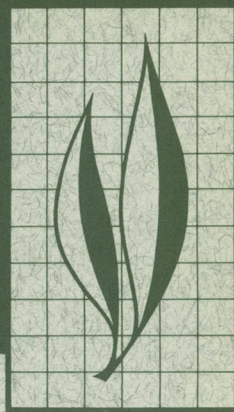


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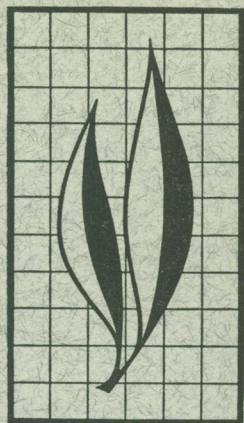
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## A Laboratory Study of Three Strains of Codling Moth, *Carpocapsa pomonella* (Linnaeus), Exhibiting Tolerance to DDT in the Field

J. Blair Bailey and Harold F. Madsen





Three field strains of DDT-resistant codling moths from different areas in northern California were mass reared in the laboratory. The degree of DDT resistance in both larvae and adults was determined by using two methods of exposure to the insecticides. Topical applications ( $LD_{50}$ ) were made to the moths, and the cage technique ( $LC_{50}$ ) was used for the larvae. A DDT-susceptible laboratory strain of codling moth was used as the standard for comparison.

The tests showed that all three of the field strains were resistant to DDT, but further tests showed no cross-resistance to either the carbamate or phosphate insecticides tested.

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# A Laboratory Study of Three Strains of Codling Moth, *Carpocapsa pomonella* (Linnaeus), Exhibiting Tolerance to DDT in the Field<sup>1</sup>

## INTRODUCTION

THE CODLING MOTH, *Carpocapsa pomonella* (Linnaeus), is a world-wide pest of pome fruits. Because of its importance, the pest control program on a number of deciduous fruits is designed around a schedule of codling moth sprays.

After 1945, when DDT became the standard insecticide against the codling moth, satisfactory control was obtained for nearly ten years. In 1954, a few reports were received indicating difficulty in controlling codling moths with DDT. Investigations by Madsen (1956) in one orchard showed that the problem was due to a difference in the flight habits of the adults rather than resistance to DDT.

The first case of codling moth resistance to DDT in California was reported by Madsen and Hoyt (1958). Their field data were confirmed in the laboratory by Barnes (1958b). Since that time, reports of resistance in individual orchards increased and field plots demonstrated that DDT was no longer providing the control obtained in past seasons (Madsen and Falcon, 1960).

Field plots to determine resistance have been limited since they cannot provide precise data on the effect of the insecticide in question. They can demonstrate whether the suggested compound and dosage rate will provide control

and eliminate the possibility that control failure is due to poor spray application or improper timing. They cannot, however, provide data on the degree of resistance, the difference in susceptibility of the various developmental stages of the pest, or solve the problem of possible cross-resistance to other compounds. The above data can be developed only in the laboratory under controlled conditions, where sufficient replication and precise dosages can be applied.

In order to provide information on the above problems, strains of codling moths from areas where field studies indicated the presence of DDT resistance were established in the laboratory. With these colonies and a strain of codling moth that had not been exposed to DDT, it was possible to initiate a project to determine the degree of resistance of each strain, the relative susceptibility of the larvae and adults, and the possibility of cross-resistance to other insecticides.

Several reviews of resistance to chemicals by insects and other arthropods appear in the literature. Two of the more complete reviews are by Glass (1960) and Brown (1960). The first covers the resistance problems relating to deciduous fruit pests. The paper by Brown is a general review of arthropod

<sup>1</sup> Submitted for publication, March 14, 1963.

resistance. It categorizes resistance both according to chemicals and to classes and species of arthropods, and gives the sequence in which resistance was reported. In addition, Brown indicates whether the resistance is of importance to agriculture or public health.

Melander (1914) mentioned an earlier warning on resistance issued in 1908—almost 50 years after the publication of Darwin's *The Origin of Species*. The article pointed out that the selective action of insecticides may leave populations which could develop into resistant strains. It also reported resistance of the San Jose scale, *Aspidiotus perniciosus* Comstock, to lime sulfur. Twenty years later the codling moth was reported to have become resistant to lead arsenate by Hough (1928). In later experiments Hough (1934) showed that this was not true resistance, but that the newly hatched larvae were larger and more tolerant to desiccation. As a result, the larvae could survive longer on sprayed apples until an untreated point of entry could be found. This behavior is referred to as "vigor tolerance" by Hoskins and Gordon (1956).

Resistance to lead arsenate by the codling moth was later reported from Washington, Virginia, South Africa, and California. A total of thirteen species of arthropods was reported to have exhibited resistance in the pre-DDT era, that is, before 1945.

DDT was introduced and used in the Armed Forces in 1945, but it was not until 1946 that it was used extensively in agriculture. After a period of six years of extensive use, Cutright (1954)

reported that control of the codling moth was unsatisfactory in one Ohio orchard. Within two years DDT completely failed to control the insect in this orchard.

Following Cutright's report, a series of papers dealing with codling moth resistance in various areas appeared in the literature: Glass and Fiori—New York (1955); Smith—Australia (1955); Hamilton—Indiana (1956); Madsen and Hoyt—California (1958); Barnes—California (1958b); Myburgh—South Africa (1958); Michelbacher and Ortega—California (1958); Barnes—California (1959); Asquith—Pennsylvania (1959); and Marshall—Canada (1959). Glass (1960), in correspondence with other entomologists, stated that there was "evidence of resistance in many areas of the United States as well as in other parts of the world."

Most of the above reports were concerned with codling moths on apples. In 1957, the first instance of codling moth resistance to DDT on pears was observed by Madsen and Hoyt (1958) in California. Madsen and Falcon (1960) stated that "reports of poor control with DDT on pears were increasing and by the 1958 season, control had failed in several orchards following well-timed and thoroughly applied DDT programs."

Madsen and Falcon (1960), studied the codling moth in three orchards that had reported poor or no control with DDT. Replicated field plots demonstrated that resistance was present in each of the areas studied.

## MATERIALS AND METHODS

As previously noted, Madsen and Falcon (1960) established that two codling moth strains, the Christie strain from Sacramento County and the Pangborne strain from Solano County, showed definite signs of DDT resistance in the field. The third field strain was taken from the Simoni ranch in Sacramento

County, where the grower had failed to obtain control of codling moths with DDT using recommended spray practices.

In order to collect representative samples from each of the three strains for rearing in the laboratory, corrugated cardboard bands measuring about 2



inches wide were stapled around the trunk of a number of heavily infested trees in the fall of the year. During the winter the bands into which the codling moth larvae crawled for overwintering were collected and taken to Berkeley where they were held in a cold room at 1.1° C. After 6 weeks' exposure to this temperature, the bands were removed and placed in sleeve cages in the greenhouse where temperatures were maintained at approximately 26° C for optimum pupal development (fig. 1). Pupation took place within the bands and the moths emerged in the month of February. A half-pint glass milk bottle containing paper toweling immersed in water and projecting from the top of the bottle provided a supply of water for the newly emerged moths until they were removed from the sleeve cages.

The Barnes strain, which had been reared continuously in the laboratory since 1948, was obtained from the Uni-

versity of California Citrus Research Center at Riverside, California. This strain was used as the standard susceptible (S) strain with which the resistant (R) field strains were compared. Repeated tests by Barnes and his associates (1961)<sup>2</sup> had established that the (S) strain was susceptible to DDT by exposing both the larvae and adults to the insecticide.

### Rearing in the Laboratory

A constant supply of codling moths for testing and colony maintenance was obtained by using the procedure described by Dickson, Barnes, and Turzan (1952). Some modification of the Dickson technique was necessary to obtain an adequate number of insects under the laboratory conditions at Berkeley.

Moths were removed daily from sleeve cages using a tank-type vacuum cleaner modified to produce just enough suction to capture moths without injury

<sup>2</sup> Personal communication, January 26, 1961.



Fig. 1. Sleeve cages at right which hold two rearing trays each. Newly emerged codling moths were taken from these cages and transferred to the plastic oviposition cages at left.

(fig. 2). Moths were counted and records were kept on the emergence time and number of generations. Moths were immobilized with  $\text{CO}_2$  in the collecting tube and then transferred from the tube into oviposition cages. For optimum mating and oviposition, it was found that 100 to 150 moths of approximately equal sexes per cage gave the best results. Oviposition cages were similar to those described by Dickson *et al.* (1952). A modification of the removable ends of these cages was made because observations showed that hundreds of eggs were laid on both ends of the cages where the screen was affixed to the plastic. To eliminate this oviposition site, heavy mosquito netting was substituted for the plastic and screen cage ends which proved to be less attractive as a surface upon which to oviposit. Pieces of netting large enough to fit over the ends were used and held taut with

plastic hoops. A Petri dish filled with cotton and covered with plastic netting served as a container for water. Water was added daily by squirting it through the vented ends of the cages into the containers.

Moderate wrinkling of the wax paper liners provided areas where eggs were concentrated. Natural twilight in the greenhouse was satisfactory for good oviposition, although oviposition declined during the winter months.

The moths were left in the oviposition cages for varying periods depending upon the purpose of the individual experiment. Normally, a period of 7 days under Berkeley laboratory conditions was adequate. Peak oviposition occurred on the third day after introduction of the moths, and oviposition continued with a slight decline for a week to 10 days. By the end of the seventh day, the earliest laid eggs reached the black-

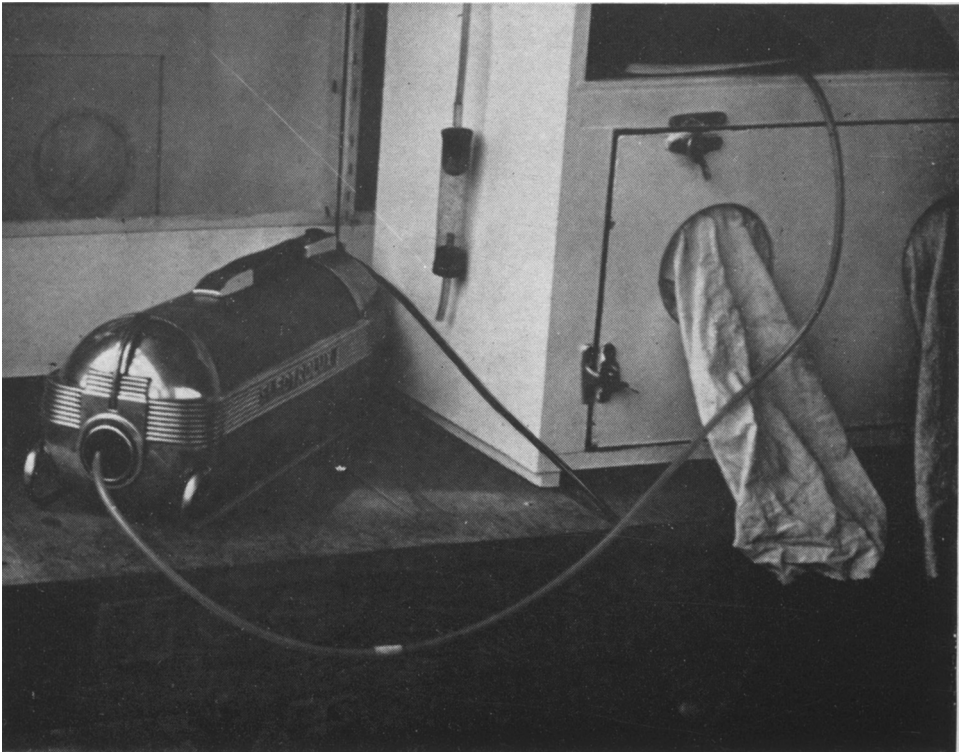


Fig. 2. Tank-type vacuum cleaner modified to produce just enough suction to capture moths without injury.



head stage of development and the wax liners were removed. At that time the live moths were transferred to another oviposition cage where they continued to oviposit to a lesser degree for approximately one more week. The wax paper liners of the oviposition cages (egg sheets) were then cut into strips, removed from the greenhouse and placed on apple thinnings in metal or plastic rearing trays (fig. 3). The rearing trays were placed in a room where cooler temperatures could be maintained.

Apple thinnings used as food were of the Yellow Newtown Pippin variety. They were selected because of their availability and keeping qualities. The apples were collected in the field during normal thinning operations when they were approximately 1 inch in diameter. The fruit was sorted and

washed in a solution of trisodium phosphate to remove spray residue, then rinsed in clean water and air dried. The fruit was packaged in polyethylene bags which were perforated to provide for ventilation; this reduced the percentage of storage rot. Bagging the fruit extended the storage life of the fruit 6 months beyond that of unbagged fruit since it prevented shriveling. The apples were stored at 2.2° C.

Apple thinnings were infested in the insectary by covering the bottom of metal rearing trays with apples and placing strips of egg sheets over the fruit. Two layers of fruit covered with egg sheets were used per rearing tray. At eclosion, the newly hatched larvae readily found the apples. Strips of corrugated cardboard were fitted around the inside periphery of the rearing trays with several strips placed midway



Fig. 3. Plastic rearing trays showing egg sheets on top of apple thinnings.

across the trays in contact with the fruit. This provided pupation sites for the larvae (fig. 4).

Rearing trays with the egg sheets in place were set in sleeve cages modified to serve as humidity chambers. Trays of water maintained the relative humidity at 85 per cent for best eclosion and first-instar survival. A hygrothermograph recorded relative humidity and air temperatures, and a lamp was placed over the cage to provide constant light. The time interval from eclosion to infestation was from 2 to 3 days. Tray tops consisting of snugly fitted aluminum frames covered with fine plastic screen prevented escape of the first-instar larvae and provided ventilation and sufficient light. Constant fluorescent light was maintained in the rearing room to prevent diapause. Larval development and pupation required approximately 3 weeks. When the first

moths emerged, the trays were removed from the rearing room and placed in sleeve cages in the greenhouse.

### Laboratory Testing Procedures for $LC_{50}$ Determinations

Determining the ability of first-instar larvae to penetrate fruit which had insecticide deposits on the surface was the purpose of the first series of tests conducted in the laboratory. Trials were run to find the lethal concentration which would result in 50 per cent mortality ( $LC_{50}$ ) of the first-instar larvae of the various strains of codling moth. Varying concentrations, both above and below the 50 per cent level were used. The resulting dosage-mortality points provided data from which regression lines were statistically calculated, using the two-variable analysis.

Some of the techniques and procedures used in this work were modifi-



Fig. 4. Metal rearing tray showing infested apple thinnings and corrugated cardboard strips in which larvae pupate.



cations of work by Steiner (1939), Carman and Fleschner (1944), and Barnes (1958b). A Latin square design was selected for these tests in order to compensate for any variation which might have existed in the constant-temperature cabinet. Three test boards were constructed from  $\frac{5}{8}$ " plywood, each measuring 16"  $\times$  16". On each of the boards, three 2"  $\times$  2" wood strips were firmly affixed. Three holes were drilled in each of the strips into which removable metal spikes could be inserted. One apple was impaled on each spike. On each test board the strips were labeled ABC, BCA, and CAB respectively (fig. 5). The three fruits on each of the three boards labeled "A" were unsprayed controls or checks; the first two spikes on the "B" boards held the fruits sprayed with the lowest concentration of insecticide; the first two fruits on the "C" boards were sprayed with a higher concentration, and the last fruits on the "B" and "C" boards received the heaviest dosage. A total of twenty-seven mature Yellow Newtown Pippin apples, varying from 2 $\frac{1}{2}$  to 3 inches in diameter, was used in each test. The procedure used for washing and spraying the fruits was similar to that described by Barnes (1958a).

The following commercial grade insecticides were used in these tests: 50 per cent W.P. DDT (dichloro-diphenyl-trichloroethane); 50 per cent W.P. Sevin (1-naphthyl N-methylcarbamate); 25 per cent W.P. Guthion [O-O-dimethyl S-(4-oxo-1,2,3-benzotriazinyl-3-methyl)phosphorodithioate]. Stock suspensions of the insecticides were prepared on a weight-volume basis and succeeding dilutions to the desired concentrations were made on a volume-volume basis starting with the basic stock suspensions.

In the spraying operations, the lowest concentrations were applied first and the spray equipment was thoroughly rinsed after each concentration was applied. The number of experiments carried out at the various concentrations

is indicated by the number of points appearing on the graphs shown in figures 8A and 9. Each point on the graphs represents thirty first-instar larvae. In each experiment, three different concentrations were used, with thirty larvae exposed to each concentration. In addition, there were forty-five control larvae. Percentage mortalities were corrected by Abbot's formula (Abbot, 1925). In any one experiment, if the per cent mortality in the controls exceeded 20 per cent, the entire experiment was discarded. Moribund individuals were counted as dead since observations showed that these individuals did not feed, nor did they survive for more than 24 hours after the test was checked. The sexes were not separated in these tests.

Five paraffin-coated metal cages (cells) were affixed to the equator of each apple after the spray deposits had dried. A molten mixture of paraffin and beeswax (50-50) was applied with a  $\frac{1}{2}$ -inch paint brush to the base of the cell and the surface of the fruit which it contacted.

About 6 hours prior to introducing larvae into the cells, several egg sheets on which most of the eggs were in the blackhead stage of development were selected and placed in empty rearing trays lined with wax paper. The tray was then put in a humidity chamber which was maintained at 29.4° C and about 85 per cent relative humidity. This procedure provided enough newly hatched larvae within a 6-hour age group for introduction into each cell. The larvae were transferred from the egg sheet into the cells with a camel's hair brush containing a single hair.

In order to avoid bias in selection of the hardiest or the weakest larvae, the selection and introduction of the larvae into the cells was done in replicate fashion. One fruit of each treatment group or untreated control was inoculated at a time, in series.

After the cells were affixed and the larvae had been introduced, a 2"  $\times$  2"

piece of nylon organdy, cut with pink-ing shears to prevent fraying, was stretched taut over each cell and held in place with a small rubber band. The porosity of the organdy was sufficient to prevent any noticeable fumigation effect

which could have resulted from the commonly utilized broadcloth covers.

The test boards with fruits in place were then held for 72 hours in a constant-temperature cabinet maintained at 26° C and 85 per cent relative hu-



Fig. 5. Three  $LC_{50}$  (lethal concentration, 50 per cent mortality) test boards showing test apples with metal cells affixed and covers in place. Each cell contains one first-instar codling moth larva. The constant-temperature cabinets were maintained at 26°C and 85 per cent relative humidity.



midity (fig. 5). Repeated checks for uniformity of temperature throughout all portions of the cabinet were made using six leads of a potentiometer. Variation was  $\pm 1/2^\circ$  C. Humidity was maintained at 85 per cent for the 72-hour period by placing water-saturated cellulose sponges on the test boards between the fruits. In addition, three small trays of water plus a humidity rack made of absorbent cotton batting placed in a wood frame and held in place with plastic mesh screen were used.

The test boards were then removed from the cabinet and the individual apples were examined to determine if the larvae had entered or succumbed to the insecticide. An entry was defined as penetration of the fruit by the larva for a distance of over  $1/4$  inch.

### Laboratory Testing Procedures for LD<sub>50</sub> Determinations

The topical application method was used to determine the LD<sub>50</sub> of DDT and other insecticides to adult codling moths of the various strains under test.

Adult codling moths were obtained by rearing the insects following the methods previously described. Newly emerged adults were collected daily and put in oviposition cages for aging. The moths were aged for 3 days before being used in the topical application tests. After the 3-day period, the moths were collected using the suction method previously described. The moths were then immobilized in the collecting tube by exposing them to CO<sub>2</sub> for approximately 30 seconds. They were then transferred into a 3-inch Buchner funnel which was covered with a glass Petri dish. To facilitate handling, the moths were inactivated by introducing a constant low volume of CO<sub>2</sub> into the Buchner funnel. The CO<sub>2</sub> was first run through a 1,000 ml water filtering flask to prevent desiccation of the moths (fig. 6).

As the moths were treated, they were

removed singly with a pair of forceps, and placed on their backs under a dissecting microscope. Insecticides were applied with a 1-microliter micropipette to the ventral thoracic region of each moth. The same three chemicals, DDT, Sevin, and Guthion, were used in these tests as in the LC<sub>50</sub> tests except that these were in a chemically pure crystalline form. Stock solutions of each of the insecticides were prepared on a weight-volume basis and succeeding dilutions to the desired concentrations were made on a volume-volume basis starting with the basic stock solutions. Acetone was used as the solvent.

The control moths were treated first in each run with acetone. In the treated groups, the insecticides were applied in ascending order of concentration and the micropipette was rinsed thoroughly with acetone between each concentration.

In order to obtain sufficiently high mortality with DDT in the Simoni strain, it was necessary to make several applications of lower concentrations to avoid clogging the micropipette. The pipette was rinsed between each application in these experiments, and the same number of applications of acetone was applied to each of the control moths as to the treated moths.

In order to determine whether differences in response to DDT would occur between male and female moths, a series of eight LD<sub>50</sub> tests was run in which the moths were separated by sex. The resulting regression lines, not shown in this paper, were parallel and differed only .1 of a microgram per microliter between the sexes, which was not significant. As a result of these tests, all subsequent tests included both males and females.

For convenience of handling and counting, approximately twenty moths were placed in each holding cage for a period of 24 hours. A constant temperature of  $21.1^\circ$  C and a relative humidity of 70 per cent were maintained in the holding room.

The holding cages were constructed from 1-point cylindrical ice cream cartons with both ends replaced by wire screen. In the center of one end, a circular hole was cut and a  $\frac{1}{2}$ -inch rubber grommet was inserted which would hold a 1-dram patent lip vial. The vial was filled with water and a 2-inch length of dental cotton was doubled over and inserted to provide a source of water for the caged moths.

Each point on the  $LD_{50}$  graphs (see figs. 8B, 10, and 11) represents the average per cent mortality corrected to Abbot's formula for that dosage. Each

point also represents an average of 125 adult moths treated over a period of several days in groups of 20 or more plus an average of 42 control moths.

In any one run, if the per cent mortality in the controls exceeded 20 per cent, the entire run was discarded, but mortality rarely reached even the 10 per cent level.

As in the  $LC_{50}$  experiments, individual moths which appeared to be moribund were included with the dead individuals. A total of over 10,000 codling moth larvae and adults were treated in this study.

## RESULTS

The results of the laboratory tests are of two types: one, the  $LC_{50}$  values, obtained from codling moth larvae, and the other, the  $LD_{50}$  values, obtained from adult codling moths treated by

topical application. The 50 per cent level of toxicity will be used as a reference point when discussing the effect of the various insecticides on both first-instar larvae and adult moths. With

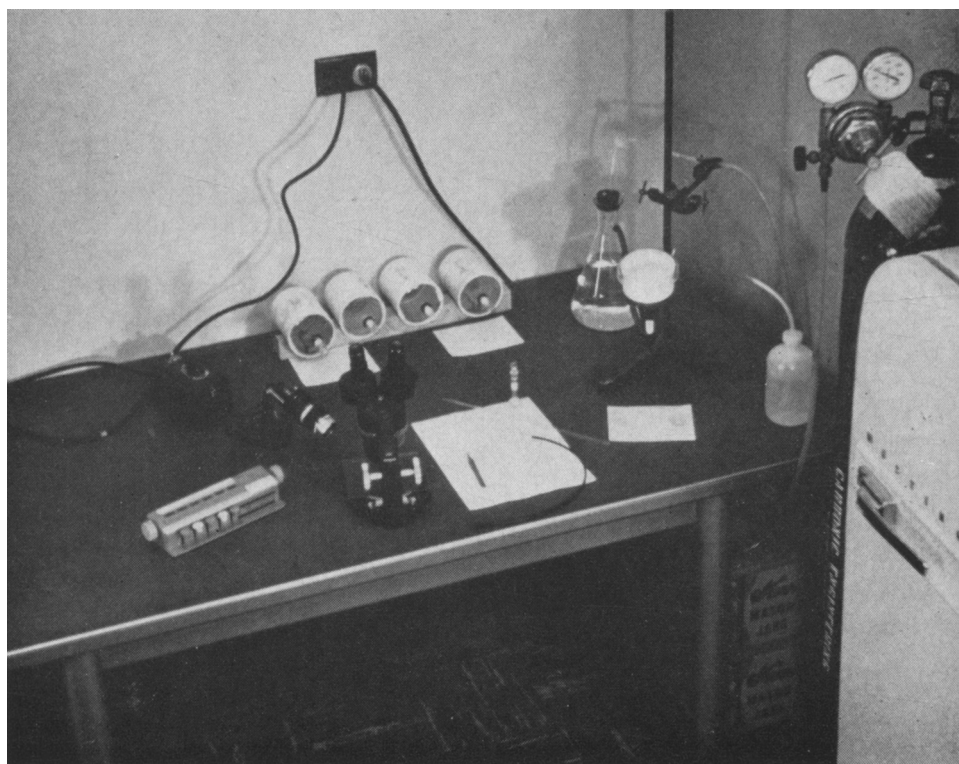


Fig. 6. Equipment and holding cages used in topical application  $LD_{50}$  (lethal dosage, 50 per cent mortality) tests.



TABLE 1  
LC<sub>50</sub> VALUES IN GRAMS OF ACTUAL INSECTICIDE PER LITER  
FOR FIRST-INSTAR CODLING MOTH LARVAE

Strain	DDT	Sevin	Guthion
Barnes.....	0.42	.03	.004
Christie.....	0.9	...	.006
Pangborne.....	1.5	.04	....
Simoni.....	5.0	...	.009

both LC<sub>50</sub> and LD<sub>50</sub> tests, the four strains of laboratory-reared codling moths were used and were subjected to three insecticides—DDT, Sevin and Guthion.

The Barnes strain will be referred to as the (S) or DDT-susceptible strain in all tests where DDT was used, since its susceptibility to DDT has been established by Barnes (1958a). In tests where Guthion or Sevin was used, the strain will be referred to as the “Barnes strain” rather than the (S) strain, since there was no evidence that the response of this strain to other insecticides dif-

fered significantly from the response of the three strains obtained from the field.

LC<sub>50</sub> Tests

Table 1 shows the LC<sub>50</sub> values converted to grams of pure insecticide per liter of water for each chemical used and for each strain of codling moth larvae. The values were taken directly from the ld-p (log dosage-probit) lines which were statistically calculated using the two-variable analysis and which are shown in figures 7, 8A, and 9.

The DDT LC<sub>50</sub> for the (S) strain was found to be 0.42 gram per liter, which

DDT SUSCEPTIBILITY OF CODLING MOTH LARVAE—LC<sub>50</sub> TESTS

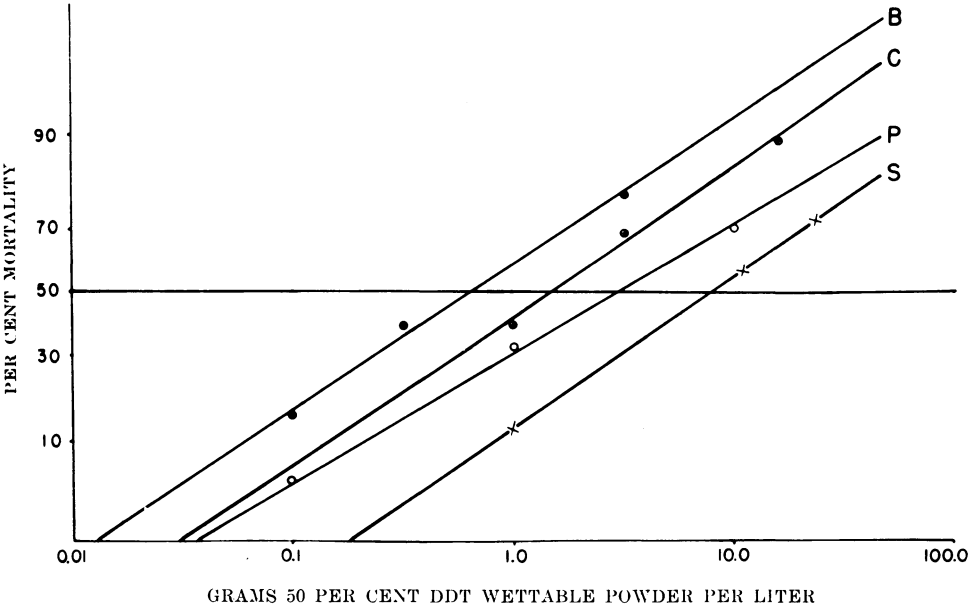


Fig. 7. Log dosage-probit (ld-p) lines for four strains of codling moth larvae, *Carpocapsa pomonella* (L.), showing the relative degree of resistance. B = Barnes, C = Christie, P = Pangborne, S = Simoni. These ld-p lines were established in LC<sub>50</sub> (cage technique) tests using DDT.

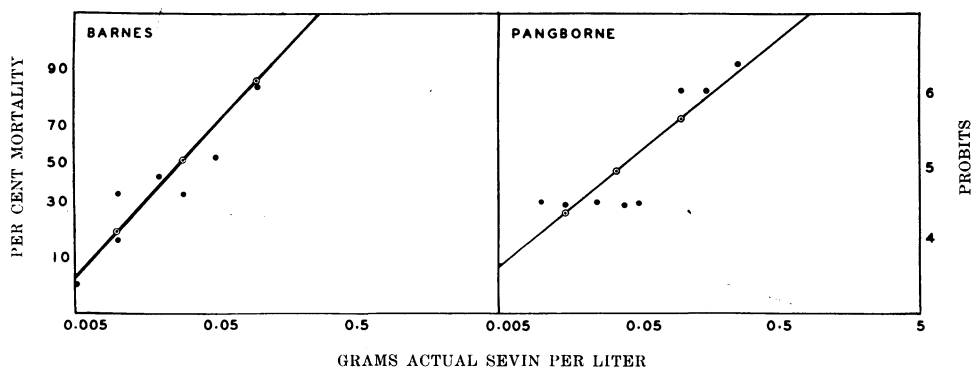
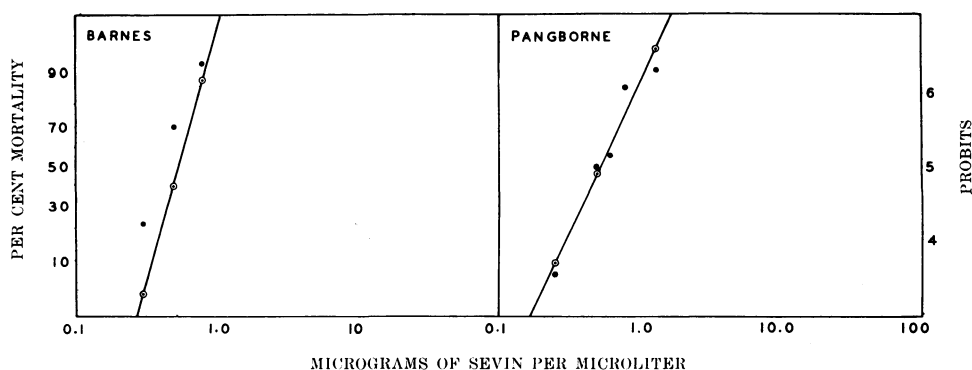
A. SEVIN SUSCEPTIBILITY OF CODLING MOTH LARVAE— $LC_{50}$  TESTSB. SEVIN SUSCEPTIBILITY OF CODLING MOTH ADULTS— $LD_{50}$  TESTS

Fig. 8. A. Log dosage-probit lines for Barnes and Pangborne strains of codling moth larvae, *Carpocapsa pomonella* (L.), established in  $LC_{50}$  (cage technique) tests using Sevin insecticide. Each point represents thirty first-instar larvae, plus 45 control larvae.

B. Log dosage-probit lines for Barnes and Pangborne adults established in  $LD_{50}$  (topical application) tests using Sevin insecticide. Each point represents 125 treated adult moths plus 42 control moths.

is comparable to the values obtained by Barnes (1958). The Christie strain required 0.9 gram per liter to obtain an  $LC_{50}$  value, which was approximately twice the concentration for that of the (S) strain. The Pangborne strain required 1.5 grams per liter, which is over three times that for the (S) strain, while the  $LC_{50}$  for the Simoni strain was 5.0 grams per liter, which exceeded the (S) strain by twelve times (fig. 7).

Only two strains, Barnes and Pangborne, were subjected to concentrations of Sevin insecticide. Both strains showed similar susceptibilities. For the Barnes strain an  $LC_{50}$  value was reached

at 0.03 gram per liter, and for the Pangborne strain at 0.04 gram per liter (fig. 8A).

Guthion was used against three of the strains and the results showed an  $LC_{50}$  of 0.004 gram per liter for the Barnes strain, and values of 0.006 and 0.009 for the Christie and Simoni strains respectively (fig. 9).

The results indicate that there was no apparent cross-resistance to either Sevin or Guthion in any of the DDT-resistant strains since similar values were obtained when the Barnes strain was compared to the three DDT-resistant strains. In addition, these data show

GUTHION SUSCEPTIBILITY OF  
CODLING MOTH LARVAE—LC<sub>50</sub> TESTS

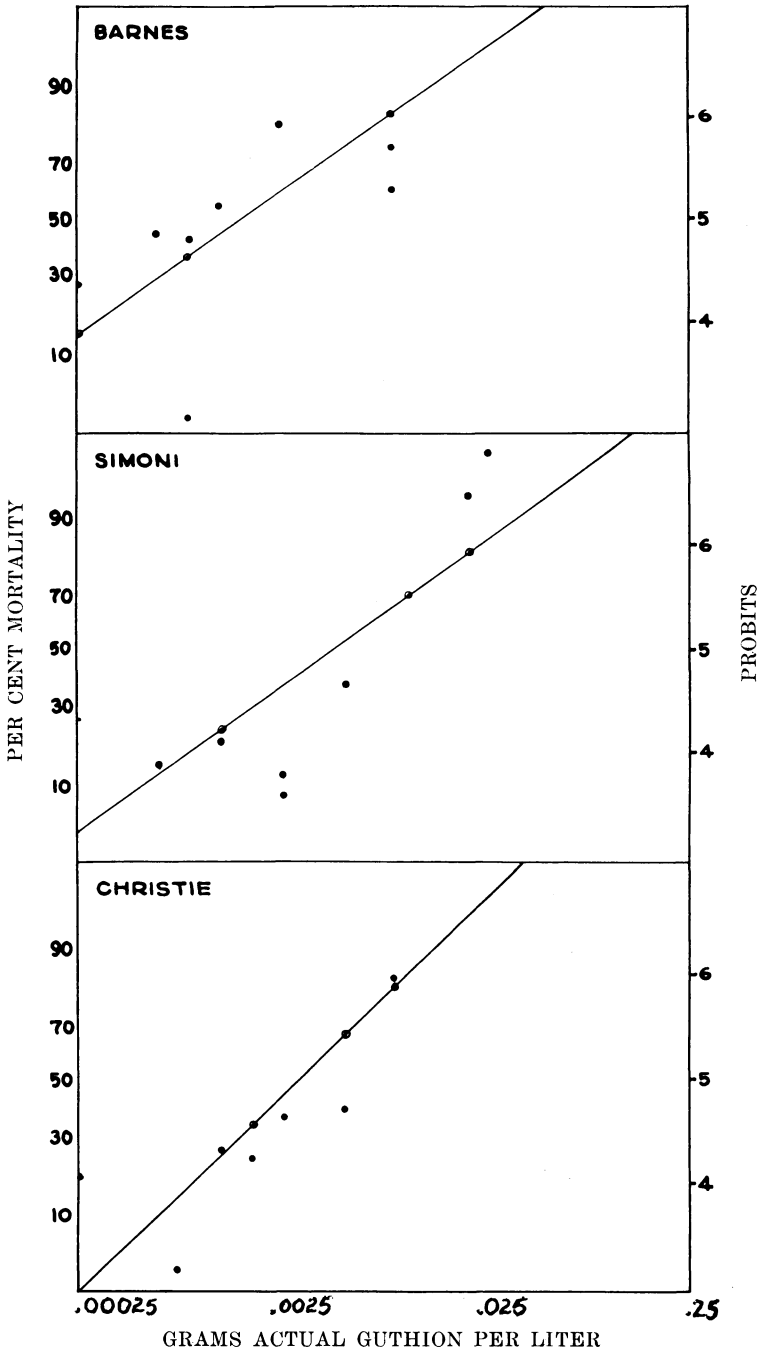


Fig. 9. Log dosage-probit lines for Barnes, Simoni and Christie strains of codling moth larvae, *Carpocapsa pomonella* (L.), established in LC<sub>50</sub> (cage technique) tests using Guthion. Each point represents thirty first-instar larvae plus 45 control larvae.



TABLE 2  
LD<sub>50</sub> VALUES IN MICROGRAMS OF ACTUAL INSECTICIDE PER  
MICROLITER FOR THREE-DAY-OLD CODLING MOTH ADULTS

Strain	DDT	Sevin	Guthion
Barnes.....	0.55	.54	.18
Christie.....	1.9	...	.10
Pangborne.....	7.0	.54	...
Simoni.....	14.0	...	.10

that Sevin was considerably more lethal than DDT to both the susceptible and resistant strains. Guthion showed an even higher toxicity to the strains, as evidenced by the low LC<sub>50</sub> values obtained in the tests.

### LD<sub>50</sub> Tests

Table 2 shows the LD<sub>50</sub> values for each chemical used in  $\mu\text{g}/\mu\text{l}$  of pure insecticide for each strain of codling moth adults. These values were taken directly from the ld-p lines which were statistically calculated and which are shown in figures 8B, 10, and 11.

Sevin was topically applied to only the Barnes and Pangborne strains in these tests. The LD<sub>50</sub> values were identical for both strains at 0.54  $\mu\text{g}/\mu\text{l}$  (fig. 8B).

A dose of 0.18  $\mu\text{g}/\mu\text{l}$  of Guthion was needed to obtain an LD<sub>50</sub> for the Barnes strain, while 0.10  $\mu\text{g}/\mu\text{l}$  was needed for both the Christie and Simoni strains (fig. 10).

All four of the codling moth strains were treated with topical applications of DDT. The LD<sub>50</sub> value for the (S) strain was 0.55  $\mu\text{g}/\mu\text{l}$ , while those for the Christie, Pangborne, and Simoni were 1.9, 7.0, and 14.0  $\mu\text{g}/\mu\text{l}$ , respectively (fig. 11). In other words, the data indicate that the Christie strain is approximately three times more resistant to DDT than the (S) strain. The Pangborne strain is greater than eleven times as resistant, and the Simoni strain is twenty-four times more resistant to DDT than the (S) strain.

A different trend was noted in the LD<sub>50</sub> values for Guthion. In these tests,

a slightly greater dose was required to reach 50 per cent mortality with the Barnes strain. This is probably of little significance since the dosages of Guthion were so low compared to those of DDT and Sevin.

### Comparison of LC<sub>50</sub> Values with LD<sub>50</sub> Values

A direct comparison of LC<sub>50</sub> values with LD<sub>50</sub> values is not possible because of the different techniques employed as well as the different stages of development of the insects tested. However, two observations are of significance. In every test the LD<sub>50</sub> values are higher than the LC<sub>50</sub> values when one analyzes the data for one strain at a time and for any one of the insecticides used. Secondly there is greater variation in the degree of resistant to DDT within the LD<sub>50</sub> tests than within the LC<sub>50</sub> tests. Referring to LC<sub>50</sub> values (table 1) it will be noted that the first-instar larvae of the Simoni strain show twelve times the resistance, while in the LD<sub>50</sub> tests (table 2) the adults of the Simoni strain are twenty-four times more resistant to DDT than the (S) strain.

### Slope Data from ld-p Lines for Each Strain

The ld-p lines for both LC<sub>50</sub> and LD<sub>50</sub> tests are graphically shown in figures 7 to 11. The numerical value for the slope of each of these lines is shown in table 3.

When dosage is expressed logarithmically and mortality is expressed in

GUTHION SUSCEPTIBILITY OF  
CODLING MOTH ADULTS—LD<sub>50</sub> TESTS

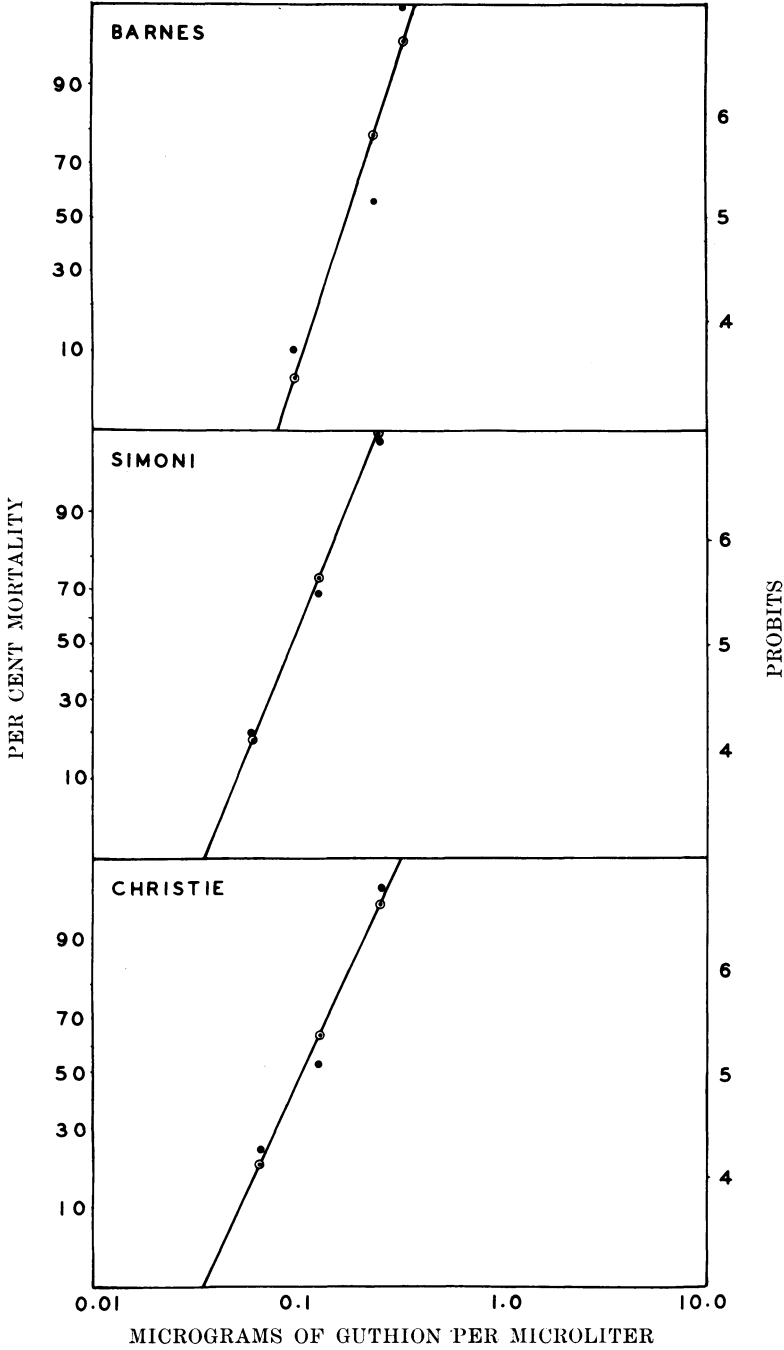


Fig. 10. Log dosage-probit lines for Barnes, Simoni and Christie strains of codling moth adults, *Carpocapsa pomonella* (L.), established in LD<sub>50</sub> (topical application) tests using Guthion. Each point represents about 125 treated adult moths plus 42 control moths.

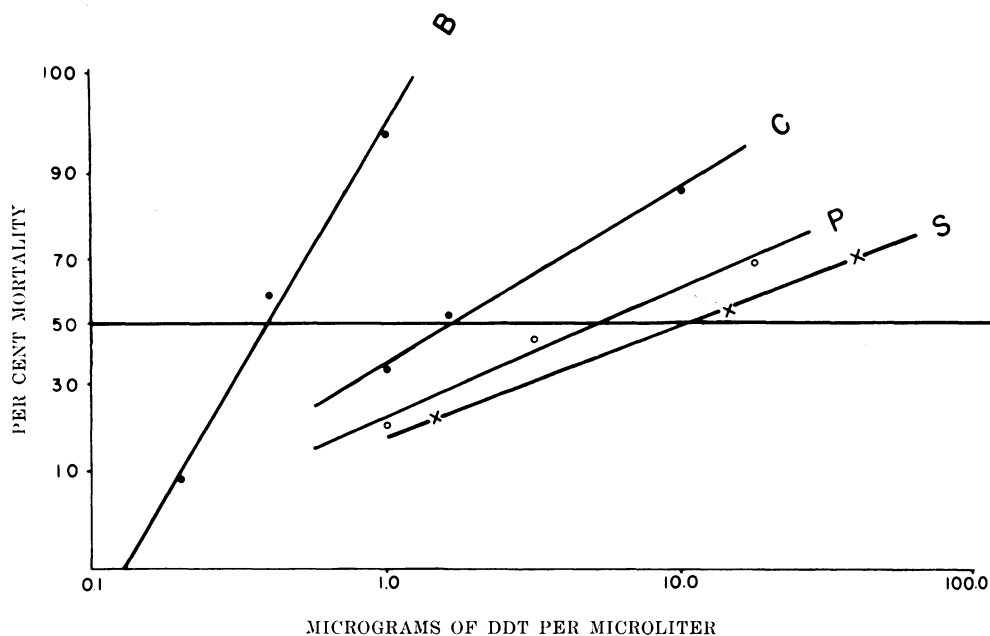
DDT SUSCEPTIBILITY OF CODLING MOTH ADULTS—LD<sub>50</sub> TESTS

Fig. 11. Log dosage-probit lines for four strains of codling moth adults, *Carpocapsa pomonella* (L.), showing the relative degree of resistance. B = Barnes, C = Christie, P = Pangborne, S = Simoni. These ld-p lines were established in LD<sub>50</sub> (topical application) tests using DDT.

standard deviations, or probits, the slope of the resulting ld-p line is a measure of the variability of the population as well as the variation in response to a toxicant (Hoskins, 1960). The slope is the coefficient,  $b$ , in the regression equation  $Y = b(X - \bar{x}) + a$ , used in statistical tests of bioassay procedures. As the value becomes greater the slope becomes steeper.

It will be noted from table 3 that the slope values from LC<sub>50</sub> tests using DDT for all four strains are not significantly different. Inspection of figure 7 graphically shows the similarity of these slopes. They indicate that the degree of variation within each strain was very similar, but this does not mean that the strains are genetically similar.

Analysis of the LD<sub>50</sub> slope values where DDT was used for all four strains (fig. 11) shows that there is considerable difference between the slope of the Barnes (Susceptible) strain and that of the Christie strain, which exhibited the

lowest degree of resistance to DDT of the three field strains. The difference between the Barnes strain and the Pangborne and Simoni strains is even greater. These values indicate that there is considerable difference in the genetic makeup of the Barnes strain as compared with the field strains.

The slopes of both the Barnes and Pangborne strains are steeper for Sevin than for DDT. Although the slope for the Barnes strain is steeper than that for the Pangborne strain, indicating a more uniform Barnes population, the difference is not great.

The same pattern is found in the LD<sub>50</sub> tests of Sevin for these two strains as was found in the LC<sub>50</sub> tests. In the LD<sub>50</sub> tests there is little variation in slope between the Barnes strain and the Pangborne strain (table 3 and fig. 8B).

In the Guthion tests the slopes for the LC<sub>50</sub> values in Barnes and Simoni strains are identical and differ only slightly from that of the Christie slope.



TABLE 3  
SLOPE VALUES FOR EACH 1d-p LINE RESULTING FROM THE  
REGRESSION EQUATION  $Y = b(X - \bar{x}) + a$  IN WHICH  
THE SLOPE IS THE COEFFICIENT  $b$

Strain	Slope values	
	LC <sub>50</sub> tests	LD <sub>50</sub> tests
	DDT	
Barnes.....	1.1	5.2      5.0
Christie.....	1.2	1.5
Pangborne.....	1.0	0.9
Simoni.....	1.1	0.9
	Sevin	
Barnes.....	2.1	4.8
Pangborne.....	1.5	4.1
	Guthion	
Barnes.....	1.4	6.0
Simoni.....	1.4	4.9
Christie.....	1.9	4.2

The slopes for the LD<sub>50</sub> values with Guthion for Barnes, Simoni and Christie strains are all steep, indicating that

even a slight change in dosage evokes a sizable change in response to this chemical when topically applied.

## DISCUSSION

Because the Barnes DDT (S) strain was available, basic 1d-p lines could be constructed before exposure to DDT became general throughout the entire codling moth population. These basic 1d-p lines indicated that resistance did exist in the three field strains, and it was possible to establish the degree of such resistance in terms of LC<sub>50</sub> and LD<sub>50</sub> values. The slope of these 1d-p lines also provided references for further comparison in this study.

The LC<sub>50</sub> and LD<sub>50</sub> values as well as slope information compared very favorably with data by Barnes (1958b, 1959). Standardized rearing procedures made it possible to move the Barnes strain from one laboratory to another without greatly affecting the 1d-p lines. Food, temperature, humidity, and light were held fairly constant. Variations in tech-

niques often make it difficult if not impossible to compare the results obtained here with those reported in other studies.

Reynolds (1959) points out several advantages of the topical technique used to obtain LD<sub>50</sub> values, such as low initial cost, use of small amounts of toxicant, rapidity of treatment, and consistent and reproducible results from laboratory to laboratory.

### Causes of Variation

Although every effort was made to rear the codling moths under optimum and uniform conditions, occasionally during the summer months temperatures rose considerably above the optimum. Humidity varied from day to day as indicated by a hygrothermograph, and efforts to moderate these variations

were made. However, it is generally conceded that humidity does not affect a population to the same extent as temperature. Water dishes were available to the moths and moths were observed using them extensively, especially on hot days. In the early stages of rearing the Barnes strain, there was a shortage of the apple thinnings used as larval food. This caused a reduction in the total number of individuals, but no other effect was noted. The results from tests with this strain were almost identical with those of Barnes (1958), which indicated that the population had not changed in any discernible manner.

In any attempt to determine susceptibility or resistance to insecticides, it is desirable to reduce variables so that only physiological resistance is measured. It is also preferable to test both the susceptible and suspected resistant strains at the same time so that factors affecting one strain will affect the other. In both types of bioassays conducted in the present investigation, every effort was made to follow these precepts.

Although standardized techniques were used, there are chances for errors which could contribute to variations in results. A bias selection of a sample of insects from laboratory-reared strains to be tested could result in the use of only the most active or hardiest individuals. The response which they exhibit to a chemical may be quite different from that shown by less active or weaker individuals. On the other hand, even weaker insects may survive longer in the laboratory. Brown (1950) pointed to this problem when he referred to inferior individuals in laboratory-reared populations which may not be eliminated under the less stringent environment. The data obtained from these experiments may give the false impression that the wild or field population is more resistant than it actually is.

The handling of delicate insects such as the first-instar codling moth larvae may cause inadvertent injury. Considerable practice was necessary before the

transfer of these tiny larvae could be made with little or no injury.

Although a single source and grade of chemicals was used throughout all of the tests, the possibility that the chemicals varied should be considered in evaluating the results. Precise measurement of insecticide concentrations is of the utmost importance. In any series of tests where various concentrations are used, this is likely to account for one of the most frequent sources of error. It is possible that many investigators have interpreted adventitious points on a graph as variability in the population when the cause was due to a mistaken concentration.

Solvents influence the spread and adhesive properties of an insecticide. In addition, some solvents exhibit certain degrees of toxicity in themselves. The rapidity with which a solvent volatilizes may also influence its effect on both the chemical and the insect. Acetone seems to be the most desirable solvent for laboratory work, and it was used in all of the topical application tests. There was no evidence that acetone was toxic to the moths when it was applied to the check groups.

One problem was encountered when high concentrations of DDT were topically applied to the Simoni strain of moths which exhibited the highest degree of resistance to DDT. As the concentration was raised above 10  $\mu\text{g}/\mu\text{l}$ , the pipette clogged because of the rapid volatilization of acetone at the tip. For heavier doses, it was necessary to apply 10  $\mu\text{g}/\mu\text{l}$  several times to the same area of the moths. When this was necessary, the check moths received an equal number of applications. No ill effects from the acetone could be observed.

Uneven deposits of insecticide on the surface of fruit in  $\text{LC}_{50}$  tests could leave areas without a toxicant. If a cage containing a larva was inadvertently placed on such an area, a false reading could be obtained. In the topical application tests, uneven deposits on the moths could result in a pile-up in one

area. This could reduce the amount of chemical absorbed and give data indicating a high degree of resistance.

Certain insecticides are more volatile than others and when insects are confined in small spaces, fumes may cause mortality. Preliminary tests conducted during this study indicated that fumigation was a factor when first-instar larvae were placed in the test cages. A modification of the individual metal cage covers from broadcloth to nylon-organdy reduced this effect. A standard exposure time was used for both the  $LC_{50}$  and  $LD_{50}$  tests to reduce variability.

Overcrowding in a cage may result in reduced contact with a sprayed surface or, in topical applications, may cause the moths to come in contact with the chemical on other moths more frequently. The twenty-five moths per holding cage in the topical tests did not seem to produce excess contact between moths.

Other factors such as treatment of moribund individuals, and proper reading of data can also influence results. Throughout the tests, efforts were made to reduce variability by considering all of the above items.

### Comparison of $LC_{50}$ and $LD_{50}$ Values

A review of the DDT  $LD_{50}$  values for all four of the codling moth strains shows a pattern similar to that of the  $LC_{50}$  values (tables 1 and 2). It is apparent that all of the  $LD_{50}$  values are higher than comparable  $LC_{50}$  values. It is also evident that the degree of resistance is greater in adults if the data for each strain are compared to the Barnes strain. For example, when  $LC_{50}$  and  $LD_{50}$  values for the Christie strain are examined, the data show that the Christie moths are three times as resistant as the Barnes moths while the larvae are only twice as resistant as the Barnes larvae. Inspection of the two values for the Pangborne strain shows that the

adult moths of this strain were 11 times as resistant as those of the Barnes strain, while the larvae were only three times as resistant.

A similar pattern of resistance in both the  $LC_{50}$  and  $LD_{50}$  tests indicates that they were consistent and sensitive enough to bring out the differences between the strains. Both show the same sequence or order of resistance regardless of degree. Since the  $LC_{50}$  values are consistently lower than the  $LD_{50}$  values one might interpret this to mean that the larvae require less toxicant than the adults to bring about mortality. They are also exposed to the toxicant both by contact and ingestion, which may cause a higher mortality with less actual chemical.

In the  $LD_{50}$  tests the chemical enters the moths through only one avenue, the integument, and thus the insects are influenced by contact action only. As mature adults, their integument may be less permeable. Since the moths possess scales which may act as mechanical barriers to the insecticide, more insecticide may be required to bring about a comparable degree of mortality. When the chemical dries and becomes powdery or crystalline on the surface of the scales, it is likely that some is brushed off when the moths are active.

Another explanation may be that the adults are more resistant than the larvae, possibly possessing a greater amount of a detoxifying enzyme, if such an enzyme exists in either the larvae or adults. There appears to be no great difference between the larval and adult response of the Barnes strain. However, in the DDT-resistant strains of moths, the Christie, Pangborne and Simoni strains show a two-, four- and almost threefold increase respectively. It should also be pointed out that the amount of chemical which the moths receive in topical applications is more precise than in the  $LC_{50}$  tests, for reasons previously mentioned. Because of this, the topical application tests should reflect the genetic make-up of each strain with



greater accuracy than do the  $LC_{50}$  tests.

Adult codling moth control is probably of greater significance than larval control in the field. Although it is the larvae that cause direct fruit injury, control of the adults would reduce the size of the population in succeeding generations since each mated female is capable of laying from 150 to 200 eggs.

The causes of the differences between resistant field strains as well as the differences in apparent degrees of resistance found in the field and the laboratory are open to speculation. Based on the field data of Madsen and Falcon (1960) and field observations of Bailey and Madsen during the 1959 season, one would have predicted that the Christie strain should have exhibited the highest degree of resistance in the laboratory. However this strain exhibited the lowest degree of resistance of the three field strains tested.

Field counts to determine the per cent of infested fruits show only whether the local population is or is not resistant and the relative number of individuals present. It makes little difference to the grower, from a control standpoint, whether the local population is twice as resistant or one hundred times as resistant as a susceptible strain. If they are resistant they will be able to penetrate the spray residue on the surface and infest the fruit. From here on, it is only a matter of how many fruits will be infested and this will depend on the size of the local population. Thus the per cent of fruits infested is a better indication of the size of a resistant population than the degree of resistance.

Environmental factors in the local area influence the size of the population. In the Christie orchard, conditions were very favorable for the codling moth. The orchard was in a protected location with very little wind. The bark of the tree trunks was soft and had deep crevices which provided ideal pupation and overwintering sites. This orchard was not under clean cultivation and thus considerable litter and debris were avail-

able for further pupation sites. In the Pangborne orchard, the bark of the trees was hard and relatively smooth with fewer good pupation and overwintering sites than in the Christie trees. This orchard was clean cultivated and provided relatively few areas for larval overwintering. The area also has a prevailing wind which may have influenced codling moth flight and oviposition.

It is possible that laboratory rearing may affect the resistance pattern. When organisms are reared under laboratory conditions, there is selection, and the possibility that resistance could either be increased or decreased. The three strains used in this study were reared for the following number of generations: Christie—21, Pangborne—13, and Simoni—8. The relative degrees of resistance were Christie, lowest; Pangborne, intermediate; and Simoni, highest. One could speculate that resistance decreases the longer a strain is reared in the laboratory. However, since no reference points were obtained on the field strains prior to rearing, this question can be answered only by further testing of future laboratory generations.

In addition, when the field strains were selected to bring into the laboratory, only a small segment of the population was involved. It is a matter of pure chance whether the sample was representative or one with a high or low degree of resistance.

Regardless of the considerations previously discussed, it is significant that the laboratory data supported the field conclusions that DDT resistance was present. This is of importance, since a change to a new chemical may create side effects that may not be desirable. If the laboratory results had shown no resistance, further investigation would have been necessary to determine why destructive populations were present. Since the field data can only indicate a problem of resistance, laboratory tests are necessary to determine the precise degree of resistance in both larvae and adults.

The reason for using the two insecticides Sevin and Guthion, was to determine whether cross-resistance to a carbamate compound or a phosphate compound was present in the DDT-resistant strains.

The Pangborne strain was selected for the Sevin tests even though there was no evidence of resistance to these compounds in the field experiments of Madsen and Falcon (1960). In the  $LC_{50}$  tests, both the DDT (S) strains and the Pangborne strain showed a similar response and no resistance to Sevin. The  $LC_{50}$  value for the Pangborne strain was almost the same as for the Barnes, and the  $LD_{50}$  values for both strains of moths were identical. In the topical application tests, the moths required a higher dosage of Sevin to reach 50 per cent mortality than the larvae, just as they did when DDT was used. However, the  $LD_{50}$  values were more than ten times greater than the  $LC_{50}$  values. It is interesting to note that the  $LD_{50}$  values for the Barnes moths with both DDT and Sevin are almost identical, which indicates almost equal susceptibility to both chemicals by the standard laboratory strain. The  $LC_{50}$  values for the larvae of both strains exposed to Sevin showed that the larvae were more than ten times as susceptible to this insecticide as to DDT. The data on the Pangborne strain show there was no cross-resistance to Sevin.

The Guthion tests indicated that the moths of both the Barnes and DDT-resistant strains were equally susceptible to this compound. There was no evidence of any cross-resistance in the field strains. The  $LD_{50}$  and  $LC_{50}$  values for Guthion on the Barnes strain indicate that the codling moths are more susceptible to this insecticide than to either DDT or Sevin.

The lack of cross-resistance temporarily is encouraging, as this provides substitute materials when DDT resistance is present. It is quite likely that codling moths resistant to Sevin and Guthion will be selected in time, but a

means of obtaining control for the present is at hand.

### Interpretation of Slopes

No genetic studies of the codling moth have been made to date, so no definite statements concerning their genetic makeup can be made. It can be stated, based on the slope data (table 3) for the  $LC_{50}$  tests in which DDT was used, that the genetic variability within each of these strains is quite similar (fig. 7). However, if one looks at the slope values for the  $LD_{50}$  tests (table 3) and at figure 11, variation is apparent. Since the topical tests are more reliable than the  $LC_{50}$  tests, they are probably better indicators of the makeup of each strain than the  $LC_{50}$  tests. The slope values taken from the  $LD_{50}$  tests indicate that the Barnes strain is very homogenous. The Christie strain exhibits some heterogeneity while the Pangborne and Simoni strains are very heterogeneous. These statements refer only to the variation within these strains. For the degree of resistance, one must refer to the  $LD_{50}$  values.

The slope values for Sevin in the Barnes and Pangborne strains (fig. 8) and the graphic illustrations of these slopes are somewhat different from those for DDT slopes. In both the  $LC_{50}$  and  $LD_{50}$  tests with Sevin, the slope values are higher or steeper than for tests in which DDT was used. The slopes of the  $LD_{50}$  tests were higher than for the  $LC_{50}$  tests, although even the  $LC_{50}$  slopes were fairly steep. This indicates less variation between strains for this chemical than for DDT. It will be noted that the  $LD_{50}$  slope for the Pangborne moths did not vary greatly from that for the Barnes moths in these tests. The significance of this probably lies in the fact that both of these strains show almost equal susceptibility to Sevin, and there are probably few if any genes in either of these strains for resistance to Sevin. This is further substantiated by the  $LC_{50}$  and  $LD_{50}$  values with Sevin.

As in the tests with DDT and Sevin,

the  $LD_{50}$  slope values for Guthion were higher than  $LC_{50}$  slope values for the reasons given previously. If the slopes of the  $LD_{50}$  tests for Sevin and Guthion are compared, one can see that they are all steep, in contrast to the variation which occurs within the slopes of  $LD_{50}$  DDT tests. One may interpret this contrast to mean that all four of the strains are relatively equal in variation to Sevin and Guthion, because there are no genes in any of these strains for resistance to either of these chemicals. It is quite obvious that the variation in the slopes of  $LD_{50}$  DDT tests indicates that resistant genes were present in the field strains when tested.

## Resistance

Resistance to insecticides has been variously defined by many individuals, but there are two definitions which seem to apply to this study. Hoskins and Gordon (1956) stated that "resistance" is the added ability to withstand an insecticide which is acquired by breeding from those individuals which survive exposure to that particular toxicant insufficient to wipe out the whole colony. Brown (1958) defined it as the term used in instances where insecticide dosages that were formerly effective, now meet with control failures.

The question of the origin of insecticide resistance always arises. For example, one might ask whether the codling moth in the three orchards from which the field strains were taken (Christie, Pangborne and Simoni) suddenly developed resistance because DDT acted as a mutagenic agent or because the factors that cause DDT resistance were already present and had only to be exposed through the use of this insecticide. There is strong evidence that the development of resistance is preadaptive rather than postadaptive.

Crow (1959) presents considerable support for this theory. It seems likely that the codling moths in each of these areas possessed genes for DDT resistance even though they may have been present only in very small numbers. Under the influence of chemical pressure from DDT which had been used for approximately 12 years, those codling moths in possession of genes for resistance were able to survive and pass these genes on to their progeny. Those codling moths without genes for resistance were reduced in number year after year until 1958, when economic control in the field was no longer possible with DDT. Attempts were made by the growers to control the codling moth by increasing the dosage of DDT to twice the normal amount and by reducing the time interval between applications, but economic control was still not attained.

The next question that arises is whether the Christie, Pangborne and Simoni strains can be called DDT-resistant. Based on the definition of Brown (1958), all three strains are resistant since none of the growers was able to control this pest any longer with DDT at dosages which were formerly effective. In the Christie orchard, even double the normal dosage did not provide control. Knippling (1950) discussed what he said constituted resistance. His statement was that "if the amount of insecticide for equivalent mortality is five times or more than that needed for a normal or standard strain, this is considered to constitute resistance in the house fly." Admitting that various standards are used for other insects, he stated that "a five- or tenfold increase in the  $LD_{50}$  of the resistant strain to that of the susceptible strain is enough to constitute failure of control in the field."



## SUMMARY

In 1958, several reports from fruit growers in Northern California were received concerning their inability to control codling moth in pear orchards with DDT. Replicated field plots were set up to determine whether DDT resistance existed or if such factors as poor timing, improper dosage, or inadequate coverage were responsible for lack of control.

Field samples were taken for rearing and testing in the Berkeley laboratory. Two of the field strains were taken from the Christie and Pangborne orchards in which DDT resistance by codling moths had been confirmed. A sample of a third field strain was taken from the Simoni orchard where the grower reported loss of codling moth control. In collecting the field strains, every effort was made to take good representative cross-sections of the local codling moth populations. Each of the field strains was mass-reared in the laboratory by a standard procedure, modified to suit local conditions.

A standard laboratory strain of DDT-susceptible codling moths was obtained from the Citrus Research Center at Riverside. The strain, known as the Barnes strain, was brought to the Berkeley laboratory and mass-reared, as were the field strains. The Barnes strain was used as a reference strain with which the three field strains were compared.

Two types of testing procedures were used in the laboratory to determine the degree of resistance to DDT exhibited by the larvae and adults of the codling moth. In addition, tests were run in which Sevin and Guthion were used to determine whether cross-resistance to either a carbamate or a phosphate insecticide existed.

The first type of test used is known as the  $LC_{50}$  test (lethal concentration—50 per cent mortality). In these tests,

apples were sprayed with various concentrations of the insecticides and first-instar codling moth larvae were caged on the surface. This procedure provided mortality data on the larvae which were used to establish ld-p (log dosage-probit) lines for each of the codling moth strains. From these regression lines,  $LC_{50}$  values for each of the strains were determined.

The second type of test used is known as the  $LD_{50}$  test (lethal dosage—50 per cent mortality). In these tests, adult codling moths were treated topically with various concentrations of the three insecticides. This procedure provided mortality data on the adult moths which were used to establish ld-p lines for each of the codling moth strains.

Over ten thousand codling moth larvae and adults were tested during the course of this investigation. Results show that the adults of each of the strains tested were generally more resistant to each of the chemicals used than were the larvae. Greater reliability was placed on the topical application tests than on the  $LC_{50}$  tests. Based on these data, the Christie strain was three times more resistant to DDT than the Barnes DDT (S) strain, while the Pangborne and Simoni strains were eleven and twenty-four times more resistant respectively than the Barnes DDT (S) strain.

The results also showed that there was no cross-resistance to either Sevin or Guthion by any of the strains of codling moth larvae or adults tested. All of the strains were highly susceptible to both Sevin and Guthion.

A comparison of the slope of each ld-p line for each test run indicated that the Barnes strain was very homogeneous. The Christie strain exhibited some heterogeneity while the Pangborne and Simoni strains were very heterogeneous.

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## LITERATURE CITED

- ABBOT, W. S.  
1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* **18**:265-67.
- ASQUITH, D.  
1959. Insects, eternal orchard problems. *Pennsylvania Fruit News* **38**(3):33-37.
- BARNES, M. M.  
1958a. Technique for testing insecticide deposits with newly hatched codling moth larvae. *J. Econ. Entomol.* **51**(4):547-49.  
1958b. A strain of codling moth in California resistant to DDT. *J. Econ. Entomol.* **51**(5):693-94.  
1959. Deciduous fruit insects and their control. *Ann. Rev. Entomol.* **4**:343-62.
- BROWN, A. W. A.  
1950. Annual report of Entomological Society of Ontario [Canada]. **81**:46-47.  
1958. Insecticide resistance in arthropods. World Health Organization. Monograph Ser. **38**, 240 p.  
1960. Mechanisms of resistance against insecticides. *Ann. Rev. Entomol.* **5**:301-26.
- CARMAN, G. E., and C. A. FLESCHER  
1944. Laboratory tests on the Oriental fruit moth with special reference to DDT. *J. Econ. Entomol.* **37**(1):122-23.
- CROW, JAMES F.  
1960. Genetics of insecticide resistance. *Entomol. Soc. Amer., Misc. Pub.* **2**(1):69-74.
- CUTRIGHT, C. R.  
1954. A codling moth population resistant to DDT. *J. Econ. Entomol.* **47**(1):189-90.
- DICKSON, R. C., M. M. BARNES, and C. L. TURZAN  
1952. Continuous rearing of the codling moth. *J. Econ. Entomol.* **45**(1):66-68.
- GLASS, E. H.  
1960. Current status of pesticide resistance in insects and mites attacking deciduous orchard crops. *Entomol. Soc. Amer., Misc. Pub.* **2**(1):17-25.
- GLASS, E. H., and B. FIORI  
1955. Codling moth resistance to DDT in New York. *J. Econ. Entomol.* **48**(5):598-99.
- HAMILTON, D. W.  
1956. Resistance of the codling moth to DDT sprays. *J. Econ. Entomol.* **49**(6):866-67.
- HOSKINS, W. M.  
1960. Use of the dosage mortality curve in quantitative estimation of insecticide resistance. *Entomol. Soc. Amer., Misc. Pub.* **2**(1):85-91.
- HOSKINS, W. M., and H. T. GORDON  
1956. Arthropod resistance to chemicals. *Ann. Rev. Entomol.* **1**:89-122.
- HOUGH, W. S.  
1928. Relative resistance to arsenical poisoning of two codling moth strains. *J. Econ. Entomol.* **21**(2):325-29.  
1934. Colorado and Virginia strains of codling moth in relation to their ability to enter sprayed and unsprayed apples. *J. Agr. Res.* **48**(6):533-53.
- KNIPLING, E. F.  
1950. Insecticidal resistant flies and mosquitos. *Soap and Sanitary Chemicals* **26**(6):87-88.
- MADSEN, HAROLD F.  
1956. Codling moth control on pears, 1954-55. *J. Econ. Entomol.* **49**(4):467-70.
- MADSEN, H. F., and LOUIS FALCON  
1960. DDT resistant codling moths on pears in California. *J. Econ. Entomol.* **53**(6):1083-85.
- MADSEN, H. F., and S. C. HOYT  
1958. Investigations with new insecticides for codling moth control. *J. Econ. Entomol.* **51**(4):422-24.
- MARSHALL, J.  
1959. Resistance to DDT in the codling moth in British Columbia. *Proc. Entomol. Soc. British Columbia* **56**:59-63.

MELANDER, A. L.

1914. Can insects become resistant to sprays? *J. Econ. Entomol.* 7:167-72.

MICHELbacher, A. E., and J. C. ORTEGA

1958. A technical study of insects and related pests attacking walnuts. California Agr. Ext. Sta. Bull. 764, 87 p.

MYBURGH, A. C.

1958. A decade of DDT on codling moth. *Deciduous Fruit Grower* 8(7):168-69. (South Africa)

REYNOLDS, H. T.

1960. Standardized laboratory detection methods for resistance determination in agricultural arthropod pests. *Entomol. Soc. Amer., Misc. Pub.* 2(1):103-111.

SMITH, L. C.

1955. DDT-resistant codling moth, a report on the 1954-55 control trials. *J. Dept. Agr. S. Australia* 59(1):12-15.

STEINER, L. F.

1939. The laboratory-field method for testing codling moth insecticides. *U. S. Bur. Entomol. and Plant Quarantine* E488:1-10.



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