THE OPTIMUM RANGE OF WEIGHTS OF THE TOBACCO BUDWORM (*HELIOTHIS VIRESCENS)* FOR USE IN THE THIRD INSTAR TOPICAL BIOASSAY

A. A. Dubbeldam and A. R. McCaffery
The University of Reading
Reading, UK

Abstract

Tolerance to cypermethrin is negatively correlated with weight in third instar larvae of the susceptible SUR strain of *H. virescens*. The relationship is not linear, but decreases sharply above a transitional weight of 19mg. Comparisons of the results of third instar topical bioassays between larvae greater than and smaller than the transitional weight leads to 10-fold differences in the estimation of cypermethrin potency. The reasons for the transitional weight are discussed.

Introduction

The third instar topical bioassay is commonly used to estimate levels of resistance to pesticides in the tobacco budworm *Heliothis virescens* (Luttrell et al. 1987; McCaffery et al. 1991; Perrin and Lower 1994). Since the first definition of the third instar topical bioassay using larvae weighing 30-40mg (Anon 1970), a number of authors have suggested the use of more appropriate weight ranges of larvae. While the primary argument for a change in weight range is an avoidance of individuals near a moult, tolerance has been shown to decrease late in the third instar. Mullins and Pieters (1982) detected a lower tolerance to methyl parathion and permethrin at 30mg compared to 20 and 40mg, and Roush and Wolfenbarger (1985) identified a similar reduction in tolerance to methomyl at 21mg.

Firko and Hayes (1990) using a system for scoring the degree of debilitation, refute the evidence for a reduction in tolerance with increasing weight. With this evident confusion about the relationship between tolerance and insect weight, we studied this in our susceptible strain to determine a suitable weight range for our bioassays. As a comparison with the work of Firko and Hayes (1990), we weighed survivors at the same time as scoring for mortality at four days. Survivor weight is a measure of growth rate over the four day period, and therefore directly related to the degree of debilitation caused by cypermethrin.

It is important in the third instar bioassay that a weight range is used with minimal response variation. A significant change in pesticide tolerance midway through the instar, would reduce the accuracy of tolerance measures if the weight range straddles a transitional weight. As demonstrated here, when a transitional weight is detected, a revised weight range for larvae in the bioassay should be used which avoids this.

Methods

Insects.
Susceptible *Heliothis virescens* (SUR), were bred from a cross between BRC (Zeneca) and LSU (Louisiana State University). Cultures were maintained at 25±1^{0}C, with a 14:8, light:dark regime. Larvae were kept in individual 30ml pots with standard Lepidopteran artificial diet, until pupation. Adults were fed on a sugar/honey solution with supplemented vitamins, and kept in large ventilated perspex boxes.

The effect of third instar weight on mortality to a fixed dose of cypermethrin.
Batches of 30 third instar *H.virescens* were individually weighed, and a 1µl drop of 0.01mg/ml technical cypermethrin in acetone was applied to the dorsal meso-thorax with a Hamilton micro-applicator. Mortality was assessed after four days, with death classified as the inability to make co-ordinated movements when probed with a blunt seeker.

The effect on growth of survival from cypermethrin exposure. Survivors of the fixed dose of cypermethrin were re-weighed four days after dosing.

Range of weights and head capsule widths in third instar *H. virescens*.
Thirty larvae were weighed at each of six times during a three day period, and then individually stored in the freezer. Head capsule widths of recently thawed larvae were measured using a calibrated compound microscope.

Relative potency of 12-19mg versus 19-25mg third instar *H. virescens*. Parallel third instar topical bioassays were performed on two consecutive days on larvae in the weight ranges 12-19 & 19-26mg. Mortality was assessed at 48h & 96h, and the results analysed using POLO-PC.

Results

Mortality due to cypermethrin is positively correlated with weight at dosing (Figure 1), but the plot suggests a proportionately greater increase in susceptibility above 19mg. Two hypotheses are compared. In the first, the expected mortality value is calculated from linear regression of all the data, and in the second the expected values are derived from separate linear regression for data <19mg and >19mg. On comparison by ANOVA the differences between the two sets of pairs of data gave $P = 0.47$ (35 d.f.). The mean difference between the expected and observed values for the first hypothesis was slightly larger at 0.059% compared to 0.048% in the second hypothesis; these differences are not statistically significant.

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Four-day-old survivor weight is positively correlated with weight at dosing, with a slope of 5.82 (Figure 2). Survivor weight increases sharply in larvae which were >18-19mg at dosing.

The correlation between head capsule width and body weight confirms that head capsule widths are constant within each instar (Figure 3). For the susceptible SUR strain the mean head capsule width at third instar is 0.89mm, with 95% of the values in the range 0.75-1.02mm. From more limited data for second and fourth instar larvae the head capsule width ranges are 0.39-0.57 & 1.09-1.79mm respectively. Third instar weights range between 4.8-40.2mg in the sample of 119 third instar weighed for the comparison with head capsule width. The larvae fall into two distinct groups with weights either above or below 18mg (Figure 3).

The LD_{50} for cypermethrin is 7-fold higher at 48h and 6-fold higher at 96h in larvae in the range 12-19mg than in 19-26mg (Table 1) and at both 48 and 96h the slope is steeper in the lighter larvae. The relative potency of cypermethrin to 12-19mg larvae compared to 19-26mg larvae when the results are adjusted for the difference in weight, is 10.46µg/mg insect at 48h and 9.27µg/mg insect at 96h (Table 2).

Discussion

Cypermethrin tolerance in third instar larvae is shown to be negatively correlated with weight at dosing. There is also limited evidence for a transitional weight at 19mg, above which there is a particular decrease in tolerance to cypermethrin. The evidence was sufficient to warrant parallel bioassays on third instar both heavier and lighter than the transitional weight.

Survivor weight as a function of weight at dosing, increases more rapidly in heavy than in light third instar larvae. The slope of 5.82 indicates that growth is accelerated during the instar, with a particular increase in growth rate above a weight of 18mg.

Head capsule width is a discrete property of the instar, with third instar larvae of the SUR susceptible strain having a range at 0.75-1.02mm, similar to that described previously (Anon 1970) with a value of 0.76-0.96mm. Third instar larvae of the SUR strain range in weight from 5-40mg, with the moult occurring in some larvae at less than 30mg. In order to avoid the moult therefore, the maximum weight of larvae in the test has to be considerably less than 30mg.

Cypermethrin was significantly more toxic to larvae above than below the transitional weight of 19mg, which could lead to the mis-classification of resistance. Large differences in potency would only occur when results are compared between tests carried out on different sides of the transition weight. It is unlikely that whole tests would be performed on larvae of different weight ranges, but differences in the proportion of larvae from each side of the transition weight would increase variability in the response.

In both replicates of the parallel bioassays, relative potency differences were slightly lower at 96 than 48h, suggesting that larger larvae have a greater ability to recover from cypermethrin poisoning. The failure by Firko and Hayes (1990) to detect a reduction in tolerance with increased weight, may be because of a difference in symptom expression with differences in weight.

The reasons for the rapid onset of reduced cypermethrin tolerance, may be because of a change in growth pattern. The transitional weight for SUR is approximately coincidental with the change in larval growth from longitudinal growth to an increase in girth (personal observation). At the same time growth rate is accelerating, and it may be that there is either a metabolic change reducing tolerance, or thinning of the cuticle as it stretches.

The evidence for a transitional weight is sufficiently convincing to recommend a third instar larval bioassay weight range for SUR of 12-19mg. The shallow slope for the bioassay on 19-26mg larvae may be because 19mg is the lower limit for the transitional weight. In this case a weight range avoiding both the transitional weight and moult would be very narrow, possibly 21-25mg, leading to difficulties in obtaining sufficient numbers of insects for the test.

The results of this experiment come from a single strain of susceptible insects. The relevance both to other susceptible strains and to resistant strains requires further investigation.

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Table 1. Mortality at 48h and 96h of third instar larvae of *H. virescens* in the weight ranges 12-19 & 19-26mg, following topical treatment with cypermethrin.

	48h			96h		
Weight class	**LD_{50} (µg/ml)**	**LD_{50} 95% CI**	**Slope**	**LD_{50} (µg/ml)**	**LD_{50} 95% CI**	**Slope**
12-19mg	9.28	5.59-23.6	0.957	11.05	5.74-52.0	1
19-26mg	1.29	1.04-1.57	1.79	1.73	1.38-2.16	1.60

Table 2. Relative potency at 48 & 96h of topically applied cypermethrin to third instar larvae of susceptible *H. virescens* in the weight ranges 12-19 & 19-26mg, expressed as dose per insect and per mg body weight.

	48h		96h	
	ng/insect	**ng/mg insect**	**ng/insect**	**ng/mg insect**
Potency	7.20	10.46	6.39	9.27

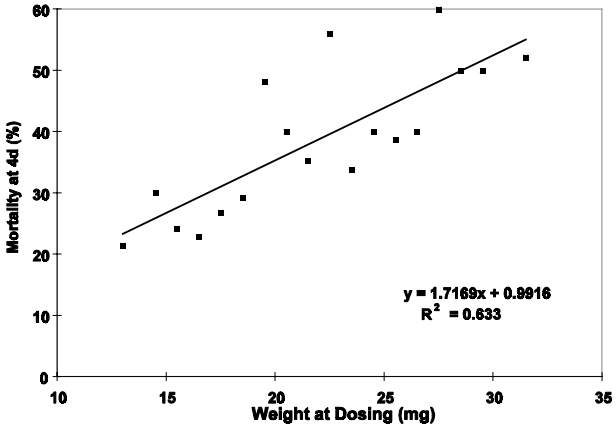

Figure 1. Correlation between mortality and weight at dosing for third instar larvae of the SUR strain of *H. virescens* treated with a fixed cypermethrin dose.

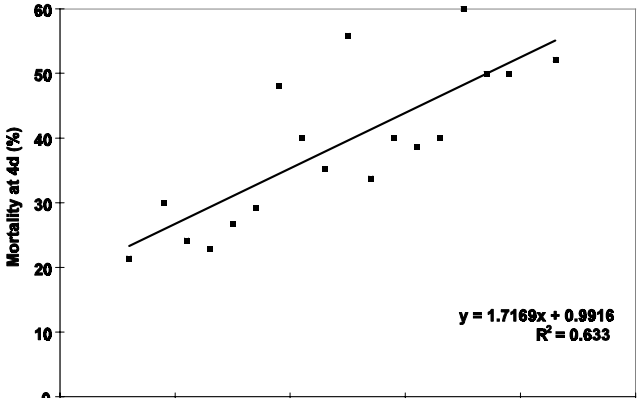

Figure 2. Effect of weight at dosing on four-day-old survivor weight in third instar larvae of the SUR strain of *H. virescens* exposed to a fixed dose of cypermethrin.

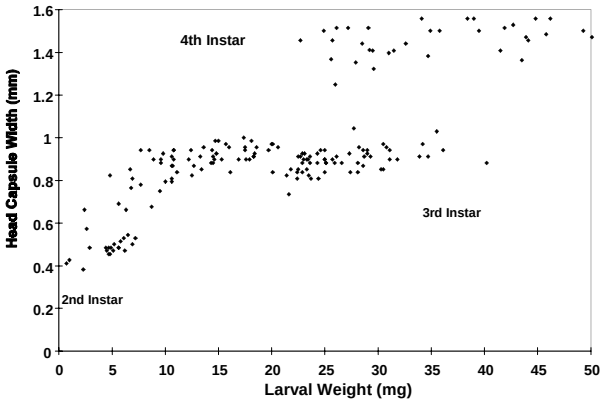

Figure 3. Correlation between head capsule width and third instar weight in larvae of the SUR strain of *H. virescens*.