Baseline Susceptibility of Tobacco Hornworms (Lepidoptera: Sphingidae) to Acephate, Methomyl and Spinosad in Georgia[1]

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Abstract The tobacco hornworm, *Manduca sexta* (L.), is a serious defoliating pest of flue-cured tobacco in Georgia. Isolated producer reports of increased difficulty in controlling this pest with standard insecticides have created concern. Therefore, a topical application technique was used to determine the dosage-mortality responses of *M. sexta* to three commonly-used insecticides for Georgia tobacco: acephate, methomyl and spinosad. Larvae, 4 to 5 days old (second instar) and weighing 20 to 40 mg, were collected from tobacco plants. Serial concentrations of selected insecticides were applied topically to the larvae. The larvae were subsequently examined for mortality up to 72 h after exposure. Hornworm larvae were highly susceptible to spinosad, with LD_{50}'s of 0.059, 0.002, and 0.0004 μg/larva at 24, 48, and 72 h, respectively. Susceptibility to methomyl was intermediate, ranging from 0.123 to 0.176 μg/larva at 72 h, and acephate was the least toxic, with an LD_{50} of approximately 1.0 μg/larva. The LD_{50} values for methomyl and acephate remained constant from 24 to 72 h exposure. Location of the hornworm population within the state (south-central, eastern, and southeastern) did not appear to influence the overall susceptibility of the larvae. Baseline data are now established for the three commonly used insecticides for hornworm control, and can be used to document insecticide resistance if it occurs.

Key Words *Manduca sexta,* tobacco hornworm, dosage-mortality responses, insecticide resistance, methomyl, spinosad, acephate

The tobacco hornworm, *Manduca sexta* (L.), is an annual economic threat to Georgia's tobacco crop, causing more than $1 million in losses in many years (McPherson and Jones 2000). This pest consumes large amounts of leaf tissue during its larval stages, particularly during the fifth, and final larval stadium (McPherson et al. 1997).

Although the tobacco hornworm reaches a length of 7.5 to 10 cm before pupating (Reich 1995), this pest has been controlled with low rates of many commonly-used insecticides, including methomyl, acephate, spinosad and commercial formulations of *Bacillus thuringiensis* Berliner (McPherson et al. 1996, Taylor et al. 1996). Tobacco is a high-value crop that must be protected from insect-induced injury during the entire growing season. In Georgia, as many as three insecticide applications are made during a single season for tobacco hornworm control (Jones and McPherson 1997), which places this pest under a great deal of selective pressure for development of

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insecticide resistance. Insecticide resistance of tobacco hornworms was reported for endrin and endosulfan in the early 1960's (Rabb and Guthrie 1964). Thus, this potential pest control problem remains a concern for the currently labeled insecticides. In 1998, some tobacco producers expressed concern that the currently recommended tobacco insecticides had become less effective in controlling hornworms. Currently, there is no baseline information on the susceptibility of tobacco hornworms to insecticides in Georgia tobacco. Thus, this study was undertaken, using topical application procedures, to develop baseline dosage-mortality of hornworms to the currently labeled and commonly used tobacco insecticides. Field populations from three counties in south-central, eastern, and southeastern Georgia were examined to compare the susceptibility among areas.

Materials and Methods

In 1999 tobacco hornworm eggs and neonate larvae were collected in June and July from tobacco foliage in Tift (south-central), Jeff Davis (east), and Ware (southeast) counties in Georgia. In 2000, eggs and larvae were only collected from Tift Co. These field-collected eggs and larvae were placed in paper bags along with the foliage they were on and returned to the laboratory. Larvae from each location were maintained in these bags on freshly-collected, untreated tobacco foliage obtained from the Entomology Farm at the University of Georgia, Coastal Plain Experiment Station in Tifton. Fresh foliage was added as needed. As larvae reached 4 to 5 days old (second instar) and weighed between 20 to 40 mg, they were placed into Petri dishes (100 × 15 mm) that contained a 62-cm^2 diam tobacco leaf disk that covered the entire bottom of the dish. No filter paper or moisture was added to the dishes because preliminary observations revealed that the tobacco leaf alone was adequate to rear larvae for 72 h. Five larvae were confined to each Petri dish to serve as an experimental unit.

Hornworm larvae were treated individually by applying a 1-µl drop of an acetone solution of the desired chemical on the dorsal surface of the thorax. An Isco (Instrumentation Specialties Co., Lincoln, NE) microapplicator was calibrated to administer the insecticide-acetone solution using procedures modified from Brazzel (1970). Insecticides were serially diluted to desired concentrations to obtain mortality ranges suitable for probit analysis. Six to eight dosages were used to establish log-dose probit lines, the median lethal dose, and associated parameter estimates. A total of 30 to 40 larvae were used to establish each point (6 to 8 reps of 5 larvae each). Following treatment, each Petri dish with 5 larvae was labeled as to chemical, dosage and date of exposure and held in an insect growth chamber at 25 ± 2°C, 14:10 L:D, and 60 to 70% relative humidity. Mortality was assessed 24, 48, and 72 h after exposure. Fresh, untreated tobacco foliage was placed into each Petri dish at each observation. Criterion of death of a larva was failure to respond when touched with a probe. A moribund larva would move slightly when touched but was obviously dying, thus it was counted as dead. Percent mortality was corrected for natural mortality (which was less than 5%) in acetone-treated controls. The three insecticides evaluated in this study included technical grade acephate (Valent USA Corp., Walnut Creek, CA), an organic phosphate insecticide; methomyl (DuPont, E.I. de Nemours Inc., Wilmington, DE), a carbamate insecticide; and spinosad (Dow Agrosciences, Indianapolis, IN), a toxin derived from fermentation products of actinomycete bacterium. From the data obtained, LD_{50}'s, 95% confidence limits, slopes, and Chi-square values were obtained through probit analysis (Daum 1970). Log dose-probit (ld-p) lines were generated using regression analyses (SAS Institute 1990).

Results and Discussion

The responses of tobacco hornworm larvae to acephate are presented in Table 1. The LD_{50}'s for both the Tift Co. and Evans Co. populations were similar in 1999, both approximately 1.0 µg/larva. The response of the 2000 population was slightly greater than the 1999 population (0.89 vs 1.0), but the confidence intervals overlapped indicating no significant differences between the 2 yrs. The slopes of the ld-p lines also were similar among the three tobacco hornworm populations examined (Fig. 1). The LD_{50} values for acephate at 24 and 48 h after exposure were almost identical to those reported at 72 h, with very little mortality observed after the 24 h count.

No significant differences in LD_{50}'s among the four populations in response to methomyl were detected. The values ranged from 0.123 (Tift Co.) to 0.176 (Ware Co.) µg/larvae. As with acephate, the LD_{50} values for methomyl at 24 and 48 h after exposure were nearly identical to those reported at 72 h (Table 2). Although the LD_{50} values were similar for methomyl for the four hornworm populations examined, the LD_{80} and LD_{90} value in the ld-p lines were significantly different among populations (Fig. 2).

The Tift Co. tobacco hornworm population was susceptible to spinosad with an LD_{50} at 24 h after exposure of 0.059 (± 0.021) µg/larva. This is 2.4× lower than methomyl (mean of 0.144 µg/larva) and 17× lower than acephate (1.0 µg/larva). At 48 and 72 h, spinosad was even more active on hornworms, with LD_{50}'s of 0.002 and 0.0004 µg/larvae, respectively (Table 3). Because spinosad was so toxic to hornworm larvae, the 1d-p lines show very little change in LD_{10} to LD_{60} values at 48 h and from LD_{10} to LD_{80} at 72 h (Fig. 3). The slopes of the 1d-p lines for spinosad were low compared to the slopes for acephate and methomyl. The toxicity of spinosad 24 h after exposure was unexpected. This naturalyte insecticide (e.g., naturally-derived from bacteria) is primarily a stomach poison that disrupts the insect nervous system

Table 1. Susceptibility of second-instar tobacco hornworm larvae collected from two locations in southern Georgia to topical applications of acephate 72 h after exposure, 1999-2000

Collection site	LD_{50} (95% CI)*	Slope ± SE	Chi-square	df
	1999			
Tift Co.	1.007 (0.877-1.159)	2.90 ± 0.29	244.5**	5
Evans Co.	1.055 (0.580-2.445)	2.86 ± 0.99	23.3	3
	2000			
Tift Co.	0.891 (0.399-1.597)	3.11 ± 0.68	20.9	3

* Dosage of acephate (µg/larva) that kills 50% of the hornworm larvae, calculated by non-linear regression fitted to a probit model with 95% confidence intervals.

** All Chi-square values significant ($P < 0.05$).

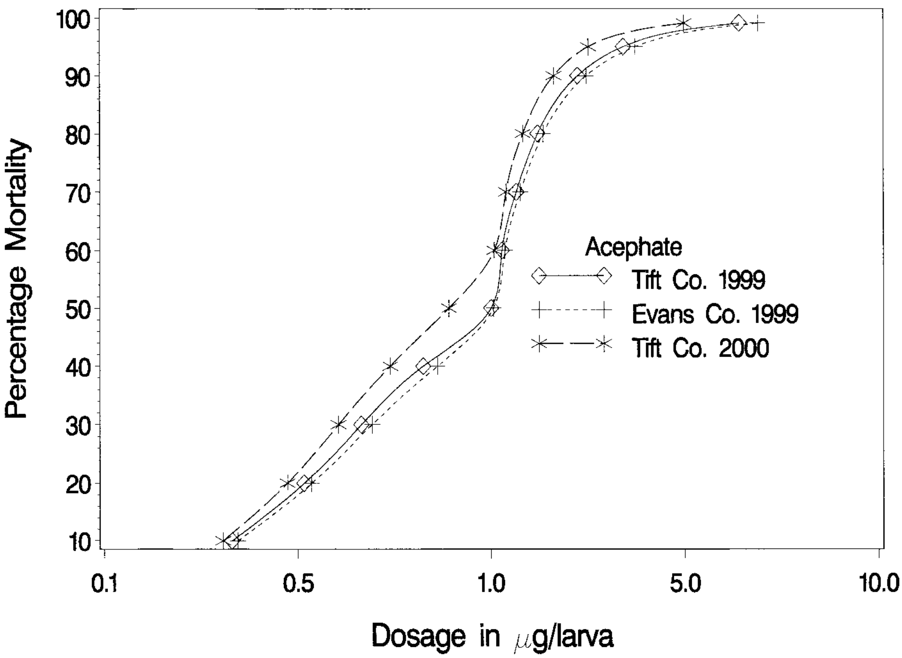

Fig. 1. Log dose-probit lines showing responses of three field populations of second instar (4 to 5-day-old) tobacco hornworm larvae to topical applications of acephate at 72 h after exposure.

Table 2. Susceptibility of second-instar tobacco hornworm larvae collected from three locations in southern Georgia to topical applications of methomyl 72 h after exposure, 1999-2000

Collection site	LD_{50} (95% CI)*	Slope ± SE	Chi-square	df
	1999			
Tift Co.	0.123 (0.083-0.171)	1.18 ± 0.16	36.8**	7
Evans Co.	0.164 (0.001-0.367)	0.91 ± 0.43	31.5	3
Ware Co.	0.176 (0.124-0.247)	1.72 ± 0.28	248.1	4
	2000			
Tift Co.	0.111 (0.083-0.152)	2.79 ± 0.54	74.0	3

* Dosage of methomyl (μg/larva) that kills 50% of the hornworm larvae, calculated by non-linear regression fitted to a probit model with 95% confidence intervals.

** All Chi-square values significant ($P < 0.05$).

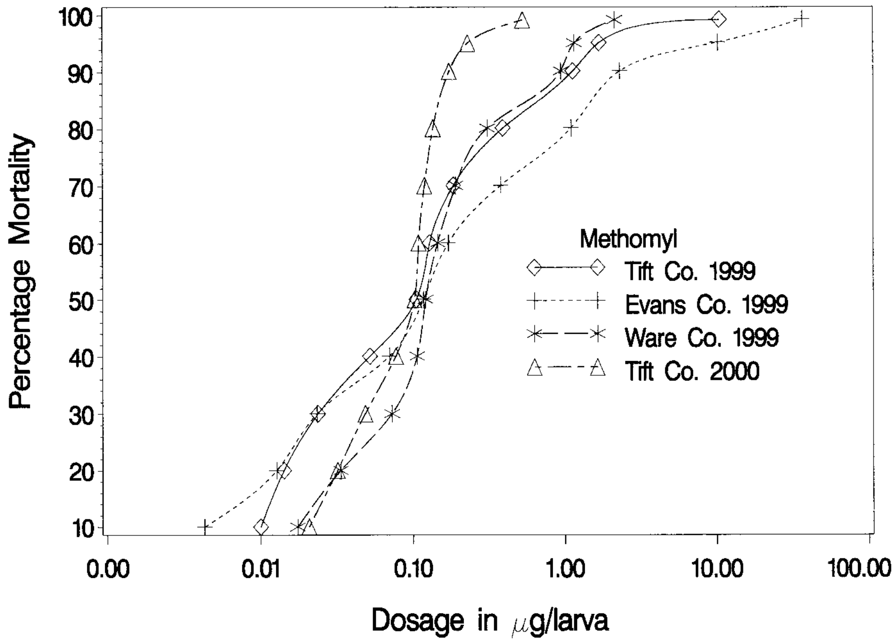

Fig. 2. Log dose-probit lines showing responses of four field populations of second instar (4 to 5-day-old) tobacco hornworm larvae to topical applications of methomyl at 72 h after exposure.

(Anonymous 1999). In comparison to the two other insecticides, spinosad was far more toxic at 72 h after exposure. At that time, spinosad had an LD_{50} that was 360× lower than methomyl (a very toxic material to tobacco hornworms in field trials) and 2500× lower than acephate (another good standard hornworm control material in field tests).

Table 3. Susceptibility of second-instar tobacco hornworm larvae collected from Tift County Georgia to topical applications of spinosad 24, 48, and 72 h after exposure, 2000

Exposure	LD_{50} (95% CI)*	Slope ± SE	Chi-square	df
24 h	0.059 (0.028-0.126)	0.49 ± 0.05	129.7**	6
48 h	0.002 (0.0003-0.007)	0.58 ± 0.09	40.1	6
72 h	0.0004 (0.0002-0.0007)	0.81 ± 0.10	41.4	6

* Dosage of spinosad (μg/larva) that kills 50% of the hornworm larvae, calculated by non-linear regression fitted to a probit model with 95% confidence intervals.

** All Chi-square values significant ($P < 0.05$).

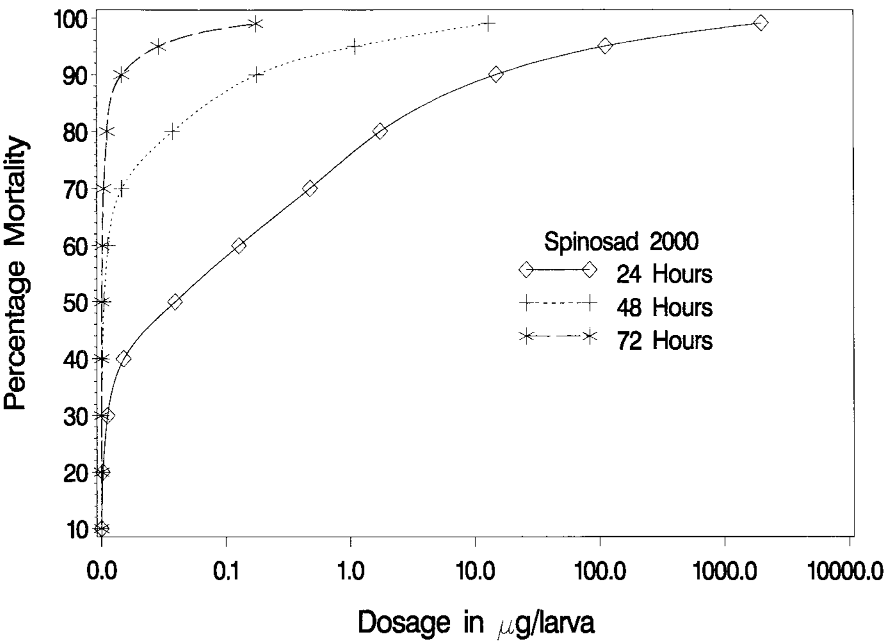

Fig. 3. Log dose-probit lines showing responses of second instar (4 to 5-day-old) tobacco hornworm larvae to topical applications of spinosad at 24, 48, and 72 h after exposure.

The LD_{50} values reported in this study for acephate, methomyl and spinosad were much lower than LD_{50} values reported by Rabb and Guthrie (1964) for endrin and endosulfan. However, the Rabb and Guthrie study reported LD_{50}'s as μg/fifth-instar larva plus the two insecticides they evaluated were chlorinated hydrocarbons. Interestingly, the LD_{50} values in their study went from 131 μg/larva (susceptible) to 2200 μg/larva (resistant) for hornworms exposed to endrin and from 650 μg/larva (susceptible) to over 2000 μg/larva (resistant) for hornworms exposed to endosulfan. A fifth-instar hornworm larva can weigh between 7500 and 9000 mg before pupating, thus the high LD_{50} values for susceptible fifth-instar hornworm larvae compared to LD_{50} values for second-instar larvae (weighing between 20 to 40 mg) is not surprising.

Development of these baseline susceptibility data reported herein for acephate, methomyl, and spinosad is the first step toward implementation of an insecticide resistance monitoring program to document tobacco hornworm resistance on flue-cured tobacco. If the control of hornworms becomes, or appears to become, less effective with these currently recommended and commonly-used materials, then dosage-mortality response curves can once again be established from these "suspect populations" and compared to the baseline data reported herein, to confirm or deny the development of insecticide resistance.

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