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Source: Florida Entomologist, 96(2) : 554-559

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.096.0221

SUSCEPTIBILITY OF *CHRYSODEIXIS INCLUDENS* (LEPIDOPTERA: NOCTUIDAE) TO REDUCED-RISK INSECTICIDES

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Abstract

Field populations of soybean looper, *Chrysodeixis includens* (Walker) (Noctuidae), were collected from soybean, *Glycine max* (L.) Merr., fields in Mississippi and Louisiana during 2010 and 2011 to determine their susceptibility to novel insecticides. Flubendiamide and chlorantraniliprole are diamide insecticides that have recently been registered for use in field crops. Baseline data were collected for each of these insecticides as well as for methoxyfenozide, which has been the recommended insecticide for soybean looper in Mississippi soybeans prior to the introduction of these new novel insecticides. Mean LC_{50} values for flubendiamide and chlorantraniliprole were similar among the populations tested, and susceptibility was higher for methoxyfenozide compared to flubendiamide and chlorantraniliprole. Diet incorporated assays determined a 9.4-fold variation in susceptibility to flubendiamide among the 7 soybean looper populations tested. Variation to chlorantraniliprole was 6.25-fold and variation for methoxyfenozide was 5.37-fold. Variation in the diamide insecticides was higher than methoxyfenozide with less exposure to soybean looper populations. Documenting variability along with baseline data will be useful in the future for resistance monitoring of soybean loopers to diamide insecticides.

Key Words: Soybean, soybean looper, flubendiamide, chlorantraniliprole, methoxyfenozide

Resumen

Se recolectaron poblaciones del medidor de la soja, *Chrysodeixis includens* (Walker), en campos de soja, *Glycine max* (L.) Merr. en Mississippi y Louisiana en el 2010 y el 2011 para determinar su susceptibilidad a los insecticidas novedosos. El flubendiamida y clorantraniliprol son insecticidas diamidos que recientemente han sido registrados para su uso en cultivos en el campo. Se recogieron los datos de la linea de referencia para cada uno de estos insecticidas, así como para metoxifenozida, que ha sido el insecticida recomendado contra el medidor de la soja en los campos de soja en Mississippi antes de la introducción de estos nuevos insecticidas. El promedio de los valores de CL_{50} para flubendiamide y clorantraniliprol fue similar entre las poblaciones analizadas, y la susceptibilidad fue mayor para metoxifenozida en comparación con flubendiamide y clorantraniliprol. El ensayo de dietas incorporadas determinó una variación de 9.4 veces en la susceptibilidad a flubendiamida entre las siete poblaciones del medidor de la soja probadas. La variación a clorantraniliprol fue 6.25 veces y la variación a metoxifenozida fue 5.37 veces. La variación de los insecticidas diamidos fue mayor que en metoxifenozida con menos exposición a las poblaciones del medidor de la soja. La documentación de variabilidad junto con los datos de la linea de referencia será útil en el futuro para la monitoreo de resistencia del medidor de la soja hacia los insecticidas diamidos.

Palabras Clave: soja, medidor de la soja, flubendiamida, clorantraniliprol, metoxifenozida

The soybean looper, *Chrysodeixis includens* (Walker), has become one of the most costly pests to manage in soybeans because of their ability to consume massive amounts of foliage (Mascarenhas & Boethel 1997). It is a migratory species and populations peak in the southern United

States in mid-Aug to Sep (Carner et al. 1974). Reported annual losses from soybean looper can exceed 10% with regard to crop yield and crop damage plus control costs (Mascarenhas & Boethel 1997). Musser et al. (2010) documented 16.3% of total insect losses in soybean (including control costs) in Mississippi were from soybean looper during 2009. Pyrethroid resistance in soybean looper has been documented where soybean and cotton are grown in the same area (Felland et al. 1990; Leonard et al. 1990; Mink & Boethel 1992). During the mid-1980s, control failures with pyrethroids were commonly reported, even when properly applied at recommended use rates (Felland et al. 1990). Diet overlay experiments were conducted in 1995 on Louisiana strains of soybean looper, and the LC_{50} for permethrin treated diet ranged from 14.69 to 60.87 ppm (Mascarenhas & Boethel 1997). The authors reported all field populations tested in this experiment had significantly higher LC_{50} values than a susceptible USDA strain (LC_{50} = 1.59 ppm). Thus, insecticide resistance evolution in soybean looper populations is a concern.

Recently, 2 novel insecticides were registered in soybean and other crops for control of soybean looper and other lepidopteran pests. Flubendiamide (Belt® 4SC, Bayer CropScience, Research Triangle Park, North Carolina) and chlorantraniliprole (Coragen® 1.67SC, DuPont Crop Protection, Wilmington, Delaware) represent the diamide class of insecticides that react with ryanodine receptors in the muscle cells, causing channels to open and release calcium (Ca^{2+}) into the cytoplasm, leading to muscle paralysis and eventual death (Cordova et al. 2006; Lahm et al. 2007). Data on baseline responses of soybean looper to these compounds are lacking, and establishing initial toxicity ranges for field strains of target pests provides an important reference for future resistance monitoring efforts.

Prior to the introduction of chlorantraniliprole and flubendiamide, methoxyfenozide, (Intrepid® 2F, DowAgrosciences, Indianapolis, Indiana), a member of the diacylhydrazides class of insect growth regulators (IGR), was used extensively to control soybean loopers in Mississippi (Catchot et al. 2010). Diacylhydrazide insecticides mimic the molting hormone ecdysone in lepidopteran insects. Ecdysone is a natural hormone that induces molting and metamorphosis at low levels. In the absence of this hormone, the insect will remain at the larval or immature stage (Sparks 1996). Baseline data for this insecticide on Mississippi soybean looper populations were never established. Control problems were reported during 2009 and 2010 and the lack of baseline data made determining resistance development to methoxyfenozide in soybean looper populations difficult.

Resistance monitoring relies on initial quantification of baseline responses to susceptible populations (Robertson et al. 2007). Therefore, the objectives of this study were to estimate the responses of field-collected populations of soybean looper to chlorantraniliprole, flubendiamide and methoxyfenozide, and to establish baseline response data for flubendiamide and chlorantraniliprole.

Materials and Methods

Insects

Soybean looper larvae were collected from 5 soybean fields during 2010 and 2011 (Table 1) using a 38.1 cm diam sweep net and taken to the laboratory. Larvae were placed individually into 30 mL cups containing an artificial wheat germ-based diet prepared in the laboratory with the addition of linseed oil at 25 mL/3.79 L of diet (BioServ, *Heliothis* diet dry mix USDA item # F9915, Vitamin premix USDA item # 6265 and pure linseed oil USDA item # 5680). Soybean looper colonies were maintained at 24 °C with 60-80% RH and 16:8 L:D. Soybean loopers were allowed to pupate and transferred into a 3.79 L cardboard bucket (approximately 50 per bucket) and allowed to emerge as adults. Adults were fed 20% honey water solution and transferred to clean buckets every 2 days. Eggs were collected every 2 days and allowed to hatch. After eclosion, individual neonates were immediately transferred to 30 mL diet cups using a #000 paint brush. Bioassays

Table 1. Description of soybean looper soybean, *Chrysodeixis includens*, field strains by identification code and collection site.

Code	Year	Location
LAB10[1]	2010	Mississippi State University, Mississippi State , Mississippi
LA10	2010	Franklin Parish; Winnsboro, Louisiana
ST10	2010	Washington County; Stoneville, Mississippi
LAB11[1]	2011	Mississippi State University, Mississippi State , Mississippi
LA11	2011	Franklin Parish; Winnsboro, Louisiana
GW11	2011	Leflore County; Greenwood, Mississippi
TCH11	2011	Holmes County; Tchula, Mississippi

[1]Field reference lab strain; initial collection was made in Tchula, Mississippi during Aug 2009.

were conducted on 3rd instar larvae (20-30 mg) from the F_2 or F_3 generations for each field strain. A field reference lab colony was established from a wild population of soybean loopers because no known sources of laboratory colonies could be obtained. The field reference colony was collected in Tchula, Mississippi in 2009 and kept in laboratory conditions for approximately 2 years. Wang et al. (2010) determined that a field reference colony of the diamondback moth, *Plutella xylostella* (L.), kept in the laboratory for extended periods without exposure (26 and 80 generations) was as susceptible to chlorantraniliprole as a susceptible lab colony.

Bioassays

The artificial diet already described was prepared in the laboratory immediately prior to infestation of larvae. The semi-solid diet was prepared following the manufacturer's standard protocol. One milliliter of each insecticide was added to an appropriate amount of clean wheat germ diet to make a 1 mg a.i./mL stock diet based on the amount of active ingredient in the formulated commercially available insecticide (Table 2). Each insecticide stock diet was prepared in a 500 mL beaker and agitated for 45-60 s with a handheld mixer (Black and Decker, Mirimar, Florida). A total of 6 insecticide concentrations for methoxyfenozide ranged from 0.15-5 µg/mL (Table 2) plus an untreated control. In order to obtain the 5 µg/mL concentration, 2.5 mL of the prepared methoxyfenozide stock diet was added to 500 mL of clean diet. Eight insecticide concentrations for flubendiamide and chlorantraniliprole ranging from 0.15-20 µg/mL (Table 2) were made using serial dilutions. Diet without any insecticide was used as an untreated control. Approximately 9 mL of diet were dispensed into each of 30 plastic diet cups (Solo Cup Co., Highland Park, Illinois) for each concentration and each insecticide. Preliminary assays were used to determine the effective dose range for each compound on the reference lab strain. Each soybean looper colony collected was subjected to the same effective dose range for each insecticide to determine the lethal concentration to kill 50% of the test population (LC_{50}). Thirty 3rd instar larvae (20-30 mg larval weight) from each field-collected strain were subjected to each insecticide dose for 96 h and mortality was recorded. Larvae were considered dead if they had no coordinated movement and were not able to right themselves in 5 s after being flipped onto their dorsal side. Data were recorded as number of individuals alive and number of individuals dead for each concentration. Dose mortality curves were analyzed using probit analysis (SAS Institute 2009), and lethal concentration (required to kill 50% of a test population) estimates were produced for each colony and insecticide. Data were corrected for control mortality (Abbott 1925) and non-overlapping confidence limits (95%) were used to determine differences among populations.

Results and Discussion

Prior to the registration of flubendiamide in soybean, methoxyfenozide was the primary insecticide recommended for soybean looper control in soybeans. However, baseline data for this insecticide were never produced for Mississippi populations. Nevertheless, it is critically important to document susceptibility of field populations prior to the occurrence of field control failures. Responses of soybean looper populations exposed to methoxyfenozide varied by 5.37 fold (LC_{50} = 0.27-1.45 µg/mL diet) (Table 3). No significant differences in LC_{50} were observed among the colonies tested. Overall LC_{50} values of methoxyfenozide were significantly lower than those for chlorantraniliprole based on non-overlapping confidence intervals (Tables 3 and 4).

Soybean looper larvae collected from different soybean fields within Mississippi and Louisiana showed varying levels of susceptibility to flubendiamide (Table 5). Susceptibility of soybean loopers to flubendiamide varied by 9.2 fold (1.02-9.4 µg/mL diet). Mortality for all tested populations indicated a good fit to a probit model (Pearson's X^2 test; $P > 0.05$). The ST10 and LAB10 populations had LC_{50} values of 3.12 and 3.02 µg a.i./mL diet, respectively, and the LA10 colony had an LC_{50} of 9.4 µg a.i./mL diet. However, these differences among the 2010 colonies were not significant (Ta-

Table 2. Insecticide information for products evaluated against soybean loopers, *Chrysodeixis includens*.

Trade Name	Active ingredient	kg ai/ L	Formulation	mL diet in stock[a]	Manufacturer	Dose Range (µg/mL)
Belt®	flubendiamide	0.48	SC[1]	480	Bayer CropScience	0.15-20
Coragen®	chlorantraniliprole	0.2	SC[1]	200	DuPont	0.15-20
Intrepid®	methoxyfenozide	0.24	F[3]	240	Dow Agrosciences	0.15-5

[1]One mL of insecticide was added to clean diet to obtain a 1 mg/mL concentration based on amount of active ingredient in the commercially available insecticide.

[2]Suspension concentrate

[3]Flowable

TABLE 3. SUSCEPTIBILITY OF SOYBEAN LOOPERS, *CHRYSODEIXIS INCLUDENS*, TO METHOXYFENOZIDE (INTREPID®) IN DIET INCORPORATED BIOASSAYS 96 H AFTER EXPOSURE.

Colony	Gen.[1] Tested	N^2	LC_{50} (95% CL) µg/mL	LC_{90} (95% CL) µg/mL	Slope Ln(rate) ± SE	*Pearson's* X^2
LAB10	F12+	180	0.92 (0.34-1.97)	17.30 (6.6-119.82)	0.43 ± 0.09	5.5
ST10	F2	180	1.44 (0.67-2.72)	17.42 (7.73-88.05)	0.50 ± 0.11	2.2
LA10	F2	180	1.45 (0.46-4.75)	7.95 (2.95-397.85)	0.75 ± 0.18	11*
GW11	F3	180	0.47 (na)	4.82 (na)	0.55 ± 0.29	1.1
LA11	F3	180	0.87 (0.33-1.74)	11.78 (5.31-51.61)	0.49 ± 0.10	2.5
TCH11	F3	180	0.62 (0.14-1.57)	16.11 (5.5-151.65)	0.39 ± 0.09	3.5
LAB11	F24+	180	0.27 (0.0008-1.33)	5.28 (0.89-24.86)	0.43 ± 0.14	0.77
Overall[3]		**900**	**0.96 (0.47-1.61)**	**12.18 (6.82-30.03)**	**0.49 ± 0.04**	**31.51**

[1]Number of generations in the laboratory when tested.
[2] Number of individuals tested excluding controls.
[3]LAB colony data excluded from overall analysis
*Indicates a significant chi square value.

TABLE 4. SUSCEPTIBILITY OF SOYBEAN LOOPERS, *CHRYSODEIXIS INCLUDENS*, TO CHLORANTRANILIPROLE (CORAGEN®) IN DIET INCORPORATED BIOASSAYS 96 HRS AFTER EXPOSURE.

Colony	Gen.[1] Tested	N^2	LC_{50} (95% CL) µg/mL	LC_{90} (95% CL) µg/mL	Slope Ln(rate) ± SE	Pearson's X^2
LAB10	F12+	210	0.8 (0.18-1.85)	9.12 (4.42-21.38)	0.53 ± 0.09	1.4
ST10	F3	240	5.01 (3.54-7.08)	29.84 (18.01-70.68)	0.71 ± 0.11	4.9
LA10	F3	210	2.7 (1.78-4.48)	25.50 (13.20-75.50)	0.57 ± 0.08	6.8
GW11	F3	240	1.8 (0.86-3.15)	23.20 (12.46-58.91)	0.50 ± 0.07	4.6
LA11	F3	240	0.71 (0.17-1.62)	14.4 (6.91-41.22)	0.42 ± 0.07	2.5
TCH11	F3	240	2.5 (0.41-5.98)	68.08 (27.81-496.75)	0.39 ± 0.09	0.97
LAB11	F24+	240	0.83 (0.18-1.89)	12.02 (6.22-28.81)	0.48 ± 0.0.09	0.86
Overall[3]		**1170**	**1.92 (0.73-2.71)**	**34.19 (20.72-69.02)**	**0.51 ± 0.03**	**22.6**

[1]Number of generations in the laboratory when tested.
[2]Number of individuals tested excluding controls.
[3]LAB colony data excluded from overall analysis.

TABLE 5. SUSCEPTIBILITY OF SOYBEAN LOOPERS, *CHRYSODEIXIS INCLUDENS*, TO FLUBENDIAMIDE (BELT®) IN DIET INCORPORATED BIOASSAYS 96 HRS AFTER EXPOSURE.

Colony	Gen.[1] Tested	N^2	LC_{50} (95% CL) µg/mL	LC_{90} (95% CL) µg/mL	Slope Ln(rate) ± SE	Pearson's X^2
LAB10	F12+	180	3.02 (0.41-7.6)	49.7 (20.31-287.76)	0.45 ± 0.11	0.6
ST10	F2	140	3.12 (1.29-5.9)	37.34 (17.54-147.34)	0.51 ± 0.10	4.2
LA10	F2	210	9.4 (5.5-19.1)	128.54 (50.14-738.47)	0.49 ± 0.08	8.5
GW11	F2	240	2.19 (1.2-3.8)	34.60 (16.70-105.96)	0.46 ± 0.06	9.3
LA11	F2	240	1.67 (0.64-2.99)	19.03 (10.82-46.70)	0.53 ± 0.09	1.7
TCH11	F2	180	1.02 (0.44-1.80)	9.32 (4.91-29.42	0.58 ± 0.11	1.5
LAB11	F24+	240	2.05 (0.11-6.59)	101.94 (33.24-1428)	0.32 ± 0.09	0.56
Overall[3]		**1010**	**2.89 (1.39-5.09)**	**15.17 (8.83-28.98)**	**0.45 ± 0.03**	**32.2**

[1]Number of generations in the laboratory when tested.
[2]Number of individuals tested excluding controls.
[3]LAB colony data excluded from overall analysis.

ble 4). Similarly in 2011, none of the field-collected soybean looper populations were significantly different from the reference lab colony. Overall, susceptibility of soybean looper populations tested against flubendiamide was not significantly different than soybean looper susceptibility to methoxyfenozide and chlorantroniliprole.

Soybean looper populations exposed to chlorantraniliprole had LC_{50} values that ranged by 6.25 fold (0.8 to 5.01 µg/mL diet) (Table 4). Overall, susceptibility of soybean looper populations to chlorantraniliprole did not differ from that of flubendiamide. ST10 was less susceptible to chlorantraniloprole than LAB10 and LAB11. However, susceptibility of ST10 was not different from LA10. Susceptibility of LA10 to chlorantraniliprole was not different than LAB10 or LAB11 colonies. Susceptibility of the field collections from LA varied by 3.8 fold (Table 4).

Susceptibility of insect populations to stomach poisons, such as chlorantraniliprole and flubendiamide, has been documented previously. Ashfaq et al. (2010) documented 5.18 fold variation to chlorantraniliprole in field populations of *Choristoneura roseceana* (Harris) (Tortricidae) with limited exposure collected from orchards in Washington State. The authors suggested that this variation in susceptibility could lead to more rapid resistance development after widespread exposure in the field. Temple et al. (2009) found that susceptibility of bollworm, *Heliocoverpa zea* Boddie (Noctuidae), populations to chlorantraniliprole varied by 4.5 fold. However, Wang et al (2010) reported variation in susceptibility of the diamondback moth (*Plutella xyllostella* (L.); Plutellidae) in China was less than 5-fold for chlorantraniliprole. Variation in pyrethroid susceptibility among soybean looper populations was also reported previously by Leonard et al. (1990), along with reduced field efficacy of the pyrethroids commonly used at that time. Currently, pyrethroids are not recommended for soybean looper management in soybean fields because of the level of resistance they have developed. Therefore, documenting evidence of variation in susceptibility to novel insecticides, such as flubendiamide and chlorantraniliprole, prior to their widespread use is important so that resistance management techniques can be implemented to preserve these insecticides.

Feeding cessation was not quantified in this experiment; however, it was observed at every concentration of flubendiamide and chloratraniliprole utilized in the bioassays. Insecticide concentrations as low as 0.15 µg/mL of diamide insecticides reduced feeding and decreased the overall size of insects when assays were rated. Consistent reduction in the amount of feeding and rapid feeding cessation from chloratraniliprole has been documented in various lepidopteran species (Hannig et al. 2009). Time after exposure to chlorantraniliprole to stop feeding for cabbage looper, *Trichloplusia ni* (Hübner) (Noctuidae) was 23.4 min, whereas, feeding ceased 408.8 min after exposure to methoxyfenozide. The effectiveness of these insecticides in field applications could be due to a reduction in feeding, causing larvae to become weak and fall from the plant. An effective dose within a field may not be enough to kill the insect immediately but prevent further feeding. Control failures with non-selective nerve poisons, such as pyrethroids and carbamates, were not hard to document because failures in the field were easily detected in the laboratory. Documenting control failures with diamides may not be easy because of the difference between the amount required to kill the insect and the dose needed to subdue or stop feeding and growth.

Delaying or preventing resistance development to insecticides is important to the sustainability of integrated pest management (IPM) in soybeans. Soybean loopers have developed resistance to pyrethroid insecticides (Felland et al. 1990; Portillo et al. 1993), and the number of labeled insecticides effective against soybean loopers is limited. Therefore it was important to document the variation in susceptibility of soybean looper populations to the diamide class of insecticides for future reference in the event of control failures. Few control failures of soybean looper to methoxyfenozide have been reported to date in Mississippi. However, in the event of a control failure, the overall LC_{50} for field populations collected in Mississippi and Louisiana was 0.96 µg/mL diet with 5.37 fold variation. These data will be useful in determining resistance ratios if control failures are reported in the future.

Flubendiamide and chloratraniliprole will likely be applied to many soybeans in the future. Monitoring the susceptibility of pest populations to these insecticides is important. Results presented here determined a 6.25 and 9.2-fold variation in soybean looper populations collected in Mississippi and Louisiana for chloratraniliprole and flubendiamide, respectively. However, overall susceptibility of soybean looper to flubendiamide and chlorantraniliprole was the same (LC_{50} = 2.89 and 2.61, respectively). Resistance management strategies and documenting variability of natural populations to an insecticide prior to its widespread use can influence resistance management decisions for these novel insecticides and should be taken into consideration when insecticide applications are made.

Summary

A 6.25 and 9.2 fold difference in susceptibility to flubendiamide and chlorantraniliprole was observed from field populations of soybean loopers collected from Louisiana and Mississippi, respectively. However, the overall susceptibility of

flubendiamide and chlorantraniliprole among the tested populations was not different. Overall LC_{50} for field populations collected in Mississippi and Louisiana to methoxyfenozide was 0.96 µg/mL diet with 5.37 fold variation.

Acknowledgments

We thank Kathy Knighten and Teresa Zeigelemann for their assistance in rearing field populations and maintaining a soybean looper colony in the laboratory.

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