

A MASS REARING TECHNIQUE, AND DOSAGE-MORTALITY DATA ON THE
BOLLWORM, Heliothis zea, AND THE TOBACCO BUDWORM,
Heliothis virescens, IN OKLAHOMA

By

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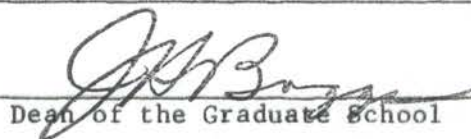
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PREFACE

It has been reported that bollworms and tobacco budworms have developed a resistance to DDT in several areas of the Cotton Belt. During 1960 cotton growers in Oklahoma were giving frequent reports of poor control with DDT. This could have been due to climatic conditions, poor methods of application, species difference, or possibly resistance to insecticides.

In order to evaluate these species for resistance, reference dosage-mortality data were needed for purposes of comparison. Consequently, dosage-mortality curves for both species from three locations in the State were established and are reported herein.

In view of the fact that large numbers of larvae are necessary for this type of study, a mass rearing technique was developed using an artificial diet developed by Adkisson et al. (1960) and modified by Berger (1962).

The author wishes to express appreciation to his major adviser, Dr. D. E. Bryan, for thoughtful guidance and encouragement throughout the experimentation and in the preparation of this manuscript. Special appreciation is expressed to Dr. Robert D. Morrison, Professor of Mathematics and Statistics, for advice and aid in analysis of data; to Drs. R. R. Walton, Professor of Entomology, and William A. Drew, Associate Professor of Entomology, for their constructive criticism of the thesis manuscript and to Mrs. Evelyn Louise Hamilton, Machine Superintendent of the Oklahoma State University Computing Center, and her staff for assistance in

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A Mass Rearing Technique and Dosage-Mortality Data on the
Bollworm, Heliothis zea, and the Tobacco Budworm,
Heliothis virescens, in Oklahoma

P. D. Lingren

During the years of 1960 and 1961 cotton growers in central and southwestern Oklahoma gave frequent reports that DDT was not giving satisfactory control of the cotton bollworm, Heliothis zea (Boddie). Control procedures which had given good results in the past appeared now to be inadequate. This indicated either that the bollworm might be becoming resistant to DDT or that what appeared to most growers to be the bollworm in reality might be the tobacco budworm, H. virescens (F.).

Bollworms are the dominant species on cotton in Oklahoma, but from 10-40% of an infestation may be budworms, depending upon the time of the year and climatic conditions. Budworm infestations on cotton have been reported by Folsom (1936) in Louisiana, Brazzel et al. (1953) in Louisiana and Arkansas, Gast et al. (1956) in North Carolina, and Brazzel (1963) in Texas. Laboratory tests by Brazzel et al. (1953), Gast et al. (1956), McPherson et al. (1956), Brazzel (1962), and Brazzel (1963), have shown the budworm to be more tolerant of DDT than the bollworm.

In order to evaluate these species for resistance, reference dosage-mortality data were needed for comparison with other work and work to be done in the future. Thus, adult moths were collected from the north-central, central, and the southwestern areas of the State. The progenies

of these adults were treated topically to get the needed dosage-mortality data.

Since very little labor was available, a time saving method of rearing large numbers of the test insects was initiated and is reported herein.

METHODS AND MATERIALS - Bollworm and budworm moths to be used for oviposition purposes were collected from a light trap at Stillwater, Oklahoma. A gasoline lantern and nets were used to trap moths from cotton fields at Chickasha and Altus. With the aid of the lantern light, the moths were easily captured while flying or resting on the cotton plants. Many of the moths collected were copulating pairs. The larval progeny of the strains collected at Stillwater, where there is very little pressure from insecticide application, was used as a reference to be compared with the suspect strains from Chickasha and Altus.

Larval Rearing - After collection the moths were transferred to oviposition cages. Usually 15 pairs of moths were put into each cage. The cages were made from white 1-gallon ice cream cartons of which the tops had been replaced with nylon tulle to provide an oviposition surface and a means of observing the moths. Three 1-dram vials were fitted with cotton plugs and inserted horizontally into the sides of the cages. Two of the vials contained a solution of honey and water to provide food for the moths. The other contained only water. The vials were cleaned daily and new plugs were added to prevent fermentation of the honey and consequent bloating of the moths.

Most of the moths used herein had already mated in the field; however, since they may mate more than once (Vanderzant et al. 1962), a

small floor lamp was placed in the rearing room to produce diffused lighting to aid the moths in initial or further copulation.

Eggs were deposited upon the lower surface of the tulle and on the cotton plugs. Before the eggs hatched, the oviposition cages were placed upon a white enamel surface. Upon emerging, the larvae were easily picked from this surface and from the oviposition cages with a No. 00 sable brush. Thus twice daily, the newly-emerged larvae were transferred in groups of 15-20 to one-half pint ice cream cartons containing one heaping tablespoon of an artificial diet developed by Adkisson et al. (1960) and modified by Berger (1962). The larvae required from 5-7 days to reach the third instar using this method of rearing. This was the stage of development desired for use in topical treatments.

Only one pair of budworm moths was collected at Chickasha and their progeny was reared to the adult stage to obtain a population adequate for producing sufficient larvae for test purposes. At the end of the third instar, the larvae were transferred individually to Lily No. 134 ice cream cups and one teaspoon of diet was added. The resulting pupae, in groups of 30, were placed in oviposition cages for emergence. Folds of paper toweling were located in the cages to provide the emerging adults a place to rest while spreading their wings. Feeding, mating, and oviposition were accomplished in the same manner as previously described.

Testing Procedures - At least 200 larvae from the F-1 generation of approximately 30 pairs of moths were used for each assay except for the budworms from Chickasha. These larvae were obtained from the F-2 generation of only one pair of moths which was captured while copulating in

the field.

Upon reaching test size, the larvae were transferred individually to Lily No. 134 ice cream cups to which approximately one-fourth teaspoon of diet was added to provide food for the test period. Each larva was then weighed to the nearest .0001 gram on a Mettler model H5 balance. The larvae were then transferred to another room for treatment. Only larvae weighing between .01-.04 gram were used in these tests.

Reagent grade DDT, Sevin (1 naphthyl N-methylcarbamate), and Telodrin (1,3,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanophthalan) were used to treat the larvae. Scalar amounts of these insecticides, from 125 to 3,600 milligrams, were dissolved in 100 milliliters of acetone and used as stock solutions for each test. One microliter of solution was applied to the dorsum of the thoracic region of each larva. This was accomplished by means of a hand manipulated micro-applicator driving a calibrated syringe.

Mortality counts were made 24 and 48 hours after treatment. Observations were recorded as alive, dead, or moribund. A larva was considered dead if it made no movement when touched with a sharp object. Sluggish larvae were recorded as moribund, but for the purpose of analysis they were listed as being alive. An acetone treated check was included for each test.

All rearing and treating was done in the laboratory at temperatures of approximately 80° F.

Analysis of Data - Probit analysis by Finney (1952), was the method used to determine the dosage-mortality curves. The dosage for each insect treated was computed as micrograms of insecticide per gram larva. Data for the 48-hour post-treatment observations were punched on IBM

cards and processed on an IBM 650 digital computer using a program proposed by Sokal (1958). This program estimated the median lethal dose, (MLD), the intercept, a , and the slope, b , for the response curve $Y = a + bx$ where Y is the probit response and x is the log dose in micrograms of insecticide per gram body weight. Confidence limits were set at the 95% level of probability. At least six points with 30 larvae per point were used to establish each dosage-mortality curve. No adjustment for natural mortality was made since the acetone treated checks showed less than 1% mortality.

RESULTS AND DISCUSSION - The rearing technique described herein can be used to produce larvae in large numbers with relatively little labor and expense. This method of rearing required approximately three man hours per day to produce enough larvae for 16 man hours of weighing and treating.

During the summer months it is a relatively simple process to collect pre-mated moths from the field and rear their progenies in the laboratory. Larvae can be reared to test-size within 7-10 days after collection of the adults. Newly-hatched larvae can be transferred (in groups of 20) to rearing cartons containing enough diet to rear them to test size with 90% survival. The larvae can be reared on to pupation with only one change of the diet and less than 5% mortality. Since very little cannibalism occurs before the fourth instar, there is some advantage in waiting until the third instar to transfer the larvae into individual containers for use in testing or in maintaining a laboratory culture.

The toxicological responses of the bollworm and budworm to Telodrin at three locations in Oklahoma are presented in figure 1. These data

include dosage-mortality curves and median lethal doses with confidence limits. All median lethal dosages for the bollworm were below 20 micrograms per gram larva and there appeared to be no significant differences among locations. Budworms from all three locations were harder to kill than bollworms. It took approximately four times as much Telodrin to kill Altus budworms (80 micrograms) as compared to Altus bollworms (18 micrograms); however, the steep almost parallel slopes of the dosage-mortality curves with relatively low MLDs indicate (Hoskins and Gordon, 1956) that both species were quite susceptible to Telodrin. The lower slope of the curve for the Chickasha bollworms can be explained in that the dosages used to establish the dosage-mortality curve were too high. This means that the 650 computer program had to estimate the lower dosages, and in doing so, it over-estimated the a value which in turn lowered the slope of the response line.

The Altus budworms appeared to be significantly different from the other strains in their response to Telodrin. This brings to light the possibility of cross-resistance initiated by the use of large quantities of closely related compounds for boll weevil and bollworm-budworm control in that area; however, there seemed to be a relatively large amount of variation in the response to the same insecticide for both species from different locations.

The toxicological responses of the bollworm to Sevin at three locations in Oklahoma and the budworm at two locations are presented in figure 2. These data include dosage-mortality curves and median lethal doses with confidence limits. There appear to be no significant differences in MLDs between species for any of the locations. Both species at all three locations appear to be relatively susceptible to Sevin; however,

the dosage-mortality curves for Chickasha and Altus bollworms and Stillwater budworms were flatter with higher MLDs. Thus, it may be that an increased use of Sevin for controlling bollworms in the Chickasha and Altus areas has produced enough selection pressure to induce low levels of resistance. Since there has been no selection pressure from Sevin on the Stillwater budworms, their tolerance to this insecticide must have been in the original population.

The toxicological responses of the bollworm and budworm to DDT at three locations in Oklahoma are presented in figure 3. These data include dosage-mortality curves and median lethal doses with confidence limits. The MLD for the Chickasha bollworms is approximately 400 micrograms as compared to less than 200 micrograms for the Stillwater and Altus strains. The MLD of the Chickasha bollworms appeared to be significantly different from the Stillwater and Altus strains. This indicates that the Chickasha bollworms have become resistant to DDT. In fact, the MLD for the Chickasha bollworms was larger than any of the other bollworm or budworm strains. Usually budworms are more tolerant of DDT, but in some cases, (Brazzel, 1963) the opposite response has occurred.

The relative effectiveness of Telodrin, Sevin and DDT on the bollworm at each location is presented in figure 4. These data indicate that the reference strains from Stillwater are more susceptible to all three insecticides than either the Chickasha or Altus strains. It is interesting, also, that the MLD for Telodrin at Altus appears to be only one-twentieth of that of DDT at Chickasha. This indicates that Telodrin could be used as a substitute for DDT provided health hazards or cost were not prohibitive.

The relative effectiveness of Telodrin, Sevin and DDT to the budworm at each location is presented in figure 5. The MLD for the Chickasha budworms for all three insecticides is less than 30 micrograms. These budworms were extremely susceptible; however, it should be remembered that the results came from the F-2 generation of a single pair of moths. These data indicate that the budworms are more susceptible to each of the three insecticides, but if the Chickasha bollworms (figure 4) and the Chickasha budworms (figure 5) are omitted it can be seen that the budworms are generally less susceptible.

There seems to be much variation among different strains of the bollworm-budworm complex in Oklahoma and as indicated by the Chickasha budworms (figure 5) there could be much variation among individuals from the same field. Other workers, (Brazzel et al. 1961, Brazzel 1962, Brazzel 1963, and Graves et al. 1963) have reported dosage-mortality data on the bollworm and budworm for DDT and other insecticides for other areas. Their data were obtained by using a standardized method of rearing and treatment comparable to the method used herein and these data also indicate a considerable amount of variation among different strains.

REFERENCES CITED

- Adkisson, P. L., Erma S. Vanderzant, D. L. Bull, and W. E. Allison.
1960. A wheat germ medium for rearing the pink bollworm. Jour.
Econ. Ent. 53: 759-61.
- Berger, Robert. 1962. Personal Communication.
- Brazzel, J. R., L. D. Newsom, J. S. Roussel, Charles Lincoln, F. J.
Williams, and Gordon Barnes. 1953. Bollworm and tobacco budworm
as cotton pests in Louisiana and Arkansas. Louisiana Tech. Bull.
482: 1-47.
- Brazzel, J. R., Howard Chambers, and P. J. Hamman. 1961. A laboratory
rearing method and dosage-mortality data on the bollworm, Heliothis
zea. Jour. Econ. Ent. 54: 949-52.
- Brazzel, J. R. 1962. Toxicity of certain insecticides against bollworms
and tobacco budworms. Texas Agric. Expt. Sta. Prog. Rept. 2236.
- Brazzel, J. R. 1963. Resistance to DDT in Heliothis virescens. Jour.
Econ. Ent. 56: 571-74.
- Finney, D. J. 1952. Probit analysis. Cambridge University Press.
318 pp.
- Folsom, J. W. 1936. Notes on little known cotton insects. Jour. Econ.
Ent. 29: 282-5.
- Gast, R. T, F. E. Guthrie, and J. D. Early. 1956. Laboratory studies
on Heliothis zea (Boddie) and H. virescens (F.). Jour. Econ. Ent.
49: 408-9.
- Graves, Jerry B., John S. Roussel and Jacob R. Phillips. 1963.
Resistance to some chlorinated hydrocarbon insecticides in the
bollworm Heliothis zea. Jour. Econ. Ent. 56: 442-44.
- Hoskins, W. M. and H. T. Gordon. 1956. Arthropod resistance to
chemicals. Ann. Rev. Ent. 1: 89-122.
- McPherson, J. E., L. D. Newsom and J. S. Roussel. 1956. Response of
Heliothis zea (Boddie) and H. virescens (F.) to DDT and endrin in
laboratory toxicity studies. Jour. Econ. Ent. 49: 368-71.
- Sokal, Robert R. 1958. Probit analysis on a digital computer. Jour.
Econ. Ent. 51: 738-739.

Vanderzant, Erma S., Christine D. Richardson, and Sam W. Fort Jr. 1962.
Rearing of the bollworm on an artificial diet. Jour. Econ. Ent.
55: 140.

APPENDIX

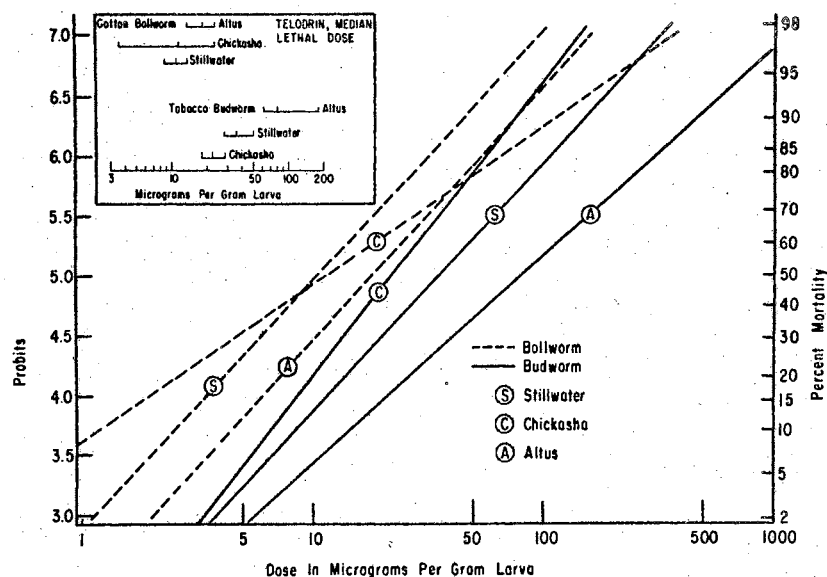


Fig. 1. - Dosage-mortality curves for third-instar bollworm and tobacco budworm larvae from three locations in Oklahoma 48 hours after topical treatment with Telodrin. Inset at upper left of graph gives median lethal dose and 95% confidence limits for each curve.

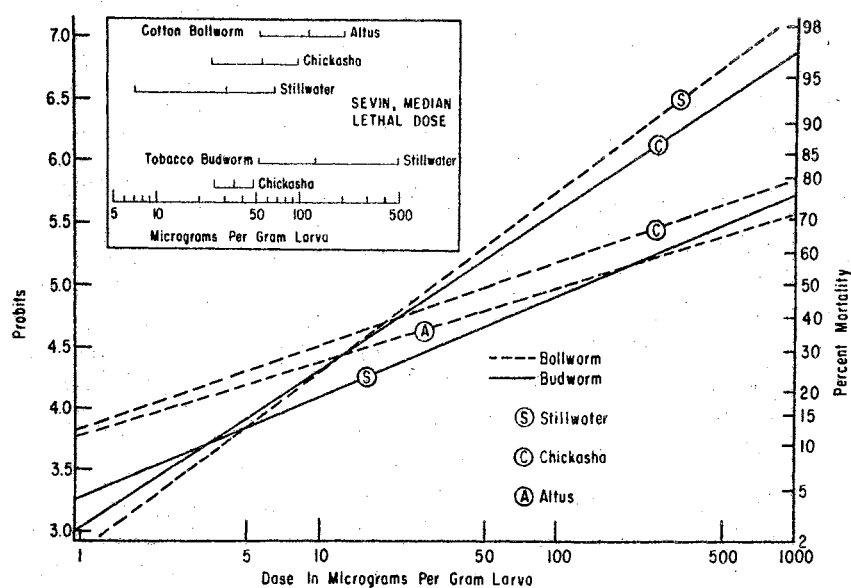


Fig. 2. - Dosage-mortality curves for third-instar bollworm larvae from three locations and tobacco budworm larvae from two locations in Oklahoma 48 hours after topical treatment with Sevin. Inset at upper left of the graph gives median lethal dose and 95% confidence limits for each curve.

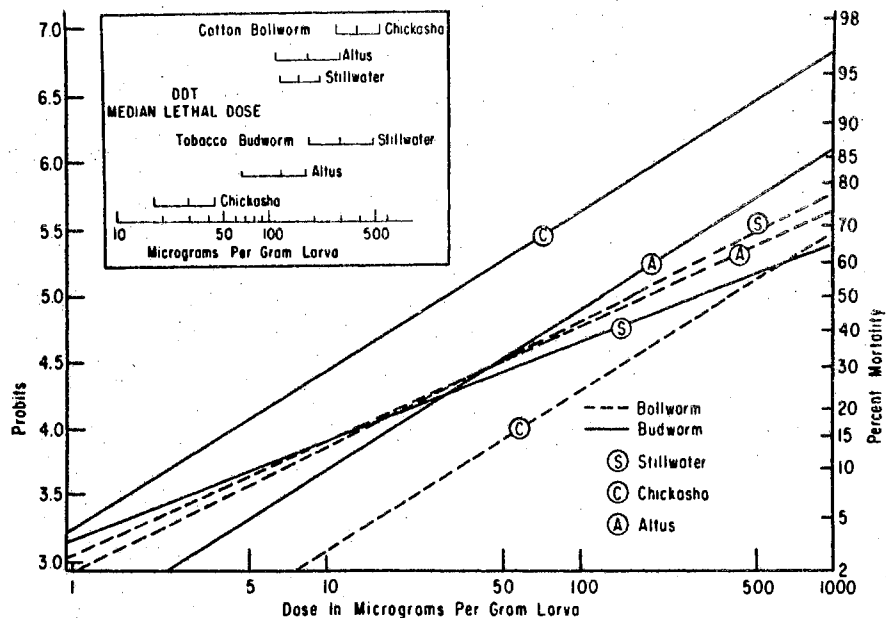


Fig. 3. - Dosage-mortality curves for third-instar bollworm and tobacco budworm larvae from three locations in Oklahoma 48 hours after topical treatment with DDT. Inset at upper left of the graph gives median lethal dose and 95% confidence limits for each curve.

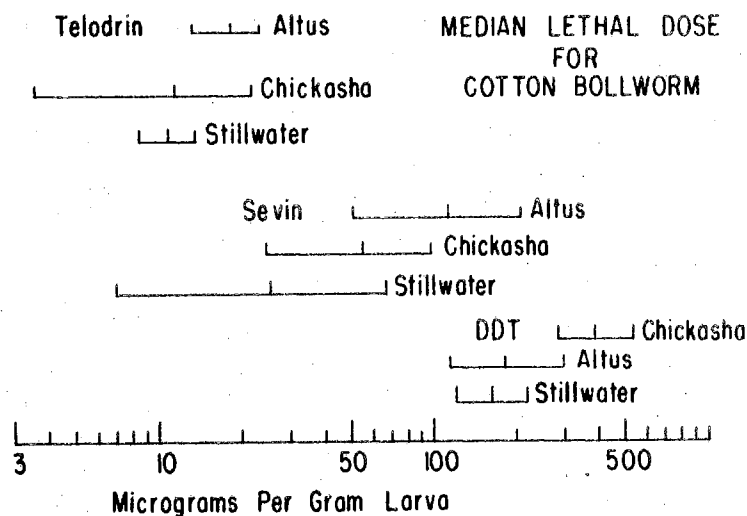


Fig. 4. - Median lethal doses with confidence limits showing the relative effectiveness of topically applied Telodrin, Sevin, and DDT on third-instar bollworm larvae from three locations in Oklahoma.

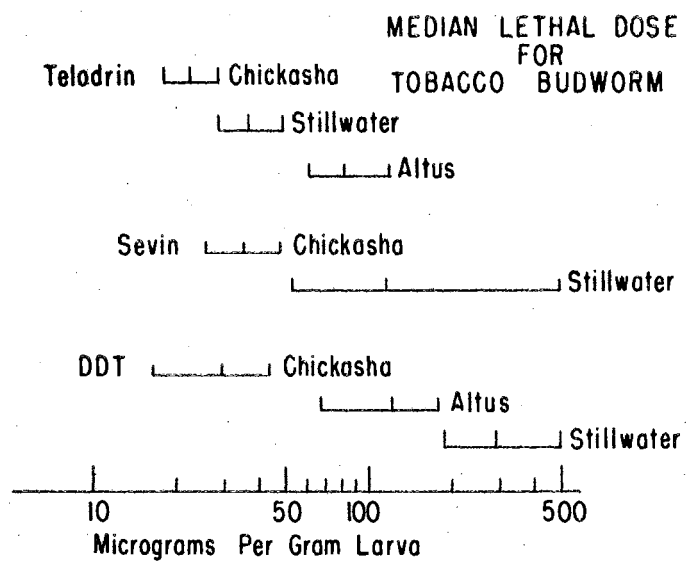


Fig. 5. - Median lethal doses with confidence limits showing the relative effectiveness of topically applied Telodrin, Sevin and DDT on third-instar tobacco budworm larvae from three locations in Oklahoma.

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Thesis: A MASS REARING TECHNIQUE AND DOSAGE-MORTALITY DATA ON THE
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