RYNAXYPYR (DPX-E2Y45) AND CYPERMETHRIN: SUSCEPTIBILITY OF SELECTED LEPIDOPTERAN INSECT PESTS

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Abstract

Susceptibility levels of >2,200 bollworm, *Helicoverpa zea* (Boddie), moths to a pyrethroid, cypermethrin, was determined using the adult vial test (AVT) during May to Sep of 2006 and 2007 in Louisiana. In 2006, adult survival at the cypermethrin discriminating dose of 5 µg/vial during May, Jun, Jul, Aug, and Sep was 24%, 57%, 48%, 33%, and 47%, respectively, with a mean annual survival of 42%. In 2007, survival at the 5 µg/vial dose during May, Jun, Jul, Aug, and Sep was 13%, 29%, 33%, 28%, and 44%, respectively, with a mean annual survival of 30%. During 2007, bollworm survival was lower when compared to survival levels recorded during 2005 and 2006. The LD_{50}'s of a field-collected bollworm colony from Winnsboro, Louisiana in topical bioassays were 6.12, 3.48, 3.50, and 2.80 µg/g larval weight, respectively in 2004, 2005, 2006, and 2007. The LD_{50} for the 2004 Winnsboro colony was the highest observed for a field collection from Louisiana and demonstrated resistance ratios (RR) of 7 to 22-fold above a LD_{50} range of previously established pyrethroid-susceptible bollworm colonies. The cypermethrin LD_{50}'s of two pyrethroid-susceptible colonies (LSU and DuPont) were determined to be 0.20 and 0.21 µg/g larval weight, respectively. These results clearly show field populations of bollworms are becoming less susceptible to pyrethroids in laboratory tests. In the rynaxypyr treated-diet assay, several bollworm colonies had similar LC_{50}'s ranging from 0.038-0.089 µg/ml diet. These bollworm colonies were collected from five states and represent a broad geographical range across the United States. The Louisiana colony which demonstrated moderate tolerance to pyrethroid insecticides also expressed a similar level of susceptibility to rynaxypyr when compared to pyrethroid susceptible colonies (LSU lab and DuPont lab). Preliminary studies would indicate a lack of cross-resistance between pyrethroids and rynaxypyr in bollworm.

Introduction

Insecticide resistance in key arthropod pests remains a critical issue for producers and crop consultants in the cotton industry. Initial laboratory bioassays and surveys of field insect populations for changes in insecticide susceptibility are integral components of an insecticide resistance management (IRM) program. Baseline toxicity of novel insecticides in field and laboratory insect strains establishes an important historical reference for future surveys of susceptibility after commercial products are used in crop production systems.

The bollworm, *Helicoverpa zea* (Boddie), remains a primary caterpillar pest of cotton in spite of the release of transgenic *Bacillus thuringiensis* var. k*urstaki* Berliner (Bt) cotton varieties. Resistance to organochlorines, DDT, organophosphates, and carbamates (Sparks 1981, Elzen et al. 1992) has been reported in bollworm populations across the United States. Pyrethroids are still the principal insecticides used for bollworm control in cotton, soybeans, grain sorghum, and many vegetable crops. Transient instances of pyrethroid resistance in bollworm have occurred in South Carolina (Brown et al. 1998) and Texas (Pietrantonio et al. 2005). Changes in pyrethroid

susceptibility of bollworm also have been recorded in Louisiana (Temple et al. 2006) and Mid-West populations (Hutchison et al. 2005, 2007). In Louisiana, mean annual bollworm survival at a discriminating dose of 5 µg cypermethrin in the AVT has increased from 2% in 1988 to 34% in 2002 (Cook et al. 2003).

Novel insecticidal chemical molecules are being constantly discovered and evaluated against selected target pests, especially those such as bollworm, which has the potential to be a consistent economic pest across a wide range of crops. Rynaxypyr (DPX-E2Y45) is classified as an anthranilic diamide insecticide and is currently in development by DuPont Crop Protection. The primary targets for this compound include a complex of lepidopteran pests. The proposed mode of intoxication is unique and the actual target sites within the insect are the ryanodine receptors in the calcium channels of muscles (Lahm et al. 2005). Physical symptoms in intoxicated insects include feeding cessation, lethargy, paralysis, and death. Anthranilic diamides appear to provide plant protection at relatively low application rates compared to many other available commercial insecticides (Cordova et al. 2006). Rynaxypyr has a very low mammalian toxicity with several target insect species demonstrating 500-fold greater susceptible than that of mammals (Cordova et al. 2006). Currently, DuPont is pursuing labels for this insecticide in many field and vegetable crops. This compound has also been licensed to Syngenta in pre-mixes with several of their proprietary products.

To date in Louisiana, there have been no bollworm control failures after pyrethroid applications which could be related to a change in susceptibility of those populations. This report summarizes the results of bollworm monitoring efforts using cypermethrin in AVT and laboratory topical bioassays during 2006 and 2007. In addition, the baseline responses of bollworm colonies to rynaxypyr in a diet-incorporation assay are included.

Materials and Methods

Adult Vial Tests (Cypermethrin)

Male bollworm moths were collected using wire cone traps baited with synthetic pheromone lures (Zealure®, Hercon Environmental, Emigsville, PA) from May through September during 2006 and 2007. Moths were collected from ten and seven sites within Louisiana cotton production areas during 2006 and 2007, respectively. The most intensive sampling occurred at four research stations located in Bossier, Franklin, Rapides, and Tensas parishes. Adult vial bioassays (AVT) similar to those described by Plapp et al. (1987), Cook et al. 2003, and Temple et al. 2006 were utilized to monitor the susceptibility of field-collected bollworm moths to cypermethrin (Chem Services, West Chester, PA).

Topical Application Procedure (Cypermethrin)

Bollworm larvae were collected from cotton and sweet corn fields at the Macon Ridge Research Station near Winnsboro, LA, during 2004-2007. These larvae were placed on diet (Heliothis Pre-Mix, Ward Natural Science., Rochester, NY) in 1 oz cups (Solo Cup Co., Urbana, IL) and allowed to complete one generation in the laboratory to achieve adequate numbers of larvae for laboratory topical bioassays. Pyrethroid-susceptible colonies (LSU and DuPont) maintained in laboratories also were tested to confirm cypermethrin toxicity. Technical grade cypermethrin was dissolved in acetone and serial dilutions were used to yield the desired insecticide concentrations. One µl of insecticide solution was applied to the thoracic dorsum of each larva using a Hamilton micro-syringe. Control (non-insecticide treated) larvae were treated with 1 µl of acetone. A minimum of 30 larvae (20-30 mg larval weight; average of 25 mg) per dose were utilized in all bioassays. Mortality was determined at 72 hours after treatment (HAT). A larva was considered dead if it could not right itself after being placed on its dorsal surface. Data were corrected for control mortality and analyzed with probit analysis using Polo PC (LeOra Software 1987). LD_{50} values were obtained from probit analysis and resistance ratios (RR) were calculated from pyrethroid-susceptible reference colonies (Louisiana Lab and Field colonies 1998). Non-overlapping confidence limits (95%) were used indicate significant differences among colonies.

Diet Incorporation (Rynaxypyr)

Bollworm larvae were collected from several states including Georgia, North Carolina, Virginia, Delaware, and Louisiana during 2007. These larvae were placed on diet (Heliothis Pre-Mix, Ward Natural Science, Rochester, NY) in 30 ml plastic cups (Solo Cup Co., Urbana, IL) and allowed to complete one generation in the laboratory to achieve adequate numbers of larvae for laboratory insecticide treated-diet bioassays. Pyrethroid-susceptible

colonies (LSU and DuPont) also were tested as negative controls to compare with the results generated for the field collections of larvae. A meridic semi-solid diet was prepared following the manufacturer's suggested protocol. Formulated rynaxypyr (35WG) was dissolved in distilled water to create a stock solution of 100 µg/ml. Serial dilutions were used to develop the desired concentrations of rynaxypyr were diluted, but were standardized to a total insecticide/water volume of 40 ml. The selected concentrations of rynaxypyr were mixed with the meridic diet to yield 200 ml of a diet/insecticide mixture. The mixtures then were agitated for 30-45 s in a 2 liter bowl using a hand mixer. Insecticide-treated diet was then placed in 30 ml cups with approximately 7 ml of diet per cup. Insecticide concentrations ranged from 0.01 µg/ml to 0.75 µg/ml of diet. The insecticide-treated diet was used within 7 d of preparation. Three to four replicates (20-50 larvae per dose) were used for each colony. Third instar bollworm larvae (similar size range as used in topical bioassays) were placed on insecticide-treated and non-treated (control) diet. Insects were evaluated at 96 HAT for mortality. A larva was considered dead if it could not right itself after being placed on its dorsal surface. Data were corrected for control mortality and analyzed with probit analysis using Polo-Plus (LeOra Software 2006) to obtain LD_{50} values.

Results and Discussion

Over 2,200 bollworm moths were assayed for pyrethroid (cypermethrin) susceptibility during 2006 and 2007 with the AVT. In 2006, survival at the cypermethrin discriminating dose of 5 µg/vial during May, Jun, Jul, Aug, and Sep was 24%, 57%, 48%, 33%, and 47%, respectively, with a mean annual survival of 42% (Table 1). In 2007, survival at 5 µg/vial during May, Jun, Jul, Aug, and Sep was 13%, 29%, 33%, 28%, and 44%, respectively, with a mean annual survival of 30%. During 2007, bollworm survival was the lower when compared to that observed during 2004, 2005, and 2006. Mean annual bollworm survival at a cypermethrin dose of 10 µg/vial was 20% and 17% during 2006 and 2007 respectively, (Figure 1).

The cypermethrin LD_{50}'s of Winnsboro field-collected bollworm derived from topical bioassays were 6.12, 3.48, 3.50, and 2.80 µg/g larval weight during 2004, 2005, 2006, and 2007, respectively, (Table 2). The LD_{50} value for the 2004 Winnsboro field colony was the highest historical value observed for a Louisiana field collection and demonstrated a range of resistance ratios (RR) from 7 to 22-fold above a LD_{50} range previously established for pyrethroid-susceptible bollworm colonies. The LD_{50}'s of two pyrethroid-susceptible colonies maintained in LSU and DuPont laboratories were 0.20 and 0.21 µg/g, respectively. All field collections from 2004-2007 had cypermethrin LD_{50}'s that were significantly higher than that for pyrethroid-susceptible laboratory colonies. The LD_{50} values of the 2004-2007 colonies ranged from 2.80 to 6.12 µg/g larval weight and had RR's of 4 to 22 fold.

In the rynaxypyr-treated diet assay, preliminary results indicate that field cultures of bollworm collected during 2007 had similar responses to Rynaxypyr (Table 3). All colonies had similar LC_{50}'s ranging from 0.038-0.089 µg/ml diet. These bollworm colonies were collected from five states and represent a broad geographical range across the United States. The Louisiana field colony expressed some tolerance to cypermethrin, but susceptibility to rynaxypyr was similar to that of pyrethroid-susceptible colonies (LSU and DuPont). Preliminary studies indicate no cross-resistance between rynaxypyr and pyrethroids in bollworm.

No field control failures of bollworms with pyrethroids have been reported in Louisiana to date, but crop consultants have reported reduced efficacy against late-season infestations in several crops. The low cost of pyrethroids allows them to be viable option for insect pest problems in many crops that are grown adjacent to cotton in Louisiana. Bollworms are possibly receiving inadvertent selection pressure from pyrethroid sprays in field corn, grain sorghum, and soybeans. A reduction in pyrethroid susceptibility of bollworms is probably due to fewer non-treated (pyrethroid) crop hosts.

Rynaxypyr in laboratory studies has shown to be a very potent insecticidal molecule (Temple et al. 2007). This insecticide has demonstrated very good activity at extremely low use rates on several important caterpillar pests of cotton including bollworm, tobacco budworm, *Heliothis virescens* (F.) and fall armyworm, *Spodoptera frugiperda* (J. E. Smith). Rynaxypyr has performed as well as or better than the standard insecticides for control of the Heliothine complex in cotton field trials (Bachelor et al. 2007, Hardke et al. 2007). This product could be an excellent option for insect resistance management strategies by offering an alternative class of chemistry for lepidopteran pest management in cotton.

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Table 1. Percent survival by month of male bollworm adults exposed to 5 µg/vial cypermethrin.

	Month-Percent Survival					Annual	No.
Year	May	Jun	Jul	Aug	Sep	Mean	Tested
1988	0	0	3	2	3	2	439
1989	-	-	5	6	3	4	170
1990	0	0	6	1	2	2	561
1991	2	5	7	4	8	5	1666
1992	3	2	11	7	12	8	932
1993	-	0	7	7	9	7	483
1994	3	9	10	8	-	8	500
1995	3	0	8	7	-	6	580
1996	4	3	9	5	-	7	3697
1997	4	4	14	7	-	9	1821
1998	12	14	27	19	-	18	1950
1999	18	13	15	16	-	16	809
2000	13	20	19	14	-	16	1445
2001	10	6	32	13	16	21	829
2002	-	28	42	26	28	34	1119
2003	50	27	33	34	-	31	1050
2004	9	16	30	21	30	23	1010
2005	23	34	43	43	38	38	1300
2006	24	57	48	33	47	42	1200
2007	13	29	33	28	44	30	1000

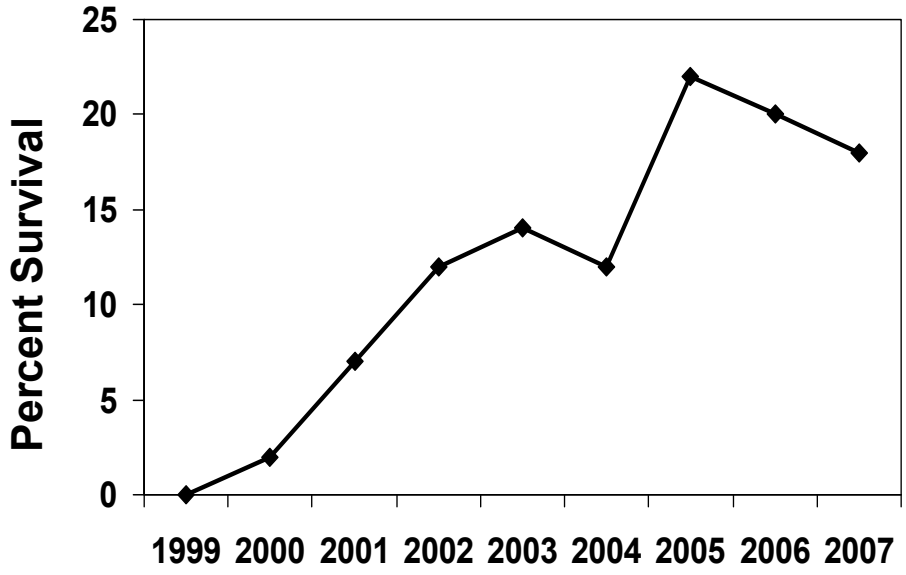

Figure 1. Mean percent survival by year for male bollworm adults collected in Louisiana and exposed to 10 µg/vial cypermethrin.

Table 2. Response of bollworm larvae exposed to cypermethrin in topical bioassays.

Colony	N	LD_{50}[a]	95% CL[b]	Slope	X^2	RR
Winnsboro-04	159	6.12	4.92-7.76	2.92±0.37	4.65	7-22
Winnsboro-05	115	3.48	2.36-4.80	1.99±0.33	3.82	4-12
LSU Lab-06	240	0.20	0.12-0.25	1.82±0.22	3.80	NA
Winnsboro -06	220	3.50	2.76-4.36	2.04±0.25	1.46	4-12
Winnsboro-07	190	2.80	2.08-3.72	1.80±0.26	2.54	3-10
DuPont Lab-07	240	0.21	0.16-0.28	1.70±0.20	3.32	NA

Resistance Ratios (RR) calculated from LD_{50}[a] data (0.28 to 0.88 µg/g larval weight) derived from Louisiana lab and field collections 1998 (Unpublished Data, J. Adamcyck and J. Gore LSU Dept. of Entomology).
[a]µg/g larval weight.
[b]Confidence limits.

Table 3. Bollworm larval susceptibility to rynaxypyr in an insecticide–treated diet assay.

Colony	Replicates	N	LC_{50}[a]	95% CL[b]	Slope	X^2
LSU Lab BW	4	280	0.069	0.049-0.099	2.85+-0.31	11.82
Georgia BW	3	240	0.041	0.034-0.049	3.15+-0.41	1.80
North Carolina BW	3	180	0.050	0.039-0.063	2.56+-0.36	3.43
Virginia BW	3	240	0.038	0.031-0.047	2.69+-0.34	2.90
Delaware BW	3	210	0.089	0.065-0.123	2.23+-0.28	4.27
Louisiana BW	3	210	0.061	0.049-0.073	2.83+-0.41	3.12
DuPont Lab BW	3	240	0.039	0.031-0.047	2.78+-0.35	2.69

[a]µg/ml diet.
[b]Confidence limits.