LABORATORY SELECTION OF A TRACER®-RESISTANT STRAIN OF THE TOBACCO BUDWORM AND COMPARISONS WITH FIELD STRAINS FROM THE SOUTHEASTERN US

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Abstract

A field strain of the tobacco budworm, *Heliothis virescens*, was selected for eleven successive generations by topical application with technical spinosad (a mixture of spinosyns A and D), the active ingredient in the insecticide Tracer®. The first indication of resistance was noted in the sixth generation. By the ninth generation a maximum dose of 0.8 *u*g of spinosad per third instar produced no mortality at 12 d after application, and in the eleventh generation, 60 *u*g per larva produced less than 40% mortality. Compared to the LD_{50} of the parental strain, the selected budworms in the eleventh generation demonstrated a resistance ratio >355-fold. The selected insects are also resistant when fed spinosad formulated as Tracer. The topical LD_{50}'s for three tobacco budworm field strains collected from NC, LA and MS in 1998 were similar to the unselected parental strain and to LD_{50}'s previously reported for field strains (Leonard et al., 1996).

Introduction

Spinosad, the active ingredient in Tracer, is an insect control agent derived from the actinomycete bacteria, *Saccharopolyspora spinosa*. The principle components of spinosad, spinosyns A and D, are large, relatively apolar polycyclic molecules of MW 732 and 741, respectively. Spinosad has a narrow range of target species, being highly toxic to lepidopteran larvae and less toxic to non-target and beneficial insects (Borth et al., 1996). For this reason spinosad has been used for controlling lepidopteran pests, particularly tobacco budworm and cotton bollworm in cotton. Spinosad has only recently (1996) come into widespread use and, prior to our study, no resistance to spinosad had been reported in either field or laboratory studies. A previous survey of field-collected strains did find a >20-fold range in susceptibility of the budworm to Tracer (Leonard et al., 1996). This indicated a natural variation among field populations.

Tracer (spinosad) is a novel insecticide chemistry with a novel mode of action. The insecticide simultaneously acts on the nicotinic acetylcholine receptors and GABA-gated chlorine channels. Because of the unique characteristics of spinosad, its importance as an insect control agent in agriculture, and minimal information available on potential resistance mechanisms, laboratory selection studies were conducted in an attempt to develop a spinosad-resistant strain of the tobacco budworm. It is the long-range goal of our research to examine the mechanisms, genetics and molecular biology of spinosad resistance. These studies are aimed toward improving resistance management and developing alternative chemistries for insect control.

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Materials and Methods

Insects and Spinosad Selection

All insects were routinely reared and toxicity determinations carried out at 27±1ºC with a 14:10 (light:dark) cycle on a standard artificial diet (Gould et al., 1995; Rose et al., 1995). Three field populations of the tobacco budworm were collected in the summer of 1998 and reared for <4 generations prior to spinosad topical LD_{50} determinations. Hv Johnston was collected as larvae on tobacco in Johnston Co., NC, Hv Franklin was collected as larvae on velvetleaf in Franklin Parish, LA and Hv Washington was collected as eggs on geranium in Washington Co., MS.

A laboratory colony of the tobacco budworm was originally established and is annually supplemented with field collections from North Carolina. The last supplementation was made in 1997 with insects from Martin, Johnston and Wake Co., NC. This colony is referred to in this paper as HvSpP (*H. virescens* Spinosad Parental) and is reared in the absence of insecticide pressure.

Tobacco budworms from the parental (HvSpP) strain were selected with technical spinosad (88.0% active ingredient, Dow AgroSciences, Indianapolis, IN) starting in November of 1997 and designated HvSpS (*H. virescens* Spinosad Selected). The doses in these experiments are indicated as *u*g of technical material. In these selections, spinosad was applied in 1 *u*l of acetone with a repeating Hamilton syringe (Hamilton Co., Reno, NV) to the dorsal thorax of ice-chilled third stadium larvae. Insects topically selected were in the range of 15-45 mg/larva. Preliminary experiments indicated that a dose of 0.05 *u*g/larva resulted in 70-80% mortality. Treated insects were reared singly in 30-ml plastic cups (Solo Cup Co., Urbana, IL) on standard artificial diet. Treatment dose was increased after the 5th generation in order to maintain uncorrected mortality between 70-80%. From 1300 to 2300 larvae were selected in each generation. Acetone controls typically had 5-10% mortality. Larval mortality in all experiments is defined by a lack of movement after 10 s when teased with a blunt needle.

Topical Toxicity

LD_{50}'s resulting from topical application of spinosad were obtained for each of the 1998 field strains (Hv Johnston, Hv Franklin and Hv Washington) as well as the parental (HvSpP) and 6th generation spinosad selected (HvSpS-G6) strains. A topical LD_{50} could not be obtained for later generations because of the increase in resistance. Mortality

was assessed at 5 doses of technical spinosad, with 1 *u*l acetone applications used to correct for control mortality (Abbott, 1925). Treatments and controls were replicated 3 times, with 25 larvae per replicate. Larvae chosen for LD_{50} assessments were in the third stadium, 30±5 mg as determined by weighing representative insects (Zhao et al., 1996). Mortality was assessed at 6 d post-application, and data were analyzed using probit analysis (PROC PROBIT, SAS 1995) with logistic regression (SAS 1995).

Oral Toxicity

Second to fourth stadium larvae from the HvSpP and 11th generation HvSpS strains were fed on 100 *u*l cylindrical (6 mm diameter) plugs of artificial diet containing either 0.05 or 0.5 mg spinosad formulated as Tracer per ml of diet. The assays were conducted in 30-ml plastic cups with a cardboard top (1 larva and 1 diet plug/cup) for 18 h under standard insect rearing conditions and 80% relative humidity. These large cups provided sufficient space for the larvae to avoid the small diet plugs when not feeding, therefore minimizing contact exposure. The dose ingested was determined by weighing the diet at the beginning and end of the assay and correcting for water loss. In these tests, 45 larvae from both the HvSpP and HvspS strains were assayed at each concentration.

Results and Discussion

The history of selection of the original HvSpP strain is summarized in Fig. 1. The goal was to select the HvSpP strain for successive generations with technical spinosad (topical) at 70-80% mortality. Mortality was reported at 12±1 d after treatment; control larvae at this time are undergoing pupation. The selection of the parent strain with 0.05 *u*g of technical spinosad per third instar produced approximately 75% mortality (generation 1 or G1, Fig. 1) and mortality remained greater than 70% at this dose through G4. An increase in dose to 0.10 *u*g/larva in G5 produced approximately 81% mortality. A decline in mortality in G6 to <40% at a dose of 0.075 *u*g/larva suggested that a decrease in spinosad susceptibility had occurred in the selected strain. To examine this question further, dose-mortality lines were generated for both the HvSpP and HvSpS-G6 strains (Fig. 2). There was an apparent increase in resistance in the HvSpS strain consistent with our toxicological data for G6 in Fig. 1 but no statistically significant differences were noted between the LD_{50}'s for these two strains (Table 1).

The selection dose was increased to 0.2 *u*g/larva in G7 and to 0.3 in G8 in order to maintain >60% mortality. Multiple treatment levels were used in G9-G11 in an attempt to determine a dose that would provide greater than 50% mortality. These treatments all failed to produce high mortality. In G10d (Fig. 1), 10 *u*g/larva and in G11c, 60 *u*g/larva produced 21 and 39% mortality, respectively. The first indication of resistance occurred in the sixth generation with high levels of resistance by at least the ninth generation. The dose of 0.05 *u*g/larva used for G1, as compared to 60 *ug*/larva in G11, represents a 1,200-fold increase in the selection dose over 11 generations, while mortality decreased from 78 to 39%, respectively. The LD_{50} for the HvSpP strain was 0.169 *u*g/larva (Table 1). Based on the LD_{50} for HvSpP, the resistance ratio for the HvSpS-G11 strain is greater than 355-fold. Because of this high level of resistance and the limited solubility of spinosad in acetone, a topical LD50 for HvSpS-G11 could not be determined.

Field strains of the tobacco budworm were collected in the summer of 1998 from three regions of the Southeast US. The dose-mortality lines (Fig. 3) were similar and the LD_{50}'s (Table 1) were not significantly different among strains. The LD_{50}'s for these field strains were also not significantly different from the HvSpP strain. All LD_{50}'s obtained for these strains were within the range of variation determined by Leonard et al. (1996) for field strains from the Southeastern US. It is apparent from these comparisons that our parental strain (HvSpP) is similar in spinosad susceptibility to other field strains and was not any more predisposed to resistance to spinosad than field populations either now or in the past.

Feeding studies were conducted with Tracer (formulated spinosad) in order to evaluate whether resistance in HvSpS-G11 was solely the result of reduced penetration. As indicated in Table 2, resistant budworms fed Tracer in the dose ranges of 0.001-0.050 and 0.051-0.500 *u*g of spinosad/larva demonstrated corrected mortality of 0% at 48 h while the HvSpP strain exhibited 26.2 and 65.4% mortality, respectively. At 96h, the HvSpP-G11 strain again had no mortality while the parental strain demonstrated 78.5 and 88.5% mortality at the low and high dose ranges, respectively. These results do not rule out the possibility that reduced cuticle penetration is a contributing factor in resistance, but indicate that other mechanisms are involved in spinosad resistance in HvSpS-G11. Further studies to better characterize the mechanism(s) of resistance to spinosad are being conducted.

Summary

We have topically selected a North Carolina strain of the tobacco budworm for 11 generations with the insecticide, spinosad (Tracer) and have developed a highly resistant strain. The resistance ratio is greater than 355-fold. This is the first report of any insect resistant to this class of chemistry. These laboratory studies do not reflect on the effectiveness of Tracer in current field applications or necessarily project what may happen in field conditions. Understanding the possible resistance mechanisms for spinosad in insects will be important to preserving the efficacy of this control agent in the future.

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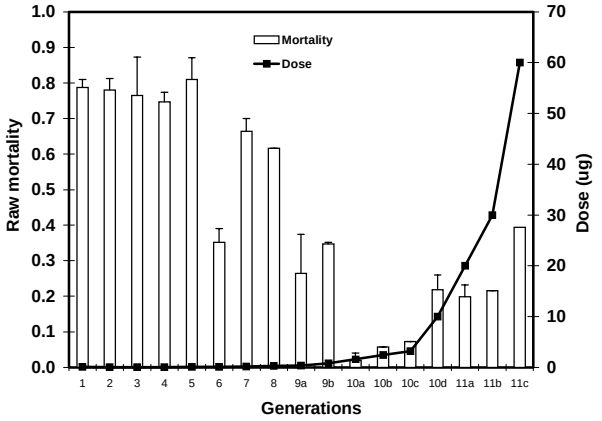

Figure 1. History of selection with spinosad (Tracer). Tracer susceptible tobacco budworms (HvSpP) were selected for 11 generations by topical application of technical grade spinosad. Mortality assessments were at 12±1 d post-treatment. Doses were increased in the later rounds of selection (solid line), while mortality decreased (bars) with the onset of resistance. In generations 9, 10, and 11 more than one dose was used. Error bars represent 1 SE. Categories without error bars are single data sets.

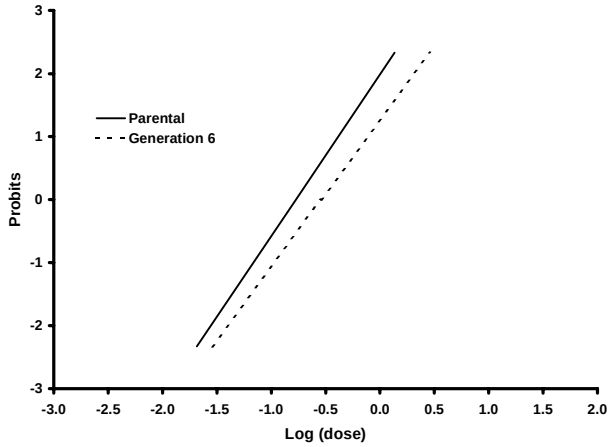

Figure 2. Probit analysis of toxicity resulting from topical spinosad application to 3rd stadium larvae of parental (HvSpP) and spinosad selected 6th generation (HvSpS) strains of the tobacco budworm. Mortality was assessed at 6 d post-treatment.

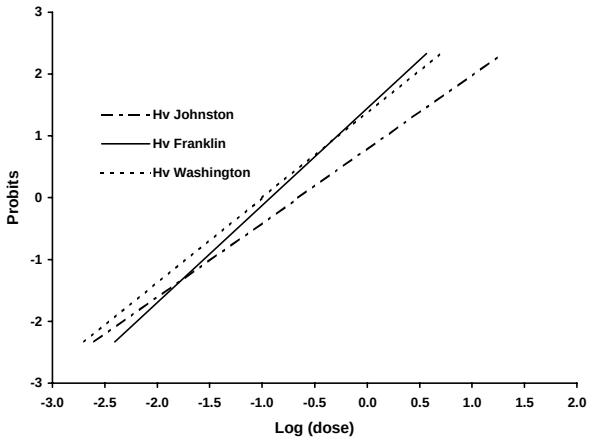

Figure 3. Probit analysis of toxicity resulting from topical spinosad application to 3rd stadium larvae of 3 strains of the tobacco budworm collected in the summer of 1998 from Johnston Co., NC, Franklin Parish, LA, and Washington Co., MS. Mortality was assessed at 6 d post-treatment.

Table 1. Comparison of LD_{50}'s for topical application of spinosad to 5 strains of the tobacco budworm. LD_{50}'s are expressed as *u*g of technical spinosad/larva.

Strain*	6 d LD50	95% CI	Slope (+1 SE)	*n*	RR†
HvSpP	0.169	0.072-0.676	2.55 (0.65)	450	-
HvSpS-G6	0.287	0.192-0.638	2.32 (0.39)	450	-
Hv Johnston	0.220	0.109-2.442	1.20 (0.29)	450	-
Hv Franklin	0.120	0.098-0.149	1.57 (0.17)	450	-
Hv Washington	0.100	0.075-0.133	1.37 (0.21)	450	-
HvSpS-G11	>60.0	-	-	132	>355

* HvSpP = parental unselected; HvSpP-G6 = spinosad selected, generation 6; HvSpP-G11 = spinosad selected, generation 11; Hv Johnston = Johnston Co., NC 1998; Hv Franklin = Franklin Parish, LA 1998; Hv Washington = Washington Co., MS 1998

†resistance ratio: Highest dose applied to HvSpS-G11 / LD_{50} HvSpP.

Table 2. Oral toxicity in G11 spinosad selected (HvSpS) and parental (HvSpP) strains of the tobacco budworm fed artificial diet containing Tracer.

	Dose		Corrected % Mortality	
Strain	(*u*g/mg insect)	*n*	48 h	96 h
HvSpP	0.001-0.050	42	26.2	78.5
	0.051-0.500	52	65.4	88.5
HvSpS-G11	0.001-0.050	47	0	0
	0.051-0.500	49	0	0