

Studies of Resistance of Beet Armyworm (*Spodoptera exigua*) to Spinosad in Field Populations From the Southern USA and Southeast Asia

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Abstract

Susceptibility to spinosad (Success®/Tracer®) of beet armyworm (Spodoptera exigua) from the southern U.S.A. and Southeast Asia was determined through exposure of third instar larvae to dipped cotton leaves. LC₅₀ values of field populations ranged from 0.6 to 14 µg spinosad/ml. Field populations were 3.0 to 70-fold less susceptible to spinosad than was a susceptible reference population. The least susceptible population was collected from Thailand. We hypothesized that this population was resistant to spinosad because: (1) it was significantly less susceptible to spinosad than all other populations evaluated and than any other reports in the literature; (2) it regained susceptibility while in culture; (3) it was collected from a region of very intensive insecticide use and severe insect resistance problems; and (4) it exhibited significant survivorship on field-treated cabbage leaves. Piperonyl butoxide (PBO), diethyl maleate (DEM), and S,S,S tributyl-phosphorothiolate (DEF) failed to synergize spinosad in this resistant Thailand population, and PBO failed to do so in the least susceptible domestic population evaluated, the Parker, AZ, field strain. However, the synergist and field residue studies were conducted using a Thailand population that had levels of resistance that declined while in laboratory culture.

Introduction

Beet armyworm, *Spodoptera exigua*, is a widely distributed (CAB 1972) polyphagous pest of numerous cultivated crops, including cotton, tomato, celery, lettuce, cabbage, and alfalfa (Metcalf & Flint 1962). It is generally considered a secondary pest. Populations generally build after natural enemy populations have been reduced through application of broad spectrum insecticides (Stoltz & Stern 1978; Smith 1989, 1994; Ruberson 1993; Ruberson et al. 1994; Graham et al. 1995). In the past two decades in the United States, *S. exigua* has emerged as a serious pest of cotton throughout the southern states, with nearly annual outbreaks occurring in Alabama, Georgia, Mississippi, and Texas (Smith 1989a; Mascarenhas et al. 1998).

Due to its polyphagous nature, this pest has a long history of exposure to a broad array of insecticides. Not surprisingly, beet armyworm has developed resistance to many of these, including chlorinated hydrocarbons, organophosphates, carbamates, pyrethroids, and benzoylphenylureas (Meinke & Ware 1978; Chaufaux & Ferron 1986; Delorme et al. 1988; Brewer & Trumble 1989; Van Laecke & Degheele 1991; Layton 1994).

Given this broad range of insecticide resistance by *S. exigua*, it is vital to study the potential for resistance development to new compounds. In the past decade, several experimental insecticides have emerged that show great promise for controlling beet armyworm. A partial list of these includes chlorfenopyr (Farlow et al. 1992; Burris et al. 1994; Wier et al. 1994; Wiley et al. 1995), tebufenozide (Rohm & Haas Co. 1989; Smaghe & Degheele 1994), emamectin benzoate (Dybas 1988; Dybas et al. 1989; Jansson et al. 1996), indoxacarb (DuPont 1998), and spinosad (Thompson et al. 1995, 1996; Yee & Toscano 1998). This paper deals exclusively with studies on spinosad.

Spinosad is the first member of Dow AgroScience's naturalyte class of insecticides (Sparks et al. 1995; Thompson et al. 1996). Spinosad is comprised primarily of two macrocyclic lactones, spinosyn A and D, secondary metabolites produced by the actinomycete, *Saccharopolyspora spinosa*, under natural fermentation conditions. Although superficially similar to avermectins in structure, the modes of action, toxicological profiles, and cross-sensitivity spectra of the two are quite different (Thompson et al. 1996). The mode of action of spinosad is two-fold; the primary target site is the nicotinic acetylcholine receptor, but the GABA receptor is also affected to some degree (Salgado 1997). Routes of entry include both topical and ingestion (Thompson et al. 1995). Signs of spinosad poisoning include initial flaccid paralysis, followed by tremors and eventual death (Thompson et al. 1995).

In this paper we investigate baseline susceptibilities to spinosad of *Spodoptera exigua*, including populations that have reduced susceptibility to this insecticide. It is our hope that this information will assist pest managers in monitoring resistance in beet armyworm to this compound, thereby preserving its efficacy. Additionally, we hope this research will serve as a springboard for more in-depth investigations of the underlying mechanisms responsible for differences observed in susceptibility among BAW populations.

Materials and Methods

Cultures

Field populations were established from samples collected by members of our laboratory, Rohm and Haas Company, and Dow AgroSciences. Arizona field populations were established from larvae swept from alfalfa, brought to the Extension Arthropod Resistance Management Laboratory (EARML), and placed onto artificial diet (*Heliothis* Premix, Stonefly Industries, Bryan, TX) to complete development. Field populations from elsewhere were shipped to EARML as surface sterilized eggs under the terms of USDA-APHIS permit number 16461, and the resulting neonates were allowed to complete development by placing them onto artificial diet. The susceptible reference strain was established from egg sheets shipped to EARML from the USDA-Western Cotton Research Laboratory (WCRL) in Phoenix, AZ.

Rearing

All cultures of *Spodoptera exigua* larvae were reared on artificial diet containing 5 ml of formalin and 1.5 gm of aureomycin (Chlortetracycline HCL, Fort Dodge Animal Health, Fort Dodge, IA) added to each 2000 gm of prepared diet (500 gm-diet, 1500 ml-water) to prevent pathogen growth. Groups of 40-50 neonates were placed into 6 oz. plastic cups and kept in an incubator set at 27°C, with a 16 hr. photoperiod. Pupae were collected from these cups after 14-21 days and placed into one gallon glass jars with wire mesh lids, for adult emergence. Adults were provided 10% sucrose solution and wax paper sheets on which to oviposit. Egg sheets were collected daily, once oviposition commenced, washed in 10% formalin for 10 min., and rinsed in tap water for 10 min.

Bioassays

Leaf-dip. Fully expanded, first true leaves of 2-3 week old cotton plants were dipped for 5 sec. in deionized water solutions containing 0, 0.1, 0.3, 1.0, 3.0, 10, 30, and 100 µg spinosad/ml (Dow AgroSciences) and allowed to air dry under a fume hood. After drying, one leaf each was placed into a 100 x 15 mm Petri plate. Five ca. 1 cm (= 5-7 day old, early-mid third instar) larvae were placed into each Petri plate. Groups of five Petri plates were then sealed inside one gallon zip-lock plastic bags containing a damp paper towel, and placed into a 27°C incubator (16 hr. photoperiod) for 48 hrs. Larvae were scored as "affected" if noticeable paralysis, typically in the posterior abdomen, was present or if they were dead. Bioassay data were analyzed using probit analysis (POLO-PC) (LeOra 1987).

Field residues. The activity of spinosad on field treated and weathered residues was estimated using our most and least susceptible strains, as determined by our leaf-dip laboratory bioassay. Two rows of pre-head stage cabbage plants in a small field plot at the Maricopa Agricultural Center were sprayed with a pneumatic, back-pack sprayer. A rate equivalent to 6 oz. of active ingredient per acre was applied in a total volume equivalent to 30.9 gallons per acre (1.52 ml of 2F Success® in 2 L of water) at 2.4 mph and 55 psi. Air and soil temperatures were 51.2 °C and 47.3 C, respectively, and wind speed at ground level was 3.5 mph. Control leaves were taken prior to application to assess survivorship of the strains and the absence of pesticide residues on the control leaves. Because control mortality was negligible, uncorrected values were reported and responses after time zero were based on comparisons of survivorship of the two strains on field treated leaves only. This field experiment was conducted 12 months after the Thailand population was placed into culture in our laboratory. At that time the intensity of resistance had declined as described subsequently herein.

48 hr. exposure - Eighty leaves (40 control/40 treated, 20 leaves/ population/exposure) were collected at time zero (T_0) and 40 treated leaves (20 leaves/population) were collected 1, 2, 3, and 5 days post-application. Two 1-1.5 cm larvae were placed on each leaf, and the plates were placed in zip-lock bags and kept at 27°C for 48 hrs. The moribundity criterion was the same as for the leaf dip assays of third instar larvae.

Continuous exposure - After 48 hr. exposure bioassays were scored, they were placed back into the incubator and observed until such time that all larvae had either pupated or died. Percent pupation was scored for each strain and residue.

Synergism experiments

These studies were identical to the leaf dip bioassay method outlined above, with the exception that larvae were treated with synergists prior to exposure to spinosad. Solutions comprised of 10 µg/ml of piperonyl butoxide (PBO), diethyl maleate (DEM), or S,S,S – tributylphosphorothiolate (DEF) were made in acetone. One hour prior to exposure to leaves dipped in spinosad, 2 µl of synergist solution was applied to the thoracic dorsum of individual third instar larvae (ca. 1 cm). Groups of synergist-treated larvae were held at room temperature (ca. 20°C) for 1 hr. prior to placement on spinosad-treated leaves, after which they were held in the incubator for 48 hrs. at 27°C. Controls comprised groups treated with 2 µl of acetone and groups not treated with any substance.

Results and Discussion

Leaf-dip bioassays

Results of the leaf-dip assays are summarized in Tables 1 and 2. LC_{50} estimates of susceptibility to spinosad of field populations ranged from 0.6 to 14 µg/ml. These were all significantly different from the 0.20 µg/ml LC_{50} of the susceptible reference strain. Based on these same statistics, ratios of field population LC_{50} s divided by the LC_{50} of the reference strain ranged from 3.0 to 70 µg/ml (Table 1). LC_{90} values for field populations ranged from 2.1 to 68 µg/ml. Fiducial limits (95%) of LC_{90} for the most susceptible field population, Maricopa, Arizona, were not significantly different than the reference strain (Table 2).

Field residues

48-hr. exposure. The susceptible reference strain (USDA-WCRL) incurred 100% mortality on leaves picked immediately after application of spinosad (T_0), whereas the Thailand strain incurred 90% mortality, i.e., 4 of 40 larvae survived (Table 3). A similar pairwise exposure to leaves picked 1 day after application (T_1) produced 62% mortality in the USDA strain and 42% mortality in the Thailand strain. These differences were highly significant in Chi-Square analyses (T_0 ; $p < 0.0001$ & T_1 ; $p < 0.001$). No mortality was observed among the 40 larvae (20/population) in the T_0 controls.

Continuous exposure. Response of the Thailand strain to continuous exposure to field-treated residues of spinosad differed significantly (X^2 , $0.05 > p > 0.025$) from that of the susceptible reference strain (Table 4). On average, approximately twice as many Thailand larvae survived to pupation throughout the course of the study. No survivors were observed from the susceptible reference strain on leaves collected at T_0 and T_1 , whereas 7.5% (3 of 40) of the Thailand larvae survived to pupation on similar residues.

Synergism experiments

PBO, DEM, and DEF failed to synergize spinosad in the Thailand field strain, and PBO failed to do so in the Parker, AZ strain (Table 5). In all instances synergists reduced mortality slightly. However, at the time that these studies were conducted the levels of resistance in the Thailand population had declined 5-fold from the intensity observed when it was first evaluated (Tables 1, 2).

Conclusions

Our findings are consistent with the hypothesis that the Thailand population is resistant to spinosad. This population was collected from an area of intensive vegetable production near Bangkok and has been found to be highly resistant to a broad range of other insecticides (J. K. Moulton, unpublished data). In leaf-dip laboratory bioassays, the Thailand population exhibited LC_{50} 's that were 70-fold higher than our reference population and significantly higher than all of the other field populations we evaluated from the U.S. These differences are considerably greater than those previously reported. Sparks et al. (1996) observed up to a 9-fold difference between two colonies originating from the lower Rio Grande Valley. Although they tested similarly sized larvae, their bioassay differed in that they delivered spinosad topically in 1 μ l of acetone. Mascarenhas et al. (1998), using a diet-overlay bioassay, observed only a 3.5-fold difference between eight strains tested.

The results of our field experiment corroborated the conclusion from leaf-dip bioassays, that the Thailand population possessed a significant resistance to spinosad. Significantly greater numbers of the Thailand population survived exposure to spinosad and pupated. Moreover, as previously noted, the field residues of spinosad were tested against a Thailand population in which resistance levels had declined over the 12 months it had been in laboratory culture. We suspect that the survivorship of the Thailand strain would have been even greater had the higher intensity of resistance previously observed been expressed.

All three synergists employed, PBO, DEM, and DEF, failed to increase the toxicity of spinosad to the Thailand strain and PBO failed to do so in the Parker, AZ strain (Table 3). These data suggest that the differences in spinosad susceptibility in these BAW strains are not the result of enzymatic degradation by MFOs, GSTs, and esterases. Further studies are underway to re-evaluate synergists against a Thailand population selected with spinosad to re-establish the highest possible intensities of resistance.

The Parker, AZ, field strain was the second least susceptible tested. LC_{50} and LC_{90} ratios for it were 24-fold and 19-fold greater than those of the susceptible reference strain, respectively. This population was taken in an area of intensive agricultural production. Given that spinosad is now being used in cotton (Tracer®) and winter vegetables (Success®) in Arizona, this reduced susceptibility should be investigated further to determine its ramifications. We will continue to closely monitor beet armyworm susceptibility to spinosad in this region.

We had no reason to believe, *a priori*, that the resistance risk of beet armyworm to spinosad was any greater or lesser than that of any other new insecticide. In order to promote pro-active management of resistance to this very important new class of IPM-compatible insecticides, we evaluated populations that we obtained from around the world. Isolation of resistance from Southeast Asia now allows us to move ahead to better understand this phenomenon prior to the onset of problems in Arizona.

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Table 1. Probit regression analysis of BAW response to spinosad leaf dip bioassays (LC50s), expressed as μg spinosad/ml. *Ratios based upon comparisons to USDA-WCRL strain.

Population	n	slope	LC50 (95% FL)	Ratio*
USDA-WCRL	515	1.3	0.2 (0.1-0.3)	N/A
AZ-Parker	504	1.5	4.8 (3.8-6.2)	24
AZ-Maricopa	358	2.4	0.6 (0.5-0.8)	3.0
FL-Belle Glade 1	1200	2.2	4.2 (3.4-5.0)	21
FL-Belle Glade 2	602	2.0	2.0 (1.4-2.5)	10
MS-Wayside	878	1.8	2.7 (2.0-3.4)	14
SC-Florence	200	1.8	2.6 (1.8-3.7)	13
Thailand (3-5/98)	541	1.9	14 (11-18)	70
Thailand (12/98)	331	1.6	2.9 (0.7-5.5)	14

Table 2. Probit regression analysis of BAW response to spinosad leaf dip bioassays (LC90s), expressed as μg spinosad/ml. *Ratios based upon comparisons to USDA-WCRL strain.

Population	n	slope	LC90 (95% FL)	Ratio*
USDA-WCRL	515	1.3	1.8 (1.2-3.0)	N/A
AZ-Parker	504	1.5	34 (24-54)	19
AZ-Maricopa	358	2.4	2.1 (1.5-3.3)	1.2
FL-Belle Glade 1	1200	2.2	16 (12-23)	8.9
FL-Belle Glade 2	602	2.0	8.3 (6.1-13)	4.6
MS-Wayside	878	1.8	14 (10-19)	7.8
SC-Florence	200	1.8	13 (8.3-27)	7.2
Thailand (3-5/98)	541	1.9	68 (48-110)	38
Thailand (12/98)	331	1.6	19 (10-48)	11

Table 3. Responses of susceptible reference (USDA-WCRL) and spinosad-resistant (Thailand) strains after 48 hr. exposure to field-treated residues of spinosad on cabbage leaves.

Days post-application	Percent mortality (<u>n</u> =40)	
	USDA (WCRL)	Thailand
0	100	90
1	62	42

Table 4. Survival of susceptible reference (USDA-WCRL) and spinosad-resistant (Thailand) strains to pupation after continuous exposure to field-treated residues of spinosad on cabbage leaves.

Days post-application	Percent pupation ($\underline{n} = 40$)	
	USDA (WCRL)	Thailand
0	0	1
1	0	2
2	2	5
3	3	8
5	6	10

Table 5. Probit regression analysis of response of Thailand field strain to spinosad (cotton leaf dip bioassay) after prior exposure to PBO, DEM, or DEF and Parker field strain to PBO.

Population	\underline{n}	slope	LC50 (95% FL)
USDA-WCRL (12/98)	287	1.4	0.3 (0.09-0.46)
Thailand (12/98)	286	1.6	2.9 (0.7-5.5)
Thailand + acetone (12/98)	125	3.3	4.5 (2.8-6.3)
Thailand + PBO (12/98)	225	1.8	4.8 (2.4-8.0)
Thailand + DEM (12/98)	219	2.8	6.6 (4.4-8.9)
Thailand + DEF (12/98)	227	2.0	3.7 (1.4-6.4)
AZ-Parker (10/98)	215	1.6	4.7 (3.3-6.7)
AZ-Parker + PBO (11/98)	139	2.5	7.0 (2.6-10.4)