.

MASCARENHAS, R. N.; BOETHEL, D. J. Responses of field-collected strains of soybean looper (Lepidoptera: Noctuidae) to selected insecticides using an artificial diet overlay bioassay. *Journal of Economic Entomology*, v. 90, n. 5, p.1117-1124, 1997.

MOTA-SANCHEZ, D.; WISE, J. C. *The arthropod pesticide resistance database*. 2020. Available at: http://www.pesticideresistance.org. Access on: 13 Jan. 2021.

NUNES, N. R.; FERREIRA, F. R.; THIESEN, L. V.; CORASSA, J. N.; PITTA, R. M. Linha básica de suscetibilidade de *Chrysodeixis includens* (Walker, [1858]) (Lepidoptera: Noctuidae) a benzoato de emamectina. *Entomological Communications*, v. 1, ec01015, 2019.

OLIVEIRA, C. M.; AUAD, A. M.; MENDES, S. M.; FRIZZAS, M. R. Crop losses and the economic impact of insect pests on Brazilian agriculture. *Crop Protection*, v. 56, n. 1, p. 50-54, 2014.

OWEN, L. N.; CATCHOT, A. L.; MUSSER, F. R.; GORE, J.; COOK, D. C.; JACKSON, R. Susceptibility of *Chrysodeixis includens* (Lepidoptera: Noctuidae) to reduced-risk insecticides. *Florida Entomologist*, v. 96, n. 2, p. 554-559, 2013.

PALMA, J.; MAEBE, K.; GUEDES, J. V. C.; SMAGGHE, G. Molecular variability and genetic structure of *Chrysodeixis includens* (Lepidoptera: Noctuidae), an important soybean defoliator in Brazil. *PloS ONE*, v. 10, n. 3, p. 1-13, 2015.

PANIZZI, A. R.; PARRA, J. R. P. *Bioecologia e nutrição de insetos*: bases para o manejo integrado de pragas. Brasília, DF: Embrapa Informação Tecnológica, 2009.

PERINI, C. R.; ARNEMANN, J. A.; CAVALLIN, L. A.; GUEDES, G. A.; MARQUES, R. P.; VALMORBIDA, I.; SILVA, K.; FELTRIN, N. M.; PUNTEL, L.; FROEHLICH, R.; GUEDES, J. V. C. Challenges in chemical management of soybean looper (*Chrysodeixis includes*) using several insecticides. *Australian Journal of Crop Science*, v. 13, n. 10, p. 1723-1730, 2019.

QUEIROZ, L. F.; CORASSA, J. N.; RODRIGUES, S. M. M.; PITTA, R. M. Susceptibility of soybean looper to lufenuron and spinosad. *Arquivos do Instituto Biológico*, v. 87, e0062019, 2020.

ROBERTSON, J. L.; PREISLER, H. K. *Pesticide bioassays with arthropods*. London: CRC, 1992.

SAS INSTITUTE. *SAS university edition 9.4*. Cary: SAS Institute Inc., 2020.

SCHNEIDER, J. A.; SOSA-GOMEZ, D. R. Suscetibilidade de populações de *Chrysodeixis includens* e *Helicoverpa armigera* a inseticidas do grupo das diamidas. *In:* REUNIÃO DE PESQUISA DE SOJA, 35., 2016, Londrina. *Resumos*... Londrina: Embrapa Soja, 2016. p. 64-66.

SIAL, A. A.; BRUNNER, J. F.; DOERR, M. D. Susceptibility of *Choristoneura rosaceana* (Lepidoptera: Tortricidae) to two new reduced-risk insecticide. *Journal of Economic Entomology*, v. 103, n. 1, p. 140-146, 2010.

SILVA, C. S.; CORDEIRO, E. M. G.; PAIVA, J. B.; DOURADO, P. M.; CARVALHO, R. A.; HEAD, G.; MARTINELLI, S.; CORREA, A. S. Population expansion and genomic adaptation to agricultural environments of the soybean looper, *Chrysodeixis includens*. *Evolutionary Applications*, v. 13, n. 8, p. 2071-2085, 2020.

SILVA, J. E.; SIQUEIRA, H. A. A.; SILVA, T. B. M.; CAMPOS, M. R.; BARROS, R. Baseline susceptibility to chlorantraniliprole of Brazilian populations of *Plutella xylostella*. *Crop Protection*, v. 35, n. 1, p. 97-101, 2012.

SOSA-GÓMEZ, D. R.; DELPIN, K. E.; MOSCARDI, F.; NOZAKI, M. H. The impact of fungicides on *Nomuraea rileyi* (Farlow) Samson epizootics and on populations of *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae), on soybean. *Neotropical Entomology*, v. 32, n. 2, p. 287-291, 2003.

SPARKS, T. C. Insecticide discovery: an evaluation and analysis. *Pesticide Biochemistry and Physiology*, v. 107, n. 1, p. 8-17, 2013.

SPARKS, T. C.; NAUEN, R. IRAC: mode of action, classification and insecticide resistance management. *Pesticide Biochemistry and Physiology*, v. 121, n. 1, p. 122-128, 2015.

SPECHT, A.; PAULA-MORAES, S. V.; SOSA-GÓMEZ, D. R. Host plants of *Chrysodeixis includens* (Walker) (Lepidoptera, Noctuidae, Plusiinae). *Revista Brasileira de Entomologia*, v. 59, n. 4, p. 343-345, 2015.

STACKE, R. F.; GIACOMELLI, T.; BRONZATTO, E. S.; HALBERSTADT, S. A.; GARLET, C. G.; MURARO, D. S.; GUEDES, J. V. C.; BERNARDI, O. Susceptibility of Brazilian populations of *Chrysodeixis includens* (Lepidoptera: Noctuidae) to selected insecticides. *Journal of Economic Entomology*, v. 112, n. 3, p. 1378-1387, 2019.

TEIXEIRA, L. A.; ANDALORO, J. T. Diamide insecticides: global efforts to address insect resistance stewardship challenges. *Pesticide Biochemistry and Physiology*, v. 106, n. 3, p. 76-78, 2013.

WANG, X.; KHAKAME, S. K.; YE, C.; YANG, Y.; WU, Y. Characterisation of field-evolved resistance to chlorantraniliprole in the diamondback moth, *Plutella xylostella*, from China. *Pest Management Science*, v. 69, n. 5, p. 661-665, 2013.

ZHANG, S.; ZHANG, X.; SHEN, J.; MAO, K.; YOU, H.; LI, J. Susceptibility of field populations of the diamindback moth, *Plutella xylostella*, to a selection of insecticides in central China. *Pesticide Biochemistry and Physiology*, v. 132, n. 1, p. 38-46, 2016.