HOUSE FLY RESISTANCE TO *Bacillus thuringiensis* Berliner, A MICROBIAL INSECTICIDE

By

TOMMY LARKIN HARVEY

Bachelor of Science
Kansas State University
Manhattan, Kansas
1950

Master of Science
Kansas State University
Manhattan, Kansas
1951

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Thesis Approved:

D E Stonewell
Thesis Adviser

J. E. Wheeler

Harvey L. Chada

R R Walton

Doreen Zuercher

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INTRODUCTION

Resistance or tolerance to disease in insects was probably first observed in the silkworm, Bombyx mori Linn., by Pasteur. By selecting individual silkworms immune to the microsporidian responsible for pebrine, Pasteur was able to rear a resistant strain which served to save the silk industry of France (Steinhaus, 1949). In spite of this early beginning, there are few cases in which differences in susceptibility to pathogens within an insect species have been proved. One well-authenticated case involves resistance in honeybees, Apis mellifera (Linn.), to Bacillus larvae White, the causative organism of American foulbrood. This was observed for many years (Park, 1937) before experimental evidence for differential resistance in genetically different lines of honeybees was obtained (Rothenbuhler and Thompson, 1956). Hall (1961) stated that there was no positive evidence that insects have become resistant to infestion from applied microorganisms or their byproducts. Resistance to virus infections in natural populations of insects was reviewed by Martignoni and Schmid (1961).

No instance of resistance to the commercially available microbial insecticide, Bacillus thuringiensis Berliner, has been reported. Interest in the commercial use of this pathogen for the control of insects has greatly increased since Steinhaus (1951) reported on its effectiveness against the alfalfa caterpillar, Colias eurytheme Boisduval. The widespread use of B. thuringiensis for control of

several insect pests would increase the pressures which may bring about resistance to this pathogen. Theoretically, there is little reason why resistance to B. thuringiensis should not develop, but it appeared to be of economic as well as academic interest to determine whether an insect could actually become resistant to this pathogen or its toxin following selection in the laboratory.

The house fly, Musca domestica Linn., has readily become resistant to chemical insecticides as a result of selection in both field and laboratory. The ease of rearing the house fly and its economic importance also contribute to its widespread use in laboratory studies involving resistance. Since house fly larvae are susceptible to B. thuringiensis (Hall and Arakawa, 1959), it appeared to be the most expedient insect to use in laboratory selection for resistance to this pathogen.

The primary objective of this study was to apply selection pressure from B. thuringiensis to house fly larvae and record changes in relative susceptibility of selected strains compared with that in normal or unselected parent strains.

Some secondary objectives were: to evaluate the stability of resistance to B. thuringiensis; to compare the effects of B. thuringiensis on various sizes of larvae; to test for cross resistance from B. thuringiensis in a "wild" strain compared with a laboratory strain; to determine the effect of the duration of the larval period on resistance; and to consider the effect of autoclaved B. thuringiensis on resistant and susceptible strains of house flies.

REVIEW OF LITERATURE

Insect resistance to B. thuringiensis apparently has not been previously studied, and there were few publications involving its use on house flies. The literature, which would have been included in this section, was cited in the introduction and discussion of results.

There were several excellent review articles involving literature to B. thuringiensis and insect resistance to insecticides which were useful in preparation of this thesis (Brown, 1958; Heimpel and Angus, 1960; Hall, 1961; Cameron, 1963).

MATERIALS AND METHODS

HOUSE FLIES. Selection for resistance to B. thuringiensis involved two strains, KUN an insecticide susceptible strain which had been maintained in the laboratory at Kansas State University for about 15 years and a "wild" strain established from 500 adults collected in a dairy barn in June, 1961.

Other laboratory strains tested but not selected for resistance to B. thuringiensis were CSMA (insecticide susceptible), DDT-45 (resistant to DDT) and Bethesda-45 (resistant to malathion), Orlando #1 (resistant to DDT), Grothe (resistant to malathion) and Cradson (resistant to DDT, malathion and others). The first three strains listed above were supplied by the U. S. Public Health Service, Dept. of Health, Education and Welfare, Communicable Disease Center, Savannah, Georgia. The last three strains listed above were supplied by the U. S. Department of Agriculture, Entomology Research Division, Orlando, Florida.

Bacillus thuringiensis. All spore powder used in this investigation was from a single batch obtained in May, 1960, from Rohm and Haas Co., Washington Square, Philadelphia 5, Pa. The spore material was designated Bakthane L-69, batch 9A, mix 16, and was rated at 75 billion spores per g by the supplier. The spores were held in the original plastic lined shipping carton at room temperatures during the 30-month period of this study.

EQUIPMENT. Adult flies were held in screened cages (about 1 ft^3) on powdered milk, sugar, and water. Oviposition sites were nylon covered

sponges or paper toweling moistened with dilute solutions of milk. Waxed paper food cartons of various sizes were used as larval rearing and testing units. During the last year of the study, all rearing and testing were done in 32 oz cheese cartons (height, 5½ inches, diameter top 4¼ inches, diameter bottom 3¼ inches). The medium was mixed by a motor driven propeller, in a steel container 1 ft high and 1 ft in diameter. Dry medium was separated from pupae by aspiration.

MEDIA PREPARATION. Ingredients for a 32 oz rearing unit were 200 g dry CSMA (Chemical Specialties Manufacturer's Association) rearing medium, 350 ml water, 3 g dry yeast, and 25 ml of one part dimalt syrup and three parts water. The moisture content of medium was increased in small testing units, which were 7 oz waxed paper cups, by using two parts water to one part CSMA by weight. Yeast, malt, water, and B. thuringiensis spore powder (in treated units) were thoroughly stirred into suspension before addition of dry CSMA. The CSMA was then mixed into the liquid with a motor-driven propeller. Ingredients for 1-4 rearing units (32 oz) were mixed at one time as a single batch.

Four generations (F15-F18) were reared in medium consisting of 200 g dry wheat bran, 40 g powdered milk, and 300 ml water. Milk, spores, and water were mixed prior to addition of bran. This medium was somewhat less satisfactory than the one previously described, especially from the standpoint of pupae removal.

STATISTICAL PROCEDURES. The objectives were to estimate the median lethal dose (LD_{50}) of B. thuringiensis, DDT and malathion for various strains of house flies; to fit regression lines to the dosage mortality data; and to set confidence limits on the LD_{50} values and on the slopes of the regression lines.

The house flies were exposed to a range of dosages of B. thuringiensis, the highest of which was expected to kill more than 50%, and the lowest concentrations less than 50% of the flies. From these data the dose which would have killed 50% was predicted.

The generally accepted method of analysis, and the one used in this study, is probit analysis (Finney, 1952). This procedure starts with a provisional regression line and goes through a series of iterations which fit new regression lines to the data, each representing closer approximations to the maximum likelihood solution. The iterative cycles cease when successive regression coefficients agree within a tolerance of 1/100th standard error. This method required two iterative cycles for each test involved in this study.

The necessary information for data preparation was the doses tested, number of flies tested at each dose, and number killed at each dose. Also, tables of Students' t distribution and Chi squared were used.

Data were punched on IBM cards and processed on an IBM 650 Digital Computer using a program prepared by Sokal (1958). The program provided LD_{50} figures and b (slope) values with confidence limits set at the 95% level of probability.

REARING OR SELECTION PROCEDURES. As selection progressed and resistance increased, dosages of B. thuringiensis were gradually increased in order to maintain selection pressure. The intensity of selection from the KUN strain during 50 generations ranged from near the LD_{50} to LD_{90}. Average numbers of pupae obtained under selection pressure per 0.1 ml of eggs in generations 1-19 were 176; in generations 20-30 were 53; and in generations 31 to 50 were 112. Without exposure to B. thuringiensis an

average of 417 pupae per 0.1 ml eggs was obtained.

Eggs were washed from oviposition pads or paper into 10 ml centrifuge tubes (calibrated to 0.1 ml) in which they were measured prior to being added to medium. The standard amount of eggs per 32 oz unit was 0.3 ml without B. thuringiensis and 0.5 ml in units treated with B. thuringiensis. Although the amounts of eggs used varied somewhat according to availability, it was not usually satisfactory to use larger quantities of eggs in units heavily treated with B. thuringiensis.

Eggs were suspended in 5 ml of water and poured on the surface of medium. Each container was covered with a fine mesh nylon cloth to prevent insertion of eggs by escaped flies. The units were held at 75-80 F until inspection showed that pupation was nearly complete (about 7 days). Pupae were separated from the medium by screening and aspiration and they were counted and held in screened cages for adult emergence.

About 20 rearing units were maintained in each generation at 2-3 dosage levels of B. thuringiensis; pupae obtained were maintained separately and those most stringently selected, in so far as sufficient numbers were available, were used to produce the subsequent generation. Controls of KUN were also reared continuously without selection for resistance to B. thuringiensis. The strains being maintained under selection pressure were also reared in untreated medium in each generation to insure against the possibility of losing the strain from overdose of B. thuringiensis.

Insufficient numbers of pupae were obtained from treated medium in generations 12 and 33 to produce the next generation. Flies obtained from the selected strain reared in untreated medium were used to continue

the selection process. Since there was no selection in the 12th and 33rd generations, for numbering purposes these generations were omitted and the following generations designated F12 and F33. This accounts for the fact that a substrain initiated at F30 without selection from B. thuringiensis gained a generation on the selected strain. For example, F30-11 was tested simultaneously with F40 (Figure 7). However, F30-17 was held back one generation so that in Figure 9 F50 and F30-20 are in agreement with respect to total numbers of generations selected.

Selection in the "wild" strain, which was collected at a dairy barn, proceeded for ten generations as described for the KUN strain. However, the controls or unselected flies were split into two strains by selection of early and late pupaters. These strains were discarded after the 10th generation of selection when their response to B. thuringiensis was determined.

TESTING PROCEDURES. The larval medium for dosage mortality testing was prepared as previously described. B. thuringiensis spore powder was weighed in mg amounts and mixed into medium to provide 6-9 dosage levels. Dosages were reported in ppm based on total weight of medium constituents. Eggs or larvae were introduced into the medium immediately after preparation.

One procedure was to transfer 100 eggs to discs (2 inches in diameter) of moistened blotter paper and place one disc egg side down to a depth of 1 inch in medium held in a 7 oz wax paper cup. A pupation site was provided by adding dry CSMA to medium surface four days after infestation. Four replications at each dose for each strain was standard. Another method involved pouring 0.3 ml of eggs into medium held in a 32 oz cheese

carton. The dosages were not replicated. This method required less labor and provided larger numbers of flies; however, it introduced an additional variable in that the numbers of eggs were estimated rather than counted.

All tests initiated with larvae were conducted in 7 oz cups. Fifty larvae were transferred from standard rearing units to each 7 oz test unit. Otherwise the same methods were used as previously described for tests initiated with 100 eggs. Although use of larvae had the advantage of eliminating variability in egg hatch, it required more labor and introduced a variable of larvae size or age.

Mortality generally was based on numbers of pupae obtained from test units; however, in some tests pupae were held for emergence and results were also based on numbers of adults. The numbers of larvae assumed to be treated in each unit were based on the numbers of pupae or adults obtained from untreated controls for each strain. Unless otherwise indicated, results were based on numbers of pupae in tests initiated with eggs.

In testing adult flies of KUN and F26 for resistance to DDT and malathion, the insecticides were dissolved in acetone and topically applied to individual flies by a micrometer driven syringe. Fifty to 75 females of each strain were treated at each of five dosage levels with each insecticide. Controls at the beginning and end of each series were treated with acetone. Mortality was recorded after 24 hours. Dosages were recorded in ug/mg based on average weights of adult females randomly selected from the groups tested. Average weights in mg per fly in the DDT test were KUN-16.7, F26-13.9 and in the malathion test KUN-16.3, F26-14.9.

RESULTS

DEVELOPMENT OF RESISTANCE IN KUN STRAIN. The resistance ratios based on the LD_{50} values of KUN and the strain selected from KUN for resistance to B. thuringiensis for 50 generations are presented in Figure 1. Results are limited to tests initiated with eggs and are based on pupation rather than adult emergence. Points prior to the 20th generation were estimated from observed differences in selection medium and preliminary tests based on an insufficient number of dosages to compute a regression line. From the 20th to 50th generations the selected strain consistently had significantly (.05 level) higher LD_{50} levels than the unselected strain. The largest gain in resistance appeared between the 20th and 30th generations, and little or no increase was registered in the last 15 or 20 generations. The highest ratio recorded was 14.3 in the 27th generation, but the average ratio from generations 30 to 50 was 11.5.

Dosage mortality lines for B. thuringiensis against KUN and selected (F20, F22, F27, F28, F33, F36, F40, F41, F50) strains of house flies are illustrated in Figures 2-9, inclusive. In general, B. thuringiensis produced a positive linear relationship between the probit-per cent mortality and log-spore concentration. The 95% confidence limits on the LD_{50} values for selected and unselected strains did not overlap; however, significant differences in the slopes of the regression lines were seldom demonstrated.

The following comments are applicable to the individual tests illustrated:

F20 and F22 (Figure 2). The 95% confidence limits on the LD_{50} values were extremely wide for the selected strains (F20 and F22), but they did not overlap the interval on the KUN strain. These were the earliest generations (based on tests initiated with eggs) in which the selected strain was significantly more resistant than the unselected strain. However, since a direct comparison of KUN with F20 and F22 was not available, the curve presented for KUN is an average for eight subsequent tests. The slopes for both F20 and F22 appeared flatter than the slope for the susceptible strain but the differences were not significant.

F27 (Figure 3). This test was atypical, especially for the resistant strain, in that it was the only one in which the data were homogenous. It was also the only test in which a significant difference between the slopes of KUN and the resistant strain was demonstrated. The b values and 95% confidence limits for KUN and F27 were 1.64 (0.77 to 2.51) and 5.22 (4.75 to 5.70), respectively. The confidence limits on the LD_{50}s did not approach the point of overlap and the interval on F27 was extremely short. The relatively high resistance ratio of 14.3 (Figure 1) is largely attributable to the lower than average LD_{50} for KUN (24.5).

F28 (Figure 4). The dosage mortality lines for KUN and F28 approximate those of their counterparts in Figure 3. The LD_{50} levels were higher for KUN (34.9) and lower for F28 (270.9) than in the previous test and this provided a decline to 7.8 in the resistance ratio (Figure 1). The slope for F28 appeared steeper than the KUN slope but the difference was not significant at the .05 level.

F33 (Figure 5). The LD_{50} for F33 and KUN are both lower than in the previous two tests (Figures 3 and 4); however, the resistance ratio of 10.5 (Figure 1) was similar to that obtained for F27 and F28. The slope of F33 (3.16) exceeded KUN (2.30) but the differences did not approach significance. The curve for F30-5 will be discussed in the next section.

F36 (Figure 6). The LD_{50} of 350.7 for F36 and 30.8 for KUN gave a resistance ratio of 11.4 (Figure 1). The confidence interval was unusually wide on the high side of F36 and its b value appeared lower than in the previous 3 tests.

F40 (Figure 7). The resistance ratio of about ten, indicated no increase in resistance between F40 and F27. The slope for KUN was unusually steep and this was the only test in which the b value of KUN exceeded b of the resistant strain. The curve for F30-11 will be discussed in the next section.

F41 (Figure 8). The LD_{50} and slope for F41 were similar to those of F27 (Figure 3). KUN was not compared directly with F41, and data presented for KUN are averages of eight previous tests as presented in Figure 2. The LD_{50} resistance ratio was 10.7 as indicated in Figure 1. The curve for F30-12 will be discussed in the next section.

F46 (Figure 20). These data will be discussed later in connection with a test involving autoclaved spore powder. However, the results presented in Figure 20 for unheated spore powder indicated a resistance ratio of 13.8. This value was plotted in Figure 1.

F50 (Figure 9). The LD_{50} levels were higher for both KUN (40) and F50 (519) than those recorded in any previous test. The LD_{50} resistance ratio of 13.0 was comparable to that obtained for F27 (Figure 3) and for

F46 (Figure 20). The slope for F50 (5.6) was, as usual for the selected strain, insignificantly steeper than the slope for KUN (3.3). The curve for F30-20 will be discussed in the next section.

STABILITY OF RESISTANCE AFTER REMOVAL FROM SELECTION PRESSURE. After the KUN strain was subjected to selection pressure from B. thuringiensis for 30 generations, a substrain was reared in the absence of B. thuringiensis for 20 generations. Dosage mortality lines were established for the substrain after removal from selection pressure for 5, 11, 12, and 20 generations. These data are presented in Figures 5, 7, 8, and 9 along with curves for KUN and the strain which was maintained under selection pressure.

The LD_{50} levels of F30-5, F30-11, F30-12 and F30-20 were all lower than F33, F40, F41, and F50, respectively. The differences were significant or approached significance in all tests. Since there was little evidence that the selected strain increased appreciably in resistance during this period, it may be valid to assume a loss in resistance for the substrain removed from selection pressure. However, the probable decline in resistance was slight, and this substrain did not revert to its original susceptibility. Differences in slopes of the regression lines were not significant except for F30-20, but those of the substrain more nearly paralled the slopes of the selected strain than those of KUN.

ADULT EMERGENCE IN TESTS INITIATED WITH EGGS. All data previously presented are based on numbers of pupae obtained in tests initiated with eggs. Preliminary data indicated that adult emergence was not drastically affected by B. thuringiensis under these conditions. Although there were no significant differences in dosage mortality lines based on pupae and adults, there were tendencies for the LD_{50} values to be lower and the slopes steeper for adults than for pupae. This tendency is indicated by curves for KUN and F28 for adults and pupae shown in Figure 10. It appears

that adult emergence is reduced slightly by B. thuringiensis and that the reduction is most evident at the higher dosages. The relative decline in emergence may be slightly greater for KUN than for F28. The resistance ratio for KUN:F28 was 7.8 based on pupae and 8.5 for adults.

PUPATION AND ADULT EMERGENCE IN TESTS INITIATED WITH LARVAE. Dosage mortality curves based on pupation for KUN and F15 when tests were initiated with two-day-old larvae, are illustrated in Figure 11. The LD_{50} and slope were almost identical for both strains (KUN and F15) when curves were plotted for pupae. Also the LD_{50} levels of about 2,000 ppm of B. thuringiensis were many times higher than LD_{50} levels established in tests initiated with eggs. The range of dosages used in this test was 625 to 5,000 ppm, and adult emergence occurred only at the lowest dose. Adults emerged from 3% of KUN pupae and from 12% of F15 pupae at 625 ppm.

Dosage mortality curves based on adult emergence for KUN and F17, in tests initiated with two-day-old larvae, are presented in Figure 12. The LD_{50} for the selected strain was significantly higher and the slope significantly steeper than the LD_{50} and slope for KUN. Although the resistance ratio of 6.5 was somewhat larger than for F20 and F22 (Figure 1), the values generally were comparable to those of similar tests initiated with eggs. The range of dosages used was 63 to 375 ppm, and pupation was reduced only slightly at the highest dose.

EFFECT OF AGE OR SIZE OF LARVAE ON RESISTANCE. Dosage mortality lines established from tests initiated with 1-, 2-, 3-, and 4-day-old larvae are shown in Figure 13. The curves are all based on pupation. Emergence of adults was negligible except for 1-day-old larvae. The

selected or resistant (F15) strain was used in all four age groups; however, similar results could be expected for KUN (Figure 11) except possibly for the one-day-old larvae. The LD_{50} values increased from about four to six fold with each additional day that the larvae were reared in the absence of B. thuringiensis. The LD_{50} values and confidence limits were as follows: one-day-old larvae - 242 (196 to 287), two-day-old larvae - 1,589 (825 to 2,631), three-day-old larvae - 6,682 (5,931 to 7,571), four-day-Old larvae - 35,728 (26,372 to 58,623). The slopes ranged from 1.8 to 2.6 but did not differ significantly among the age groups.

CROSS RESISTANCE TO B. thuringiensis OF HOUSE FLIES RESISTANT TO DDT AND MALATHION. Dosage mortality lines for two insecticide susceptible (KUN and CSMA) and two insecticide resistant (DDT-45 and Bethesda-45) laboratory strains of house flies subjected to B. thuringiensis in tests initiated with eggs are illustrated in Figure 14. There were no significant differences in LD_{50} values or slopes among the strains. Since both the highest (CSMA - 34.8) and lowest (KUN - 20.6) LD_{50} figures were recorded for insecticide susceptible strains, there was no evidence for cross resistance in this test. Although regression lines were not obtained for Orlando #1, Cradson and Grothe, in preliminary tests, these insecticide resistant strains appeared comparable in B. thuringiensis susceptibility to those strains shown in Figure 14.

CROSS RESISTANCE TO DDT AND MALATHION OF HOUSEFLIES RESISTANT TO B. thuringiensis. Dosage mortality lines for KUN and F26 adults subjected to topical applications of malathion are illustrated in Figure 15. The confidence limits on both LD_{50} figures and slopes overlapped.

Dosage mortality lines for KUN and F26 adults subjected to topical applications of DDT are shown in Figure 16. The LD_{50} levels and confidence

limits in ug DDT/mg of female house fly were .095 (.044 to .128) for KUN and .021 (.015 to .026) for F26. This indicates a 4.5 fold increase in susceptibility to DDT or possibly loss in vigor for the strain selected on B. thuringiensis for 26 generations.

Regression lines were not established for DDT or malathion applied to the larval medium of KUN or the strain selected for resistance to B. thuringiensis; however, observations on single-dose tests indicated no differences in resistance among strains.

DEVELOPMENT OF RESISTANCE IN A WILD STRAIN AND EFFECT OF THE DURATION OF THE LARVAL PERIOD ON RESISTANCE. A strain established from 500 adults collected at a dairy barn was held under selection pressure from B. thuringiensis for ten generations. Two control strains (not selected for resistance) were established from selection based on long and short larval periods. After ten generations of selection, the strain designated "early" had an average larval period of 5 days; whereas, the other control strain (late) had an average larval period of 8 days (Figure 17). The strain selected on B. thuringiensis (F10) and simultaneously selected for early pupation had a larval period of 6 days.

Results of a dosage mortality test initiated with eggs involving the 3 strains (early, late, and F10) are presented in Figures 18 and 19. Chi square tests indicated significant differences between F10 and either control strain at all dosages tested except 9 ppm and a significant difference between early and late pupaters at 34 ppm (Figure 18). However, results based on probit analysis (Figure 19) indicated that LD_{50} levels and slopes for the three strains were not significantly different. The resistance ratio of F10 to the control strains was 1.7.

EFFECT OF HEAT ON TOXICITY OF B. thuringiensis. Spore powder which had been autoclaved at 15 psi for 30 minutes was compared in dosage mortality tests with unheated B. thuringiensis for effectiveness against resistant (F46) and susceptible (KUN) larvae. Results presented in Figure 20 indicate that B. thuringiensis toxicity was decreased to about the same degree for resistant and susceptible strains. The resistance ratio was 13.8 for unheated B. thuringiensis and 13.2 for autoclaved B. thuringiensis. The slopes of the regression lines for both strains appeared about equal for unheated and autoclaved spore powder.

MISCELLANEOUS OBSERVATIONS. The events described in this section were noted during the period of this study, but they were not verified by statistical analysis of data.

The house fly strain selected for resistance for 50 generations appeared to decline in egg production, and it did not survive as well in untreated medium as the unselected strain. Since observations on the selected strain were mostly based on individuals which had survived B. thuringiensis treatment, it is uncertain whether the differences noted were due to selection of a less vigorous strain or to direct injury resulting from exposure to treatment.

The selected and KUN strains did not appear to differ significantly in survival when larvae and adults were held in the absence of food and water.

In support of the observations of Dunn (1960) and Briggs (1960), adults incapable of expanding their wings and abnormally formed pupae appeared more common in treated than untreated flies. It was observed that individuals with abnormal wings produced normal F_1 progenies.

Partially emerged adults, which were unable to become free of the pupal case, appeared more frequent among flies exposed to B. thuringiensis than untreated flies.

Eggs, larvae, and pupae exposed to concentrated suspensions and pastes prepared from water and spore powder continued with normal development.

DISCUSSION

Resistance to B. thuringiensis developed slowly, at least in the house fly strains involved in this study, and it did not attain the high levels frequently associated with resistance to chemical insecticides. On the other hand, resistance to chlorinated hydrocarbon insecticides has characteristically developed slowly or not at all for several generations prior to abrupt increases ending in high-resistance levels. For example, Decker and Bruce (1952) obtained less than a 10-fold increase in resistance from chlordane pressure for 30 generations; but, after 40 generations, resistance rose to a level 1,500 times normal. This may indicate the need for additional generations of selection for resistance to B. thuringiensis; however, high levels of resistance to chemicals have not always been induced by selection. March and Metcalf (1952), obtained about a 7-fold increase in resistance to parathion after selection with that insecticide for 60 generations.

The fact that there was little or no increase in resistance to B. thuringiensis during the last 20 generations of selection, and the apparent steepness of the slopes of the regression lines for the selected strain compared with the unselected control may indicate that resistance had approached its maximum for this particular strain.

In comparing the nearly 10-fold resistance to B. thuringiensis with the extremely high values of several thousand times normal as reported for some chemical insecticides, it should be recognized that dosing of

house flies with B. thuringiensis is limited to the larval stage, whereas the preponderance of work with resistance to chemicals in house flies involved adults. Usually selection of larvae for resistance to chemicals has conferred resistance on the adults to a greater degree than when selection is limited to adults (Bruce and Decker, 1950). Also, larvae are normally more resistant than adults to topically applied DDT (Sternburg and Kearns, 1950). However, evidence that larvae of resistant strains will tolerate thousands of times the normal LD_{50} of chemical insecticide in the medium has not been found. March and Metcalf (1950) worked with larvae, initially unable to survive in media containing 1 ppm of parathion. After being bred for 11 generations in medium containing increasing amounts of parathion, the survivors were able to grow normally in 80 ppm. On the other hand, Mer and Civilich (1956) selected four generations with 0.5 ppm of diazinon in larval medium and then increased the dose to 1 ppm for 21 generations. This selected strain did not become sufficiently resistant to survive in media treated with 2 ppm of diazinon.

The resistance developed to B. thuringiensis in 30 generations of selection remained fairly stable during 20 generations reared without selection pressure; however, there was an indication that the resistance level declined slightly. With additional rearing free of B. thuringiensis the strain may revert to normal susceptibility. A house fly strain maintained its resistance to DDT for 35 generations (Metcalf, 1955) but eventually reverted to normal susceptibility (Sokal and Hunter, 1955).

Age or size of insect larvae may be an important factor in susceptibility to B. thuringiensis. Large larvae of the alfalfa caterpillar suffered a higher per cent mortality than did smaller larvae in field

tests (Stern et al., 1959). On the other hand, laboratory tests with European corn borer, Ostrinia nubilalis (Hbn.), suggest that susceptibility decreases as size or age increases (McConnell and Cutkomp, 1954). Briggs (1960) noted greater survival based on emergence of adult house flies in tests initiated with first instar larvae than in tests initiated with eggs. Dunn (1960) reported high mortality in pupal stage in tests initiated with 2-day-old house fly larvae. In the present study, as larval age or size increased, much higher dosages were required to prevent pupation; however, age or size appeared less important when results were based on adults. Evidently, most larvae sufficiently resistant to survive B. thuringiensis during their entire larval period are also likely to survive pupation; whereas, susceptible individuals first exposed to B. thuringiensis as late instar larvae may not succumb until they reach the pupal stage. Bioassays with late instar larvae could grossly underestimate the practical potency of B. thuringiensis for house fly control if results were based on pupation rather than emergence. Variations in size or age of larvae also may be more critical if results are based on pupae rather than adults.

In studies of chemical insecticides, cross-resistance has received much attention, and it was probably most common among compounds closely related in chemical structure. Cross-resistance to chemical insecticides (DDT and malathion) of house flies resistant to B. thuringiensis and vice versa was not evident with the strains compared in this study, which may indicate that the nature of the B. thuringiensis toxin and its mode of action are unlike that of DDT or malathion. However, among relatively unrelated compounds, cross-resistance to DDT of house flies resistant to parathion was well known, (March, Lewallen and Metcalf, 1956). Further evaluation of the apparent increase in susceptibility to DDT of the

B. thuringiensis resistant strain (F26) should be considered before possible causes, such as negatively-correlated cross-resistance, are discussed.

The tests for cross-resistance provide evidence that the B. thuringiensis spore powder used in this investigation was not contaminated with chemical insecticides. Borgatti and Guyer (1962) reported an instance in which a batch of B. thuringiensis was adulterated with DDT.

Different strains of house flies have varied in the degree of resistance induced by chemical insecticides. This indicates a need for selection of additional strains for resistance to B. thuringiensis in order to determine, more adequately, the resistance capacity of house flies. The possibility of a "wild" strain being more heterogeneous and thus more likely to become resistant than a laboratory-reared strain was considered in this study. Although the two strains probably did not differ greatly in resistance to B. thuringiensis after ten generations of selection, this may be an insufficient number of generations to demonstrate a strain's resistance potential.

In testing early and late pupaters for resistance to B. thuringiensis, strains having larval periods of 5 and 8 days, appeared equally susceptible. Apparently the three extra days of exposure to B. thuringiensis in the medium experienced by the late pupating strain was not an impediment to its survival. When McKenzie and Hoskins (1954) selected late pupaters of a susceptible strain they obtained an increase in DDT-tolerance of adults.

Some instances of low-level resistance have been attributed to a general increase in vigor resulting from selection rather than from any

specific defense mechanism (Hoskins and Gordon, 1956). It seems unlikely that the resistance reported here to B. thuringiensis is of the "vigor tolerance" type. The resistant strain did not exhibit "vigor tolerance" to DDT or malathion; it did not appear to survive longer than the unselected strain in the absence of food and water; and it did not survive as well as the unselected strain in untreated medium. The degree of resistance also exceeded that which generally has been attributed to "vigor-tolerance". Hoskins and Gordon (1956) showed that when specific resistance is involved, the regression line becomes flatter as the LD_{50} increases and becomes steeper again as the population reaches homogeneity for resistance. Statistically significant differences among slopes of lines in this study were seldom demonstrated, but there was a tendency for the slopes to become flatter (Figure 2) and then steeper (Figure 3).

It has been shown that spore counts may not provide a reliable index for the insecticidal activity of B. thuringiensis (Angus, 1954; Hall and Arakawa, 1959; Menn, 1960; McEwen et al. 1960). The protein crystals formed by B. thuringiensis were believed to play a vital role in toxicity (Hannay and Fitz-James, 1955); however, Heimpel and Angus (1959) reported an example of an insect which was not killed by the crystals in the absence of spores. Mortality in larvae and pupae was rarely attributable to septicemia (Hall and Arakawa, 1959; Dunn, 1960; Briggs, 1960); therefore, a vital role for the spores in toxicity to house flies seems doubtful. Since the protein crystals are insoluble in water, their toxicity was open to question when Briggs (1960) found a soluble fraction obtained from whole cultures which was toxic to larvae.

The crystals are heat labile (Angus, 1954) and since in this study autoclaving appeared to reduce but not destroy B. thuringiensis toxicity,

this may further indicate that something other than the crystals are involved. Another heat labile factor named lecithinase also may be involved in toxicity to insects (Heimpel, 1955). Since autoclaved B. thuringiensis retained much of its toxicity to house flies, some relatively heat stable factor may remain undiscovered. Perhaps the loss in toxicity due to autoclaving was indicative of the part played by the heat labile crystals or lecithinase in destroying house flies. On the other hand, possibly these factors are only partially heat labile with respect to toxicity to house flies. Chu (1949) found lecithinase of Bacillus cereus Fr. and Fr. retained 32% of its activity after standing in boiling water 10 minutes.

McConnel and Richards (1959) isolated a toxic fraction from B. thuringiensis which was heat-stable and water-soluble, but this substance was toxic to insects only when injected and its low potency probably excluded it from this study.

The possible use of B. thuringiensis as an animal feed additive for control of fly larvae in feces (Dunn, 1960; Briggs, 1960; Harvey and Brethour, 1960; Sherman and Ross, 1961; Burns et al., 1961) and its non-toxicity to vertebrates (Steinhaus, 1957 and 1959, Fisher and Rosner, 1959) indicate some potential in fly control, particularly if varieties of B. thuringiensis more effective for fly larvae are developed.

Studies pertaining to the use of B. thuringiensis for fly control should be encouraged by the fact that resistance developed slowly and to a lesser degree than would have been expected for many chemical insecticides under comparable conditions.

SUMMARY AND CONCLUSIONS

Resistance to Bacillus thuringiensis Berliner was induced in house flies (Musca domestica Linnaeus) by selection of survivors from treated larval medium during 50 generations of rearing. The degree of resistance, based on the ratios of LD_{50} values, varied from 8 to 14-fold between the 27th and 50th generations, inclusive. Prior to the 24th generation of selection the level of resistance was less than 4-fold. Resistance developed during 30 generations of selection probably declined slightly during 20 generations without selection pressure. A wild strain subjected to ten generations of selection did not become more resistant than a laboratory strain with comparable selection.

Resistance to B. thuringiensis did not induce cross-resistance of house flies to DDT or malathion or vice versa. Early and late pupating strains of house flies, with larval periods of 5 and 8 days, respectively, were equally susceptible. Spore powder autoclaved at 15 psi for 30 minutes produced LD_{50} values in both resistant and susceptible strains of house flies which were higher than comparable values for unheated material.

Mortality occurred primarily in the larval stage when tests were initiated with eggs, and it occurred mainly in the pupal stage when tests were initiated with 2-day-old larvae. Differences between resistant and susceptible strains were not evident based on pupation in tests initiated with 2-day-old larvae.

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APPENDIX

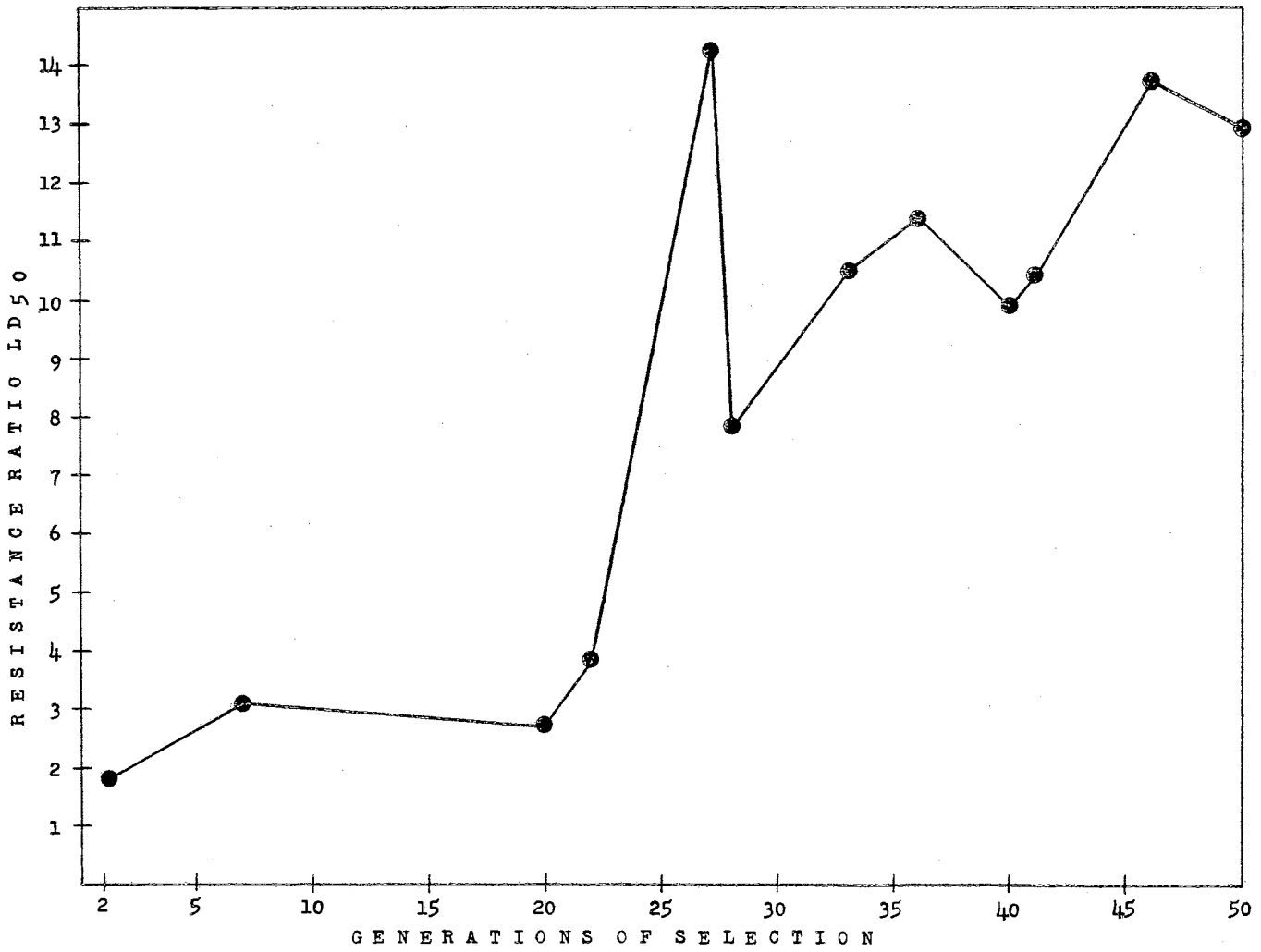


Fig. 1.--Increase in resistance of the KUN strain of house flies during 50 generations of selection from Bacillus thuringiensis. Resistance ratios based on comparisons of LD_{50} figures with normal.

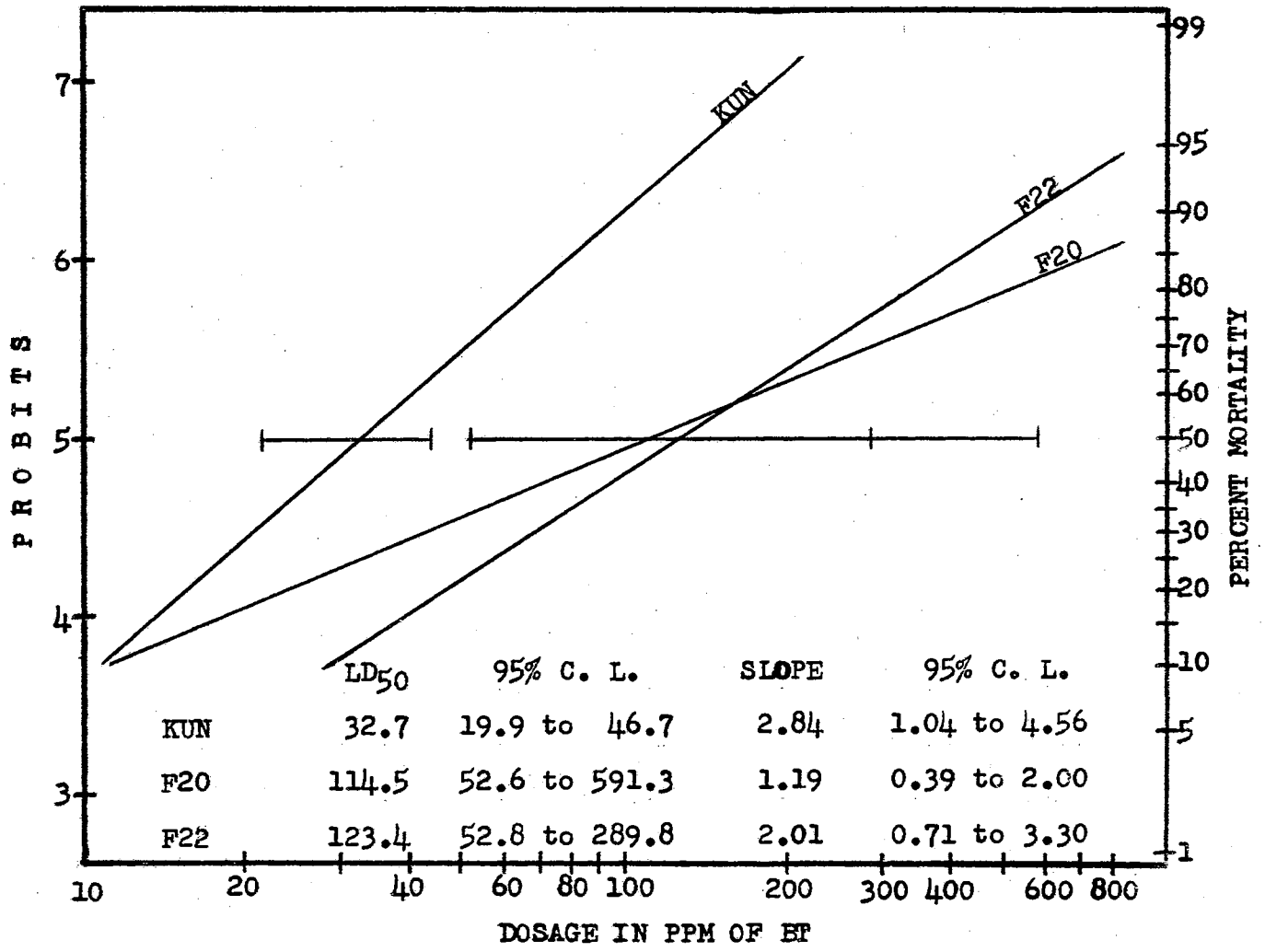


Fig. 2.--The regression of Bacillus thuringiensis concentration on per cent mortality for selected (20 & 22 generations) and unselected strains of house flies. Tests initiated with eggs and mortality based on pupation.

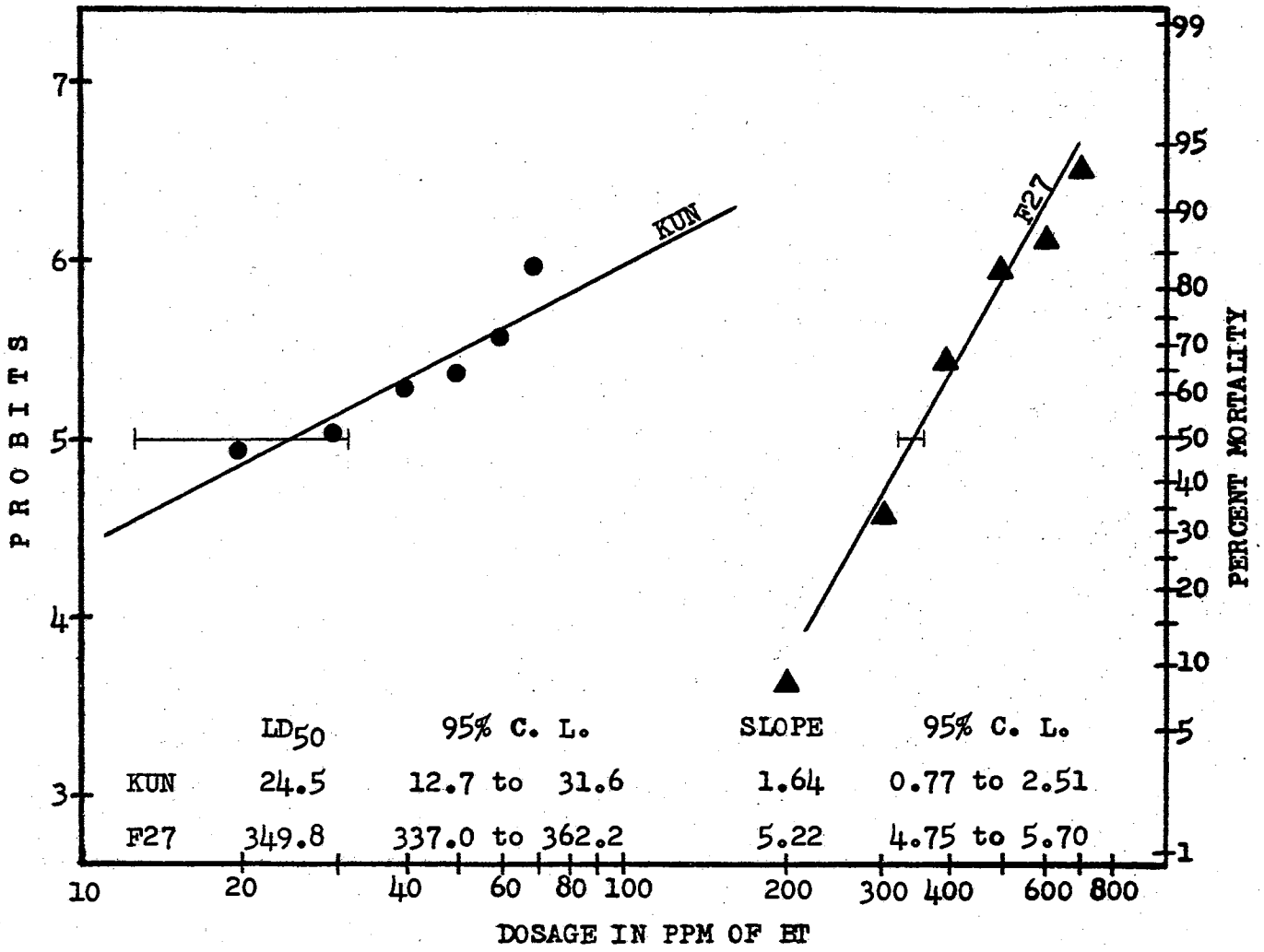


Fig. 3.--The regression of Bacillus thuringiensis concentration on per cent mortality for selected (27 generations) and unselected strains of house flies. Test initiated with eggs and mortality based on pupation.

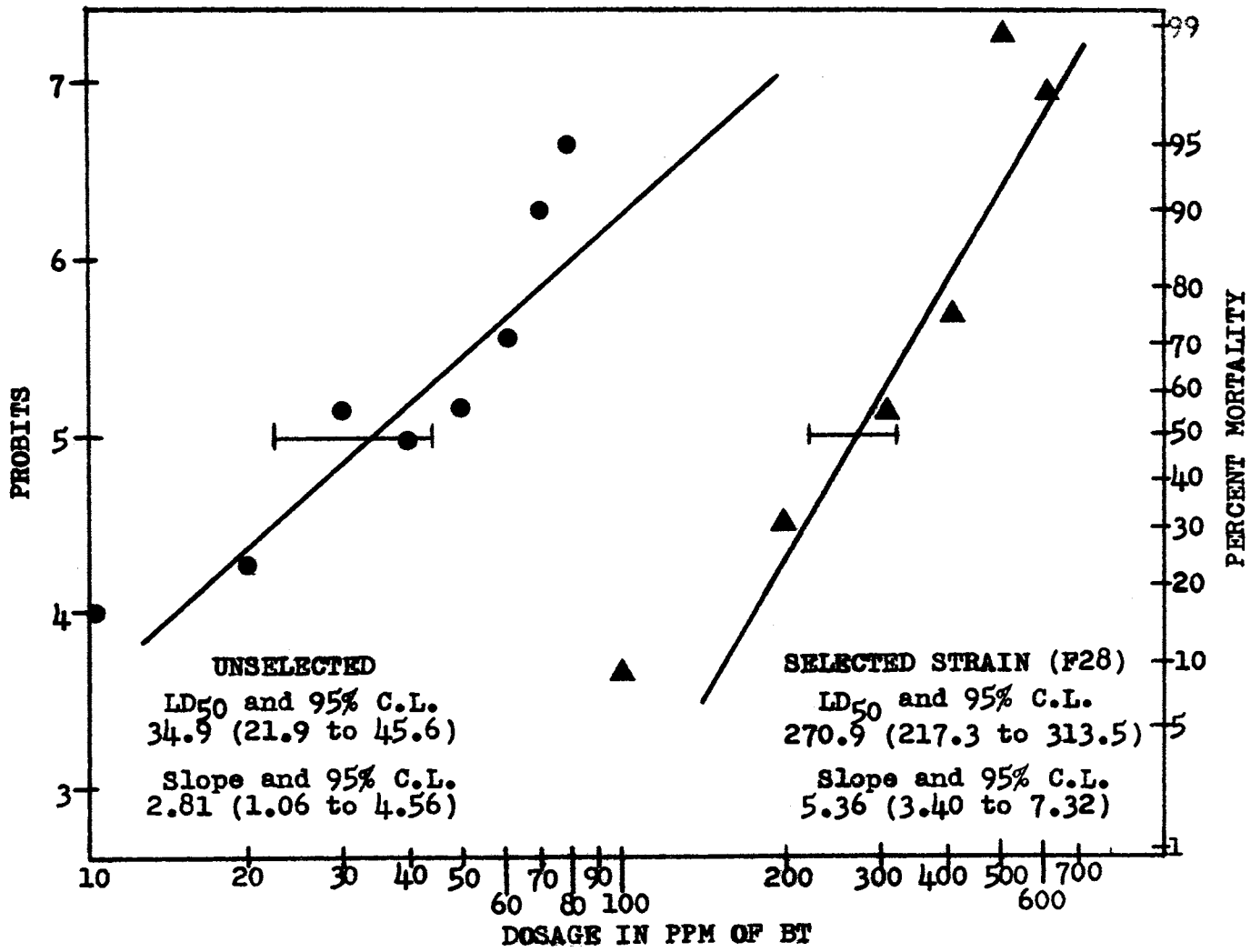


Fig. 4.--The regression of Bacillus thuringiensis concentration on per cent mortality for selected (28 generations) and unselected strains of house flies. Test initiated with eggs and mortality based on pupation.

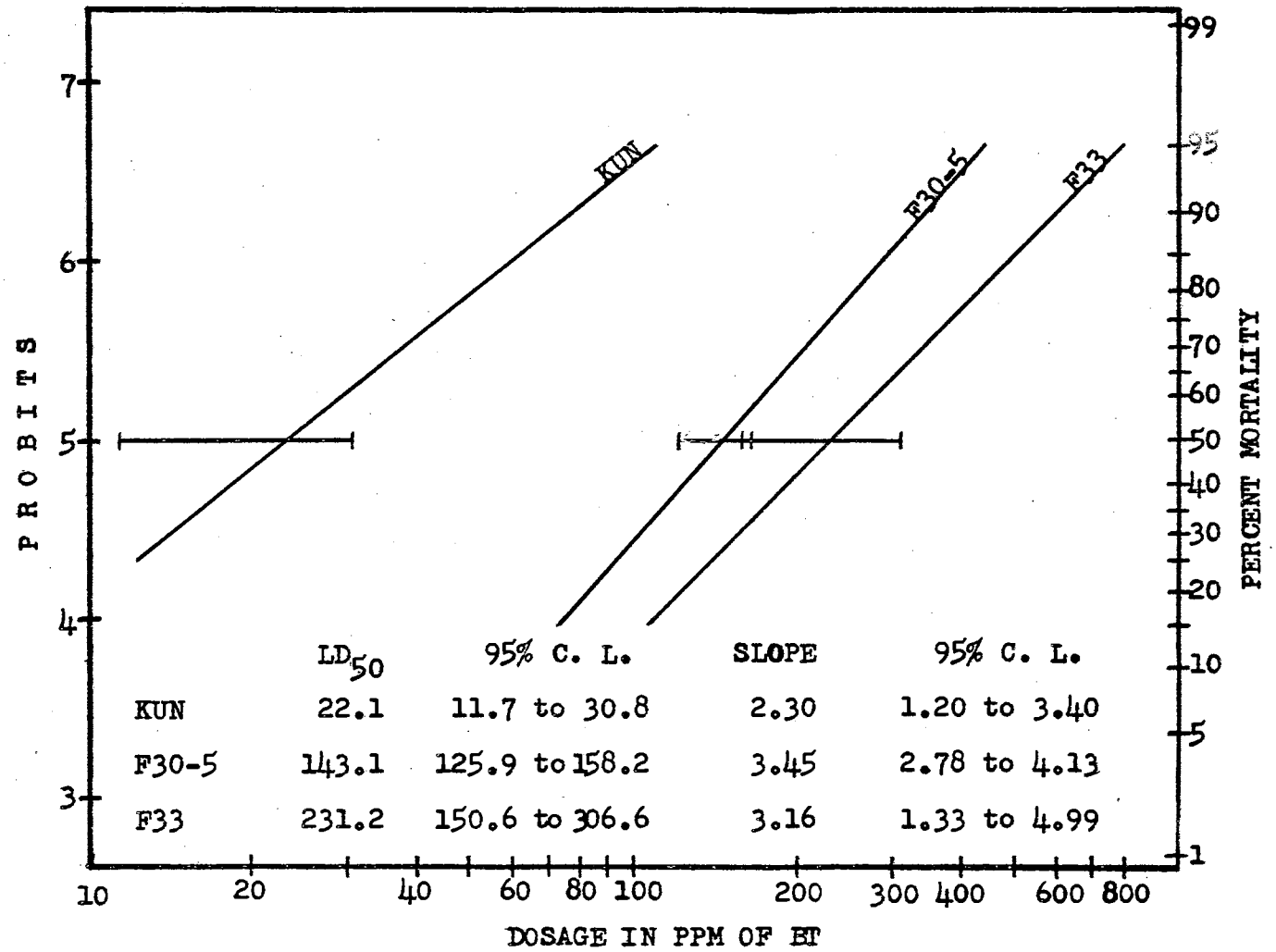


Fig. 5.--Dosage mortality curves for house flies fed spore powder in larval medium. The strains were: KUN unselected, F30-5 selected 30 generations and unselected 5 generations, F33 selected for 33 generations.

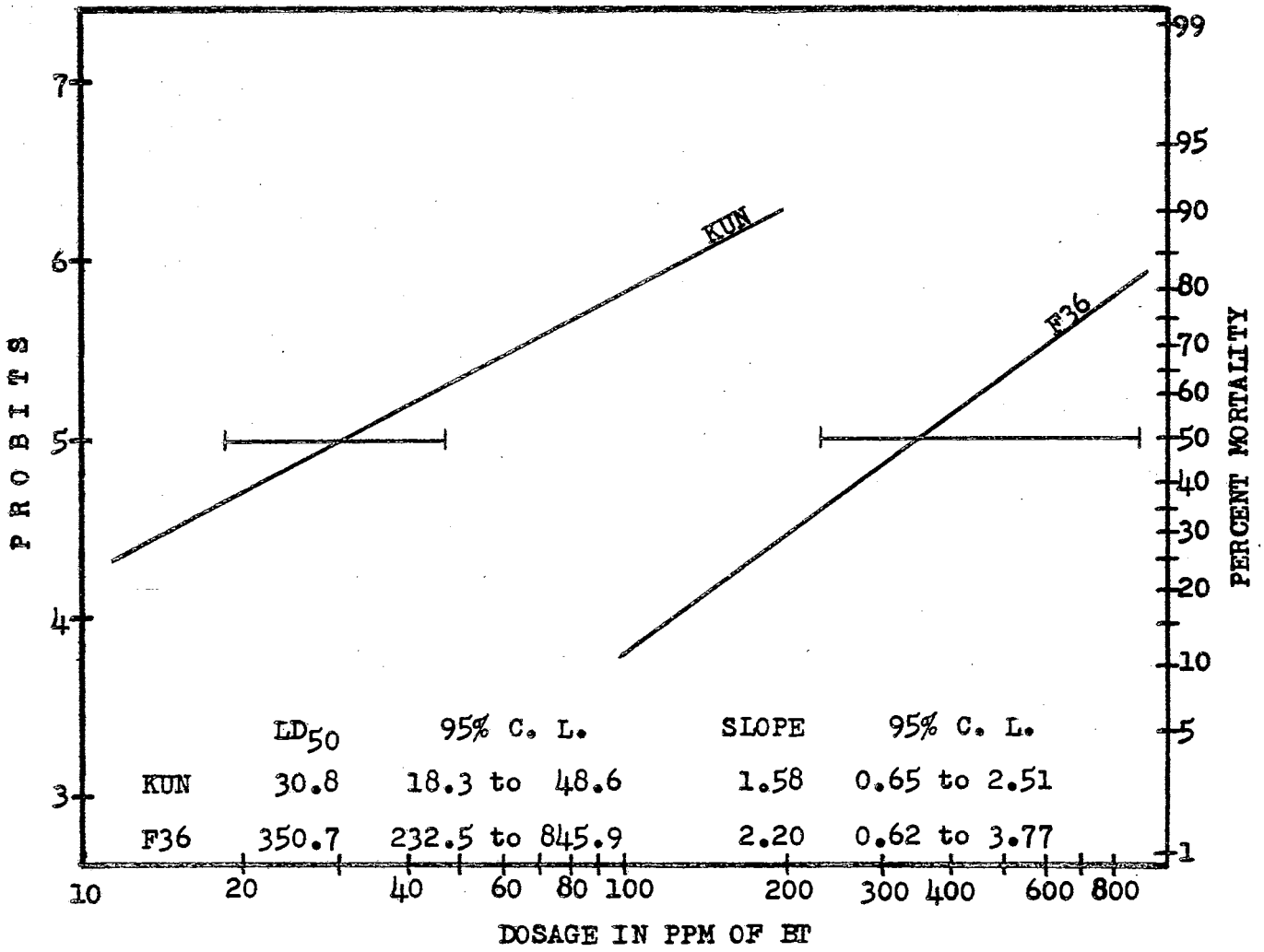


Fig. 6.--The regression of Bacillus thuringiensis concentration on per cent mortality for selected (36 generations) and unselected strains of house flies. Test initiated with eggs and mortality based on pupation.

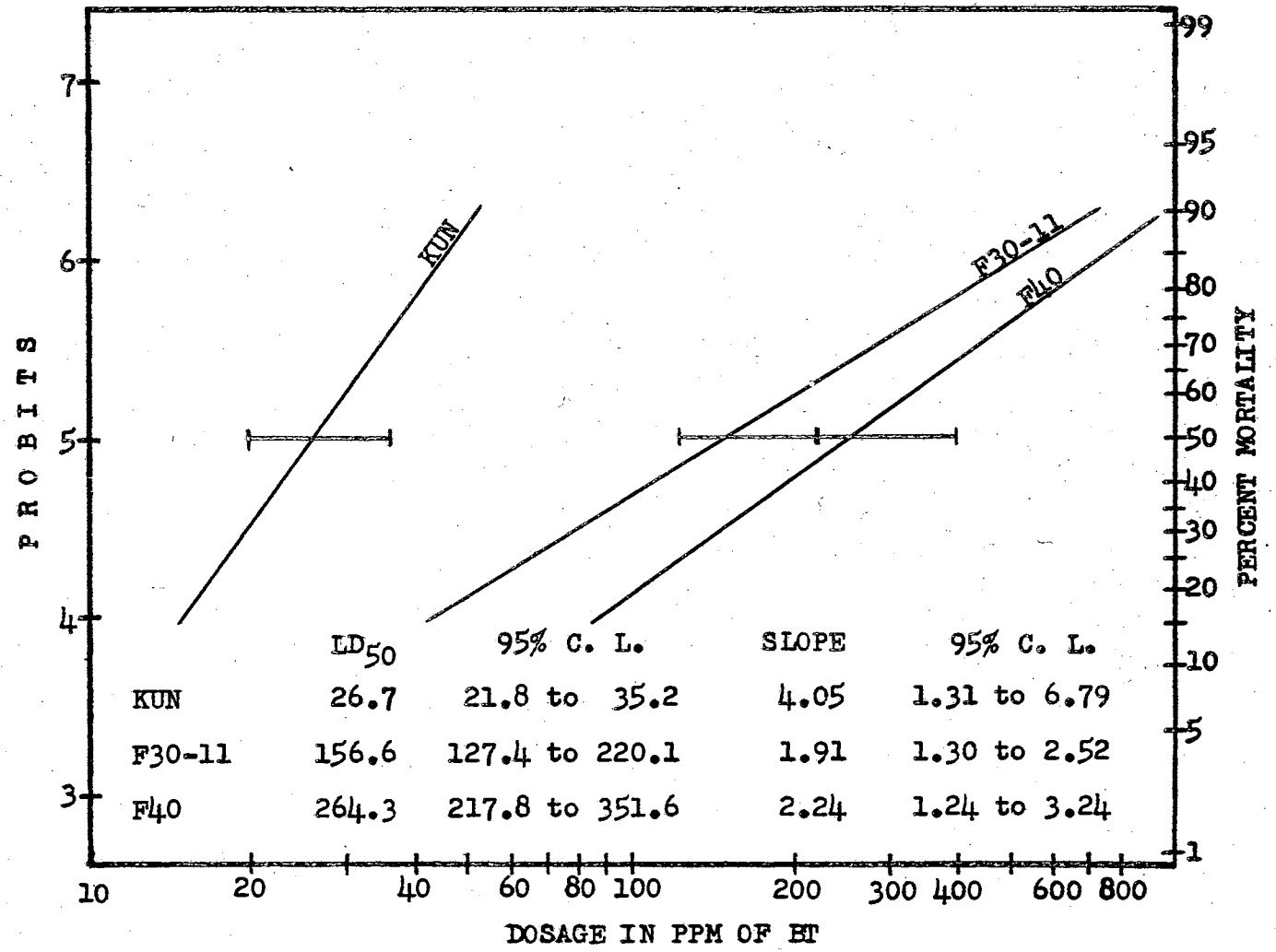


Fig. 7.--Dosage mortality curves for house flies fed spore powder in larval medium. The strains were: KUN-unselected, F30-11 selected 30 generations and unselected 11 generations, F40 selected for 40 generations.

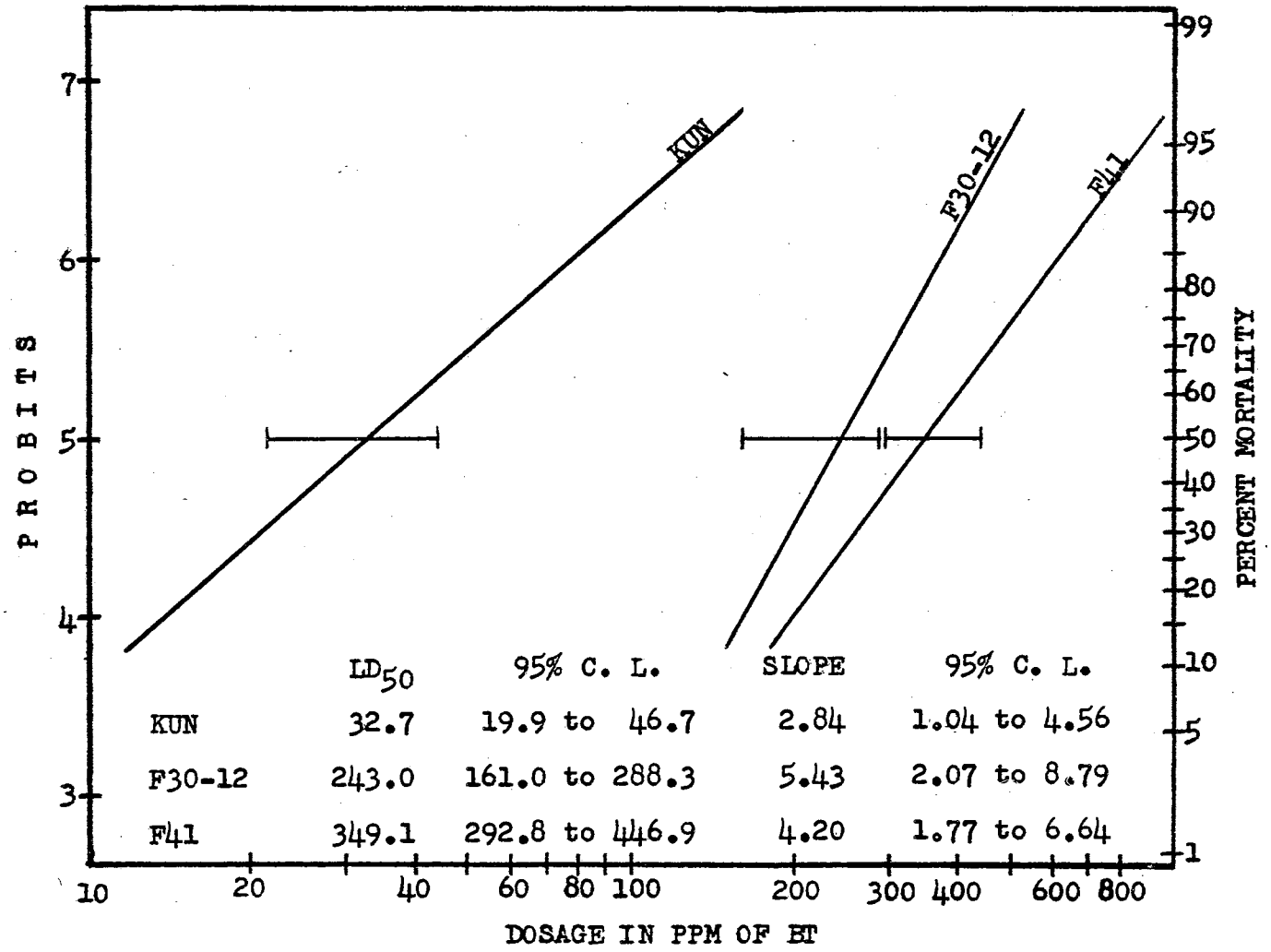


Fig. 8.--Dosage mortality curves for house flies fed spore powder in larval medium. The strains were: KUN-unselected, F30-12 selected 30 generations and unselected 12 generations, F41 selected for 41 generations.

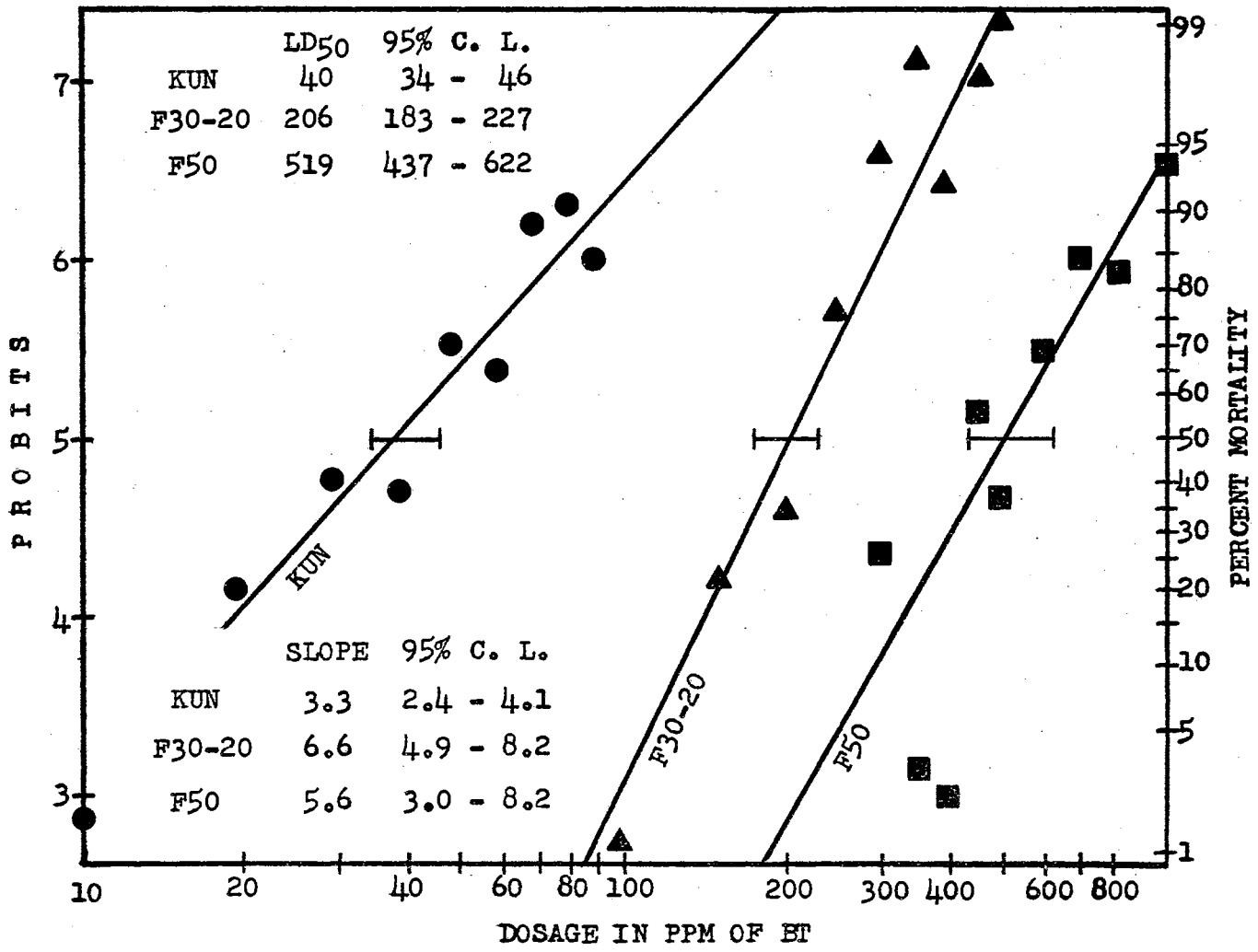


Fig. 9.--Dosage mortality curves for house flies fed spore powder in larval medium. The strains were: KUN-unselected, F30-20 selected 30 generations and unselected 20 generations, F50 selected for 50 generations.

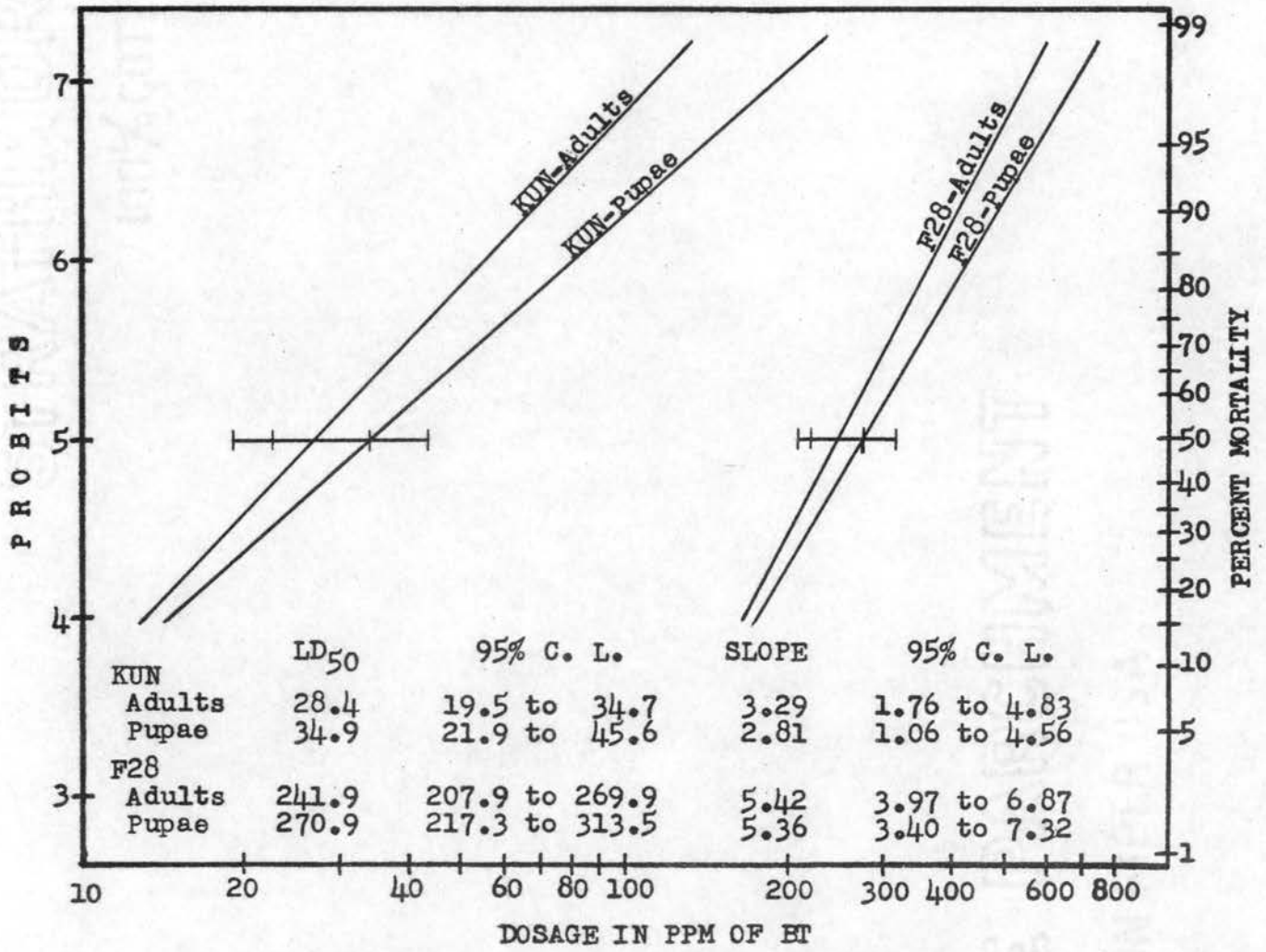


Fig. 10.--Dosage mortality lines for Bacillus thuringiensis in larval medium for selected (F28) and unselected (KUN) strains to compare results based on pupation and adult emergence in tests initiated with eggs.

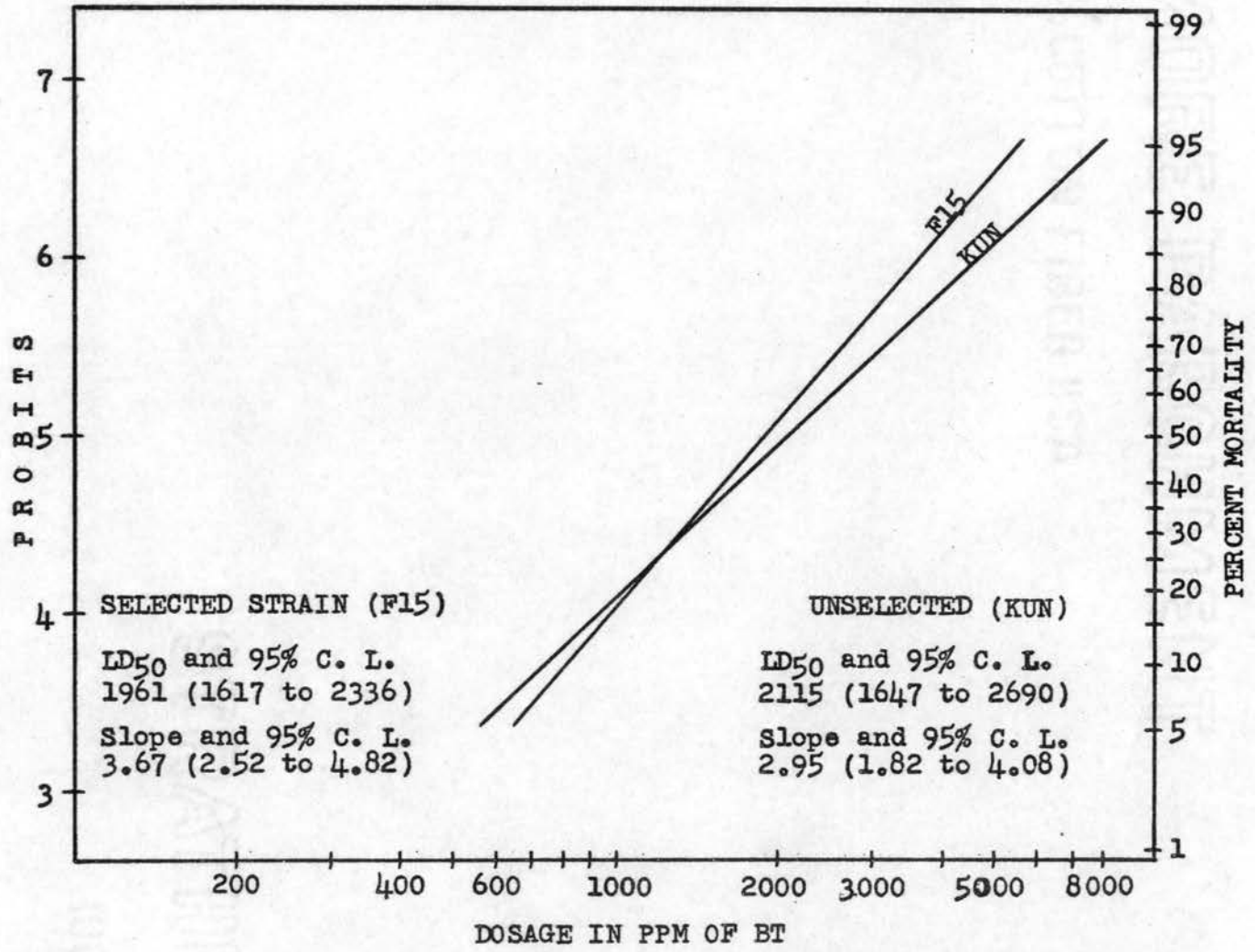


Fig. 11.--Dosage mortality lines for Bacillus thuringiensis in house fly larval medium for selected (15 generations) and unselected (KUN) strains based on pupation in a test initiated with 2-day old larvae.

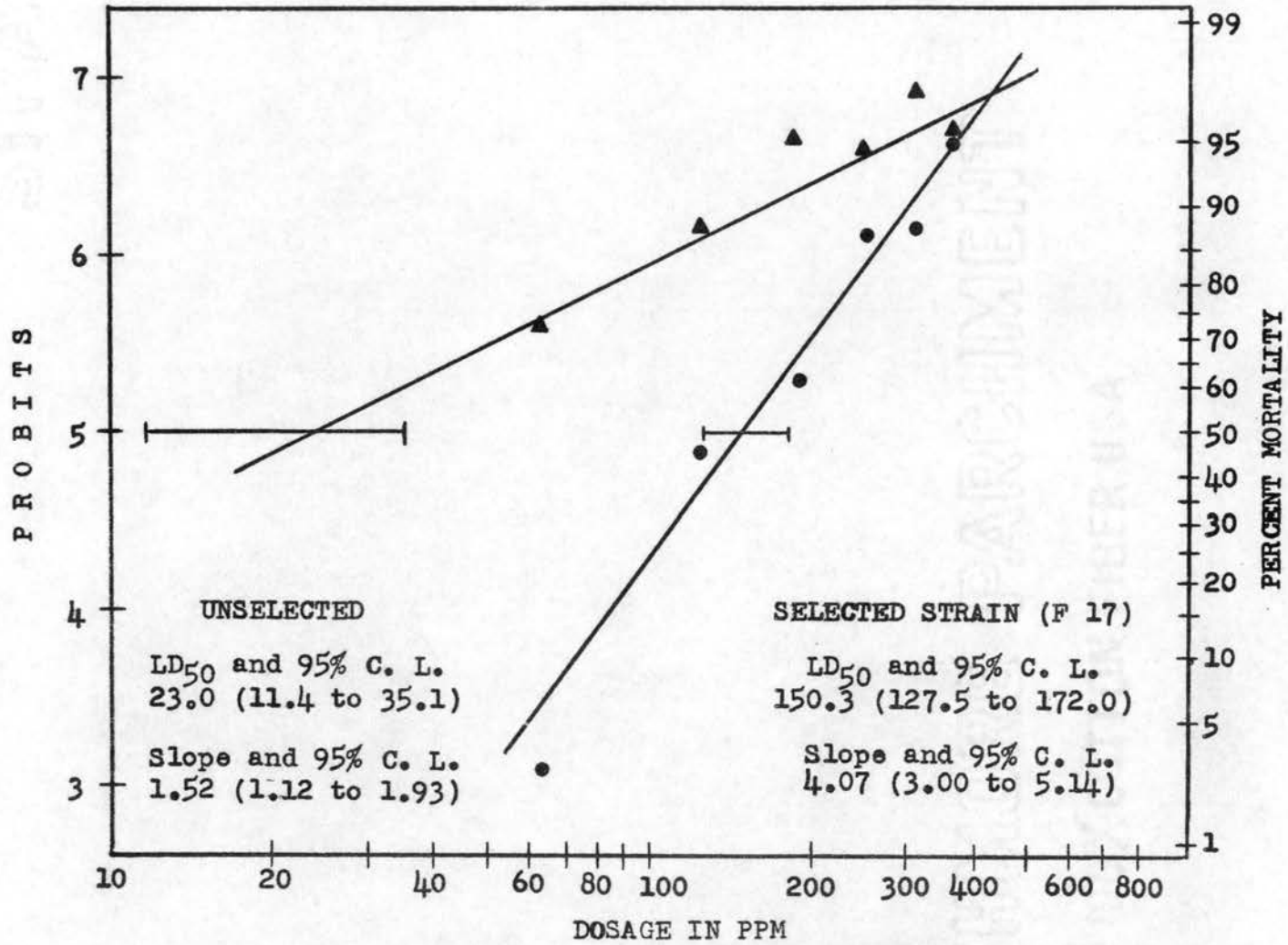


Fig. 12.--Dosage mortality lines for Bacillus thuringiensis in house fly larval medium for selected (17 generations) and unselected (KUN) strains based on emergence of adults in a test initiated with 2-day old larvae.

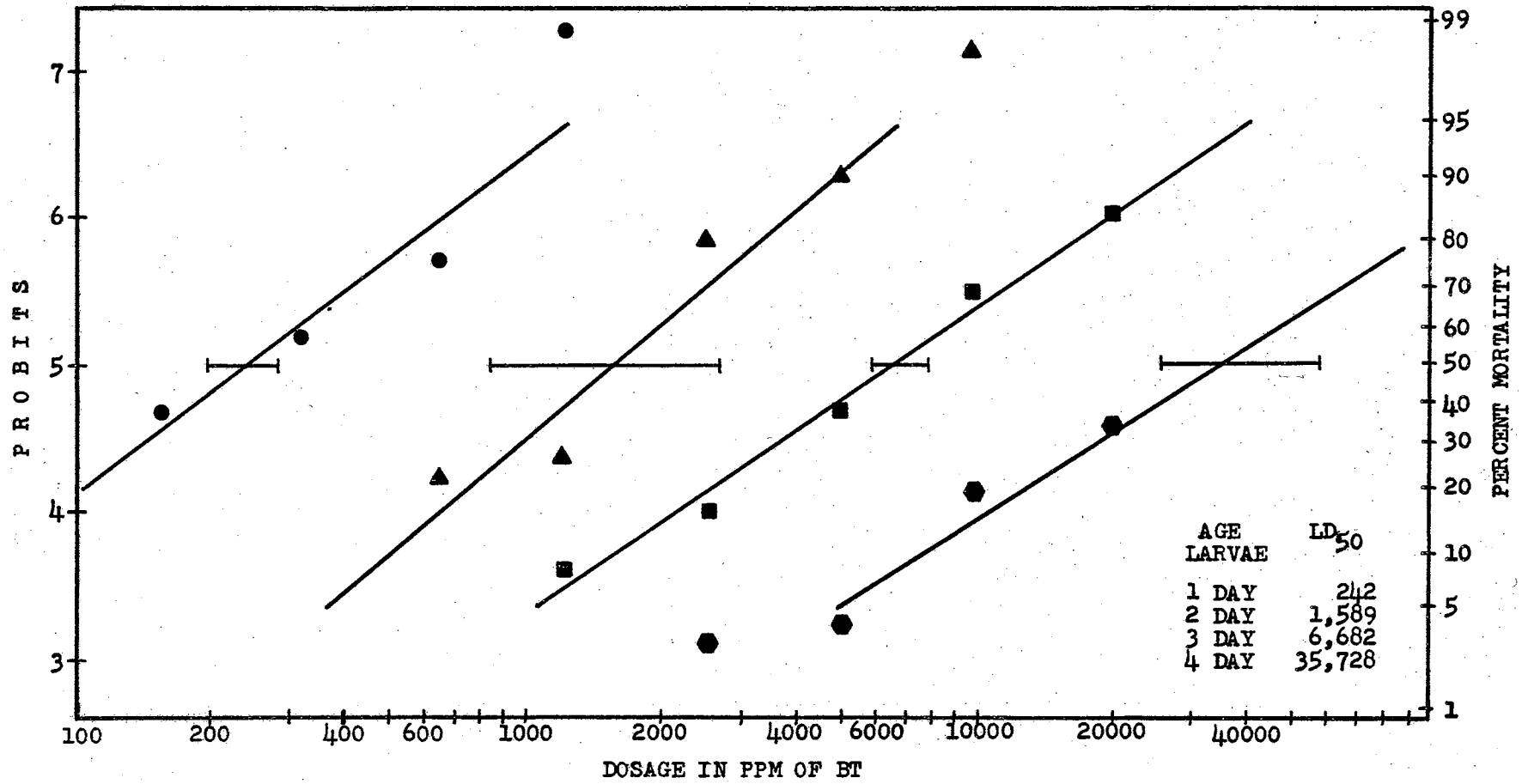


Fig. 13.--Dosage mortality lines for Bacillus thuringiensis when tests were initiated with 1, 2, 3, and 4 day old house fly larvae of a selected strain (15 generations). Results were based on pupation.

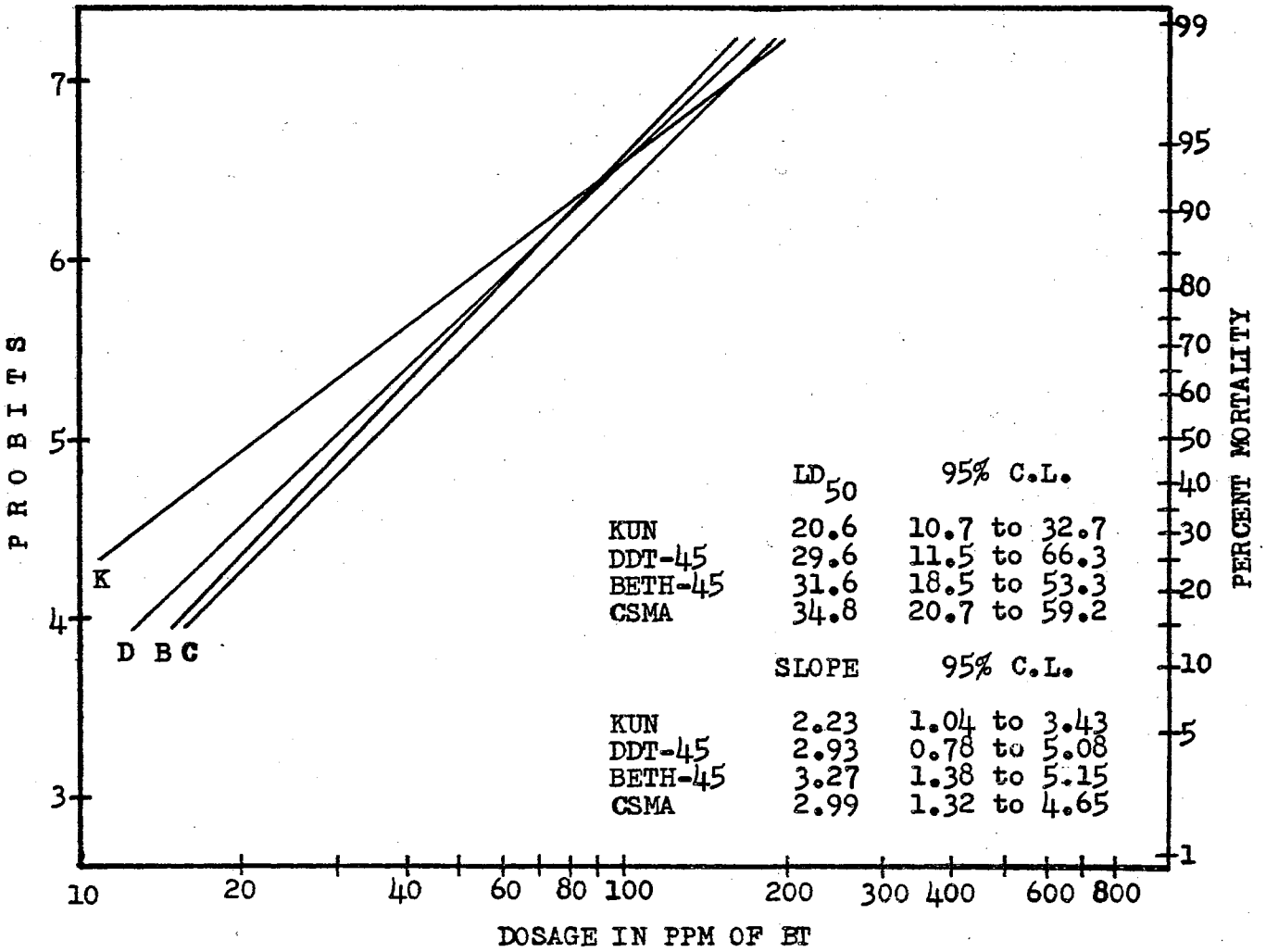


Fig. 14.--Dosage mortality curves for Bacillus thuringiensis in medium for strains of house flies resistant (DDT-45, Bethesda-45) and susceptible to insecticides. Tests initiated with eggs and mortality based on pupae.

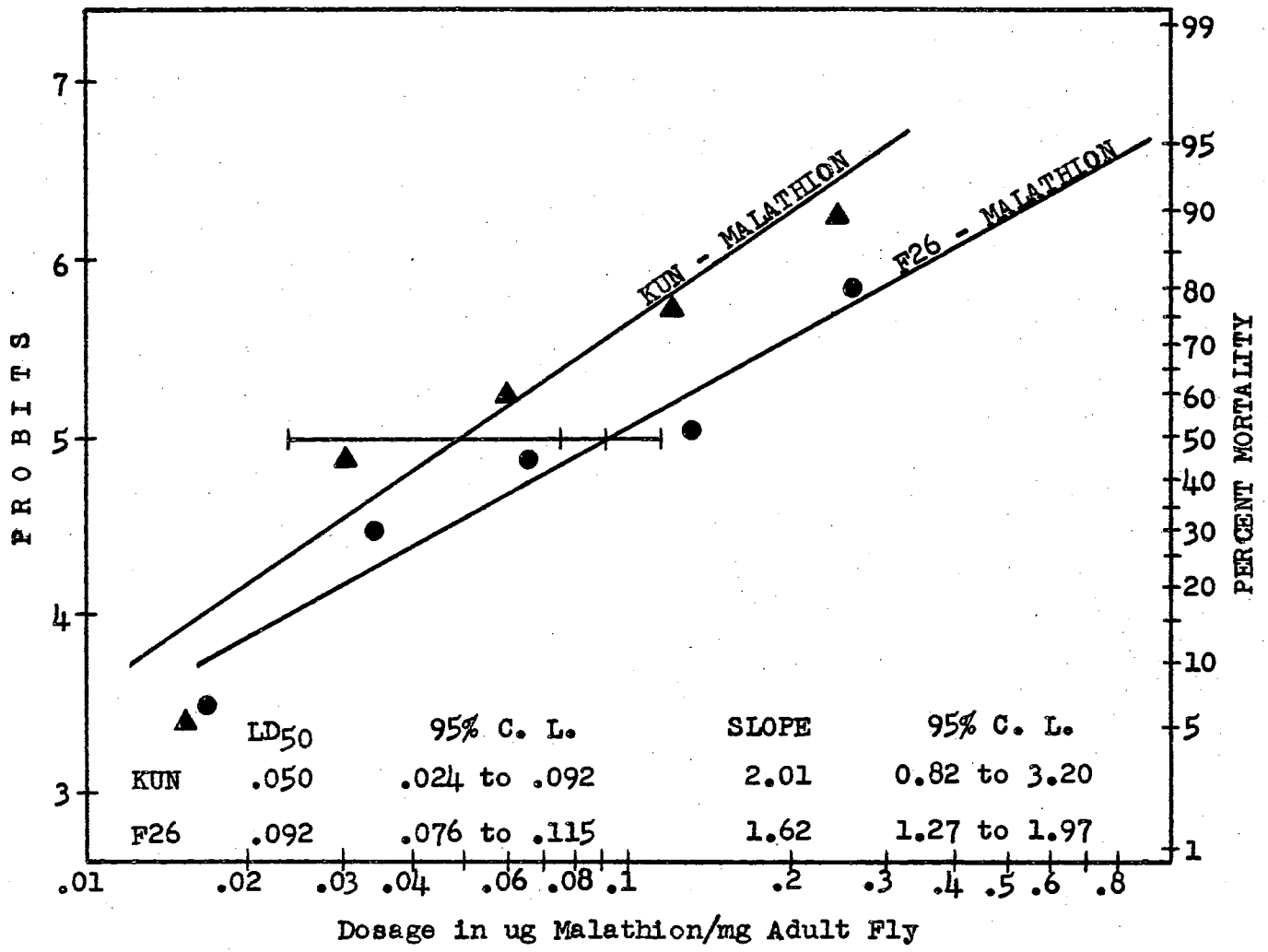


Fig. 15.--The regression of topically applied malathion on per cent mortality for strains of house flies selected (26 generations) and unselected for resistance to Bacillus thuringiensis.

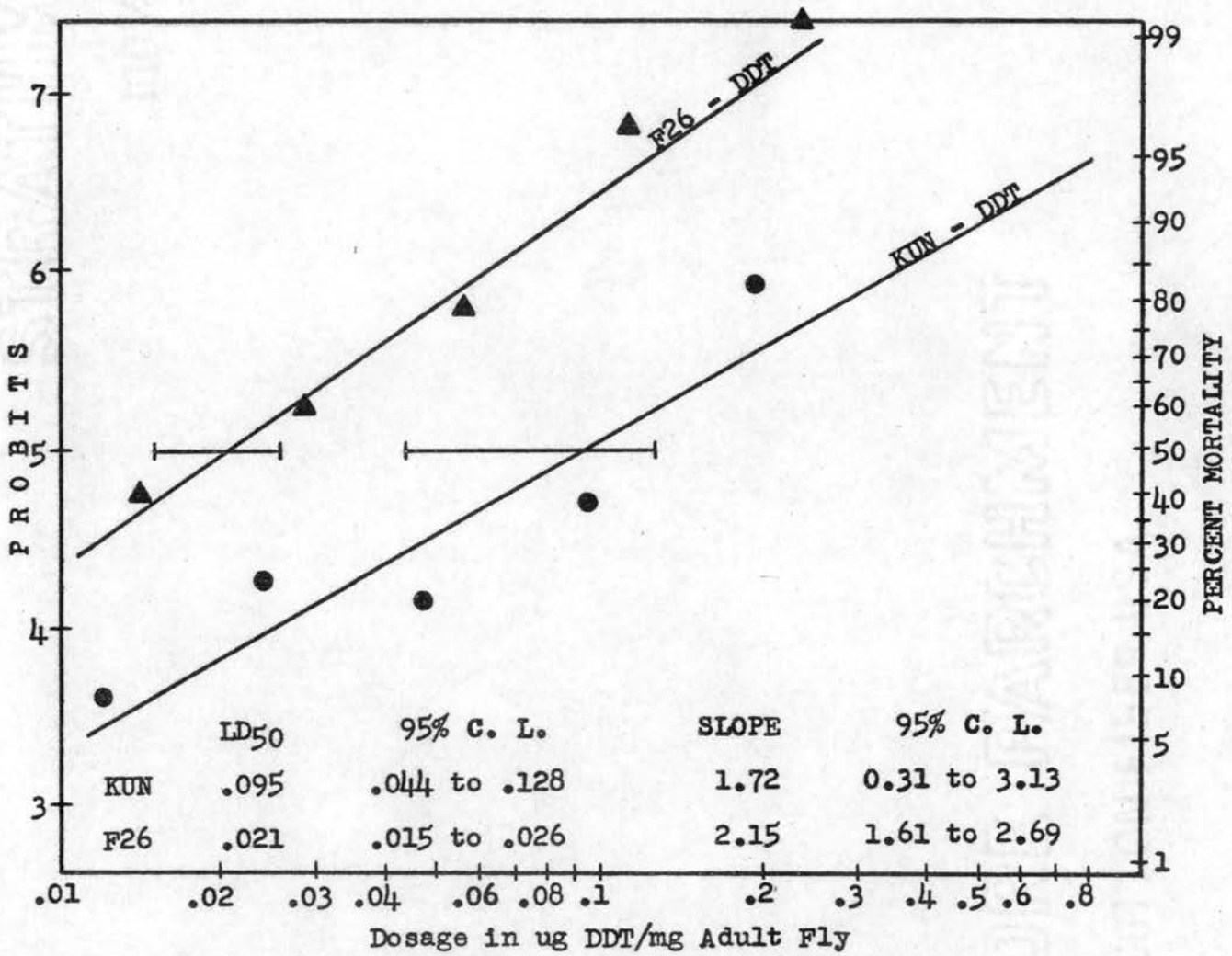


Fig. 16.--The regression of topically applied DDT on per cent mortality for strains of house flies selected (26 generations) and unselected for resistance to Bacillus thuringiensis.

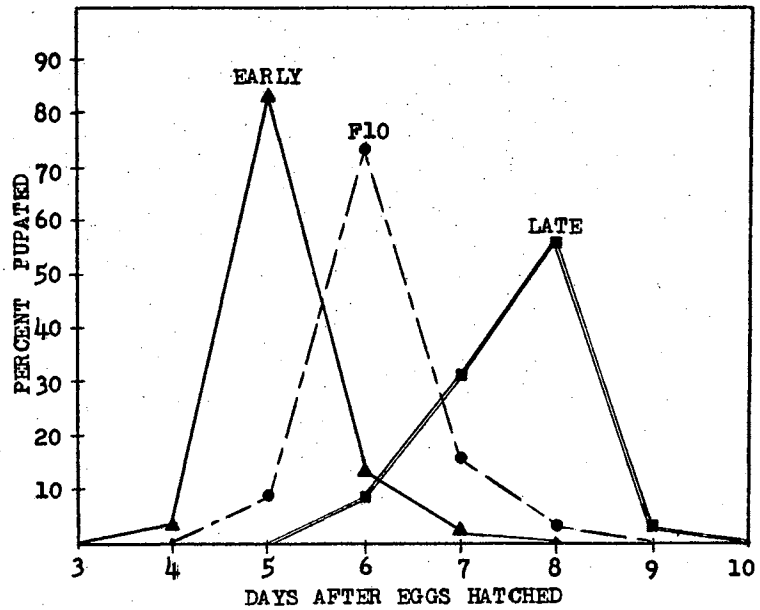


Fig. 17. Larval period for 3 strains of house flies selected for 10 generations from a field strain. (Early - selected early pupaters, late - selected late pupaters, F10 selected for early pupation and resistance to B. thuringiensis.)

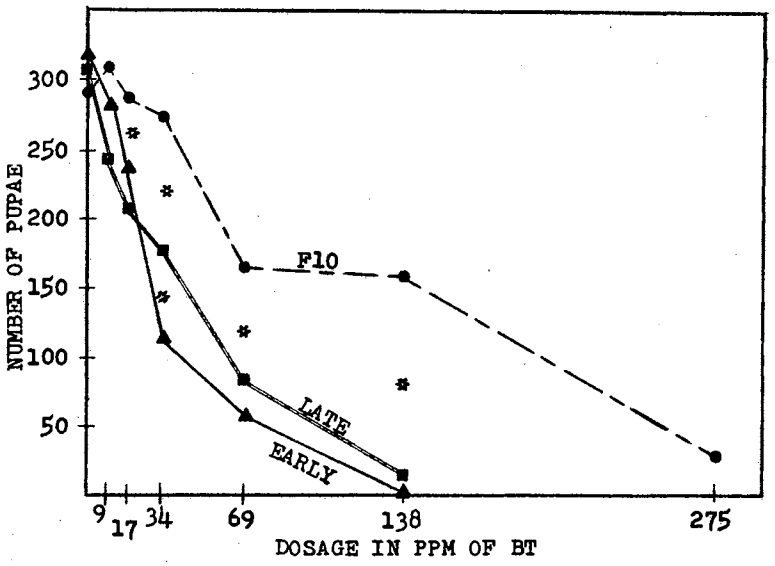
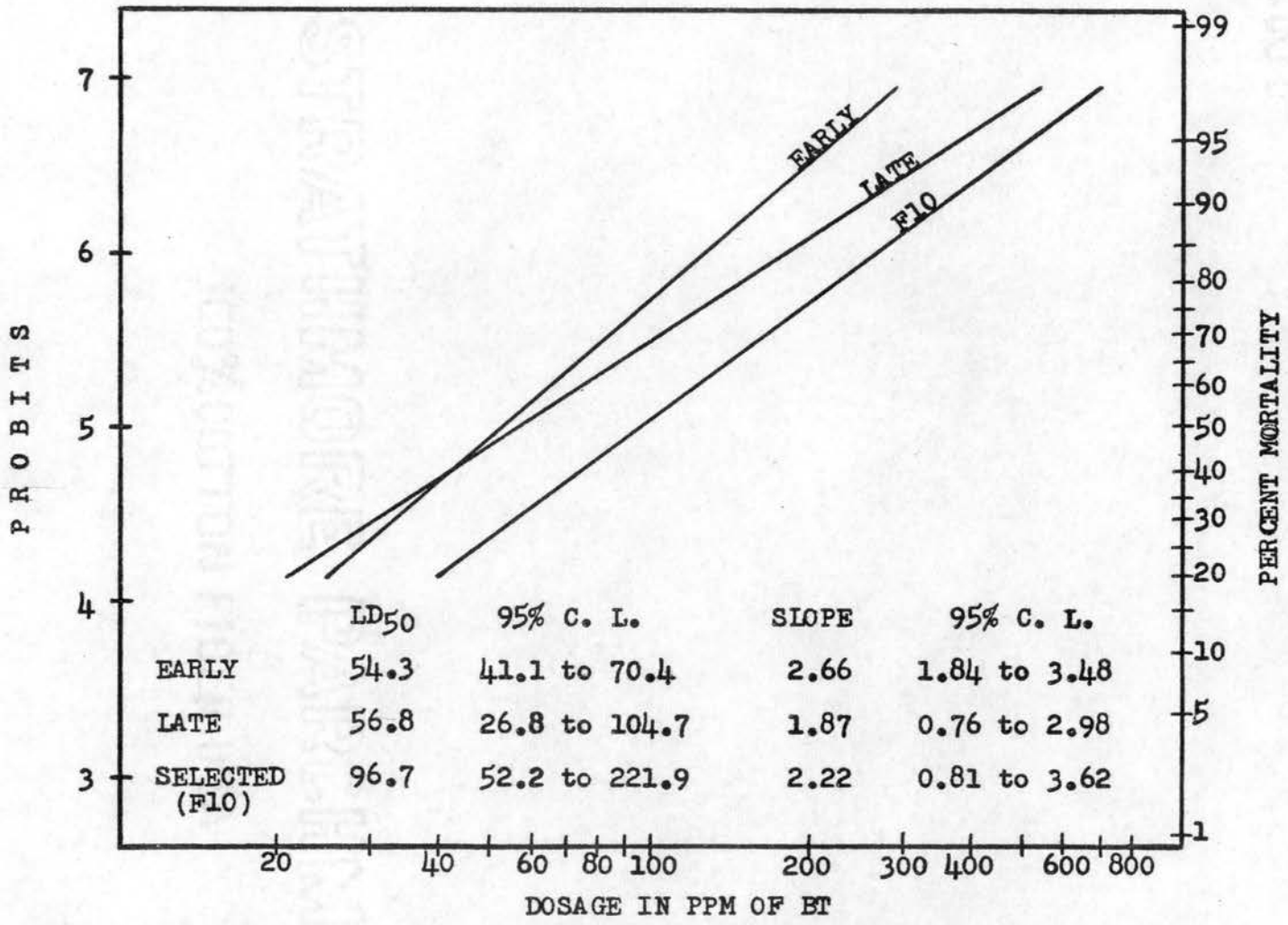


Fig. 18. Resistance of 3 strains of house flies varying in length of larval period (Fig. 17) and selection for resistance to B. thuringiensis. Asterisks separate points that were significantly different at .05 level. Test initiated with eggs and mortality based on pupation.



	LD_{50}	95% C. L.	SLOPE	95% C. L.
EARLY	54.3	41.1 to 70.4	2.66	1.84 to 3.48
LATE	56.8	26.8 to 104.7	1.87	0.76 to 2.98
SELECTED (F10)	96.7	52.2 to 221.9	2.22	0.81 to 3.62

Fig. 19.--Dosage mortality curves for Bacillus thuringiensis in house flies with larval periods of 5 days (early), 6 days (F10), and 8 days (late). F10 was selected for early pupation and resistance for 10 generations.

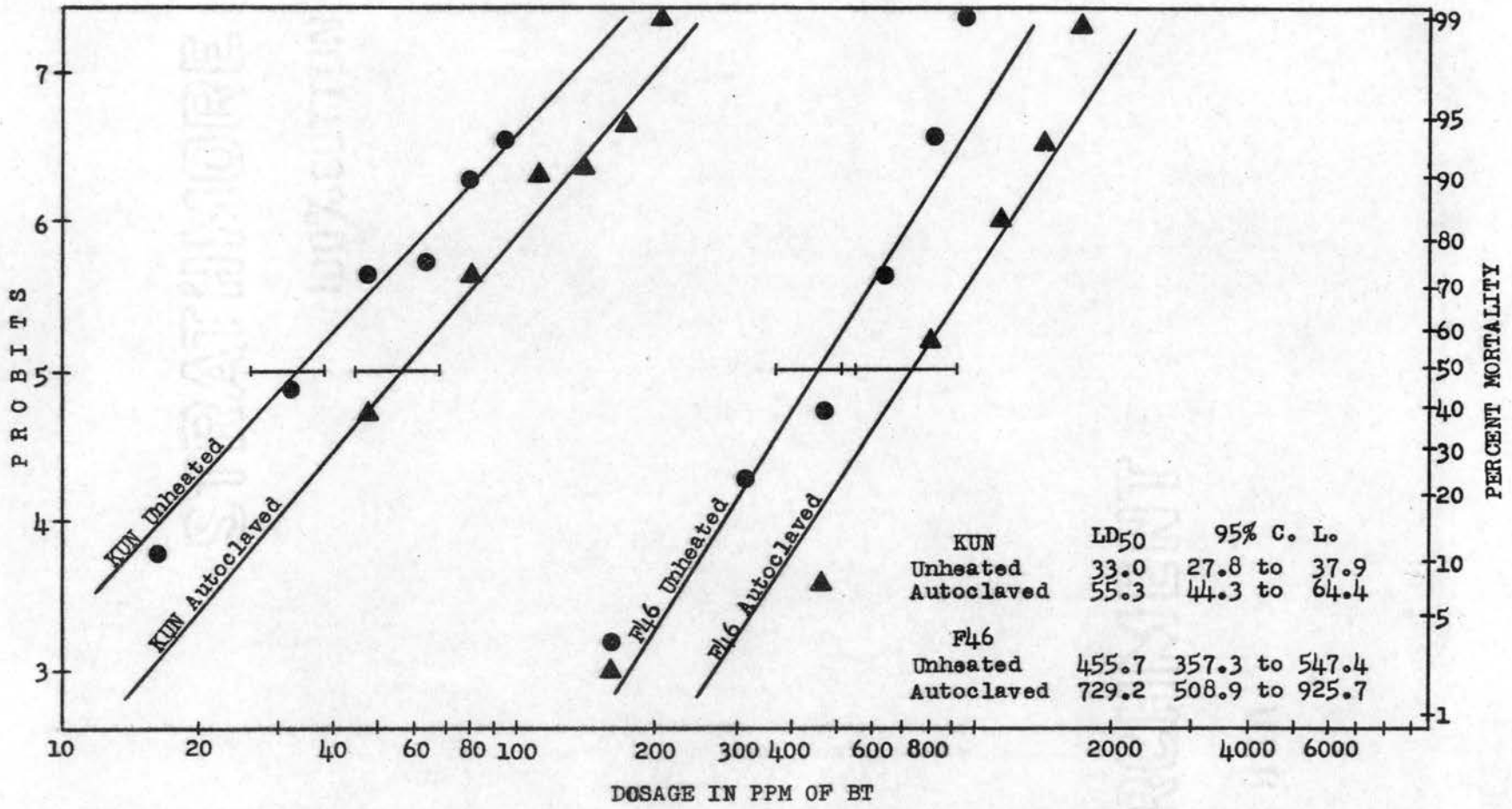


Fig. 20.--The regression of autoclaved and unheated Bacillus thuringiensis concentration on per cent mortality for selected (46 generations) and unselected (KUN) strains of house flies. Tests were initiated with eggs and mortality based on pupation.

VITA

Tommy Larkin Harvey

Candidate for the Degree of

Doctor of Philosophy

Thesis: HOUSE FLY RESISTANCE TO Bacillus thuringiensis Berliner, A MICROBIAL INSECTICIDE

Major Field: Entomology

Biographical:

Personal Data: Born in Ulysses, Nebraska, January 17, 1926, the son of H. R. and Olive Harvey.

Education: Graduated from Ulysses High School, Ulysses, Nebraska in 1943; attended University of Nebraska in 1946; received the Bachelor of Science degree in Entomology from Kansas State University in May, 1950; received the Master of Science degree from Kansas State University, with a major in Entomology and Plant Pathology, in August, 1951; completed requirements for the Doctor of Philosophy degree in August, 1963.

Experience: Served with the U. S. Army in the Philippines, 1944-1946; graduate research assistant in Entomology at Kansas State University, 1950-1951; assistant entomologist, New Mexico State University, 1952-1953; assistant professor of Entomology at Kansas State University in charge of insect investigations at the Fort Hays Branch Experiment Station, 1954-present; served on inactive duty with U. S. Naval Reserve since 1950, with present rank of Lieutenant Commander in the Medical Service Corps.

Organizations: Entomological Society of America, Sigma Xi, Phi Kappa Phi, Gamma Sigma Delta, Sanborn Entomology Club, and Oklahoma Academy of Science.