

ARTHROPOD MANAGEMENT

Susceptibility of Selected Lepidopteran Pests to Rynaxypyr®, a Novel Insecticide

J.H. Temple*, P.L. Pommireddy, D.R. Cook, P. Marçon, and B.R. Leonard

ABSTRACT

The use of foliar insecticides has historically been a common management strategy for lepidopteran pests in cotton, *Gossypium hirsutum* (L.). Bioassays to establish initial toxicity levels and surveys of changes in insecticide susceptibility for field insect populations are critical components of an insecticide resistance management (IRM) program. A novel insecticide in the anthranilic diamide class, rynaxypyr® (chlorantraniliprole), has demonstrated significant levels of toxicity to several lepidopteran targets in preliminary field screening trials. The objectives of this study were to evaluate three bioassay methods (insecticide-treated diet, topical application, and adult vial test) for potential use in future insecticide resistance surveys and to determine the baseline toxicity of rynaxypyr® to selected lepidopteran pests. Rynaxypyr® was highly toxic to bollworm, *Helicoverpa zea* (Boddie), fall armyworm, *Spodoptera frugiperda* (J.E. Smith), and tobacco budworm, *Heliothis virescens* (F.), in all three laboratory bioassay procedures. Larvae of the three species demonstrated similar susceptibility to rynaxypyr® in the insecticide-treated diet bioassay with LC₅₀ values ranging from 0.02-0.09 ppm. Rynaxypyr® susceptibility among the three species was also observed in the topical application assay with LD₅₀ values ranging from 0.52 to 1.52 µg/g larval weight. There were no significant differences in rynaxypyr® susceptibility between the laboratory tobacco budworm (LSU lab TBW, 1.21 µg/vial) and field-collected fall armyworm (Louisiana FAW, 1.71 µg/vial) colonies in the adult vial test (AVT). No evidence of rynaxypyr® cross-

resistance was detected in pyrethroid-resistant bollworm and tobacco budworm colonies. The consistent results generated with the bioassay procedures suggest that all methods can be used for future rynaxypyr® resistance surveys of these target pests.

Historically, insecticide resistance in key arthropod pests has been a major concern for producers and crop consultants in the cotton, *Gossypium hirsutum* (L.), industry because of the heavy reliance on chemical control strategies. There has been a continuing need for new insecticide molecules in cotton due to target pests' ability to develop resistance. Initial laboratory bioassays to determine toxicity of novel insecticides to target pests are important. Surveys of field insect populations for changes in insecticide susceptibility are also an integral component of an insecticide resistance management (IRM) program. Geographical susceptibility of target pests should be established before a novel insecticide compound like rynaxypyr® (chlorantraniliprole) is widely used and while the frequency of non-susceptible individuals is low (French-Constant and Roush, 1990). Establishing the initial toxicity of novel insecticides to field and laboratory strains of target insects is an important historical reference for future monitoring programs.

Lepidopteran pests are the most damaging insects of cotton in the United States. In 2007, lepidopteran pests infested 11.5 million acres, caused the loss of 245 thousand bales of cotton, and cost producers an average of 14 dollars/acre to control (Williams, 2008). The primary tools for controlling lepidopteran pests in cotton are insecticide sprays and the use of transgenic *Bacillus thuringiensis* var. *Kurstaki* Berliner (*Bt*) cotton cultivars. Several important lepidopteran pests infest cotton in the United States including: bollworm, *Helicoverpa zea* (Boddie); tobacco budworm, *Heliothis virescens* (F.); fall armyworm, *Spodoptera frugiperda* (J. E. Smith); pink bollworm, *Pectinophora gossypiella* (Saunders); beet armyworm, *Spodoptera exigua* (Hübner); and soybean looper, *Pseudoplusia includens* (Walker). Transgenic Bt cotton varieties

J. H. Temple*, P. L. Bommireddy, B. R. Leonard, Department of Entomology, Louisiana State University AgCenter, 404 Life Sciences Building, Baton Rouge, LA 70803; D. R. Cook, Mississippi State University, North Mississippi Research and Extension Center, P.O. Box 1690, Verona, MS 38879; P. Marçon, DuPont Crop Protection, 1090 Elkton Road, Newark - DE, 19711.

*Corresponding author: jtemple@agcenter.lsu.edu

(Bollgard®) introduced in 1996 effectively managed key lepidopteran pests such as the tobacco budworm and pink bollworm. However, Bollgard® has provided inconsistent control of other important lepidopteran pests of cotton such as bollworm, fall armyworm, beet armyworm, and soybean looper. Supplemental applications of insecticide sprays have been necessary to control infestations of these pests in numerous instances (Stewart et al., 2000; Leonard et al., 2003).

Historically, shifts in insecticide susceptibility have created insecticide-resistant populations of these pests. Resistance to organochlorines, DDT, organophosphates, and carbamates (Sparks, 1981; Elzen et al., 1992) has been reported in bollworm and tobacco budworm. Pyrethroid resistance has also been reported in tobacco budworm (Plapp et al., 1990; Elzen et al., 1992). A general decrease in pyrethroid susceptibility of bollworm has been reported in South Carolina (Brown et al., 1998), Texas (Pietrantonio et al., 2005), Louisiana (Temple et al., 2006; 2008) and in Mid-West populations (Hutchison, 2005; Hutchison et al., 2007). Fall armyworm resistance to pyrethroids, carbamates, and organophosphates has also been reported (Yu, 1992). The performance of insecticides against these pests is important in many cotton production systems, especially those using non-Bt cotton cultivars (41% of U.S. acreage) and in many other field, fruit, and vegetable crops that these pests may infest.

Development of insecticides that act on novel biochemical pathways is essential due to the propensity of target pest populations to develop resistance. Rynaxypyr® is a novel anthranilic diamide insecticide developed by DuPont Crop Protection and recently registered in the United States for control of a broad range of insect pests (Anonymous 2007 ; Yu,

2008). This class of insecticides targets the ryanodine receptors in muscle cells (Lahm et al., 2005). Activation of these receptors causes unregulated release of internal Ca^{2+} stores leading to depletion of calcium, muscle paralysis, and ultimate death (Cordova et al., 2007). The symptoms of rynaxypyr® intoxication in treated insects include feeding cessation, lethargy, paralysis, and regurgitation. Rynaxypyr® has demonstrated very low mammalian toxicity and a favorable eco-toxicological profile (Cordova et al., 2006). In addition, Rynaxypyr® has been highly efficacious against several lepidopteran species at relatively low application rates (Cordova et al., 2006; Lahm et al., 2007). The objectives of this study were to determine the baseline toxicity of rynaxypyr® to three major lepidopteran pests of cotton and to evaluate three bioassay methods for potential use in future insecticide resistance monitoring surveys.

MATERIALS AND METHODS

Lepidopteran Target Species. The insect colonies for these bioassays included both insecticide-susceptible laboratory strains and colonies derived from field populations. Bollworm colonies were established (minimum of 300 larvae collected) from several crops and states representing diverse geographic regions across the United States during 2007 (Table 1). A Louisiana field collection was made from sweet corn, *Zea mays* L., in 2007 (Louisiana BW), and laboratory colonies (LSU Lab BW and DuPont Lab BW) originally obtained from Bio-Serv (Frenchtown, NJ) and Chesapeake Pearl (Newark, DE) insectaries. The LSU BW laboratory colony has been in culture since 1998 and is highly susceptible to pyrethroids. The tobacco budworm laboratory colony (LSU Lab

Table 1. Description of insect species, collection site/date, and original host.

Species	Colony Name	Collection Site	Host	Date
<i>Heliothis virescens</i>	LSU Lab TBW	N.E. Louisiana	cotton	August 1977
<i>Heliothis virescens</i>	Louisiana TBW	Winnsboro, LA	garbanzo beans	July 2006
<i>Helicoverpa zea</i>	LSU Lab BW	Lab	Lab	Lab
<i>Helicoverpa zea</i>	Georgia BW	Leary, GA	peanut	July 2007
<i>Helicoverpa zea</i>	North Carolina BW	Jamesville, NC	field corn	July 2007
<i>Helicoverpa zea</i>	Virginia BW	Eastville, VA	field corn	July 2007
<i>Helicoverpa zea</i>	Delaware BW	Georgetown, DE	tomato	August 2007
<i>Helicoverpa zea</i>	Louisiana BW	Winnsboro, LA	sweet corn	August 2007
<i>Helicoverpa zea</i>	Chesapeake Pearl BW	Lab	Lab	Lab
<i>Spodoptera frugiperda</i>	Louisiana FAW	Winnsboro, LA	field corn	July 2006

TBW) has been in continuous culture since 1977 and is also susceptible to pyrethroids. A field collection of pyrethroid-resistant tobacco budworm larvae (Louisiana TBW) was obtained in early August 2007 from a garbanzo bean, *Cicer arietinum* L., field near Winnsboro, LA. The fall armyworm field colony (Louisiana FAW) utilized in these studies was collected from a conventional field corn, *Zea mays* L. hybrid, during late July 2006 near Winnsboro, LA. All larvae were reared on a laboratory meridic diet (Heliothine Premix, Ward's Natural Science, Rochester, NY). Adults were fed a 20% solution of sucrose and water. The colonies were maintained at a 14:10 (L:D) photoperiod at 80° F with a relative humidity of 70-80%. Field-collected insects were allowed to complete 1-2 generations in the laboratory before bioassays. The F₁ or F₂ progeny was then exposed to rynaxypyr® in selected bioassays.

Insecticide-Treated Diet. A meridic semi-solid diet (Heliothine Premix) was prepared following the manufacturer's suggested protocol. Formulated rynaxypyr® (35% wettable granular [WG] DuPont Crop Protection, Newark, DE) was dissolved in distilled water to create a stock solution of 100 µg/ml. Serial dilutions of desired concentrations of rynaxypyr® were diluted in 40 ml of distilled water. Desired concentrations (40 ml) of rynaxypyr® in a water solution were mixed with diet to yield 200 ml of insecticide-treated diet. The insecticide-treated diet was agitated for 30-45 s in a 2 liter bowl using a hand mixer to distribute insecticide evenly (Black and Decker, Miramar, FL). Insecticide-treated diet (7 ml/cup) was then placed in 30 ml plastic cups (Solo Cup Co., Highland Park, IL). Insecticide concentrations ranged from 0.01 parts per million (ppm) to 0.75 ppm of diet. The insecticide-treated diet was stored in the refrigerator (4°C) and used within 7 d of preparation. Three to five replicates (20-50 larvae per concentration) were used for each colony. Third instar larvae (20-30 mg) were utilized in tobacco budworm and bollworm bioassays. Third instar larvae (25-35 mg) were also utilized in fall armyworm bioassays. Larvae were placed on rynaxypyr®-treated and non-treated (control) diet. Insects were evaluated at 96 h after exposure (HAE) for mortality. Larvae were considered dead if they could not right themselves after being placed on their dorsal surface. Data were corrected for control mortality (Abbott, 1925) and analyzed with probit analysis using Polo-Plus (LeOra Software, 2006) to obtain dose mortality (LC₅₀ and LC₉₀) values. Non-overlapping confidence limits (95%) were used to detect significant differences among colonies and species.

Direct Application of Insecticide to Larvae.

Topical application bioassays generally utilize acetone as the solvent/diluent to dissolve technical grade products, but the insolubility of technical grade rynaxypyr® (99.2%, DuPont Crop Protection, Newark, DE) in acetone required an alternative solvent. Rynaxypyr® was dissolved in dimethyl sulfoxide (DMSO) and dilutions were adjusted to yield the desired insecticide concentrations. A range (0.01 µg/µl to 0.75 µg/µl) of doses was utilized in this bioassay. One µl of insecticide solution was applied to the thoracic dorsum of each larva with a Hamilton micro-syringe (Hamilton Co., Reno, NV). Control larvae were treated only with 1 µl of DMSO. Three to six replicates with 20-50 larvae per dose were used to obtain dose mortality values for the target colonies. Third instars of tobacco budworm (20-30 mg), bollworm (20-30 mg) and fall armyworm (25-35 mg) were used in the bioassays. Mortality was determined at 72 h after treatment (HAT). Larval mortality was determined in the same manner as in the insecticide-treated diet bioassays. Data were corrected and analyzed as previously described to obtain dose mortality (LD₅₀ and LD₉₀) values. LD values and confidence limits were standardized to µg/g larval weight for comparison among species. Non-overlapping confidence limits (95%) were used to indicate significant differences among colonies and species.

Insecticide Residue on Glass. Adult vial test (AVT) bioassays similar to those described by Plapp et al. (1987) were used to determine the susceptibility of selected lepidopteran adults (moths) to rynaxypyr®. Technical grade rynaxypyr® did not readily dissolve in several commercial solvents including acetone, methanol, or ethanol. Therefore, a stock solution of rynaxypyr® was prepared by dissolving formulated rynaxypyr® (35% WG, DuPont Crop Protection, Newark, DE) in a solution of distilled water (5%) and acetone (95%). Serial dilutions were generated from the stock solution to yield desired insecticide concentrations. Concentrations used in the AVT ranged from 0.1 µg/vial to 20 µg/vial. The interior surface of 20 ml glass scintillation vials was coated with 0.5 ml of the appropriate insecticide solution. Uncapped vials were then rotated on a modified hot dog roller (Star Manufacturing International, St. Louis, MO) (heating element disconnected) until all acetone/water solution evaporated leaving only the insecticide

residue. Vials were stored in a dark environment at room temperature until needed for the bioassays. Freshly emerged (1-d-old) adults (1 moth/vial) were placed into insecticide-treated and non-treated (control) vials. Mortality was determined at 24 HAE. Moths were considered dead if they were incapable of sustained flight for 1 m (Graves et al. 1988). Four to six replicates (20-50 moths per dose) were used for each colony. Data were corrected and analyzed as previously described to obtain dose mortality (LC₅₀ and LC₉₀) values. Non-overlapping confidence limits (95%) were used to indicate significant differences among colonies and species.

Cypermethrin susceptibility for several the colonies (LSU lab BW, Louisiana BW 2007, LSU lab TBW, and Louisiana TBW) was ascertained using AVT testing procedures as previously described by Temple et al. (2006) and Plapp et al. (1987).

RESULTS

Insecticide-Treated Diet. An initial experiment was conducted on the LSU Lab BW colony to determine the consistency and repeatability of these procedures. Four replications of this experiment indicated that mortality results and slopes of response lines were very similar (Figure 1). The LC₅₀'s for LSU Lab BW ranged from 0.06-0.09 ppm of treated diet among the four replicates. There were no significant differences in rynaxypyr® susceptibility between the field (Louisiana TBW) and laboratory (LSU Lab TBW) strains of tobacco budworm. The LC₅₀'s and

LC₉₀'s for the field and laboratory tobacco budworms were 0.03 and 0.22 ppm for the field and 0.02 and 0.13 ppm for the laboratory strains, respectively (Table 2). Susceptibility of all bollworm colonies was very similar with LC₅₀ values ranging from 0.04-0.09 ppm. The LC₉₀ values for the bollworm colonies ranged from 0.11-0.34 ppm. The LC₅₀ and LC₉₀ values for the Louisiana FAW colony were 0.07 and 0.21 ppm, respectively. All species exhibited similar susceptibility to rynaxypyr® with average LC₅₀'s for tobacco budworm, bollworm, and fall armyworm of 0.03, 0.06, and 0.07 ppm, respectively.

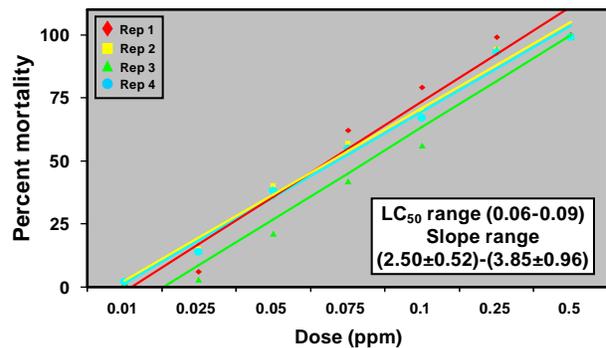


Figure 1. Dose-response and slope of the LSU Lab BW colony exposed to rynaxypyr® in an insecticide-treated diet bioassay.

Direct Application of Insecticide to Larvae. This bioassay was first performed on the LSU Lab TBW colony to validate the procedures. Mortality results and slopes of the response lines were very similar among four replications (Figure 2). The LD₅₀'s for the LSU Lab TBW colony ranged from 0.24-0.72

Table 2. Comparative susceptibility of tobacco budworm, bollworm, and fall armyworm larvae to rynaxypyr® in dose-mortality curves generated with insecticide-treated diet.

Colony Name	Species	N	LC ₅₀ ^z	95%CL ^{z,y}	LC ₉₀ ^z	95%CL ^{z,y}	Slope	χ-square
LSU Lab TBW	Tobacco budworm	240	0.02	0.01-0.03	0.13	0.09-0.23	1.52±0.31	2.74
Louisiana TBW	Tobacco budworm	215	0.03	0.02-0.04	0.22	0.13-0.51	1.39±0.23	1.94
LSU Lab BW	Bollworm	280	0.07	0.05-0.10	0.20	0.13-0.47	2.85±0.31	11.82*
Georgia BW	Bollworm	240	0.04	0.03-0.05	0.11	0.08-0.15	3.15±0.41	1.80
North Carolina BW	Bollworm	180	0.05	0.04-0.06	0.16	0.12-0.26	2.56±0.36	3.43
Virginia BW	Bollworm	240	0.04	0.03-0.05	0.12	0.09-0.17	2.69±0.34	2.90
Delaware BW	Bollworm	210	0.09	0.07-0.12	0.34	0.22-0.80	2.23±0.28	4.27
Louisiana BW	Bollworm	210	0.06	0.05-0.07	0.17	0.13-0.26	2.83±0.41	3.12
DuPont BW	Bollworm	240	0.04	0.03-0.05	0.12	0.09-0.16	2.78±0.35	2.69
Louisiana FAW	Fall Armyworm	455	0.07	0.06-0.08	0.21	0.17-0.28	2.61±0.24	3.50

^z PPM

^y Confidence Limits

* Indicates a significant chi-square value

µg/g larval weight among the four replicates. There were no significant differences in rynaxypyr® susceptibility between the field and laboratory colonies of tobacco budworms. The LD₅₀'s and LD₉₀'s for tobacco budworms were 0.68 and 9.64 µg/g larval weight for the field (Louisiana TBW) and 0.52 and 9.16 µg/g larval weight for the laboratory (LSU Lab TBW) colonies, respectively (Table 3). Bollworm and fall armyworm expressed similar susceptibility to rynaxypyr®, with LD₅₀'s and LD₉₀'s of 0.84 and 6.24 µg/g larval weight for bollworm (LSU Lab BW) and 1.43 and 20.11 µg/g larval weight for fall armyworm (Louisiana FAW), respectively. All species expressed similar susceptibility to rynaxypyr® in these bioassays. The laboratory tobacco budworm (LSU Lab TBW) colony was slightly more susceptible than the bollworm (LSU Lab BW) and fall armyworm (Louisiana FAW) colonies.

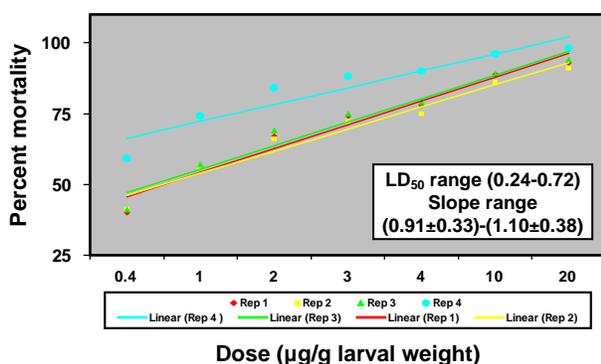


Figure 2. Dose-response and slope of the LSU Lab TBW colony exposed to rynaxypyr® in a topical bioassay.

Table 3. Comparative susceptibility of tobacco budworm, bollworm, and fall armyworm larvae to rynaxypyr® in dose-mortality curves generated with topical bioassays.

Colony	Species	N	LD ₅₀ ^z	95%CL ^z	LD ₉₀ ^{z,y}	95%CL ^{z,y}	Slope	χ-square
LSU Lab TBW	Tobacco budworm	347	0.52	0.28-0.84	9.16	5.80-19.12	1.04±0.16	5.65
Louisiana TBW	Tobacco budworm	210	0.68	0.32-1.12	9.64	5.64-26.12	1.12±0.21	5.86
LSU Lab BW	Bollworm	270	0.84	0.52-1.16	6.24	4.36-10.84	1.46±0.21	3.91
Louisiana FAW	Fall Armyworm	518	1.43	0.90-2.10	20.11	12.93-64.46	1.06±0.11	10.00

^z LD₅₀'s converted to ug/g larval weight

^y Confidence Limits

Table 4. Comparative susceptibility of tobacco budworm and fall armyworm adults to rynaxypyr® in dose-mortality curves generated with the adult vial test (AVT).

Colony	Species	N	LC ₅₀ ^z	95%CL ^{z,y}	LC ₉₀ ^z	95%CL ^{z,y}	Slope	χ-square
LSU Lab TBW	Tobacco budworm	330	1.21	0.76-1.65	8.67	6.38-13.62	1.50±0.20	2.63
Louisiana FAW	Fall Armyworm	191	1.71	1.00-2.53	22.48	12.21-69.05	1.15±0.20	0.68

^z ug/ vial

^y Confidence Limits

Insecticide Residue on Glass. Consistent mortality levels and slopes of the response lines were obtained with rynaxypyr® and the Louisiana FAW colony in the AVT across four replications (Figure 3). The LC₅₀'s for the four replicates ranged from 1.20-2.18 µg/vial for the LSU FAW. There were no significant differences between the two species. The LC₅₀'s and LC₉₀'s for the Louisiana FAW and LSU Lab TBW colonies were 1.71 and 22.48 µg/vial and 1.21 and 8.67 µg/vial, respectively (Table 4).

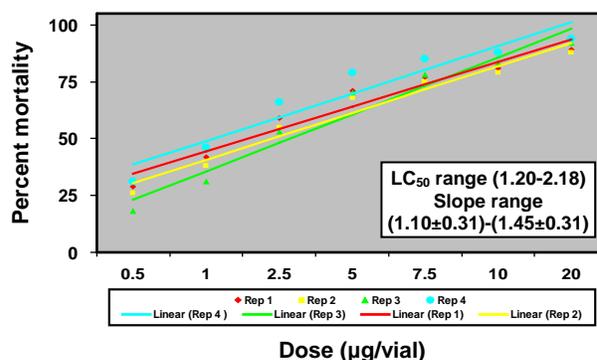


Figure 3. Dose-response and slope of the Louisiana FAW colony exposed to rynaxypyr® in the adult vial test (AVT).

Four colonies (LSU Lab BW, Louisiana Bollworm, LSU Lab TBW, and Louisiana TBW) expressed significant differences in susceptibility to a pyrethroid, cypermethrin. The laboratory strains, LSU Lab BW and LSU Lab TBW, were significantly more susceptible to discriminating doses of cypermethrin in an AVT when compared to the field

strains (Louisiana TBW and Louisiana BW) of both species (Figure 4A). No susceptibility differences to rynaxypyr® were noted between tobacco budworm (field and laboratory) colonies or bollworm (field and laboratory) colonies (Figure 4B). The results suggest that cross-resistance is unlikely between rynaxypyr® and pyrethroids for these species.

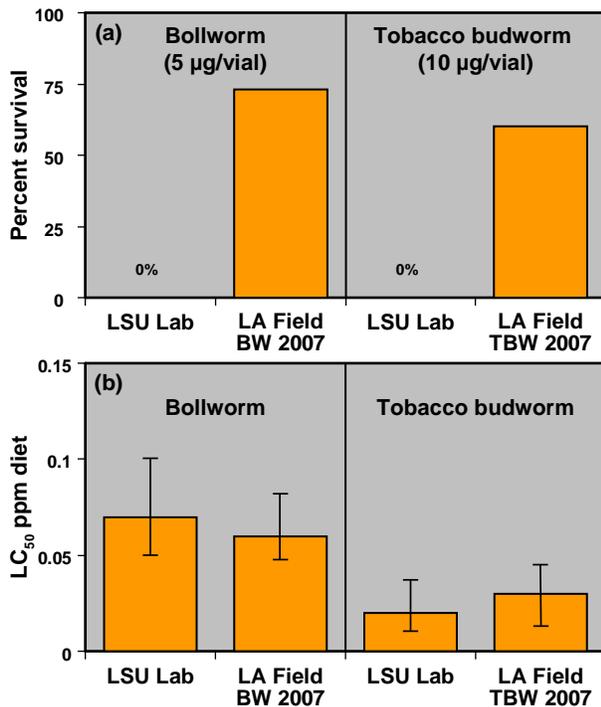


Figure 4. Bollworm and tobacco budworm (field and laboratory colonies) survival to discriminating doses of cypermethrin (A) and LC₅₀'s generated with rynaxypyr®-treated diet (B).

DISCUSSION

Rynaxypyr® demonstrated very good activity at relatively low rates against all three major caterpillar pests of cotton evaluated in this study, including tobacco budworm, bollworm, and fall armyworm. Insecticidal activity of rynaxypyr® in these studies was similar to that reported by Lahm et al. (2007) during their initial classification of rynaxypyr® toxicity to selected lepidopteran targets. In laboratory studies, LC₅₀'s for rynaxypyr® (0.1 ppm) were significantly lower when compared to two standard insecticides indoxacarb (1.5 ppm) and cypermethrin (13.5 ppm) in a insecticide-treated diet assay on a laboratory colony of tobacco budworm (Anonymous, 2007).

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., 2008). Rynaxypyr® also

has demonstrated significant residual activity, protection from wash off, resistance to photo-degradation, and translaminar movement. In a field evaluating *Helicoverpa zea* control in tomatoes, 100% mortality was reported at 18 days after a single foliar application of rynaxypyr® (50 g ai/ha) compared to 77, 70, and 13% mortality with methoxyfenozide (135g ai/ha), esfenvalerate (56 g ai/ha), and spinosad (75 g ai/ha), respectively, (Anonymous, 2007). Rynaxypyr® is effective against other lepidopteran species (beet armyworm and soybean looper) that are occasional U. S. cotton pests (Anonymous, 2007).

Rynaxypyr® could be an excellent option for IRM strategies as an additional class of chemistry and mode of action for management of lepidopteran pests in cotton. The high degree of mammalian safety, relatively low use rates compared to standard insecticides (pyrethroids, organophosphates, and carbamates), long residual properties, and broad spectrum of activity against lepidopteran pests will make rynaxypyr® an excellent control option in an overall integrated pest management system (Anonymous, 2007). Rynaxypyr® also has an excellent environmental profile with low impact on fish, birds, and mammals and has demonstrated little or no toxicity to common beneficial arthropod species such as chrysopidae, coccinellidae, nabidae, lygaeidae, and braconidae or pollinators from the apidae family (Anonymous, 2007). Rynaxypyr® should also be a candidate insecticide for control of lepidopteran pests in other row crops and may be an excellent option in pest management situations where standard insecticides no longer provide adequate control.

The results of this study show that rynaxypyr® exhibits contact (AVT and topical bioassay) and ingestion (insecticide-treated diet) toxicity to the target insects. In addition, rynaxypyr® has demonstrated significant activity on adults of these species. All three bioassay methods produced usable dose-response curves and may be used for surveying changes in the rynaxypyr® susceptibility of these target pests. Nevertheless, the bioassay using insecticide-treated diet produced tighter confidence intervals and steeper slopes and generally a better fit of the data to the probit model as indicated by the chi-square values. Therefore, insecticide-treated diet was chosen as the preferred method for expanded research and will continue to be recommended in a standard protocol for surveying the rynaxypyr® susceptibility of lepidopteran pests.

Data generated from the present study comprise initial efforts in establishing baseline susceptibility to rynaxypyr® that can be used as reference points for future monitoring programs associated with field populations of tobacco budworm, bollworm, and fall armyworm. These insects are polyphagous and feed on a wide variety of food and fiber crops. Current efforts are underway to register this product and other diamide chemistry with modes of action similar to rynaxypyr in many fruit, vegetable, and row crops. Proactive insecticide susceptibility surveys should be established against all target pests to sustain the durability and efficacy of rynaxypyr® and related products. These results will serve as baseline data for detection of changes in rynaxypyr® susceptibility to these insects, regardless of commodity or geography.

CONCLUSIONS

The purpose of this study was to evaluate the contact (larvae and adults) and ingestion (larvae) activity of rynaxypyr® against several economically important lepidopteran pests of cotton. The data from this study indicate that rynaxypyr® expresses contact and ingestion activities against tobacco budworm, bollworm, and fall armyworm at relatively low rates. These data will serve as a basis for future studies that monitor changes in susceptibility to rynaxypyr® in these pests. This novel insecticide should compliment the current foliar insecticide products used in conventional cotton and as a supplemental insecticide option in transgenic cotton cultivars.

ACKNOWLEDGMENTS

We wish to thank the numerous student workers at the Macon Ridge Research Station and the LSU Department of Entomology for their assistance in these studies. We would also like to thank Kathleen Heldreth, Glenn Hammes, Tom Kuhar, John Van Dyun, Bob Williams, Chris Williams, Joe Saienni, and Greg Hannig for their cooperation in collecting and shipping field colonies. The financial support provided by the Louisiana State University Agricultural Center, Cotton Incorporated, Louisiana's cotton producers, and DuPont Crop Protection to complete this project is also appreciated. The authors also wish to express appreciation to Fangneng

Huang, Jeff Davis, and Jarrod Hardke for providing critical reviews of this manuscript. This manuscript is published with the approval of the Director of the Louisiana Agricultural Experiment Station as manuscript number 2008-234-1848.

DISCLAIMER

Mention of a trademark, warranty, proprietary product of vendor does not constitute a guarantee by the Louisiana State University Agricultural Center and does not imply approval or recommendation of the product to the exclusion of others that may be suitable.

ABBREVIATIONS

IRM (Insecticide Resistance Management), *Bt* (*Bacillus thuringiensis*), BW (Bollworm), TBW (Tobacco Budworm), FAW (Fall Armyworm), L:D (Light: Dark) HAE (Hours After Exposure), HAT (Hours After Treatment), DMSO (Dimethyl Sulfoxide), AVT (Adult Vial Test), LC (Lethal Concentration), LD (Lethal Dose) PPM (Parts Per Million)

REFERENCES CITED

- Abbott, W.S. 1925. A method for computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265-267.
- Anonymous. 2007. DuPont Rynaxypyr® insect control technical bulletin. [Online]. Available at http://www2.dupont.com/Production_Agriculture/en_US/assets/downloads/pdfs/Rynaxypyr®_Tech_Bulletin.pdf (verified 17 Dec. 2008).
- Bachelor, J.S. and D.W. Mott. 2007. Efficacy of selected insecticides on conventional cotton vs. a Bollgard II variety against bollworm, 2006. *Arthropod Management Tests 2007* [Online]. Volume 32, F(21). Available at http://www.entsoc.org/Protected/AMT/members_only/AMT32/F/F21.pdf (verified 17 Dec. 2008).
- Brown, T.M., P.K. Bryson, D.S. Brickle, S. Pimprale, F. Arnette, M.E. Roof, J.T. Walker, and M.J. Sullivan. 1998. Pyrethroid-resistant *Helicoverpa zea* and transgenic cotton in South Carolina. *Crop Protection* 17: 441-445.
- Cordova, D., E.A. Benner, M.D. Sacher, J.J. Rauh, J.S. Sopa, G.P. Lahm, T.P. Selby, T.M. Stevenson, L. Flexner, S. Gutteridge, D.F. Rhoades, L. Wu, R.M. Smith, and Y. Tao. 2006. Anthranilic diamides: a new class of insecticides with a novel mode of action, ryanodine receptor activation. *Pestic. Biochem. Physiol.* 84: 196-214.

- Cordova, D., E.A. Benner, M.D. Sacher, J.J. Rauh, J.S. Sopa, G.P. Lahm, T.P. Selby, T.M. Stevenson, L. Flexner, T. Caspar, J.J. Raghianti, S. Gutteridge, D.F. Rhoades, L. Wu, R.M. Smith, and Y. Tao. 2007. Elucidation of the mode of action of Rynaxypyr®, a selective ryanodine receptor activator, pp.121-126. *In* H. Ohkawa, H. Miyagawa, and P. W. Lee [Eds.], *Pesticide Chemistry, Crop Protection, Public Health, and Environmental Safety*. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.
- Elzen, G.W., B.R. Leonard, J.B. Graves, E. Burris, and S. Micinski. 1992. Resistance to pyrethroid, carbamate, and organophosphate insecticides in field populations of tobacco budworm (Lepidoptera: Noctuidae) in 1990. *J. Econ. Entomol.* 85: 2064-2072.
- French-Constant, R.H., and R.T. Roush. 1990. Resistance detection and documentation: the relative roles of pesticidal and biochemical assays, pp. 4-38. *In* R. T. Roush and B. E. Tabashnik [Eds.], *Pesticide Resistance in Arthropods*. Chapman and Hall, New York, New York and London, United Kingdom.
- Graves, J.B., B.R. Leonard, A.M. Pavloff, G. Burris, K. Ratchford, and S. Micinski. 1988. Monitoring pyrethroid resistance in tobacco budworm in Louisiana during 1987: resistance management implications. *J. Agric. Entomol.* 5: 109-115.
- Hardke, J.T., G.M. Lorenz, C.K. Colwell, C.M. Shelton, and R.M. Edmunds. 2008. Evaluation of selected insecticides for control of heliothines in Bt and non-Bt cotton, 2006. *Arthropod Management Tests* 33: F23. [Online]. <http://www.entsoc.org/Protected/AMT/AMT33/Text/amt33.asp?Report=F23>. (verified 17 Dec. 2008).
- Hutchison, W.D. 2005. Vegetable insect summary for Minnesota, 2005: European corn borer and corn earworm. *Minnesota fruit and vegetable IPM news*. Vol. 2(15): 3-4. University of Minnesota, St. Paul, MN.
- Hutchison, W.D., E.C. Burkness, B. Jensen, B.R. Leonard, J. Temple, D.R. Cook, R.A. Weinzierl, R.E Foster, T.L. Rabaey, and B.R. Flood. 2007. Evidence for decreasing *Helicoverpa zea* susceptibility to pyrethroid insecticides in the Midwestern United States. *Plant Health Progress* [Online]. Available at: <http://www.plantmanagementnetwork.org/sub/php/symposium/hzea/decrease/decrease.pdf>. (verified 17 Dec. 2008).
- Lahm, G.P., T.P. Selby, J.H. Freudenberger, T.M. Stevenson, B.J. Myers, G. Seburyamo, B.K. Smith, L.Flexner, C.E. Clark, and D. Cordova. 2005. Insecticidal anthranilic diamides: a new class of potent ryanodine receptor activators. *Bioorganic & Medic. Chem. Letters* 15: 4898-4906.
- Lahm, G.P., T.M. Stevenson, T.P. Selby, J.H. Freudenberger, C.M. Dubas, B.K. Smith, D. Cordova, L. Flexner, C.E. Clark, C.A. Bellin, and J.G. Hollingshaus. 2007. Rynaxypyr®: A new anthranilic diamide insecticide acting at the ryanodine receptor, pp.111-120. *In* H. Ohkawa, H. Miyagawa, and P. W. Lee [Eds.], *Pesticide Chemistry, Crop Protection, Public Health, and Environmental Safety*. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.
- Leonard, B.R., S. Micinski, and R.D. Bagwell. 2003. Beyond Bollgard: Insect-resistant varieties. *Louis. Agric.* 46(4): 38-39. LSU AgCenter, Baton Rouge, LA.
- LeOra Software. 2006. POLO-Plus. A user's guide to Probit or Logit analysis. LeOra Software, Berkeley, CA.
- Pietrantonio, P.V., T. Juneke, R. Parker, C.G. Sansone, A. Cranmer, G. Cronholm, G. Moore, D. Mott, E. Nino, P. Porter, K. Siders, N. Troxclair, R.R. Minzenmayer. 2005. Monitoring for pyrethroid resistance in bollworm (*Helicoverpa zea*) in Texas-2004, pp. 1617-1629. *In* Proc. Beltwide Cotton Conf., New Orleans, LA. 4-7 Jan. 2005. Natl. Cotton Counc. Am. Memphis, TN.
- Plapp, F.W., G.M. McWhorter, and W.H. Vance. 1987. Monitoring for pyrethroid resistance in the tobacco budworm in Texas-1986, pp. 324-326. *In* Proc. Beltwide Cotton Conf., Dallas, TX. 5-8 Jan. 1987. Natl. Cotton Counc. Am., Memphis, TN.
- Plapp, F.W., Jr., J.A. Jackman, C. Campanhola, R.E. Frisbie, J.B. Graves, R.G. Luttrell, W.F. Kitten and M. Wall. 1990. Monitoring and management of pyrethroid resistance in tobacco budworm (Lepidoptera: Noctuidae) in Texas, Mississippi, Louisiana, Arkansas, and Oklahoma. *J. Econ. Entomol.* 83: 335-341.
- Sparks, T. C. 1981. Development of resistance in *Heliothis zea* and *Heliothis virescens* in North America. *Bull. Entomol. Soc. Am.* 27:186-192.
- Stewart, S. D., K. S. Knighten, and F. M. Davis. 2000. Efficacy of Bt cotton expressing two insecticidal proteins of *Bacillus thuringiensis* Berliner on selected caterpillar pests, pp. 1043-1049. *In* Proc. Beltwide Cotton Conf., San Antonio, TX 4-8 Jan. 2000. Natl. Cotton Counc. Am., Memphis, TN.
- Temple, J.H., D.R. Cook, P.L. Bommireddy, S. Micinski, W. Waltman, A.M. Stewart, B. Garber, and B.R. Leonard. 2006. Monitoring cypermethrin susceptibility in Louisiana bollworm 2004-2005, pp. 1236-1240. *In* Proc. Beltwide Cotton Conf., San Antonio, TX. 3-6 Jan. 2006. Natl. Cotton Counc. Am., Memphis, TN.

- Temple, J.H., P.L. Bommireddy, P. Marcon, S. Micinski, K.D. Emfinger, and B.R. Leonard. 2008. Rynaxypyr® (DPX-E2Y45) and cypermethrin: susceptibility of selected Lepidopteran insect pests, pp. 1282-1289. *In Proc. Beltwide Cotton Conf., Nashville, TN. 8-11 Jan. 2008. Natl. Cotton Counc. Am., Memphis, TN.*
- Williams, M.R. 2008. Cotton insect losses 2007, pp. 927-979. *In Proc. Beltwide Cotton Conf., Nashville, TN. 8-11 Jan. 2008. Natl. Cotton Counc. Am., Memphis, TN.*
- Yu, S.J. 1992. Detection and biochemical characterization of insecticide resistance in fall armyworm (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 85: 675-682.
- Yu, S.J. 2008. The classification of insecticides, pp. 25-84. *In The Toxicology and Biochemistry of Insecticides.* CRC Press, Boca Raton, FL.