Effects of Variable Doses of Permethrin on *Heliothis zea* (Lepidoptera: Noctuidae) Growth and Development[1]

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ABSTRACT *Heliothis zea* (Boddie) larvae reared on artificial diet were treated with permethrin using a topical or dip bioassay at 3, 5, and 7 days post-hatch. Forty-eight-hour survival and weight gain in survivors, percent pupation, and percent adult eclosion were measured in one experiment. Generally, larval weight gain after 48 hours among survivors decreased with increasing dose. A high proportion of individuals surviving after 48 hours went on to pupate and emerge as adults regardless of dose, age, and reduction in growth at 48 hours post-treatment. In a second experiment, pupal and adult weights and development times of larve and pupae were measured after topical treatment of larvae with permethrin. Pupal weights of survivors decreased with increasing dose in all age classes. Adult weights decreased with increasing dose only in the 7-day-old treatment. Development times were protracted with increasing dose in the 5-day-old and 7-day-old treatments, but not the 3-day-old treatment. In both experiments, smaller larvae were more tolerant of permethrin than larger larvae per unit body weight.

KEY WORDS *Heliothis zea*, permethrin, Ambush 2EC, sublethal effects, topical bioassay, dip bioassay, fitness.

Nonlethal doses of permethrin have been shown to cause repellent and antifeedant effects and to decrease mating efficiency in taxonomically diverse groups of insects (Boles 1974; Linn and Roelofs 1984; Armstrong and Bonner 1985; Haynes et al. 1986; Hodges and Meik 1986). Several studies have reported effects of variable doses of permethrin on development in lepidopteran larvae. For example, permethrin applied to the diamondback moth, *Plutella xylostella* (L.), at doses producing no short-term mortality was shown to be deleterious in terms of percent of larvae surviving to pupation and percent adult eclosion (Kumar and Chapman 1984). Tan (1981) continuously fed fifth instar *Pieris brassicae* [*Trichoplusia ni* (Hübner)] larvae leaf dics dipped in permethrin and found the larval period extended, foliage consumption reduced, and the maximum weight of larvae and pupae decreased.

In the field, sublethal effects of variable doses could influence pest population dynamics. Insecticide delivery systems produce a heterogenous chemical environment for insects. Leaf residue studies by Southwick et al. (1986) and Uk and Courshee (1982) demonstrated that spray deposits attenuate with increasing depth in the plant canopy. Hutchins and Pitre (1985) observed differential mortality in

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lepidopteran defoliators in soybean correlated with position in the canopy. In their study, percent larval reduction was higher in the upper than middle and lower canopy as a consequence of decreased vertical distribution of droplets to lower portions of the plant. Recent studies indicate that *Heliothis zea* (Boddie) larvae receive variable doses of insecticide during a spray on soybean or cotton depending on their position in the canopy, age, and the application technique (Follett and Gould, unpubl. data). In addition to spatial heterogeneity, residue decay will produce temporal heterogeneity in levels of pesticide experienced by the population (Daly et al. 1988). Therefore, some larvae potentially receive a nonlethal dose which may affect their developmental and reproductive performance. To my knowledge, no studies have focused specifically on the effects of variable doses of pyrethroids on the fitness of *H. zea*. Two experiments reported here were undertaken to assess the effect of variable doses of permethrin on the growth and development of surviving *H. zea*.

Materials and Methods

The first experiment was conducted to determine effects of permethrin on short-term larval weight gain and subsequent pupation and adult eclosion. In July 1988, neonate *H. zea* larvae, obtained from a large stock colony maintained at North Carolina State University, were placed individually on a modified CSM (corn-soybean-milk) diet (Burton 1970) in 1-ounce (ca. 33 ml) plastic cups with paper caps and reared at 27 ± 1°C. At 3, 5, and 7 days post-hatch, larvae within a specified weight range for each age class were treated with a range of insecticide doses in a factorial arrangement. Two delivery techniques were used, dipping and topical application. Larvae to be dipped were held with soft forceps and submersed for ca. 2 seconds in a 20-ml vial containing formulated permethrin in water (Ambush® 2EC, ICI Americas, Goldsboro, NC). Topical applications of technical grade permethrin (95%, ICI Americas, Goldsboro, NC), dissolved in acetone, were applied as two 0.5 µl droplets, one immediately following the other, delivered from a 20-µl digital microdispenser (The Drummond Scientific Co., Broomall, PA) to the dorsal surface of each larva in the rearing cup. In both cases, doses were prepared by serial dilution of the compound in solution. The range of doses was 13-863 ng permethrin in the topical bioassay and 0.1-10.0 µl Ambush 2EC/100 ml in the dip bioassay. At each dosage within an age class, 30-35 larvae were weighed and treated. A control group (n = 30 to 35) for each age class was dipped in water (dip technique) or received a droplet of acetone (topical technique). In an attempt to minimize the amount of permethrin blotting from larva to the diet surface, each larva was placed on the inside wall of the diet cup, which was laying on its side, to facilitate air-drying before righting and capping the cups.

After 48 hours, larvae were scored as dead or alive and survivors reweighed in each treatment. A larva was defined as "dead" if it was unable to right itself within 3 seconds after being flipped onto its dorsum. Two out of three responses were judged if the first larval response was questionable. Surviving larvae were returned to their diet cups and cups were inspected four weeks later for presence of pupae and adults.

A second experiment was conducted to determine effects of permethrin on larval and pupal development times, and pupal and adult weight. In August 1989, *H. zea* larvae from the N.C. State colony were reared and treated by topical

application with permethrin as described in the earlier experiment, but with three modifications: larvae were reared at 28 ± 0.5°C, photophase was constant (24:0, L:D), and 25 larvae were used in each treatment at each insecticide dose level. The range of doses was 14-450 ng of permethrin. Treated larvae were observed daily. Pupae were weighed 24 - 48 hours after becoming fully melanized and adults were weighed <24 hours after eclosion. Because a nearly 1:1 sex ratio was observed, data for the two sexes were combined for analysis.

In both experiments, LD_{50} values were determined using probit analysis (SAS Institute Inc. 1986) of mortality data. Analysis of data in the second experiment employed linear regression of untransformed data performed using MGLH (Wilkinson 1989), a procedure which also computes analysis of variance. Regression analysis is the appropriate statistical technique for evaluating the effects of quantitative factors, such as levels of pesticide (Chew 1976).

Results and Discussion

In the dip bioassay in the first experiment, the mean initial weight of 5-day-old larvae was 18.4 mg (s = 3.1, n = 159) and the mean weight of 7-day-old larvae was 144.4 mg (s = 14.4, n = 209). Topically treated 5-day-old larvae had a mean weight of 25.7 mg (s = 3.9, n = 195) and 7-day-old larvae had a mean weight of 111.4 mg (s = 15.5, n = 150). All 3-day-old larvae in each test weighed approximately 2 mg. Mean weights were calculated using all larval weights from only those dose treatments with survivors 48 hours post-treatment and included the control larvae for that age class. Control mortality was zero in all tests.

Probit analysis of mortality data in the first experiment demonstrated that tolerance to permethrin increased with larval size (Table 1). Dividing LD_{50} or LC_{50} values by mean larval weights for each age class shows that smaller larvae are actually more tolerant per mg body weight than older, larger larvae. For 3-, 5-, and 7-day-old larvae, LD_{50} values per mg body weight were 24.8 ng, 5.3 ng, and 3.9 ng, respectively, in the topical bioassay. In the dip bioassay, LC_{50} values per mg body weight were 0.15 µl Ambush 2EC/100-ml in 3-day-old larvae and 0.01 µl Ambush 2EC/100 ml in 7-day-old larvae.

Table 1. Log probit analysis of 48-hour mortality in *H. zea* larvae treated with permethrin.

Bioassay	Age		Slope	LD_{50}	95% Fiducial limits	
		n	(± SE)	(ng)*	Upper	Lower
Topical	3	99	2. 2 (0.5)	49.6	66.5	33.0
	5	125	2. 8 (0.4)	137.3	175.8	108.6
	7	75	1. 6 (1.6)	438.3	-§	-
				µl Ambush 2EC/100ml†		
Dip	3	125	1. 4 (0.2)	0.3	0.5	0.2
	5	-	-‡	-		
	7	105	1.93 (1.3)	1.0	-§	-

* Estimated dosage killing 50% of larvae.
† To convert to mg AI/ml, multiply by 0.0024.
‡ Omitted because only two levels of permethrin gave between 99% and 1% survivorship.
§ Probit analysis program unable to generate fiducial limits.

In the first experiment, weight gain among survivors after 48 hours generally decreased as insecticide dose increased within each age class (Fig. 1). Survivorship decreased with increasing treatment dosage, also (Fig. 2). A high portion of larvae that survived for 48 h went on to pupate and eclose, regardless of age, treatment dosage, and growth reduction after 48 h. For example, 3-day-old larvae receiving topical treatments of permethrin of 27 ng, 54 ng, and 108 ng exhibited mean weight gains of 4.0 mg, 2.2 mg, and 1.5 mg, respectively (converted from log values in Fig. 1A). After 48 h, survival was 72, 46 and 24%, respectively, and all survivors in the three treatments went on to pupate and eclose (Fig. 2A). By comparison, control larvae gained 17.1 mg after 48 h and all survived to the adult.

There was some tendency toward lowered success of survivors in reaching the adult stage at high dosages for each age class (Fig. 2). Considering topically treated larvae of all age classes, 75% of those survivors having received >54 ng permethrin eclosed (n = 99), compared with 99% eclosion in larvae having received ≤54 ng permethrin (n = 245). All adults appeared normal, but were not mated in this study to observe possible effects of permethrin on fertility.

The mean weights of 3-, 5-, and 7-day-old *H. zea* larvae used in the second experiment were 2 mg, 30.4 mg (S = 6.1, n = 124), and 154.9 mg (S = 37.5, n = 150), respectively. As in the first experiment, younger larvae suffered higher mortality at each level of permethrin than older larvae, and younger larvae were more tolerant per unit body weight than older larvae. For 3-, 5-, and 7-day-old larvae, LD_{50} values per mg body weight were 11.7 ng, 1.6 ng, and 0.5 ng, respectively, in this experiment.

Pupal weights decreased with increasing dose in all age classes (Figs. 3A,B,C; Table 2). Adult weights significantly decreased with increasing dose only in the 7-day-old treatment (Table 2). Increasing doses of permethrin caused significantly protracted larval development times in 5-day-old and 7-day-old treatments, but not in the 3-day-old treatments (Figs. 3D, E, F; Table 2). Pupal development time was attenuated only in the 7-day-old treatment (Fig. 3F; Table 2).

These results suggest that early instars receiving a nonlethal dose of pesticide may be able to recover, so that any detrimental effects on growth and development initially are not observed later. For example, 3-day-olds suffered slowed growth 48 hours after exposure to all but the lowest concentration of topically applied permethrin in the first experiment (Fig. 1A). However, a nearly equivalent range of treatments had no effect on larval development time (Fig. 3D) or pupal weight (Fig. 3A) among survivors in the second experiment. Later instars appear to be less able to recover from the stress associated with a nonlethal dose, resulting in delayed pupation and lower pupal and adult weights.

A laboratory study of larvae receiving a single dose of insecticide is far removed from the experiences of *H. zea* larvae inhabiting a crop. For example, many larvae succumb following a spray only after accumulating a lethal dose through contact with residues on the plant surface. A field-applied pesticide may have its full killing effect only after 72 hours or more, because larvae moving and feeding on the plant continue to accumulate a dose in addition to that received from the initial contact with spray droplets. But in crops where insects can experience complete or partial refuges (on lower canopy leaves, inside unfolded terminal leaves, inside or partly inside fruits, covered by bracts, etc.), variable, nonlethal doses may be common, and associated sublethal effects on performance may play a role in population dynamics.

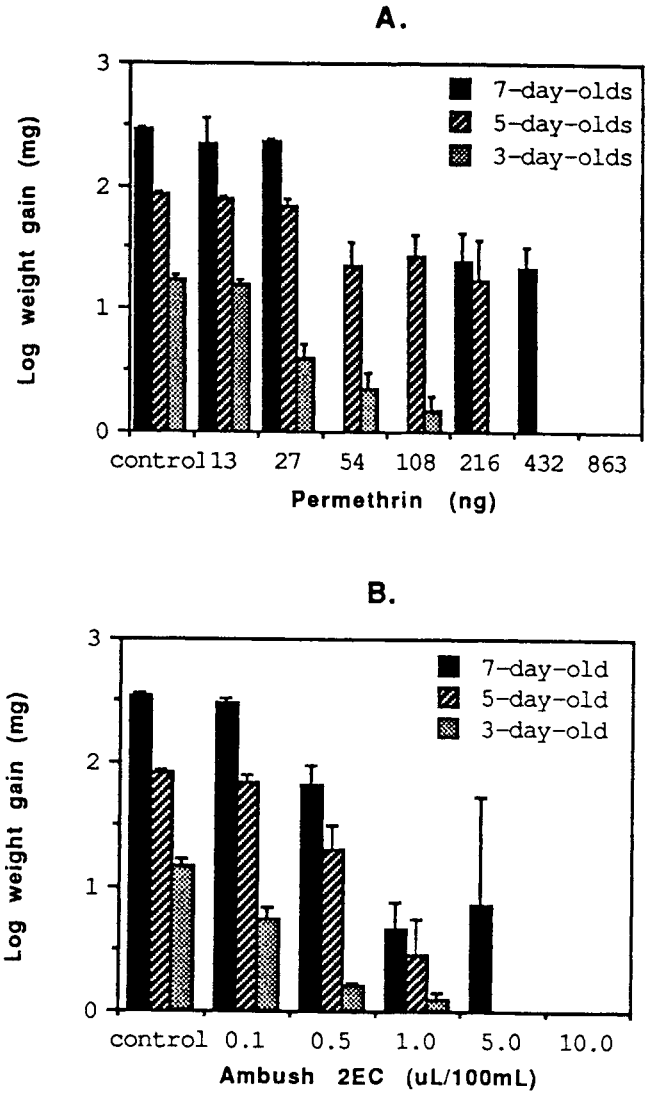

Fig. 1. Weight gain of surviving *H. zea* larvae 48 hours post-treatment with permethrin. Larvae receiving a topical application at age 3 days, 5 days, and 7 days (A). Larvae dipped at age 3 days, 5 days, and 7 days (B).

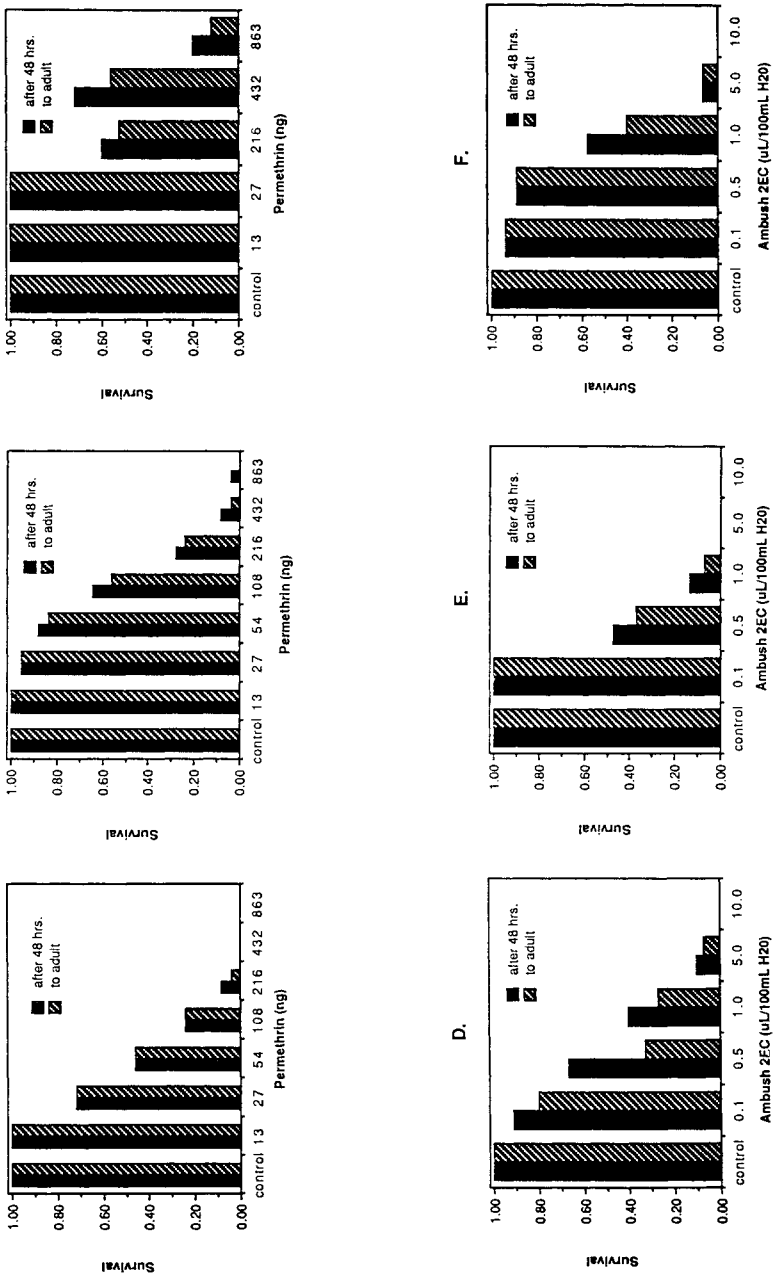

Fig. 2. Survival of *H. zea* larvae to 48 hours and to adult after treatment with permethrin. Larvae receiving a topical application at age 3 days (A), 5 days (B), and 7 days (C). Larvae dipped at age 3 days (D), 5 days (E), and 7 days (F).

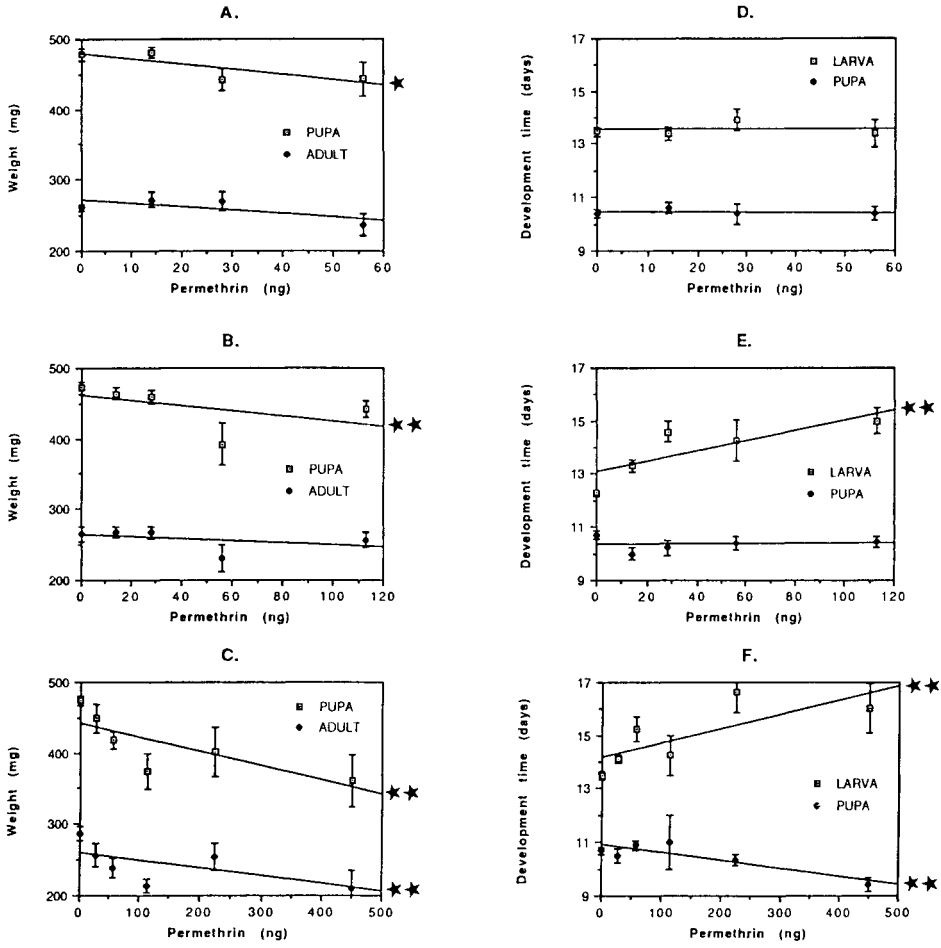

Fig. 3. Pupal and adult weights of *H. zea* which received topical treatments of permethrin as larvae at 3 days (A), 5 days (B), and 7 days (C) post-hatch. Development times of larvae and pupae of *H. zea* which received topical treatments of permethrin as larvae at 3 days (D), 5 days (E), and 7 days (F) post-hatch. See Table 2 for linear regression analysis. Slopes are significantly different from O, $P \leq 0.05$ (*) $P \leq 0.01$(**).

Table 2. Linear regression analysis of pupal weight, adult weight, larval development time, and pupal development time of *H. zea* larvae treated with permethrin.

Parameters	Age at treatment (days)	Intercept	Slope	T	P (2 tail)
Pupal weight (mg)	3	481	-0.84	-2.4	0.02
	5	467	-0.44	-2.5	0.01
	7	456	-0.25	4.1	0.00
Adult weight (mg)	3	269	-0.33	-1.1	0.28
	5	266	-0.15	-1.1	0.29
	7	269	-0.14	-2.6	0.01
Larval development time (days)	3	13.5	0.004	0.39	0.70
	5	13.0	0.023	4.51	0.00
	7	14.0	0.006	4.92	0.00
Pupal development time (days)	3	10.5	0.000	-0.03	0.98
	5	10.3	0.000	-0.11	0.91
	7	10.8	-0.003	-3.16	0.00

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