Toxicological and Metabolic Response of the Bollworm Heliothis Zea (Boddie) to Ddt.

Jerry Brook Graves

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TOXICOLOGICAL AND METABOLIC RESPONSE OF THE BOLLWORM
HELIOTHIS ZEA (BODDIE) TO DDT

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Zoology, Physiology and Entomology

by

Jerry Brook Graves
M.S., Mississippi State University, 1958
August, 1962
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ABSTRACT

A study of the toxicological and metabolic response of the bollworm larva, *Heliothis zea* (Boddie), to DDT was conducted over a three-year period. The primary objectives of this investigation were to determine if resistance to DDT had developed and to elucidate the metabolic fate of DDT in this species.

Cultures of bollworm larvae from three selected locations in Louisiana were established for this study. Larvae from Baton Rouge, where populations are seldom exposed to DDT, were compared to those from the Bunkie and Waterproof areas where DDT has been used extensively since 1947. The response to DDT of bollworm larvae from other areas of Louisiana was also investigated. In addition, a survey was made of the response of bollworm larvae to endrin from five areas and toxaphene-DDT (2-1) mixture from three areas in Louisiana.

Cultures were established by collecting adults from cotton fields by means of a light trap. Toxicological information was obtained by applying scalar doses of the insecticide to the dorsal thoracic region of individual larvae. LD-50's were determined by means of probit analysis of the 72-hour post-treatment observations.

The response of second instar bollworm larvae from all areas to DDT was approximately the same. However, the slopes of the
dosage-mortality curves indicated that the Baton Rouge larvae were somewhat more susceptible to DDT than the Bunkie and Waterproof larvae. There was a two- to five-fold increase in the LD-50 between bollworm larvae of the second and third instars.

A significant difference amounting to a two- to three-fold increase in the LD-50's of third, fourth, fifth and sixth instar bollworm larvae from Bunkie and Waterproof as compared to those from Baton Rouge was demonstrated consistently over a three-year period. The slopes of the dosage-mortality curves for the larvae of the various instars of Baton Rouge larvae showed them to be more susceptible to DDT than the corresponding instars of larvae from Bunkie and Waterproof. This indicates that the larvae from Bunkie and Waterproof were more resistant to DDT than the larvae from Baton Rouge. Whether or not this two- to three-fold difference was present before the use of DDT and other chlorinated hydrocarbons is speculative. However, this level of resistance probably accounts for some of the difficulties in bollworm control experienced by farmers from these two areas.

It was demonstrated that although the LD-50's are essentially the same for the larvae of the third and succeeding instars, about 10 times as much DDT is required in order to elicit the same response from a sixth instar larva as from a third instar larva. This increase in the amount of DDT required is a function of the increase in the weight of the larvae. The slope of the dosage-mortality curve for larvae of each instar decreased as the instar increased. Thus, a
greater variation in the response to DDT was obtained in the later instars. Furthermore, considering the larvae of each instar, there was a more variable response indicated for the Bunkie and Waterproof larvae than for the Baton Rouge larvae.

There were no significant differences in the response to endrin of bollworm larvae from five areas of Louisiana. Also, no significant difference in the response to toxaphene-DDT mixture of larvae from three different areas was demonstrated.

DDT was absorbed at approximately the same rate by bollworm larvae of the third through the sixth instars. Very little of the absorbed DDT was metabolized. Only small amounts of DDT were present in the excreta of bollworm larvae 24 hours after topical application of DDT.
INTRODUCTION

The bollworm, *Heliothis zea* (Boddie), has been recognized as a cotton pest since 1820. In Arkansas, Louisiana, and Mississippi, it is outranked in importance only by the boll weevil, *Anthonomus grandis* Boh. Prior to the widespread use of the new organic insecticides for boll weevil control, the bollworm occurred on cotton only sporadically, being held down by natural enemies. The application of these insecticides has resulted in the destruction of many predators and parasites, subsequently causing the build up of bollworms.

DDT has been recommended as the most effective insecticidal control since 1952, when the Conference of Cotton Entomologists in their report adopted 0.5 to 1 pound of technical DDT per acre depending on severity of infestation. Brazzel et al. (1953) reported effective control of bollworms with 0.5 to 1.5 pounds technical DDT per acre depending on severity of infestation and interval between applications. The studies of McPherson et al. (1956) showed that bollworm larvae of the first three instars were readily controlled by DDT applied at the recommended rate per acre, but that many of the fourth, fifth and sixth instar bollworm larvae survived.

However, since 1956, farmers in certain areas of Louisiana have experienced difficulty in controlling the bollworm with DDT, even when
a much larger dosage rate than recommended was used. At first, many of these failures were attributed to such factors as improperly timed applications, improper application techniques and substandard insecticidal formulations. However, since all reports of bollworm control failures involving the use of DDT have come from areas of Louisiana where DDT and other organic insecticides have been used extensively year after year in cotton insect control programs, bollworm resistance to DDT must be considered as a possible explanation.

Therefore, the studies reported in this dissertation were initiated for the following reasons: (1) To determine if resistance to DDT has developed or is developing in the bollworm. (2) To discover the metabolic fate of DDT in the bollworm.
Economic Importance of Heliothis zea (Boddie)

Heliothis zea (Boddie) has long been recognized as a serious cotton pest and is cosmopolitan in its distribution. It is commonly known as the bollworm, the corn earworm, or tomato fruitworm, depending on its host plant. Quaintance and Brues (1905) state that it was first reported as damaging crops in 1820 when it attacked cotton. By 1841, it had become quite prominent as a pest of cotton and corn in the southern United States. These authors considered the bollworm as the most serious insect pest of cotton in the United States prior to the entry of the boll weevil, Anthonomus grandis Boh., in 1892. They cited more than 70 host plants which included corn, cotton, and other important crops.

Hyslop (1927) rated the bollworm as the third most important insect pest in the United States. Metcalf and Flint (1939) stated that considering the United States as a whole the bollworm or corn earworm is the worst pest of corn. Brazzel et al. (1953) reported that the bollworm as a pest of cotton in Louisiana and Arkansas was outranked only by the boll weevil. It is also rated as an important pest of cotton in Alabama, Arizona, California, Georgia, Mississippi, Missouri, Oklahoma, North Carolina, New Mexico, South Carolina, Tennessee and Texas.
Relatively low infestations of the bollworm can cause sizable reduction in the yield of cotton. According to Adkisson et al. (1962), when an average of only 3.6 per cent of the bolls were damaged, losses of 627 pounds of seed cotton per acre occurred where yields amounted to 2165 pounds of seed cotton in the controls. Losses of more than 800 pounds of seed cotton per acre occurred when approximately 10 per cent of the bolls were infested.

General Concepts of Resistance

Since 1914, when Melander first reported that the San Jose scale, *Aspidiotus perniciosus* Comstock, did not respond as expected to lime-sulfur, several hundred similar instances have been noted involving numerous species and many of the newer insecticides. Poor application, lowered quality, improper timing and other simple reasons probably accounted for some of the failures in control; but careful comparative tests have revealed genuine changes in the insect's reaction to the toxicant in many cases. The term "resistance", which was first popularized in connection with survival of San Jose scale treated with lime-sulfur, rather unfortunately has been used in connection with all degrees of control failure.

In 1916, Quayle found that the California red scale, *Aonidiella aurantii* (Mask.), was resistant to hydrogen cyanide fumigation. One year later, the codling moth, *Carpocapsa pomonella* L., was no longer controlled with lead arsenate. Thus, resistance, *per se*, is not as new as it may seem. However, since the development of resistance to
DDT and many of the other synthetic organic insecticides, resistance has become the primary problem facing entomologists today.

All living organisms can carry on their vital functions with little or no impairment in the presence of a chemical, up to some level of concentration or amount. This level depends upon the species, the chemical, the method of exposure, the temperature, etc. When these factors are fixed, this level becomes a measure of the susceptibility or tolerance of the species. Tolerance, as commonly used, means the natural ability of a normal population to withstand a toxicant. The median lethal dose or LD-50 is generally used to express the level of susceptibility of a population. The LD-50 is the amount of a toxicant required to kill 50 per cent of the population.

A second term is needed to denote the added ability of a species to withstand a toxicant which appears to stem from improved nutrition, extra weight, or any other factor generally associated with what may be called extra vigor. This change from the normal tolerance is called "vigor tolerance" by Hoskins and Gordon (1956).

Finally, "resistance" means the development of an ability in a strain of a species to survive doses of a toxicant which would prove lethal to the majority of the individuals in a normal population of the same species. Resistance is a developed characteristic of a population that is attained subsequent to the application of the toxicant. For pre-existing resistance, for instance, that of the boll weevil or grasshopper for DDT, the term "refractoriness" should be used.
Two distinct types of resistance have been reported (Schoof, 1959).

Physiologic: The ability through physiological processes to withstand a toxicant after it has entered the body.

Behavioristic: The ability through protective habits or behavior to avoid lethal contact with a toxicant.

When the resistance problem is considered in toto, it is found that, with few exceptions, the difficulties experienced have been the result of physiological resistance.

Much has been said about the various physiological mechanisms of resistance. Chadwick (1955) discussed the various types of mechanisms under the headings of behavior, structure, penetration, storage, excetion, detoxification and decreased sensitivity. These various mechanisms and sometimes combinations of them have all been found at one time or another to be the device used to effect resistance. However, the most important mechanism of resistance by far has been the detoxification of a toxicant to harmless or less harmful metabolites.

Insects do not acquire resistance during their lifetime; on the contrary, exposure to small amounts of the insecticide has been found to make them more susceptible. This has been shown by several workers including Hoffman et al. (1951), Beard (1952) and Hadaway (1956). Furthermore, resistance cannot be induced by exposing a normal strain to insecticide treatments that are truly sublethal and fail to kill any of the individuals. Evidence of this nature has been reported by Harrison (1952), Luers (1953) and Cole (1956). Luers (1953) using DDT
and Pielou (1952) using BHC both reported that these insecticides evidently do not induce mutations for resistance and in fact do not increase the mutation rate at all. Crow (1957) has found that years of insecticide pressure could not produce resistance in isogenic strains of *Drosophila* which lacked the genes for resistance in the first place. Resistance may therefore be concluded to arise not from post-adaptation but from pre-adaptation, the factors making for resistance being already present before the insecticide was ever applied. Thus, development of resistance in insects constitutes a perfect example of evolution by natural selection in which the insecticide acts as the selecting agent.

**Resistance to DDT**

Since the initial occurrence of housefly resistance to DDT in Sweden in 1946 (Wiesmann), numerous reports of resistance of other insects continue to appear in the literature. The widespread development of DDT resistance among pests of both agricultural and public health importance has become a major entomological problem. According to Brown (1958), insects of major public health importance that have developed resistance to DDT include the housefly (*Musca domestica* L.), more than a dozen species of mosquitoes, the body louse (*Pediculus humanus humanus* L.) and at least four species of fleas. Other insects of minor public health concern that have developed DDT-resistance are the little house fly (*Fannia canicularis* (L.)), the fruit fly (*Drosophila melanogaster* Meig.), the bed bug (*Cimex lectularius* L.) and several species of cockroaches.
Metcalf (1960) stated that there were more than 50 agricultural pests in which significant immunity to one or more insecticides has been proved. DDT-resistance alone accounts for more than half of this total. At the Fifteenth Annual Conference on Cotton Insect Research and Control held in 1962, the following cotton pests were listed as being resistant to DDT: beet armyworm (*Spodoptera exigua* (Hubner)), boll weevil (*Anthonomus grandis* Boh.), cabbage looper (*Trichoplusia ni* (Hubner)), cotton leaf perforator (*Bucculatrix thurberiella* Busck), cotton leafworm (*Alabama argillacea* (Hubner)), several species of Lygus bugs, pink bollworm (*Pectinophora gossypiella* (Saunders)), salt-marsh caterpillar (*Estigmene acrea* (Drury)), southern garden leafhopper (*Empoasca solani* DeLong), several species of thrips and several species of spider mites.

Glass (1960) listed three orchard insects as being resistant to DDT. These were the codling moth (*Carpocapsa pomonella* L.), the red-banded leaf roller (*Argyrotaenia velutinana* (Walker)), and the grape leafhopper (*Erythronoe ura variabilis* Beamer). Chapman (1960) summarized the information available on resistance in insects attacking vegetable crops. The insects reported as being resistant to DDT were the potato flea beetle (*Epitrix cucumeris* (Harris)), the Colorado potato beetle (*Leptinotarsa decemlineata* (Say)), the imported cabbageworm (*Pieris rapae* (L.)), the cabbage looper (*Trichoplusia ni* (Hubner)) and the diamondback moth (*Plutella maculipennis* (Curtis)). He also reported possible DDT resistance in the European corn borer (*Ostrinia nubilalis* (Hubner)), the tomato hornworm (*Protoparce quinquemaculata* (Haworth)) and the bollworm (*Heliothis zea* (Boddie)).
Response of the Bollworm to DDT

The bollworm is generally used as one of the standard test insects in screening programs for evaluation of potential cotton insecticides. Usually, third or later instar larvae are used for this purpose. Brazzel et al. (1953) reported that H. zea was more susceptible to DDT and 3-5-40 dusts than was H. virescens.

In North Carolina, Babers (1953) reported the bollworm did not respond as in the past to currently recommended chlorinated hydrocarbons. However, in Texas this insect was as susceptible to DDT and toxaphene after 8 to 10 years' use as when these chemicals were initially tested (Ivy and Scales, 1954).

McPherson et al. (1956) studied the response of both the bollworm and the tobacco budworm to DDT and endrin in the laboratory. They reported that 10 per cent DDT dust readily controlled larvae of the first three instars of both species. In the fourth instar, many larvae survived the treatment. Very few fifth and sixth instar larvae of either species were killed with dosages as high as 15 pounds of 10 per cent DDT dust per acre.

Wilson (1960), in reporting on the performance of DDT for H. zea control over an eleven-year period in Florida, stated that the data did not indicate that H. zea was developing resistance to DDT.

Gast (1961) reported that full grown H. zea required more than 1,000 times as much DDT on a weight basis as small larvae in order to obtain LD-50 values for topical applications in acetone. Injected DDT in acetone was only slightly less effective on large larvae than on small ones. He proposed that lack of penetration by DDT through
the integument of the large larvae was the chief factor causing the increase in LD-50 values. Tests with different colored larvae indicated that light yellow larvae were approximately twice as susceptible as the dark red or black larvae.

Brazzel et al. (1961) found no measurable insecticide resistance in bollworms collected at College Station, Texas. In tests conducted during 1961, Brazzel (1962) reported that there was no clear-cut evidence of resistance in bollworms collected from several areas of Texas. However, he found the tobacco budworm to be highly resistant to DDT as compared to a Florida strain.

**Metabolism of DDT**

The first studies on the metabolic fate of DDT in insects were carried out on the large milkweed bug, *Oncopeltus fasciatus* (Dallas), by Ferguson and Kearns in 1949. The milkweed bug is refractory to both topical and injected doses of DDT. These workers found that sublethal doses of DDT injected in acetone solution were rapidly metabolized to unknown products. DDE (1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene) and DDA (bis(p-chlorophenyl) acetic acid), the principal products of animal metabolism according to White and Sweeney (1945), were not found in the milkweed bug.

Since 1949, numerous studies have been made concerning the metabolism of DDT in insects. The metabolism of DDT by various insect species has been admirably reviewed by Perry (1960). According to Perry, insects, like most other animals, must degrade or chemically
alter a large variety of compounds to maintain their normal body functions. Thus, it is not surprising that many foreign compounds, including poisons, are attacked in the metabolic process.

The chemical changes or alterations that take place are either activating or detoxifying in nature. Thus, a detoxifying mechanism may convert a toxicant such as DDT to non-toxic metabolites, which are either retained in the tissues or excreted rapidly. Perry in his review stated that DDT is metabolized by the housefly (Musca domestica L.), body louse (Pediculus humanus humanus L.) certain mosquitoes, American roach (Periplaneta americanus (L.)), Mexican bean beetle (Epilachna varivestis Mulsant), boll weevil (Anthonomus grandis Boh.), milkweed bug (Oncopeltus fasciatus (Dallas)), fruit fly (Drosophila melanogaster Meig.), Eastern tent caterpillar (Malacosoma americanum (Fab.)), European corn borer (Ostrinia nubilalis (Hubner)), cabbage worm (Pieris rapae (L.)), red-banded leaf roller (Argyrotaenia velutimana (Walker)), and certain grasshoppers. However, the detoxification process, depending on the species involved, may take any one of the four or five metabolic pathways thus far known. Many of these processes have been found to be enzymatically catalyzed.

The most important and most common metabolic pathway for DDT in insects is the conversion of DDT to DDE as typically found in the housefly. The conversion of DDT to DDE by houseflies was first demonstrated simultaneously but independently by Sternburg, Kearns, and Bruce (1950) and Perry and Hoskins (1950). Only three years later,
Sternburg et al. (1953) reported the isolation of the enzyme DDT-dehydrochlorinase from houseflies, which in the presence of glutathione catalyzes the dehydrochlorination of DDT to DDE. Using this enzyme, these researchers demonstrated the conversion of DDT to DDE in vitro.

Perry and Buckner (1958) reported the enzymatic breakdown of DDT in the human body louse. However, after isolating the crude enzyme, they found it to be quite different from DDT-dehydrochlorinase. Furthermore, the resulting metabolite was found to be an acidic water-soluble conjugate.

Hoskins et al. (1958) demonstrated the metabolite, p, p'-dichlorobenzophenone, in the excreta of DDT treated American roaches. A small amount of DDE was also found. Several species of DDT-resistant mosquitoes have been shown to convert large amounts of DDT to DDE in vivo. However, neither Brown et al. (1956) nor Perry (1960) succeeded in isolating the enzyme system in vitro.

The most recent addition to the study of DDT metabolism in insects is that of Tsukamoto (1959) who showed that DDT is metabolized to 1, 1-bis-(p-chlorophenyl) 2,2,2-trichloroethanol or Kelthane by DDT-resistant and susceptible strains of Drosophila melanogaster Meig. and Drosophila virilis Sturtevant. When Drosophila were reared on DDE containing media, the above metabolite was not produced, indicating that DDE was not an intermediate product in DDT metabolism.

Metabolism of DDT in the Bollworm

Although many studies on the metabolism of DDT in various insect species have been performed, there is only one such study concerning
the bollworm. Gast (1960), in studying the decrease in the susceptibility of the larger bollworm larvae to topical applications of DDT, made some metabolic studies using radioactive DDT. He found that, regardless of the size of the larvae, essentially the same amount of DDT penetrates the integument. Radiometric analysis of the integument and the viscera showed very little, if any, DDE produced. Approximately 10 per cent of the applied dose could not be accounted for in this study. Most of the recovered DDT was still in the form applied. The author concluded there was no evidence of any appreciable metabolism of DDT by the bollworm.
METHODS AND MATERIALS

Laboratory Rearing of the Bollworm

Laboratory cultures of bollworm larvae were maintained for these studies. Cultures were established by collecting adult bollworm moths directly from cotton fields by means of a light trap equipped with a General Electric (H 100, SP 4) projector spot, high intensity ultraviolet lamp. Although moths were collected from several areas of Louisiana, the three main areas investigated were Baton Rouge, Bunkie and Waterproof. These areas were selected as being representative locations. The bollworm population from the Baton Rouge area has been exposed relatively little to DDT whereas populations in the Bunkie and Waterproof areas have been exposed to DDT extensively in cotton insecticide programs since 1947.

After collection, the moths were placed in wooden oviposition cages 24 inches high, 18 inches wide and 18 inches long. One side of the oviposition cage was equipped with a removable glass pane over which white cotton cloth could be placed for deposition of eggs. Shell vials, 5.5 x 2.5 millimeters, containing cellucotton saturated with a solution of honey and water at the ratio of 1 part honey to 9 parts water, were placed in the oviposition cages to provide food.

The cloths were removed daily in order to have larvae of a known age, and were placed in wide-mouth gallon jars until hatching occurred.
Upon hatching, which usually required from 2 to 4 days, the larvae were provided with fresh cotton terminals for food. As soon as they had reached the third instar, they were placed in individual containers to prevent cannibalism. Either half-pint jelly glasses or half-pint ice cream cartons were used for this purpose. All the larvae of the third and succeeding instars were fed either cotton squares or bolls.

The sixth instar larvae were allowed to pupate in individual containers. The pupae were then placed in groups of 20 in wide-mouth gallon jars with about three inches of damp sand in the bottom. The sand was kept moist by the addition of water every three to five days. The emerging adult moths were placed in the oviposition cages where they usually mated and subsequently deposited eggs. When difficulty was experienced in obtaining mating, moths were placed over cotton plants in large square cages with four foot dimensions where they were kept for three days.

All rearing, except for the occasions when adult moths were placed in large cages over cotton in the field, was done in a walk-in temperature room held at 27°± 2°C. and a relative humidity of 50-70 per cent.

Toxicological Screening for Resistance

The p,p'-DDT used in this study was recrystallized twice from methanol and had a melting point of 108-109°C. A few tests were also conducted using endrin, (1,2,3,4,10,10-hexachloro-6,7 epoxy-1,4,4a,5, 6,7,8,8a-endo-endo-dimethanonapthalene), and a 2:1 mixture of toxaphene (67% chlorine) and DDT.
Scalar doses of the insecticide in one microliter of acetone were applied to the thoracic region of each larva. The insecticide was administered with a calibrated 0.25 milliliter syringe, the plunger of which was driven by a micrometer. An Alga brand all-glass syringe made by Burroughs Wellcome and Company of London, England and fitted with a 27-gauge hypodermic needle was utilized. The micrometer was manufactured by Shardlow Micrometers, Inc. of Sheffield, England. An acetone treated control group was included in each test. The larvae were weighed in groups of ten immediately before treatment. They were held at 27°C ± 2°C. during both pre-treatment and post-treatment periods.

The larvae were placed in individual containers previously described after treatment and provided either cotton squares or bolls. Mortality counts were made at 24, 48 and 72 hours after treatment and the observations recorded. A larva was considered dead if it made no movement when touched with a pencil point. From the data obtained at 72-hour post-treatment observations, dosage-mortality curves were plotted on log-probit paper and the LD-50's determined by probit analysis with fiducial limits set at 5 per cent (Finney, 1949). Adjustments for natural mortality were made using the method of Abbot (1925). At least four points were used to establish dosage-mortality curves and 30 or more larvae were used to establish each point.
Metabolism of DDT

The same method of treatment was used for metabolism studies as that described above except that treated larvae were held individually without food in 5.5 x 2.5 millimeter glass vials fitted with perforated plastic tops at 27° ± 2°C. However, only sublethal doses of DDT were used in order to prevent mortality and to allow metabolism of DDT to occur. At 0, 2, 7 and 24-hour intervals after treatment the larvae were analyzed by chemical methods to determine the amount of DDT that had been metabolized.

At each time interval four replicates of 10 pre-weighed larvae from the third through the sixth instar were analyzed for DDT and some of its known and possible degradation products. Each sample of 10 treated larvae was subdivided into three samples. The sub-samples were the "external rinse," "larval extract" and "excretal extract." After the treated groups had been held for the different time intervals, the feces were collected from each of the 10 vials in a group and combined as a single sample. This sample was then ground in a mortar with anhydrous sodium sulfate. The resulting powder was transferred to a four ounce screw-cap bottle with the aid of a small portion of distilled ethyl ether, and the mixture was mechanically agitated with three 15 milliliter portions of ether for one hour. The three portions were filtered and combined to give the "excretal extract".

The larvae and the vials of a group were given three successive 15 milliliter rinses with ether. These rinses were combined as the
"external rinse." The rinsed larvae were ground in a mortar with anhydrous sodium sulfate as previously described. The resulting powder was extracted on a mechanical shaker three times with 15 milliliter portions of ether. The three portions were filtered and combined to give the "larval extract."

The method of Schechter et al. (1945) for the determination of DDT and related compounds in microgram quantities was used primarily in this study. This method involves intense nitration of DDT or related compounds, followed by reaction of the nitrated products with a standardized anhydrous methanol solution of NaOCH₃. By this method, p, p'-DDT yields an intense blue color with maximum absorption of light at 596 μm. Various analogues of DDT, either known or theoretically possible as metabolites, yield red colors. The optical densities of the colored solutions were measured with a Beckman Model DU quartz spectrophotometer, using standard one centimeter Corex cells. Mixtures of DDT and DDE were analyzed by the procedure of Mattson et al. (1953). One part of DDE may be determined in the presence of 80 parts of DDT with an error of less than 2 per cent by this method.

The ultraviolet spectrophotometric technique described by Sternburg et al. (1954) was also used, especially on the "external rinse" samples. This method is specific for DDT and DDE; however, since only DDT was present in the "external rinse" samples, this method was very useful. Only a small amount of time was required to complete a test using this technique as compared to the method of Schechter et al.
The ultraviolet spectroanalysis consisted of adding three milliliters of sulfuric acid and eight milliliters of cyclohexane to a sample and shaking vigorously for 30 minutes on a mechanical shaker. An aliquot of the cyclohexane layer was taken and diluted with cyclohexane until the expected total of DDT and DDE fell within the range of 15 to 25 micrograms per milliliter. The absorbance of these solutions was then determined at 241 and 260 μm in the Beckman spectrophotometer.

The chromatographic separation described by Sternburg and Kearns (1952) was used to establish the identity of the metabolite formed and to investigate the possibility of the formation of a metabolite other than DDE.

Since the "larval extracts" of the bollworm contained sufficient fat to cause interference with the methods of analysis used, it was necessary to separate the fat from the samples. This was accomplished using the procedure outlined by Jones and Riddick (1952). This procedure is satisfactory for samples containing up to two grams of fat. The fat is removed by a series of extractions with a combination of hexane and acetonitrile. The fat is collected in the hexane portion while the DDT is collected in the acetonitrile portion.
RESULTS

Toxicological Studies with DDT

The dosage-mortality response to DDT for second instar bollworm larvae from three locations in Louisiana during 1959 is shown in figure 1. The median lethal dosages, or LD-50's, with fiducial limits set at 5 per cent and expressed as milligrams of DDT per gram of body weight are: Baton Rouge (.016 ± .0055), Bunkie (.016 ± .0062) and Waterproof (.017 ± .0093).

The dosage-mortality data for the third instar larvae from the same locations during the same year are shown in figure 2. The LD-50 for the Baton Rouge bollworm larvae is .030 ± .0075, while the LD-50's for the Bunkie and Waterproof larvae are .065 ± .018 and .0813 ± .0213, respectively. It can readily be seen that there is a considerable difference in the LD-50's between larvae of the second and third instars. Furthermore, there is a significant difference between the LD-50's of the Bunkie and Waterproof larvae and the Baton Rouge larvae within the third instar.

The dosage-mortality data for the fourth instar larvae are presented in figure 3. LD-50's of the larvae from the Bunkie and Waterproof areas are .064 ± .0196 and .083 ± .0241, respectively. Thus, they exceed by a two- to three-fold amount the LD-50 of the larvae from the Baton Rouge area which is .037 ± .0094.
Figure 1. Dosage-mortality curves showing the response to DDT of second instar bollworm larvae from three locations in Louisiana.
Figure 2. Dosage-mortality curves showing the response to DDT of third instar bollworm larvae from three locations in Louisiana.
Figure 3. Dosage-mortality curves showing the response to DDT of fourth instar bollworm larvae from three locations in Louisiana.
The response of the fifth instar bollworm larvae to DDT is shown in figure 4. The LD-50's follow the same trend as exhibited by figures 2 and 3. The LD-50's for the fifth instar larvae are: Baton Rouge \( (.029 \pm .0086) \), Bunkie \( (.068 \pm .0185) \) and Waterproof \( (.091 \pm .0333) \).

Sixth instar larval response to DDT is presented in figure 5. Again the median lethal dosages for the Bunkie and Waterproof areas \( (.072 \pm .0350 \) and \( .096 \pm .0387 \), respectively) exceed that of the Baton Rouge area \( (.038 \pm .0217) \) by a two- to three-fold amount.

The toxicological response of bollworm larvae to DDT from three locations in Louisiana over a three-year period is shown in table I. The LD-50's of bollworm larvae from the second through the sixth instar are presented for each area for the years 1959, 1960 and 1961. The LD-50's for the second instar larvae are approximately the same for the three locations involved over the three-year period. However, there is a two- to five-fold increase in the LD-50 from the second to the third instar larvae, depending on the location. From the third through the sixth instar, there is a two- to three-fold difference in the LD-50's between the Baton Rouge area and the Bunkie and Waterproof areas. This difference is consistent over the three-year period. Considering a given location, the LD-50's of the larvae of the third through the sixth instar are approximately the same.

The same data that is presented in table I is shown in table II; however, the LD-50's in this table are expressed as micrograms of DDT per mean weight of larvae. The mean weight of the larvae of each
Figure 4. Dosage-mortality curves showing the response to DDT of fifth instar bollworm larvae from three locations in Louisiana.
Figure 5. Dosage-mortality curves showing the response to DDT of sixth instar bollworm larvae from three locations in Louisiana.
Table I. Toxicological response of *Heliothis zea* (Boddie) larvae from three locations in Louisiana over a three-year period to DDT with the LD-50's expressed as milligrams of DDT per gram of body weight with fiducial limits set at 5 per cent.

<table>
<thead>
<tr>
<th>Culture or Location</th>
<th>Year</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>6th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baton Rouge</td>
<td>1959</td>
<td>.016 ± .0055</td>
<td>.030 ± .0075</td>
<td>.037 ± .0064</td>
<td>.029 ± .0086</td>
<td>.038 ± .0687</td>
</tr>
<tr>
<td></td>
<td>1960</td>
<td>.023 ± .0036</td>
<td>.019 ± .0092</td>
<td>.041 ± .0082</td>
<td>.035 ± .0095</td>
<td>.031 ± .0093</td>
</tr>
<tr>
<td></td>
<td>1961</td>
<td>.018 ± .0012</td>
<td>.033 ± .0108</td>
<td>.022 ± .0052</td>
<td>.032 ± .0086</td>
<td>.037 ± .0115</td>
</tr>
<tr>
<td>Bunkie</td>
<td>1959</td>
<td>.016 ± .0062</td>
<td>.065 ± .0183</td>
<td>.064 ± .0196</td>
<td>.068 ± .0185</td>
<td>.072 ± .0350</td>
</tr>
<tr>
<td></td>
<td>1960</td>
<td>.015 ± .0047</td>
<td>.078 ± .0121</td>
<td>.068 ± .0223</td>
<td>.091 ± .0278</td>
<td>.092 ± .0286</td>
</tr>
<tr>
<td></td>
<td>1961</td>
<td>.024 ± .0032</td>
<td>.082 ± .0262</td>
<td>.075 ± .0216</td>
<td>.076 ± .0213</td>
<td>.083 ± .0321</td>
</tr>
<tr>
<td>Waterproof</td>
<td>1959</td>
<td>.017 ± .0053</td>
<td>.080 ± .0213</td>
<td>.083 ± .0241</td>
<td>.091 ± .0333</td>
<td>.096 ± .0387</td>
</tr>
<tr>
<td></td>
<td>1960</td>
<td>.028 ± .0048</td>
<td>.084 ± .0295</td>
<td>.093 ± .0333</td>
<td>.096 ± .0358</td>
<td>.082 ± .0410</td>
</tr>
<tr>
<td></td>
<td>1961</td>
<td>.023 ± .0059</td>
<td>.072 ± .0157</td>
<td>.072 ± .0237</td>
<td>.082 ± .0285</td>
<td>.106 ± .0450</td>
</tr>
</tbody>
</table>
Table II. Toxicological response of *Heliothis zea* (Boddie) larvae from three locations in Louisiana over a three-year period to DDT with the LD-50's expressed as micrograms of DDT per mean weight larvae.\(^a\)

<table>
<thead>
<tr>
<th>Culture or Location</th>
<th>Year</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>6th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baton Rouge</td>
<td>1959</td>
<td>0.339</td>
<td>1.488</td>
<td>4.388</td>
<td>7.172</td>
<td>13.767</td>
</tr>
<tr>
<td></td>
<td>1960</td>
<td>0.488</td>
<td>0.942</td>
<td>4.863</td>
<td>8.656</td>
<td>11.231</td>
</tr>
<tr>
<td></td>
<td>1961</td>
<td>0.382</td>
<td>1.637</td>
<td>2.609</td>
<td>7.914</td>
<td>13.405</td>
</tr>
<tr>
<td>Bunkie</td>
<td>1959</td>
<td>0.339</td>
<td>3.224</td>
<td>7.590</td>
<td>16.816</td>
<td>26.086</td>
</tr>
<tr>
<td></td>
<td>1960</td>
<td>0.318</td>
<td>3.869</td>
<td>8.065</td>
<td>22.504</td>
<td>33.332</td>
</tr>
<tr>
<td></td>
<td>1961</td>
<td>0.509</td>
<td>4.067</td>
<td>8.895</td>
<td>18.795</td>
<td>30.071</td>
</tr>
<tr>
<td>Waterproof</td>
<td>1959</td>
<td>0.360</td>
<td>3.968</td>
<td>9.844</td>
<td>22.504</td>
<td>34.781</td>
</tr>
<tr>
<td></td>
<td>1960</td>
<td>0.594</td>
<td>4.166</td>
<td>11.030</td>
<td>23.741</td>
<td>29.709</td>
</tr>
<tr>
<td></td>
<td>1961</td>
<td>0.488</td>
<td>3.571</td>
<td>8.539</td>
<td>20.279</td>
<td>38.404</td>
</tr>
</tbody>
</table>

| Mean weight in milligrams | 21.2 | 49.6 | 118.6 | 247.3 | 362.3 |

\(^a\)Mean weight of each instar obtained by weighing 1000 larvae per instar.
instar, which was determined by weighing 1,000 larvae of each instar, is also presented in this table. Thus, in addition to presenting the same information as table I, this table shows that the increase in the size of the larvae in the later instars, even though the LD-50 in milligrams of DDT per gram of body weight is essentially the same, requires a much larger amount of DDT in order to obtain the same response. For example, consider the data concerning larvae from the Baton Rouge area in 1959 which is presented in table I. The LD-50 of the third instar larvae is .030 milligrams of DDT per gram of body weight while that of the sixth instar larvae is .038 milligrams. However, by referring to table II, one can see that the LD-50's in this case are 1.488 micrograms of DDT per mean weight larvae for the third instar while the sixth instar mean weight larvae LD-50 is 13.767 micrograms. Thus, when based on per unit weight, the larvae of these two instars have approximately the same LD-50. However, based on actual weight, about 10 times as much DDT is required for a sixth instar larvae as for a third instar larvae.

Response of bollworm larvae to DDT in five additional areas of the state is shown in table III. These areas include Cheneyville, Church Point, Gilliam, Gueydan, and St. Joseph. The majority of the larvae used in these studies were in the third instar. However, a few second instar larvae were also present. As can readily be seen from table III, there is considerable variation in the LD-50's depending on the location involved. The area with the lowest LD-50 was Church Point
Table III. Response of *Heliothis zea* (Boddie) larvae from additional areas of Louisiana to DDT with the LD-50's expressed in milligrams of DDT per gram body weight with fiducial limits set at 5 per cent.

<table>
<thead>
<tr>
<th>Culture or Location</th>
<th>Year Tested</th>
<th>Number Larvae Tested</th>
<th>Average Weight (mg.)</th>
<th>LD-50 (mg./gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheneyville</td>
<td>1960</td>
<td>168</td>
<td>43</td>
<td>.041 ± .0086</td>
</tr>
<tr>
<td>Church Point</td>
<td>1959</td>
<td>120</td>
<td>26</td>
<td>.039 ± .0064</td>
</tr>
<tr>
<td>Gilliam</td>
<td>1959</td>
<td>88</td>
<td>57</td>
<td>.063 ± .0111</td>
</tr>
<tr>
<td>Gueydan</td>
<td>1959</td>
<td>130</td>
<td>23</td>
<td>.046 ± .0065</td>
</tr>
<tr>
<td>St. Joseph</td>
<td>1959</td>
<td>250</td>
<td>41</td>
<td>.085 ± .0165</td>
</tr>
<tr>
<td></td>
<td>1960</td>
<td>160</td>
<td>36</td>
<td>.093 ± .0152</td>
</tr>
</tbody>
</table>

(0.039 ± 0.0064), while the highest LD-50 (0.093 ± 0.0152) was recorded with larvae from St. Joseph.

Toxicological Studies with Endrin and Toxaphene-DDT Mixture

Table IV shows the response of bollworm larvae from five different locations in Louisiana to endrin and from three locations to toxaphene-DDT (2-1) mixture. The locations surveyed were Baton Rouge, Bunkie, Gilliam, Gueydan and Waterproof. The LD-50's obtained with endrin are approximately the same for these five locations. The same is true for the toxaphene-DDT (2-1) mixture on larvae from three of these locations. A combination of second and third instar larvae were used in these tests. The average weight of each group tested is shown in table IV.

Metabolism of DDT

The data obtained in the study of DDT metabolism in bollworm larvae of the Baton Rouge culture is shown in table V. Third, fourth, fifth and sixth instar larvae treated topically with DDT were analyzed for DDT and DDE at time intervals of 0, 2, 7 and 24 hours. Ten larvae of each instar at each time interval were pooled as a sample. The results indicate that given the same amount of time, approximately the same amount DDT is absorbed by the larvae of the various instars regardless of size. Furthermore very little metabolism of DDT occurs in the bollworm larva. After 24 hours only trace amounts
Table IV. Response of *Heliothis zea* (Boddie) larvae to endrin and toxaphene-DDT mixture with the LD-50's expressed in milligrams of insecticide per gram body weight with fiducial limits set at 5 per cent.

<table>
<thead>
<tr>
<th>Culture or Location</th>
<th>Year Tested</th>
<th>Insecticide Tested</th>
<th>Number Larvae Tested</th>
<th>Average Weight (mg.)</th>
<th>LD-50 (mg./gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baton Rouge</td>
<td>1959</td>
<td>Endrin</td>
<td>150</td>
<td>46.3</td>
<td>.015 ± .0035</td>
</tr>
<tr>
<td></td>
<td>1961</td>
<td>Toxaphene-DDT (2-1)</td>
<td>128</td>
<td>53.8</td>
<td>.031 ± .0083</td>
</tr>
<tr>
<td>Bunkie</td>
<td>1959</td>
<td>Endrin</td>
<td>180</td>
<td>26.3</td>
<td>.018 ± .0047</td>
</tr>
<tr>
<td></td>
<td>1961</td>
<td>Toxaphene-DDT (2-1)</td>
<td>150</td>
<td>28.7</td>
<td>.042 ± .0095</td>
</tr>
<tr>
<td>Gilliam</td>
<td>1959</td>
<td>Endrin</td>
<td>109</td>
<td>35.8</td>
<td>.017 ± .0051</td>
</tr>
<tr>
<td>Gueydan</td>
<td>1959</td>
<td>Endrin</td>
<td>83</td>
<td>28.6</td>
<td>.010 ± .0028</td>
</tr>
<tr>
<td></td>
<td>1961</td>
<td>Toxaphene-DDT (2-1)</td>
<td>75</td>
<td>23.2</td>
<td>.033 ± .0072</td>
</tr>
<tr>
<td>Waterproof</td>
<td>1959</td>
<td>Endrin</td>
<td>120</td>
<td>45.2</td>
<td>.012 ± .0053</td>
</tr>
</tbody>
</table>

*The LD-50 for the toxaphene-DDT (2-1) mixture is expressed in terms of the DDT present.*
<table>
<thead>
<tr>
<th>Instar</th>
<th>Micrograms of DDT Applied per Larvae</th>
<th>Hours After Treatment</th>
<th>Per cent of Applied Dosage Recovered</th>
<th>Total Per cent DDT and DDE Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>External Rinse</td>
<td>Larval Extract</td>
<td>Excretal Extract</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DDT  DDE</td>
<td>DDT  DDE</td>
<td>DDT  DDE</td>
</tr>
<tr>
<td>3rd</td>
<td>3</td>
<td>0</td>
<td>97  0</td>
<td>0  0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>76  0</td>
<td>19  0</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>53  0</td>
<td>41  trace</td>
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<td>49  trace</td>
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</tr>
<tr>
<td></td>
<td></td>
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<td>79  0</td>
<td>15  0</td>
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<td>7</td>
<td>52  0</td>
<td>40  0</td>
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<td></td>
<td></td>
<td>24</td>
<td>32  0</td>
<td>57  2.4</td>
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<tr>
<td>6th</td>
<td>5</td>
<td>0</td>
<td>96  0</td>
<td>0  0</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>38  0</td>
<td>54  trace</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>28  0</td>
<td>59  3.1</td>
</tr>
</tbody>
</table>

*Ten larvae pooled as a sample for each time interval. Figures shown represent the average of at least 4 replications.
of DDE were found in the excreta of the larvae of all instars tested. However, measurable amounts of DDE were found after 24 hours in the fifth and sixth instar larval extracts. Trace amounts of DDE were found in the larval extracts of all instars tested after 7 hours. The majority of the absorbed DDT was recovered from the larval extracts with only very little DDT being found in the excreta.

The fate of DDT applied topically to third and fourth instar bollworm larvae of the Bunkie culture is shown in table VI. These results are essentially the same as those obtained from the Baton Rouge culture. Again, regardless of size, absorption of DDT, as shown by the amount of DDT recovered in the external rinse at 0, 2, 7 and 24 hours, is approximately the same for the larvae of the two instars tested. Very little metabolism occurs since only traces or very small amounts of DDE could be found either in the larval extract or in the excreta.

Considering the metabolism studies of both cultures of larvae, approximately 94 per cent of the applied DDT was accounted for by the techniques used.
Table VI. Recovery of DDT and DDE in the external rinses, larval extracts, and excretal extracts of Heliothis zea (Boddie) larvae of the Bunkie culture treated topically with DDT.

<table>
<thead>
<tr>
<th>Instar</th>
<th>Micrograms of DDT Applied per Larvae</th>
<th>Hours After Treatment</th>
<th>External Rinse</th>
<th>Per cent of Applied Dosage Recovered</th>
<th>Total Per cent DDT and DDE Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>DDT</td>
<td>DDE</td>
<td>DDT</td>
</tr>
<tr>
<td>3rd</td>
<td>3</td>
<td>0</td>
<td>96</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>71</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>58</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>45</td>
<td>0</td>
<td>46</td>
</tr>
<tr>
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\(^a\) Ten larvae pooled as a sample for each time interval. Figures shown represent the average of at least 4 replications.
DISCUSSION OF RESULTS

Toxicological Studies with DDT

During the years 1959, 1960 and 1961, an extensive study of the toxicological response of bollworm larvae to DDT was made in three selected locations in Louisiana. The response to DDT of bollworm larvae from Baton Rouge, where populations are seldom exposed to DDT or other chlorinated hydrocarbon compounds, were compared to the response to DDT of those from the Bunkie and Waterproof areas where DDT has been used extensively for bollworm control since 1947. The dosage-mortality responses of second through sixth instar larvae to DDT from these areas during 1959 are shown in figures 1 through 5, respectively.

Figure 1 shows the 72-hour dosage-mortality response to DDT for second instar bollworm larvae from three locations in Louisiana during 1959. The LD-50's with fiducial limits set at 5 per cent overlap, indicating that there is no significant difference in the response to DDT of these second instar bollworm larvae from these locations. However, the slope of the dosage-mortality curve for the Baton Rouge larvae is greater than the slope of the dosage-mortality curve for either the Bunkie or the Waterproof larvae. According to Hoskins and Gordon (1956), as the slope of the dosage-mortality curve decreases the susceptibility to the insecticide being tested
decreases. Therefore, the population of bollworm larvae from Baton Rouge appears to be more susceptible to DDT than those from Bunkie and Waterproof.

The dosage-mortality data for the third instar larvae from the same locations during the same year are shown in figure 2. In this case, the LD-50 for the Baton Rouge larvae (0.030 ± 0.0075) is significantly different from the LD-50's of the Bunkie and Waterproof larvae which are 0.065 ± 0.0183 and 0.080 ± 0.0213, respectively. By comparing the LD-50's of the third instar larvae shown in figure 2 to those of the second instar shown in figure 1, it can readily be seen that there is an increase in the LD-50 as the larvae progress to the third instar. Also, the slopes of the dosage-mortality curves decrease, thereby indicating a decrease in susceptibility. Again, the slope of the dosage-mortality curve for the Baton Rouge larvae is greater than the slopes of the dosage-mortality curves for the larvae from Bunkie and Waterproof.

The dosage-mortality response to DDT for fourth, fifth and sixth instar larvae from the three areas under investigation are shown in figures 3, 4 and 5. The dosage-mortality responses follow the same pattern exhibited in figure 2. In each of these instars, the LD-50 of the Baton Rouge larvae is significantly different from the LD-50's of the larvae from Bunkie and Waterproof. In general, the difference is two- to three-fold in magnitude. The LD-50's for the third through the sixth instar larvae from a particular area are essentially the same based on dosage of DDT per gram of body weight. However, it is
apparent that the slopes of the dosage-mortality curves decrease as the larvae pass from the earlier to the later instars. Thus, a greater variation in the response to DDT is being obtained in the later instars. Furthermore, considering each instar, there is a more variable response indicated for the Bunkie and Waterproof larvae than for the Baton Rouge larvae.

The 1959 data that were presented in figures 1 through 5 as well as the data obtained in 1960 and 1961 are summarized in table I. In general, the data gathered in 1960 and 1961 very closely parallel that already shown for 1959. The LD-50's for the second instar larvae are approximately the same for the three locations involved over the three-year period. However, there is a two- to five-fold increase in the LD-50 from the second to the third instar larvae, depending on the location and the year. From the third through the sixth instar, there is a two- to three-fold difference in the LD-50's of the larvae between the Baton Rouge area and the Bunkie and Waterproof areas. This difference is consistent over the three-year period. Considering a given location, the LD-50's of the larvae of the third instar through the sixth instar are approximately the same.

Table II, by presenting the same data shown in table I but with the LD-50's expressed in micrograms of DDT per mean weight larvae, shows that the increase in the size of the larvae in the later instars, even though the LD-50 in milligrams of DDT per gram of body weight is essentially the same, requires a much larger amount of DDT in order to obtain the same response. These data showing the relationship of
weight of bollworm larvae to the LD-50 agree essentially with that of Gast (1959). Though he demonstrated a greater increase in the LD-50, especially with very large larvae of the fifth and sixth instar, part of this difference can be explained by the fact that he conducted his experiments at 32°C rather than 27°C.

The response of bollworm larvae to DDT in five additional areas of the state is presented in table III. The majority of the larvae utilized in these tests were in the third instar. There is considerable variation in the LD-50's obtained with about a three-fold difference from the highest and the lowest LD-50.

Generally speaking, LD-50's shown in tables I through III agree with the data presented by McPherson et al. (1956), Brazzel et al. (1961) and Brazzel (1962). Most of the data presented by these authors were obtained from second, third and fourth instar larvae. However, these authors were unable to demonstrate any consistent differences in the response of larvae from various areas to DDT. According to Brown (1958), a difference in the LD-50's of two populations of only two- to three-fold, though not considered to be sufficient to be termed resistance, is great enough to be of importance in control practices where insects of public health importance are concerned. Thus, the difficulty in controlling bollworm larvae experienced by some growers in the Bunkie and Waterproof areas can probably be explained by the two- to three-fold increase in the LD-50 of larvae from these areas as compared to larvae from the Baton Rouge area. This two- to three-fold increase indicates
that the larvae from Bunkie and Waterproof were more resistant than those from Baton Rouge. Whether this difference is due to selective pressure by DDT and other insecticides or was already present in the populations before they were first used is speculative. However, since there was no difference in the response to endrin and toxaphene-DDT using larvae from these same three areas, it would appear that a change toward DDT-resistance is occurring.

Toxicological Studies with Endrin and Toxaphene-DDT Mixture

The response of bollworm larvae from five locations in Louisiana to endrin and toxaphene-DDT (2-1) mixture is shown in table IV. As previously stated, the LD-50's obtained with endrin are approximately the same for the five locations. Larvae from only three areas were treated with toxaphene-DDT and essentially the same response was obtained. Based on these results, there seems to be less variation in the LD-50's obtained from larvae from various areas using endrin or toxaphene-DDT than if DDT is used. The data obtained for endrin and toxaphene-DDT agree with the data presented by Brazzel et al. (1961) and Brazzel (1962).

Metabolism of DDT

The metabolism of DDT by various instars of bollworm larvae from two locations was investigated. The results of these studies are summarized in tables V and VI, which show the per cent of the
applied dose of DDT that was recovered as either DDT or DDE. No other metabolites are listed since preliminary investigations utilizing the analytical techniques of Schechter et al. (1945) and the chromatographic separation procedure described by Sternburg and Kearns (1952) revealed only DDT and DDE to be present in the bollworm after topical application of DDT. Approximately 94 percent of the applied DDT was accounted for by the analysis utilized.

Data obtained in the study of DDT metabolism in the third, fourth, fifth and sixth instar bollworm larvae of the Baton Rouge culture is shown in table V. The fate of DDT in third and fourth instar bollworm larvae of the Bunkie culture is presented in table VI. The results obtained with bollworm larvae from these two locations are essentially the same. First, regardless of size, absorption of DDT, as shown by the amount of DDT recovered in the external rinses at 0, 2, 7 and 24 hours, is approximately the same for all instars tested. Secondly, very little DDT metabolism occurs since only traces or very small amounts of DDE could be found either in the larval extracts or in the excretal extracts. These findings are in close agreement with the results obtained by Gast (1961), in which he reported that there was no evidence of any appreciable metabolism of DDT by the bollworm.
CONCLUSIONS

Results of this study allow the following conclusions to be made.

1. There were no significant differences in the response to DDT of second instar bollworm larvae from Baton Rouge, Bunkie and Waterproof. However, the slopes of the dosage-mortality curves show that larvae from Baton Rouge were more susceptible to DDT at the higher dosages than larvae from the other two areas.

2. There was a two- to five-fold increase in the LD-50 of the larvae of the third compared to the second instar depending on location involved.

3. There was a two- to three-fold increase in the LD-50's to DDT of third, fourth, fifth and sixth instar bollworm larvae from Bunkie and Waterproof compared to the larvae from Baton Rouge. This difference was demonstrated consistently over a three-year period and was statistically significant. This indicates that the larvae from Bunkie and Waterproof were more resistant to DDT than the larvae from Baton Rouge. Whether or not this difference was present before the use of DDT and other chlorinated hydrocarbons is speculative. The slopes of the dosage-mortality curves for the various instars of the Baton Rouge larvae indicated greater susceptibility to DDT than comparable larvae from Bunkie and Waterproof.
4. The LD-50's are essentially the same for the larvae of the third and succeeding instars. However, approximately 10 times as much DDT is required for a sixth instar larva as for a third instar larva because of the difference in size.

5. The slope of the dosage-mortality curve for larvae of each successive instar decreases. This was true for larvae from each of the three locations. Thus, the later instars exhibited greater variation in the response to DDT. There was more variation in response to the Bunkie and Waterproof larvae than the Baton Rouge larvae.

6. There were no significant differences in the response to endrin of bollworm larvae from five areas of Louisiana which included Baton Rouge, Bunkie and Waterproof.

7. There were no significant differences in the response to toxaphene-DDT (2-1) mixture of bollworm larvae from three areas of Louisiana.

8. Bollworm larvae of the third through the sixth instars absorbed DDT at approximately the same rate.

9. Very little of the absorbed DDT was metabolized.

10. Only small amounts of DDT were present in the excreta of bollworm larvae 24 hours after topical application.
SELECTED BIBLIOGRAPHY


Jerry Brook Graves was born February 28, 1935 at Tylertown, Mississippi. He attended elementary and high school at Lexie High School and graduated in 1951.

From September, 1951 to June, 1955 he attended Mississippi State University where he received the degree of Bachelor of Science in Entomology. In July, 1955 he entered the United States Army where he served until July, 1957.

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He entered Louisiana State University in September, 1958 and was granted a graduate research fellowship with the Department of Entomology Research, Louisiana Agricultural Experiment Station. In February, 1961 he was appointed to the staff of the Department of Entomology Research as an Associate.

On December 25, 1960, he married Mary Ellen Brumfield of Tylertown, Mississippi.

At present, he is a candidate for the degree of Doctor of Philosophy.
EXAMINATION AND THESIS REPORT

Candidate:  Jerry Brook Graves

Major Field:  Entomology

Title of Thesis:  Toxicological and Metabolic Response of the Bollworm, Heliothis zea (Boddie), to DDT

Approved:

[Signatures and names]

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures and names]

Date of Examination:

July 30, 1962