VARIATION IN THE RESPONSE TO ENDRIN OF PROGENY FROM PAIRS OF BOLLWORMS, *Heliothis zea* (Boddie), AND VALIDITY OF AVERAGE LARVAL WEIGHT FOR COMPUTING DOSAGE-MORTALITY CURVES

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PREFACE

Variation has been reported in the response to insecticides by bollworms from the same or different areas (Brazzel 1962) and from different host plants (Brazzel 1964). One source of this variation may be the number of pairs of bollworm moths from which progeny were selected for testing. If one pair of bollworm moths produces all resistant progeny while another produces susceptible progeny when compared to each other, then sample size would be important. To determine if response variation exists to a given insecticide, bollworm adults were paired and their progeny were treated topically with endrin.

Two methods for analysis of data to compute dosage-mortality curves have been reported. The accepted method used by Lingren and Bryan (1964) of using individual larval weights versus the method used by Brazzel (1964) of using the average weight of all larvae treated at each concentration was tested. Dosage-mortality curves from the two methods were compared.

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The bollworm, *Heliothis zea* (Boddie), has displayed variable responses to the same insecticide (Brazzel 1962, Brazzel 1964). The budworm, *Heliothis virescens* (Fabricius), has been reported to be generally more tolerant to insecticides than the bollworm (Brazzel *et al*. 1953; Gast *et al*. 1956; McPherson *et al*. 1956; Brazzel 1962; Brazzel 1963). However, Lingren and Bryan (1964) reported that the progeny from one pair of budworms were less tolerant than the bollworms.

This study was undertaken to test the hypothesis that one pair of bollworms may produce progeny that may be more or less resistant than progeny of another pair. Bollworms were paired, their progeny treated topically with endrin and dosage-mortality curves established in order to test this hypothesis.

Hand calculations made to transform the dose applied to each larva into micrograms (ug) per gram body weight, and to calculate a dose are a slow and laborious task. Easier methods utilizing computer fortran programs were initiated to calculate doses for computing dosage-mortality curves. Two methods for calculating doses, individual larval weight as

used by Lingren and Bryan (1964) and average larval weight as used by Brazzel (1964) were compared.

METHODS AND MATERIALS

Bollworm moths for use in this work were collected from light traps at Stillwater, Oklahoma the last week in September, 1965. Only moths appearing to have recently emerged were used for pairing. Crosses were also made with P_2 moths obtained from F_1 pupae. Larvae were obtained from caged P_1 moths at Chickasha and Altus, Oklahoma and allowed to pupate. The emerging P_2 adults were paired and the F_2 larvae from these matings were tested.

Larval Rearing

The paired moths were placed in oviposition cages made from one-pint ice cream cartons. The cage tops were covered with nylon tulle to provide an oviposition surface. A 1-dram vial was filled with a 10% sucrose solution and provided with a cotton ball wick. This vial was inserted into the side of the carton to provide food for the moths.

A small incandescent table lamp in the oviposition room provided diffused lighting to encourage copulation.

Eggs were deposited on the tulle, cotton plugs, and sides of the cartons. The pint oviposition cartons containing eggs in the dark ring stage were placed in 1-gal cartons. To retain the larvae upon hatching and provide the adults with air circulation and light, the gallon lids were covered with muslin.

After the larvae hatched, they were transferred in groups of 6-10 to 1-oz transparent plastic jelly cups (Premium Plastics, 465 West Cerman Road, Chicago, Illinois) containing approximately 1/2 oz artificial diet developed by Adkisson et al. (1960) and modified by Berger (1963).

A pressurized dispensing device described by Burton[1] was modified and used to dispense the diet into the 1-oz cups. The device consisted of an 8-qt pressure cooker fitted with a pressure gauge at the air inlet. A kitchen sink hose with spray assembly was fitted to the lid. The hose extended to the bottom of the cooker to allow the diet to be forced out by air pressure. Air was supplied by a tank of compressed air. The amount of diet dispensed was controlled by depressing the lever of the nozzle of the spray assembly.

Prior to placing the diet in the cooker for dispensing, the cooker was heated to keep the diet in a liquid state. In spite of the above effort, approximately 1/2 gal of diet solidified and could not be dispensed. To overcome this problem, a metal funnel was altered by removing the spout and welding a flat base to the bottom. This was placed base down in the cooker. Freshly mixed diet in the liquid state was poured into the funnel. The area around the funnel was filled with hot water to prevent solidification of the diet prior to dispensing. The use of the funnel and jacket of hot water allowed all of the diet to be dispensed in one-third of the previous time.

The cups to be filled were placed on wooden trays (16" by 24"). The cups of diet were stored on these trays in refrigerators. The use

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[1]Robert L. Burton. 1965. Personal Communication. USDA Entomol. Res. Div. Georgia Coastal Plain Exp. Sta. Tifton, Georgia.

of these trays allowed minimum handling of the cups and easy manipulation of the larvae. Refrigerator space was limited; therefore, excess cups of diet were placed in a freezer and thawed as needed.

Insecticide

Technical grade endrin dissolved in 100 ml of acetone in amounts of 62.5, 125, 250, 500 and 1000 mg was used for treating.

Testing Procedures

Prior experience showed that 200 or more larvae from each pair are needed to obtain sufficient data to compute dosage-mortality curves and provide adults for propagation of that pair.

Third instar larvae weighing between .0200 - .0400 g (weighed to the nearest .0001 g) were transferred individually to 1-oz jelly cups containing approximately 1/6 oz diet, an amount sufficient to last the length of the test. This conserved diet and reduce the time necessary for its preparation. As the larvae became available for usage, they were assigned at random, as nearly as possible, to receive one of the four (or five) concentrations of insecticide. The individual larval weight was not considered in determining the amount of insecticide it would receive. The larvae were treated by applying 1 ul of a known concentration of endrin in an acetone solution to the dorsum of the thoracic region by means of an electric micro-dispenser^{R} (Demick Enterprises, El Cerrito, California) driving a calibrated syringe.

Mortality counts were made 48 and 72 hours after treatment. Larvae were recorded as dead or alive. For the purpose of analysis, moribund (sluggish) larvae were listed as alive. The 72 hr post-treatment

observations were used for analysis. Acetone treated checks were used to determine the possible effects of the solvent on larval mortality.

The transparent cups allowed easy observation of the larvae. To avoid buildup of contaminating agents, the cups were discarded after use in rearing or treating.

Analysis of Data

Dosage-mortality curves were determined by the probit analysis method (Finney 1952). Two methods for analysis of data to compute dosage-mortality curves were conducted. The accepted method used by Lingren and Bryan (1964) of using the individual larval weight and an experimental method used by Brazzel (1964) of using an average larval weight to determine the dose were compared. To obtain the dose by the accepted method of using the average larval weight $\Sigma(c/w_i)/n$ is calculated where c = ug insecticide applied to each larva, w = weight of larva and n = the number of larvae to which the same c was applied. To obtain the dose by the experimental method of using average larval weight, $c/(\Sigma w_i/n)$ is calculated. The individual larval weights were punched on cards and an IBM 7040 digital computer was utilized to compute the doses by each method. These data were then processed on the same computer using a program written by Daum et al. (1962). This program estimates the lethal dose at the 30, 50, 70 and 90 percent levels with fiducial limits set at 95%. The intercept, a, and the slope, b, are also estimated for the response curve $Y = a + bx$ where Y is the probit response and x is the log dose in micrograms (ug) of insecticide per gram of body weight.

Four or five points were used to establish each curve. Progeny of 21 pairs were tested and curves computed for each. The number of larvae per point was consistent within each curve, but varied from 30 to 60 among curves. No mortality was noted in the acetone treated checks. Dosage-mortality curves were computed for each pair, and data for all pairs were combined to compute a common curve. These curves were plotted on log probability paper. Data from these curves were used to establish the response distribution.

Other computer programs were utilized to calculate the percent difference in the doses obtained by the experimental and accepted methods. This was also done for the lethal dose values. The average percent difference was computed for each dosage-mortality curve. The weight variance was calculated for each group and pooled for each pair.

A common slope was computed and the hypothesis tested that the slopes of the dosage-mortality regression lines were equal. The MLDs (median lethal doses) were plotted on log normal paper and a straight line eye fitted through these points.

DISCUSSIONS AND CONCLUSIONS

Rearing techniques employing a modified pressure cooker for dispensing diet into 1-oz transparent plastic cups and wooden trays for cup manipulation allowed the production of more larvae with less labor than required previously. The computer fortran programs for calculating an average dose produced results in approximately 1/8 the time required previously. This allowed dosage-mortality curves to be computed soon after mortality data were collected.

The accepted method used by Lingren and Bryan (1964) of using individual larval weights to calculate a dose produced larger doses than the experimental method used by Brazzel (1964) of using average larval weights. The lethal dose values were also larger when computed from the accepted doses. The percent difference became greater between the accepted doses and experimental doses as the variances of the weights of the larvae within the dosages increased. The pooled weight variance for progeny of each pair showed a similar relationship to the average percent difference in the accepted and experimental doses for each pair. The same was true for the lethal dose values. The accepted method produced doses that, for the 21 pairs, averaged 3.78 percent larger than the experimental doses. The lethal dose values for the 21 pairs obtained using the accepted doses were 3.74 percent larger than the lethal dose values obtained using the experimental doses. This indicates the experimental method of using the average larval weights to calculate a dose will give lower lethal dose

values. When larvae are selected from a range of weight wider than the .0200 – .0400 g used in these tests, the weight variance may increase; thus increasing the error of both the dose and lethal dose values. Use of individual larval weight corrects for the weight variance and appears to give a better estimate of the lethal dose values. However, the error encountered when using the experimental methods to determine the degree of resistance between two populations is not believed to be sufficient to warrant using individual larval weights.

The use of the experimental method employing average larval weight will shorten the time required to compute dosage-mortality curves.

The MLDs, when plotted on log probability paper, appeared log normal. A straight line fitted through these points was used to predict frequencies with which the MLDs would occur in the populations tested. These frequencies were used to draw a curve to describe the LD_{50} distribution of the populations tested (Figure 1). The distribution was approximately normal. From Figure 1, a sigmoid curve (Figure 2) was drawn to depict the percentage of the population having an LD_{50} lower than a specified values. This shows that 50 percent of the population had an LD_{50} of less than 58 ug.

The MLDs for the progeny tested from the 21 pairs of bollworms ranged from 18 to 126 ug. Little evidence was found to reject the hypothesis that the slopes of the dosage-mortality regression lines were equal; therefore, all regression lines were considered as having the same slope. The data from progeny of all 21 pairs were combined, and a common MLD of 51 ug was obtained. The mortality that would be expected if the common MLD was applied to progeny of each pair is presented in Table I. This expected mortality ranges from 25 to 80.5 percent. This shows a large

variation in the response of progeny from different pairs of bollworms. The response of progeny from any pair may be very susceptible or resistant in relation to progeny of another pair. Therefore, the hypothesis that one pair of bollworms may produce progeny which may be more or less resistant than progeny of another pair cannot be rejected on the basis of this evidence.

To determine or compare the responses of populations, test larvae must be randomly selected from progeny of several pairs. Analysis of these data indicate that such random selection should be made from progeny of at least 20-30 pairs of bollworms.

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APPENDIX

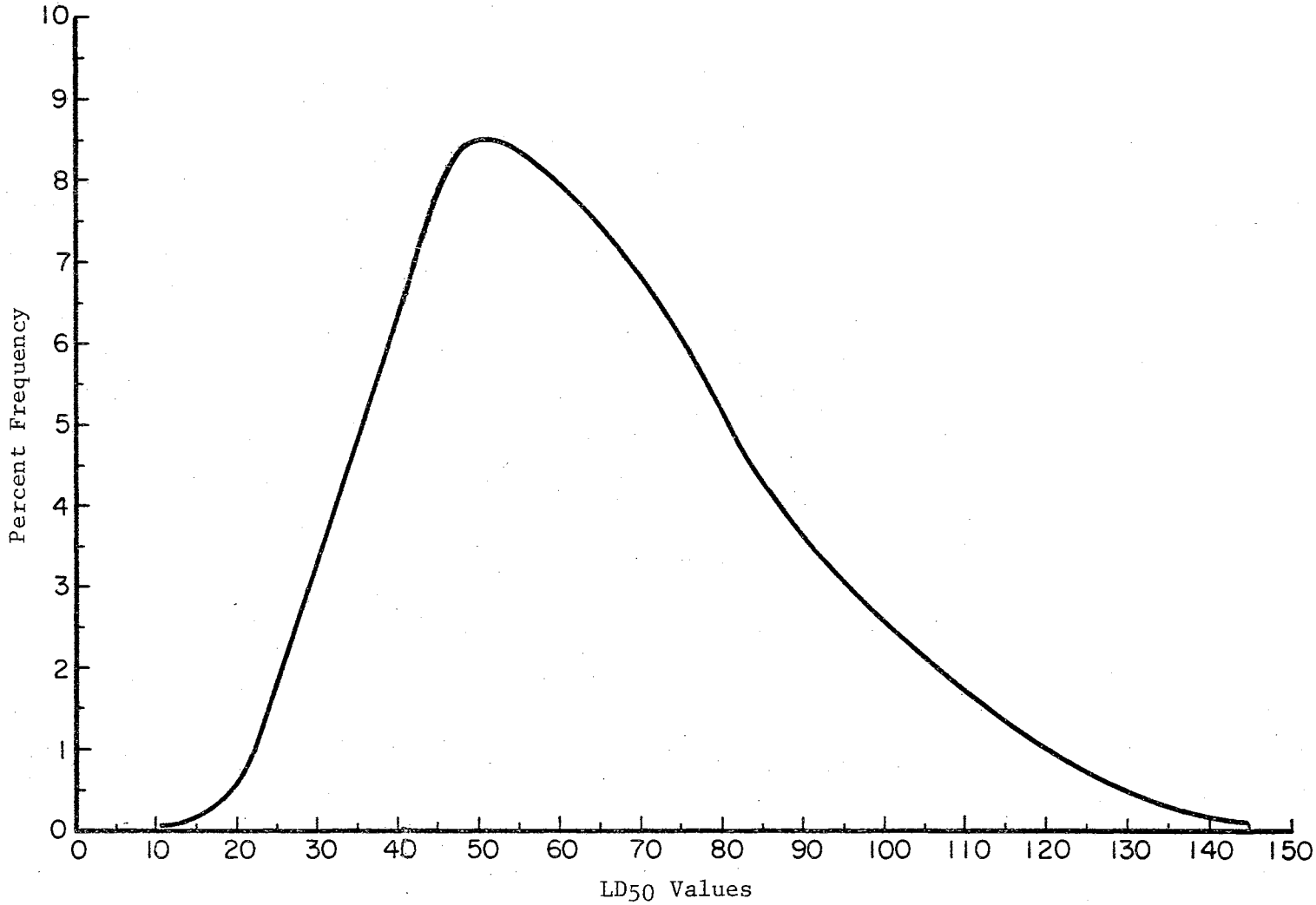

Figure 1. Distribution Curve for the LD_{50} Values of Progeny From Bollworm Pairs.

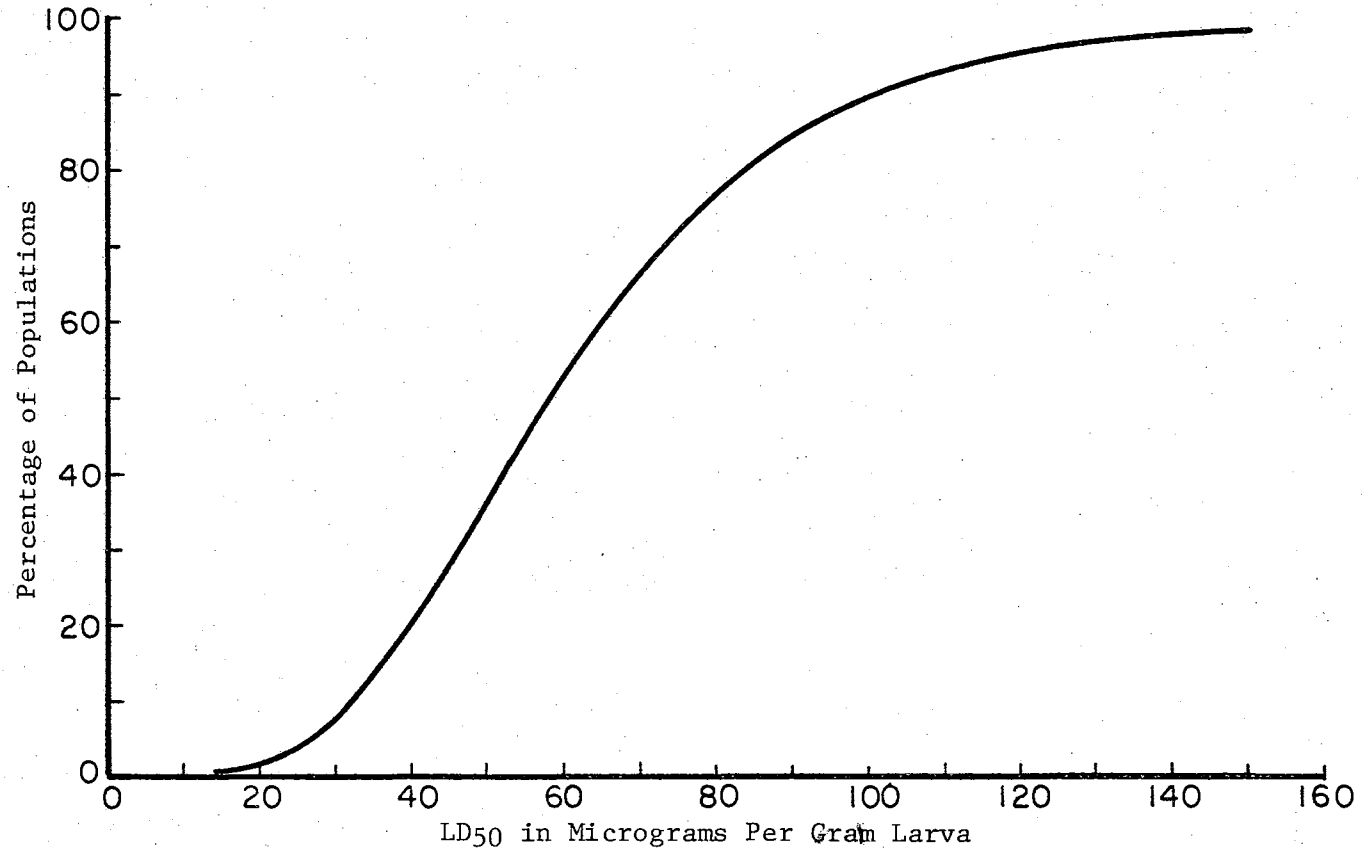

Figure 2. Sigmoid Curve Derived From Figure 1 to Show the Percentage of the Populations Tested Having an LD_{50} Less Than a Specified Value

TABLE I

EXPECTED MORTALITY OF PROGENY FROM BOLLWORM PAIRS IF TREATED WITH THE COMMON MLD OF 51 ug* OF ENDRIN

Origin	Brood	Pair No.	%	Origin	Brood	Pair No.	%
Stillwater	F_1	15	80.5	Stillwater	F_1	27	52.5
Altus	F_2	3	76.0	Stillwater	F_2	32	50.5
Stillwater	F_2	28	66.0	Stillwater	F_2	29	45.0
Stillwater	F_1	16	64.0	Stillwater	F_1	13	40.5
Stillwater	F_1	22	64.0	Stillwater	F_1	1	38.5
Stillwater	F_1	26	60.5	Altus	F_2	4	37.5
Stillwater	F_1	10	59.5	Stillwater	F_2	31	33.2
Stillwater	F_1	6	57.5	Stillwater	F_1	3	30.5
Stillwater	F_1	2	56.1	Stillwater	F_1	9	26.7
Chickasha	F_2	3	54.5	Stillwater	F_2	32	25.0
Chickasha	F_2	1	53.7				

*Obtained from combined data from progeny of all pairs.

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