EVALUATION OF SPINOSAD, INDOXACARB, AND S-1812 AGAINST SELECTED LEPIDOPTERAN PESTS

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Abstract

Spinosad was evaluated against laboratory colonies of tobacco budworm, *Heliothis virescens* (F.), and beet armyworm, *Spodoptera exigua* (Hübner), using the adult vial test (AVT). Tobacco budworm adults were more susceptible (>12-fold) to spinosad than beet armyworm adults. Bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm field populations also were monitored for susceptibility to spinosad in Louisiana during 2000. An increase in survival of male tobacco budworm moths exposed to the 5 µg/vial concentration was observed compared to data from 1991 to 1993. Survival at the 15 µg/vial concentration during 2000 was similar to that observed during 1991 to 1993. Studies also were conducted to determine the response of laboratory colonies of pest insects to indoxacarb and S-1812 using the AVT and diet overlay procedures. Dose-mortality values of indoxacarb and S-1812 for bollworm, tobacco budworm, beet armyworm, and fall armyworm, *Spodoptera frugiperda* (J.E. Smith), were higher than the respective values for cypermethrin in the AVT. In response to these findings, indoxacarb and S-1812 were evaluated in a diet insecticide overlay bioassay against 2-day old first instar larvae of each species. S-1812 demonstrated greater activity against tobacco budworm (4.9-fold) and beet armyworm (6.2-fold) larvae compared to indoxacarb. Bollworm larvae (4.2-fold) were more sensitive to indoxacarb than S-1812. Both compounds were equally toxic against fall armyworm larvae. These studies indicate that the AVT is not appropriate for use with indoxacarb or S-1812. These studies also provide valuable information for use in developing insect resistance monitoring programs.

Introduction

Resistance of key insect pests to insecticides is an important issue in cotton production. Two of the most important cotton pests in the Mid-South and Southeastern United States are the bollworm, *Helicoverpa zea* (Boddie), and the tobacco budworm, *Heliothis virescens* (F.). The tobacco budworm has developed resistance to organochlorines, DDT, organophosphates (Sparks 1981), carbamates (Elzen et al. 1992), and pyrethroids (Plapp et al. 1990, Elzen et al. 1992). Resistance to DDT and organochlorine insecticides has been reported in bollworm (Sparks 1981). Pyrethroid-resistant populations of bollworm have been reported in South Carolina by Brown et al. (1998). However, in most areas organophosphates, carbamates, and pyrethroids have generally remained effective against bollworm (Kharboutli et al. 1999, Brickle et al. 2000). Numerous states have developed insecticide resistance monitoring programs for bollworm and tobacco budworm.

Beet armyworm, *Spodoptera exigua* (Hübner), is a pest that is difficult to control. No coordinated monitoring program in cotton has been implemented in the United States due to its sporadic nature and the lack of activity of many insecticides used in cotton production against this pest. However, Martínez-Carrillo et al. (1998) reported no differences between field colonies of beet armyworm from Northern Mexico and a laboratory colony in their responses to emamectin benzoate. In the Mid-South, beet armyworm infestations have become more common in recent history. Also, some of the newer insecticides, including spinosad, indoxacarb, and S-1812 have been shown to be efficacious against beet armyworm (Gore et al. 1999, Leonard unpublished).

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Monitoring of insect populations for changes in susceptibility to insecticides is an integral component of any insecticide resistance management program. Monitoring efforts ideally should be initiated before a compound is widely used and or while resistance is rare (ffrench-Constant and Roush 1990). Determination of initial resistance frequencies allows for early detection of changes in susceptibility. This in turn allows time for implementation of resistance management strategies before field control failures occur. Baseline responses of laboratory and field strains must be determined so that discriminating concentrations can be established to initiate a monitoring program.

Spinosad and indoxacarb are new compounds for control of lepidopteran cotton pests. A monitoring program is already being conducted with spinosad for bollworm and tobacco budworm (Tracer 4SC, Dow Agrosciences, 9330 Zionsville Rd., Indianapolis, IN 46268), which was released commercially in 1997. Leonard et al. (1996) and Bailey et al. (1999) found no differences in the response of field populations of tobacco budworm compared to a laboratory colony. Indoxacarb (Steward 1.25SC, E.I. DuPont de Nemours and Company, Walker's Mill, Banley Mill Plaza, Wilmington, DE 19898) was available for use in Texas, Louisiana, and Mississippi during the 2000 growing season under a Section 18 label, and was recently granted full registration during November, 2000. Valent's S-1812 (Valent USA Corporation, 1333 N. California Blvd., Walnut Creek, CA 94596) is another compound that is expected to be commercially available in the near future.

The objectives of these studies were to determine an appropriate testing method for indoxacarb and S-1812. Both compounds were evaluated against male and female moths using the AVT, and against larvae using diet overlay bioassays. Another objective was to generate baseline dose-mortality responses of bollworm, tobacco budworm, beet armyworm, and fall armyworm, *Spodoptera frugiperda* (J.E. Smith), to these compounds. These data will serve as a reference for future monitoring programs in Louisiana.

Materials and Methods

Insects

The insects were taken from laboratory colonies maintained at the Louisiana State University Department of Entomology Baton Rouge, LA. Bollworms utilized in these studies were collected from clover, *Trifolium* spp., and sweet corn, *Zea maize* L., during 1998 and 2000. Bollworms were reared in the laboratory for at least five generations before testing. The tobacco budworm colony utilized in these studies was the LSU-Lab colony originally established in 1977 by collections from cotton fields in Louisiana (Leonard et al. 1988). The beet armyworm colony used in these studies was obtained from Ecogen, Inc. (Langehorne, PA). The fall armyworm colonies utilized in these studies were established by collections from field corn in 1997 and 1999. Larvae were fed a laboratory meridic diet, with rearing conditions consisting of a 14:10 light-dark photoperiod, 75 to 85º F, and 80% relative humidity. Prior to testing, insects were segregated by sex based on dimorphic pupal characters. Pupae were placed into 1-gal cardboard cartons containing a thin layer of vermiculite on the bottom. Eclosed adults were removed daily and placed into polypropylene cages (11.8 x 11.8 x 11.8 inches) (BugDorm, Megaview Science Education Services CO. Ltd., Taichung, Taiwan).

Adult Vial Bioassays

Adult vial bioassays (AVT) similar to those described by Plapp et al. (1987) were utilized to evaluate the activity of spinosad, indoxacarb, and Valent's S-1812 against bollworm, tobacco budworm, beet armyworm, and fall armyworm adults. Stock solutions of spinosad, indoxacarb, and Valent S-1812 were made by dissolving technical grade insecticide in acetone. Serial dilutions were made from each stock solution to yield the desired insecticide concentrations. The interior surface of 20 ml scintillation vials

was coated with insecticide by pipetting 0.5 ml of the appropriate insecticide solution into the vials. These vials were placed on a modified hot dog roller (heating element disconnected) until all of the acetone had evaporated. Vials were stored in a dark environment until used.

Male and female moths were held in separate cages for ca. 24-h and provided 10% sugar water as a food source. After 24-h, moths were placed into insecticide treated vials (1 moth/vial) and mortality was determined after 24-h of exposure (HAE). Moths were considered dead if they were incapable of sustained flight for 3 ft. Data for males and females of the respective insect species were pooled.

Male bollworm and tobacco budworm moths were collected and monitored at the Macon Ridge location of the Northeast Research Station (Winnsboro, LA) and at the Red River Research Station (Bossier City, LA) for spinosad susceptibility during Jul, Aug, and Sep 2000. Wire cone traps (Harstack et al. 1979) baited with artificial sex pheromone lures (Hendricks et al. 1987) were utilized to collect male moths. Only moths that were in good condition were used for testing. Moths were placed into insecticide treated vials and mortality was determined after 24-h of exposure (HAE). Insects were considered dead if they were incapable of sustained flight for 3 feet.

Diet Overlay Bioassays

Diet overlay bioassays similar to those described by Joyce et al. (1986) and Mascarenhas et al. (1998) were utilized to evaluate the activity of indoxacarb and Valent S-1812 against first instar 2-day old bollworm, tobacco budworm, beet armyworm, and fall armyworm larvae. Meridic diets used in these studies included those described by King and Hartley (1985) for beet armyworm and fall armyworm, Shour and Sparks (1981) for tobacco budworm, and a commercial diet from Stonefly Industries, Bryan, TX (Heliothis Premix) for bollworm. Three ml of hot liquid diet were transferred into 30 ml plastic cups (Solo Cup Company, Urbana, IL 61801) using an Eppindorf (Brinkman, Westbury, NY) repeating pipette. This procedure was utilized for all assays, except for those on bollworm. The commercial bollworm diet has a low viscosity, therefore for these assays, 5 ml of diet were transferred into 30 ml cups using a volumetric spoon.

Each insecticide was diluted in distilled water based on the percentage of active ingredient of the formulated product. One hundred µl of solution was applied to the diet surface in each cup. Diet treated with distilled water was utilized as a control. Cups were rotated to distribute the solution evenly over the diet surface. Treated diet was allowed to dry (evaporation of the distilled water carrier) for approximately 1-h. One larva was placed in each cup and capped to prevent larval escape. A minimum of 30 larvae per concentration was utilized in all bioassays. Mortality was determined 72 HAE. Larvae were considered dead if they could not right themselves after being rolled onto their dorsal surface.

Data Analysis

All data were corrected for control mortality (Abbott 1925) and analyzed by probit analysis using Polo PC (LeOra Software 1987).

Results and Discussion

Adult Vial Bioassays

Spinosad was more toxic to the tobacco budworm (LSU-Lab) colony (3.58 µg/vial) compared to the beet armyworm laboratory colony (45.58 µg/vial) based on LC_{50}'s (Table 1). The LC_{90} value for tobacco budworm (8.68µg/vial) was within the range of the two concentrations (5 µg and 15 µg/vial) utilized for susceptibility monitoring of field populations of tobacco budworm. The LC_{90} value for beet armyworm exceeded the highest concentration (100 µg/vial).

The average survival of field collected male tobacco budworm moths to the 5 µg and 15 µg concentrations was 29.3% and 3.4%, respectively during 2000 (Table 2). These values are 3.5-fold and 3.2-fold higher than the average of responses determined before spinosad was commercially released (data from 1991, 1992, and 1993). Average survival of male bollworm moths at the 5 µg and 15 µg concentrations of spinosad was 53.7% and 19.2%, respectively, during 2000.

The LC_{50} value of indoxacarb against bollworm was 15.65 µg/vial (Table 3). Tobacco budworm adults were more sensitive to indoxacarb (LC_{50} 76.72 µg/vial) than S-1812 (LC_{50} 143.02 µg/vial) (Table 4). Dose-mortality values for beet armyworm (Table 5) and fall armyworm (Table 6) exceeded the maximum concentration (100 µg/vial) tested in these studies. Dose-mortality values for indoxacarb and S-1812 were at least 9.7-fold, 511-fold, 3-fold, and 3-fold higher than the cypermethrin values for the bollworm, tobacco budworm, beet armyworm, and fall armyworm, respectively.

The AVT is a useful tool in the pyrethroid resistance monitoring program for tobacco budworm and the IRAC-US project monitoring pyrethroid susceptibility of bollworm (Martin et al. 1999). Also the AVT works well for monitoring susceptibility of tobacco budworm and bollworm to spinosad. However, for indoxacarb and S-1812, this procedure is not likely to be used. The LC_{50} values obtained for the four insect species were either near or exceeded 100 µg/vial, with one exception. Dose-mortality values obtained in our studies are extremely high in comparison to the discriminating concentrations established for cypermethrin (5 µg – 10 µg/vial) (Plapp et al. 1987, Graves et al. 1989), methomyl (2.5 µg and 10 µg/vial), profenofos (10 µg, 20 µg, 25 mg, and 40 µg/ vial), and endosulfan (3 µg and 10 mg/vial) (Kanga et al. 1995, Graves et al. 1994). The discriminating concentrations for these compounds in the AVT, if they could be established, would probably be cost prohibitive. Contact exposure to residues does not appear to be as important in intoxication with indoxacarb and S-1812 compared to pyrethroids.

Diet Overlay Bioassays

Indoxacarb and S-1812 were also evaluated using diet overlay bioassays against first instar (2-day old) larvae. Indoxacarb (0.34 ppm) was more active against bollworm than S-1812 (1.43 ppm) (Table 7). However, tobacco budworm larvae were more sensitive to S-1812 (1.31 ppm) than indoxacarb (6.46 ppm) (Table 8). Similar results were observed for beet armyworm, dose-mortality values for S-1812 (3.35 ppm) were ca. 6.2-fold lower than for indoxacarb (20.73 ppm) (Table 9). These values are similar to those for tebufenozide (4.65 ppm), chlorpyrifos (18.58 ppm), chlorfenapyr (5.61 ppm), and spinosad (3.57 ppm), but much lower than thiodicarb (356.36 ppm) (V. J. Mascarenhas unpublished). Dose-mortality values of fall armyworm for indoxacarb (0.59 ppm) and S-1812 (0.57 ppm) were similar based on overlap of 95% confidence limits (Table 10).

The LC_{50} values of indoxacarb and Valent S-1812 for tobacco budworm, beet armyworm, and fall armyworm are considerably higher than those reported for thiodicarb (tobacco budworm, 0.09-0.32 ppm; beet armyworm, 0.18 ppm; fall armyworm 0.07-0.27 ppm) tested against third instar larvae of the same species (Joyce et al. 1986). However, the LC_{50} values of indoxacarb and Valent S-1812 at 72 HAE for beet armyworm are similar to those for chlorpyrifos (28.0 ppm), chlorfenapyr (15.1 ppm), emamectin benzoate (2.4 ppm), methoxyfenozide (8.7 ppm), spinosad (52.2 ppm), and tebufenozide (17.6 ppm) at 120 HAE (Mascarenhas et al. 1998). Andaloro et al. (2000) reported LC_{50} values >100 ppm for bollworm, tobacco budworm, and beet armyworm larvae exposed for 1-h to surfaces treated with indoxacarb. Also, Andaloro et al. (2000) reported that ingestion was one of the major modes of entry for indoxacarb that resulted in intoxication. These data suggest that the diet overlay bioassay is likely to be suitable for monitoring susceptibility to indoxacarb and S-1812.

Summary

These data provide a comparison of the relative toxicity of these compounds against several important insect species. These studies also indicate that testing methods other than the AVT must be utilized with indoxacarb and Valent's S-1812, as opposed to pyrethroids, which perform very well in the AVT. This information is also the first step in developing baseline data for the determination of discriminating concentrations used in monitoring of field populations of bollworm, tobacco budworm, beet armyworm, and fall armyworm to spinosad, indoxacarb, and S-1812.

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Table 1. Response of tobacco budworm and beet armyworm adults to spinosad in the adult vial test (AVT).

	LC_{50}	95% C.L.	LC_{90}	95% C.L.
Tobacco Budworm	3.58	2.87-4.32	8.68	6.79–13.12
Beet Armyworm	45.58	39.92-52.49	>100[1]	NA

LC values expressed in µg insecticide per vial.
[1] Values exceeded highest concentration tested.

Table 2. Responses of field collected male bollworm and tobacco budworm moths to spinosad in AVT Louisiana.

		Bollworm		Tobacco Budworm	
		5µg	15µg	5µg	15µg
1991	Aug	-[1]	-	5.1	2.7
	Sep	-	-	4.3	2.8
	Average	-	-	4.7	2.8
1992	Jul	-	-	6.6	0.5
1993	Aug	-	-	7.3	1.6
	Sep	-	-	18.2	0.8
	Average	-	-	12.8	1.2
2000	Jul	51.6	25.5	21.0	7.9
	Aug	57.7	5.1	29.9	2.3
	Sep	-	-	36.9	0.0
	Average	52.7	15.3	29.3	3.4

[1]No data collected.

Table 3. Response of bollworm adults to indoxacarb and S-1812 in the adult vial test. (AVT).

	LC_{50}	95% C.L.
Indoxacarb	15.65	11.49-20.16
S-1812	NA	NA
Cypermethrin[1]	1.60	1.21-2.06

Concentrations expressed in µg insecticide per vial.
[1]LSU laboratory colony 1998.

Table 4. Response of tobacco budworm adults to indoxacarb and S-1812 in the adult vial test. (AVT).

	LC_{50}	95% C.L.
Indoxacarb	76.72	33.88-128.36
S-1812	143.02	98.77-250.89
Cypermethrin[1]	0.15	0.09-0.23

Concentrations expressed in µg insecticide per vial.
[1]LSU laboratory colony 1998.

Table 5. Response of beet armyworm adults to indoxacarb and S-1812 in the adult vial test (AVT).

	LC_{50}	95% C.L.
Indoxacarb	>100[1]	NA
S-1812	>100[1]	NA
Cypermethrin[2]	37.09	17.70-125.46

Concentrations expressed in µg insecticide per vial.
[1] Values exceeded highest concentration tested.
[2]LSU laboratory colony 1998.

Table 6. Response of fall armyworm adults to indoxacarb and S-1812 in the adult vial test. (AVT).

	LC_{50}	95% C.L.
Indoxacarb	>100[1]	NA
S-1812	>100[1]	NA
Cypermethrin[2]	31.02	24.56-42.44

Concentrations expressed in µg insecticide per vial.
[1] Values exceeded highest concentration tested.
[2]LSU laboratory colony 1998.

Table 7. Response of bollworm larvae to indoxacarb and S-1812 in diet overlay assays.

	LC_{50}	95% C.L.
Indoxacarb	0.34	0.16-0.56
S-1812	1.43	1.11-1.75

Values expressed in ppm insecticide.

Table 8. Response of tobacco budworm larvae to indoxacarb and S-1812 in diet overlay assays.

	LC_{50}	95% C.L.
Indoxacarb	6.46	3.52-11.07
S-1812	1.31	0.71-1.86

Values expressed in ppm insecticide.

Table 9. Response of beet armyworm larvae to indoxacarb and S-1812 in diet overlay assays.

	LC_{50}	95% C.L.
Indoxacarb	20.73	13.22-25.61
S-1812	3.35	1.73-5.31
Tebufenozide[1]	4.65	2.68-7.29
Chlorpyrifos[1]	18.58	10.00-44.16
Chlorfenapyr[1]	5.61	4.59-6.56
Spinosad[1]	3.57	2.06-5.57
Thiodicarb[1]	356.36	272.96-537.56

Values expressed in ppm insecticide.
[1]V. J. Mascarenhas, LSU Department of Entomology unpublished.

Table 10. Response of fall armyworm larvae to indoxacarb and S-1812 in diet overlay assays.

	LC_{50}	95% C.L.
Indoxacarb	0.59	0.15-0.85
S-1812	0.57	0.22-0.86

Values expressed in ppm insecticide.