THE DETERMINATION OF LOG-TIME MORTALITY CURVES OF THE VARIOUS LIFE STAGES OF (*HYPERA POSTICA*) SUBJECTED TO CERTAIN INSECTICIDES

by

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TABLE OF CONTENTS

LIST OF PLATES

LIST OF TABLES

INTRODUCTION

The alfalfa weevil Hypera postica (Gyllenhal), is a serious pest of alfalfa in much of the western, southern, and northern United States. This insect was first discovered in the U. S. in Salt Lake County, Utah in 1904, and for fifty years its distribution was limited to approximately twelve western states (Titus, 1910). In 1952, it was discovered in Maryland and Virginia (Poos and Bissell, 1953). Evans (1959) discussed the distribution of the weevil in Virginia. Since that time it has spread rapidly, covering many states east of the Mississippi.

The damage caused by this insect is accomplished through the defoliation of the alfalfa plant by the four larval instars. They feed on the buds, growing tips, and leaves of the plant. The economic importance of this pest is such that without control, entire fields may be destroyed.

Alfalfa weevil control during the early period of this century consisted of good farming practices which would maintain vigorous stands of alfalfa, and retard the development of the alfalfa weevil (Wakeland, 1919; Newton, 1933). Cultural practices such as destruction of overwintering places, dragging or dust mulching, harrowing in the spring, sweeping, irrigating, burning, pasturing, and timely cutting were advocated as supplemental controls (Titus, 1919; Wakeland, 1919).

Early insecticides such as zinc arsenite, lead arsenate, and calcium arsenate plus sulfur were recommended by entomologists when weevil larvae caused serious damage (Hagan et al., 1918). Since the advent of DDT, the insecticidal recommendations have changed due to the development of more synthetic organic compounds. These insecticides have proven useful in controlling a variety of insect pests. Under eastern conditions, App (1954) found that dieldrin was the most effective insecticide for alfalfa weevil control, followed by heptachlor, methoxychlor, endrin, and toxaphene in descending order.

Muka (1957), while working in Virginia with granulated formulations of several insecticides, found heptachlor to be the most effective in winter or early spring. At the present time, heptachlor is being recommended for the control of the alfalfa weevil under eastern conditions. During the spring, methoxychlor and malathion are recommended (Gyrisco, 1958 and Va. Ext. Leaflet, 1962). There is, however, a residue problem involved with the use of heptachlor which limits its use to fall applications (Dogger and Bowery, 1958).

Many of the modern insecticides have relatively great mammalian toxicity; their use results in undesirable residues; or they become useless in practice due to the development of resistant insects. Even when current recommended control practices are effective, the entomologist is constantly searching for better and more economical insecticides. Involved in this search is the preliminary screening of candidate insecticides against appropriate insect pests.

These screening tests are conducted in the laboratory for several reasons. Laboratory testing is less expensive than field testing, many of the variables are eliminated in the laboratory, laboratory tests can be conducted throughout the year, and techniques and equipment used in laboratory testing are more accurate than those used in field testing. For these reasons, laboratory screening tests give reliable information about the inherent toxicities of insecticides to the test insects. This information is essential for measuring the magnitude of resistance to insecticides exhibited by insects.

The purpose of this investigation was to test three insecticides on all the life stages of the alfalfa weevil, including both naked and cocooned pupae, and to analyze the data gathered from the insecticidal tests. The specific objectives of this investigation were to obtain information on the toxicity of certain insecticides to the alfalfa weevil in the laboratory, to establish standard susceptibility curves for the life stages of the alfalfa to the insecticides tested, and to establish a basis for determining insecticide resistance. Other objectives were to compare the speed of action of various insecticides and to observe the effects of these insecticides upon molting, feeding, and other processes that may be affected.

REVIEW OF LITERATURE

Since the discovery of the alfalfa weevil in the eastern states, there have been conflicting reports on various aspects of the life history of this insect. Therefore, data on the life history and seasonal behavior patterns of the alfalfa weevil in the East are far from complete.

In the East, investigations in Maryland by Poos and Bissell (1953) and App (1954), and in Delaware by Milliron and MacCreary (1955) and Milliron (1956) show that the eggs, especially those deposited near the onset of cold weather, may survive winter temperatures and hatch the following spring. Evans (1959) observed in his studies that about 50 percent of the overwintering eggs in Virginia are viable.

Observations of Manglitz and App (1957) strongly indicate that larvae and pupae will not survive winter temperatures in Maryland. It seems quite probable, therefore, that the largest segment of the larval population for a given season develops from eggs deposited in the spring by overwintered females.

Upon hatching, the first-instar larvae move toward the terminal growth of the alfalfa plant. This is the first of the four larval instars, with the third and fourth instars being more active and feeding on any tender foliage available.

In the western states it was found that after reaching maturity the larvae crawl or fall to the ground and then spin a cocoon, (Essig and Michelbacher, 1933).

In the East, Poos and Bissell (1953) observed that many mature larvae spun their cocoons within the foliage of the host plant rather than dropping to the ground. Evans (1959) observed that the larva spins a cocoon on dead leaves or other debris on the ground or on the leaves of the alfalfa plant. Later, after cocooning occurs, the larva can be observed in a slightly curved position. In a few days the larva changes into the pupal state. In about a week or ten days the adult weevils begin to emerge.

After emergence, the adult weevils feed for awhile and then seem to undergo a period of aestivation. Titus (1913) states that "a large number of weevils in the summer flight leave the alfalfa fields." Manglitz (1958) demonstrated that the adult weevil, after a short period, can survive several months without food and apparently does so through the summer.

Evans (1959) observed that in Virginia there occurs one main generation a year with a partial second generation. App (1954) collected larvae of all sizes as late as December 8, indicating that at least a part of the population has more than one generation a year.

The larvae are responsible for most of the damage caused by the alfalfa weevil (Anon., 1956). They feed within the tips of alfalfa plants and on upper leaves as they open and then on lower foliage, all of which reduces the crop yield. To combat this loss, entomologists have investigated two means of controlling this pest. First by means of biological control, i.e., parasitism; and, secondly, by use of chemicals.

Chamberlin began the search for natural enemies of the alfalfa weevil in Italy in 1911, and in the next two years a large number of parasites - about five species - were imported and released in Utah (Clausen, 1956). The larval parasite, *Bathyplectes curculionis* (Thoms.), was the only one to become established as a result of the release at this time (Chamberlin, 1924).

Additional importations were made after 1911, resulting in the establishment of *Mymar pratensis*, an egg parasite, and *Dibrachoides dynastes* (Foerst), an external parasite of the pupa. These have since been collected in Utah, Oregon, and Washington (Clausen, 1956).

Essig and Michelbacher (1933) introduced *Bathyplectes curculiones* (Thoms.) during 1933 and 1934 as a source of biological control. They later determined that as much as 90 percent parasitization occurred in the field during this investigation.

Poinar and Gyrisco (1960) found some larvae that were parasitized by nematodes belonging to the family Mermithidae. Further observations made by these men indicated that only fourth-instar larvae were parasitized by these nematodes.

Due to the lack of effective biological control and the need for controlling insects, the entomologist must use insecticides. Koehler et al. (1959) conducted experiments using fifteen insecticides in 15 counties in New York, applied as low-pressure, low-volume sprays and concluded that heptachlor, at four ounces actual material per acre, gave excellent control of adults for two weeks after treatment.

Out of twenty-seven insecticides tested in Maryland by App (1959) for control of the alfalfa weevil larvae, heptachlor, aldrin, dieldrin, endrin, and thiodan were the most effective. These insecticides, when used in granular form or mixed with fertilizer, were less satisfactory and required heavier dosages. Mixtures proved more effective than single insecticides.

Frank (1959), experimenting in Wyoming with granulated heptachlor and dieldrin at 1.25 pounds per acre, found that heptachlor was more effective; however, dieldrin gave good control for two years.

In preliminary experiments in central Virginia with five granulated insecticides, Muka (1957) applied insecticides at different dates during the dormant period showing that heptachlor, applied at the rate of 1½ pounds of actual toxicant per acre either in the winter or in the spring, was more effective than aldrin, dieldrin, parathion, or lindane.

Phillips and Bissell (1959), experimenting with different insecticides, obtained excellent results with the two new systemic insecticides, American Cyanamid 12880 and 18706 against adult weevils. None of the several insecticides tested, however, appeared to be as effective as heptachlor combined with malathion. The new systemic compounds gave 98% and 96% control after nine days, and they gave a 97% reduction at the sixteen-day sampling date.

Like many other workers, Wolstrom and Lafgren (1957) working in South Dakota, Blackburn (1957) in Pennsylvania, and Evans (1959) in Virginia, have agreed from data gathered in their experiments that granulated heptachlor gave satisfactory control of all life stages of the alfalfa weevil.

Busvine (1957) discussed several test methods of applying residual insecticides in the laboratory. One of these methods is that of using non-volatile oils which act as vehicles for the insecticides. Another method is that of applying residual insecticides by dissolving them in a volatile solvent, usually acetone, and spreading a measured quantity over a test surface. Bishop (unpublished data, 1959) found that the latter method was more applicable because only the insecticide residue remains, with the former method not being suitable since many of the oils are toxic to alfalfa weevil larvae.

Stringer (1949); Stringer et al. (1955); Busvine and Barnes (1947), all used the impregnated filter paper method. In this case, filter paper is impregnated with a solution of insecticide calculated to give standard deposits per unit area. The volatile solvent (acetone) is rapidly evaporated, leaving only the toxicant distributed uniformly over the filter paper. Further discussion on methods, techniques, and analysis will come under the section Methods and Materials.

METHODS AND MATERIALS

All alfalfa weevils used in these tests were collected from untreated fields. Large quantities of infested alfalfa were collected and placed in plastic bags. The collected alfalfa, especially new leaf growth, was closely inspected and the larvae separated by use of a jeweler's forceps. All larvae were subjected to testing within several hours after sorting. Prior to testing, all life stages were maintained in one-gallon paper ice cream cartons containing small amounts of alfalfa.

Other larval stages were obtained by sweeping alfalfa with a standard fifteen-inch beating net. Large numbers of larvae were obtained in this manner and they were separated according to instars in the laboratory as follows: The alfalfa weevil larvae were observed through a binocular microscope containing a calibrated ocular grid. The larval instars were determined by applying head capsule measurements to Dyar's law (1890) as observed by Guerra (1960).

Pupae, both naked and cocooned, were reared from fourth-instar larvae collected in the field. All pupae were several days old when subjected to insecticidal tests.

The test chambers were standard petri dish bottoms in which Whatman #1 filter paper, 9 centimeters in diameter, had been placed. The side walls of the petri dishes were smeared with petroleum jelly in which a small amount of _para_-dichlorobenzene had been added. This was sufficient in keeping the alfalfa weevil larvae and adults within the petri dish.

A known amount of insecticide was dissolved in glass distilled acetone, so that when 2 milliliters of solution were pipetted on to the filter paper, a dosage of 100 micrograms of insecticide was applied per square centimeter. Rotation of the pipette over the surface of the filter paper gave a uniform distribution of the insecticide. Evaporation of the acetone was accelerated by placing the petri dishes in an exhaust hood for ten minutes, allowing the fan to remove all traces of the solvent vapor.

The three insecticides tested were:

(1) Dibrom -- (Dimethyl 1, 2 - dibromo - 2, 2 - dichloroethyl phosphate); 95% technical; California Chemical Corporation.

(2) Imidan -- (Pthalimidomethyl - 0, 0 - dimethyl phosphorodithioate); 98% technical; Stauffer Chemical Company.

(3) Heptachlor epoxide -- (1, 4, 5, 6, 7, 8, 8 - heptachlor - 3a, 4, 7, 7a - tetrahydro - 7, 7a - epoxy - 4, 7 - endomethanoidene); 72% technical; Velsicol Chemical Corporation.

Tests were conducted on each of the four larval instars, naked pupae, cocooned pupae, and adults. Random samples of male and female adults were used.

Each complete test of one insecticide against a particular life stage was undertaken in a series of five replicates. In each of these replicates there were twenty treated and ten untreated test chambers containing ten individuals per chamber. This resulted in a total of 200 treated individuals per replicate, or a total of one thousand for each test. The untreated chambers for control purposes held a total of 100 weevils for each test. One hour after the weevils had been introduced into the test chambers, fresh alfalfa was added. Observations were made on the relationship of time of exposure and mortality, the effects of the treatment on feeding or molting, and any other effects that might be worthy of note.

After accumulation of the data, provisional log-time-mortality curves were eye-fitted on Winthrop Logarithmic-probit graph paper. The true time-mortality curve was determined by the method of Bliss (1935). The LT_{50} and LT_{90} values were ascertained from the corrected time-mortality curve.

RESULTS AND DISCUSSION

It has become common practice to express the relationship between insect mortality and insecticide dosage in terms of logarithmic dosage-probit mortality regression lines. This practice holds true when time of exposure to insecticides is substituted for dosage. Whether this straight line relationship actually exists or not, the log time-probit mortality curves have been useful in serving as guides for reference and comparison.

Plates I through VII present time-mortality lines resulting from the work reported herein. These regression lines were fitted to the data by the method of least squares as described by Bliss. The differences in toxicities of the three insecticides are not great (Plates I - VII). However, it can be seen that longer exposures are required to kill the larvae as they become older. Pupae, possibly due to their slow metabolic rate, withstand the insecticides for a longer period of time than the larvae. It is noteworthy that adults are killed more rapidly than fourth, and in some cases, even third instar larvae. Table I gives LT_{50}, LT_{90}, (time required to kill 50% and 90% of the test weevils) and beta (slope of the line) values as found in this work. In tabular form it is again indicated that the insecticides are generally comparable. LT_{90} figures, which may be of more practical value than LT_{50} figures from the control point of view show two or three exceptions worthy of note. The comparative rapidity at which heptachlor epoxide killed fourth instar larvae and adults has practical implications, especially since the adult stage is the prime target of field control measures.

Plate I. Log time-probit mortality lines for first instar larvae of the alfalfa weevil tested against 100mg/cm^2 of each of the following insecticides:

A. ———————— Dibrom

B. — — — — — — — — Heptachlor epoxide

C. .... Imidan

PLATE I

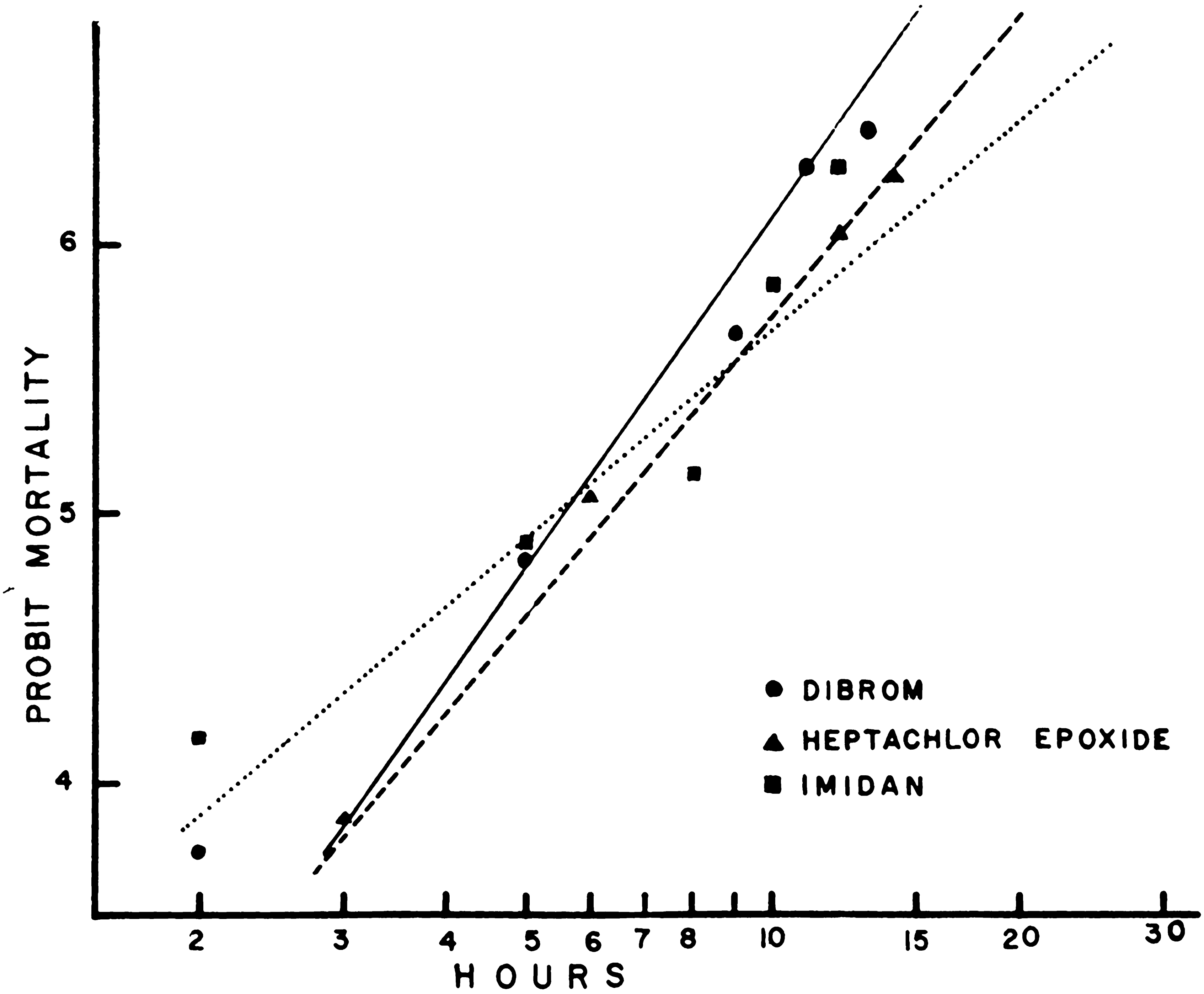
PROBIT MORTALITY
6
5
4
2
3
4
5
6
7
8
10
15
20
30
HOURS
● DIBROM
▲ HEPTACHLOR EPOXIDE
■ IMIDAN

Plate II. Log time-probit mortality lines for second instar larvae of the alfalfa weevil tested against 100mg/cm^2 of each of the following insecticides:

A. —————————— Dibrom

B. — — — — — — — — — Heptachlor epoxide

C. .... Imidan

PLATE II

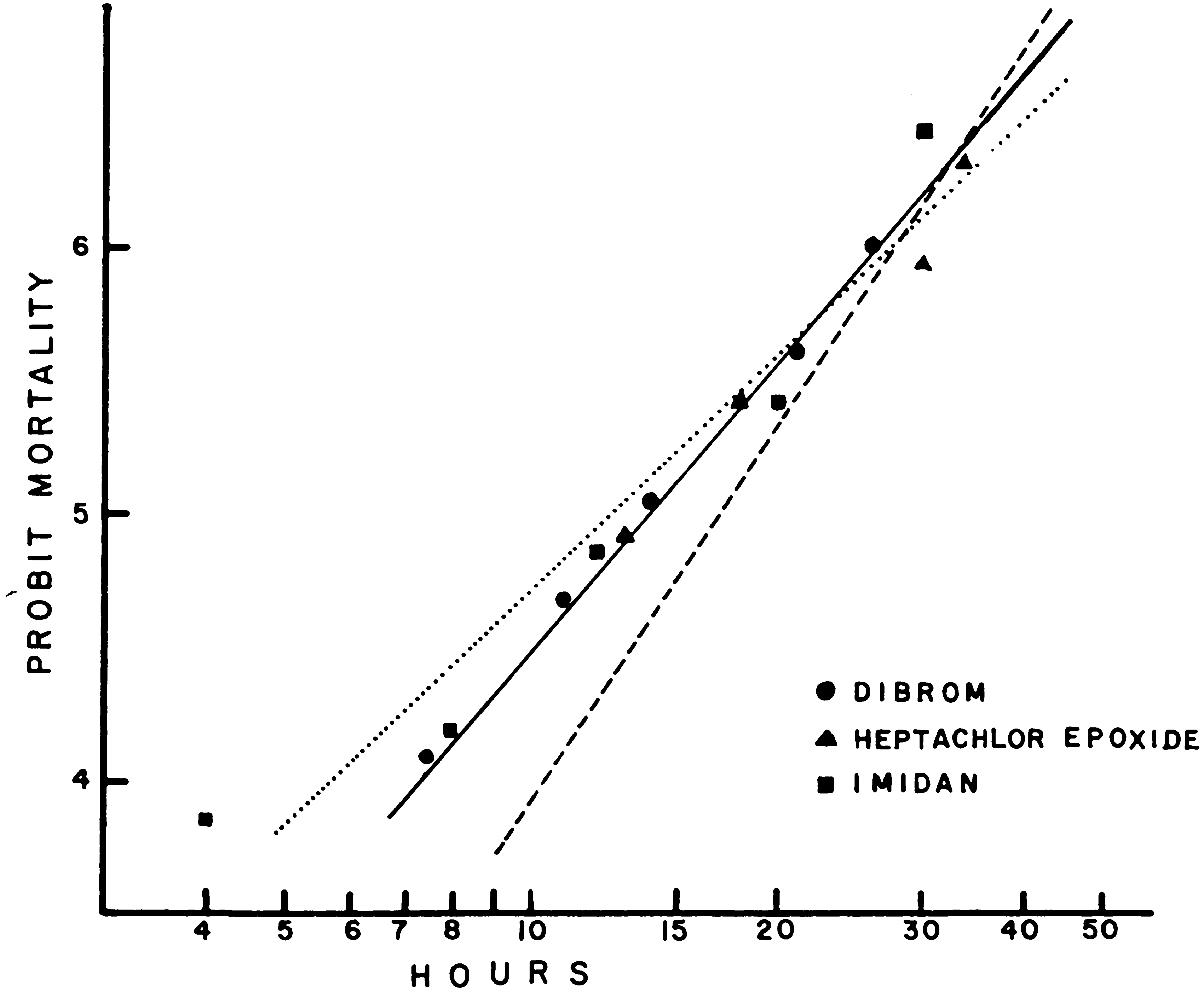
PROBIT MORTALITY
6
5
4
4
5
6
7
8
10
15
20
30
40
50
HOURS
● DIBROM
▲ HEPTACHLOR EPOXIDE
■ IMIDAN

Plate III. Log time-probit mortality lines for third instar larvae of the alfalfa weevil tested against 100mg/cm^2 of each of the following insecticides:

A. ———————— Dibrom

B. — — — — — — — — Heptachlor epoxide

C. .... Imidan

PLATE III

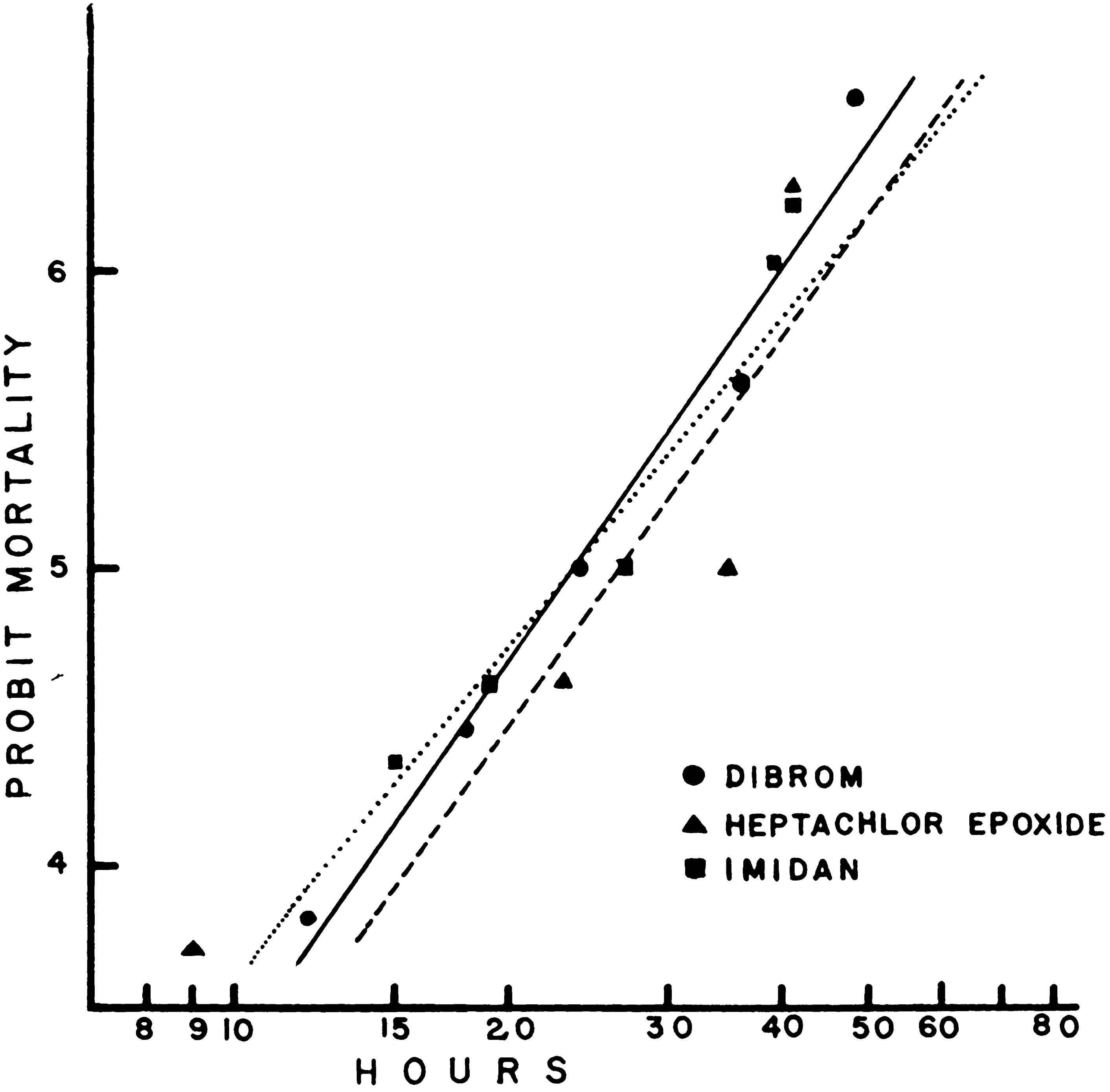
PROBIT MORTALITY
6
5
4
8
9
10
15
20
30
40
50
60
80
HOURS
● DIBROM
▲ HEPTACHLOR EPOXIDE
■ IMIDAN

Plate IV. Log time-probit mortality lines for fourth instar larvae of the alfalfa weevil tested against 100mg/cm^2 of each of the following insecticides:

A. ———————— Dibrom

B. — — — — — — — — Heptachlor epoxide

C. .... Imidan

PLATE IV

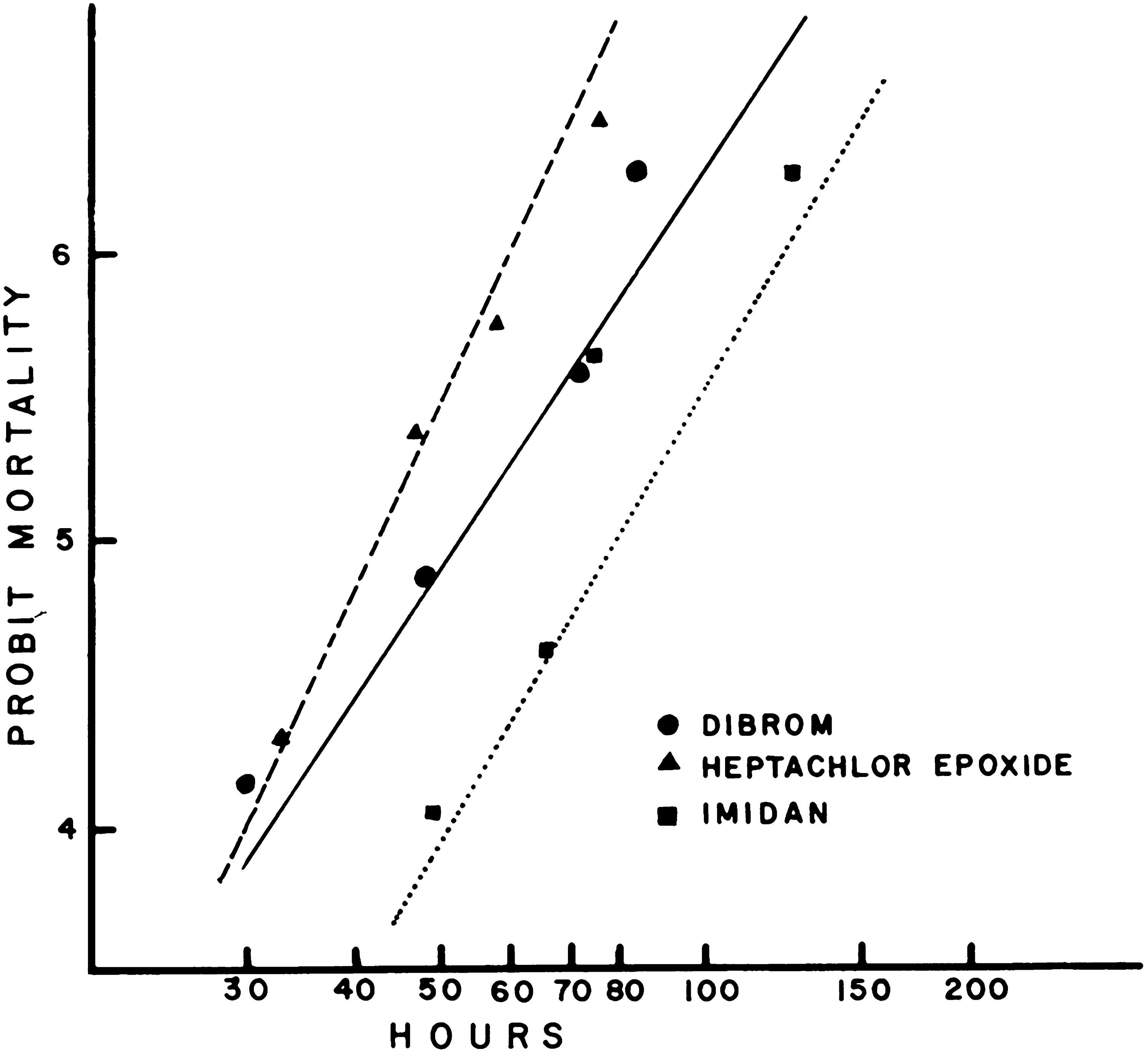
PROBIT MORTALITY
6
5
4
30
40
50
60
70
80
100
150
200
HOURS
● DIBROM
▲ HEPTACHLOR EPOXIDE
■ IMIDAN

Plate V. Log time-probit mortality lines for naked pupae of the alfalfa weevil tested against 100mg/cm^2 of each of the following insecticides:

A. ———————— Dibrom

B. – – – – – – – – – Heptachlor epoxide

C. .... Imidan

PLATE V

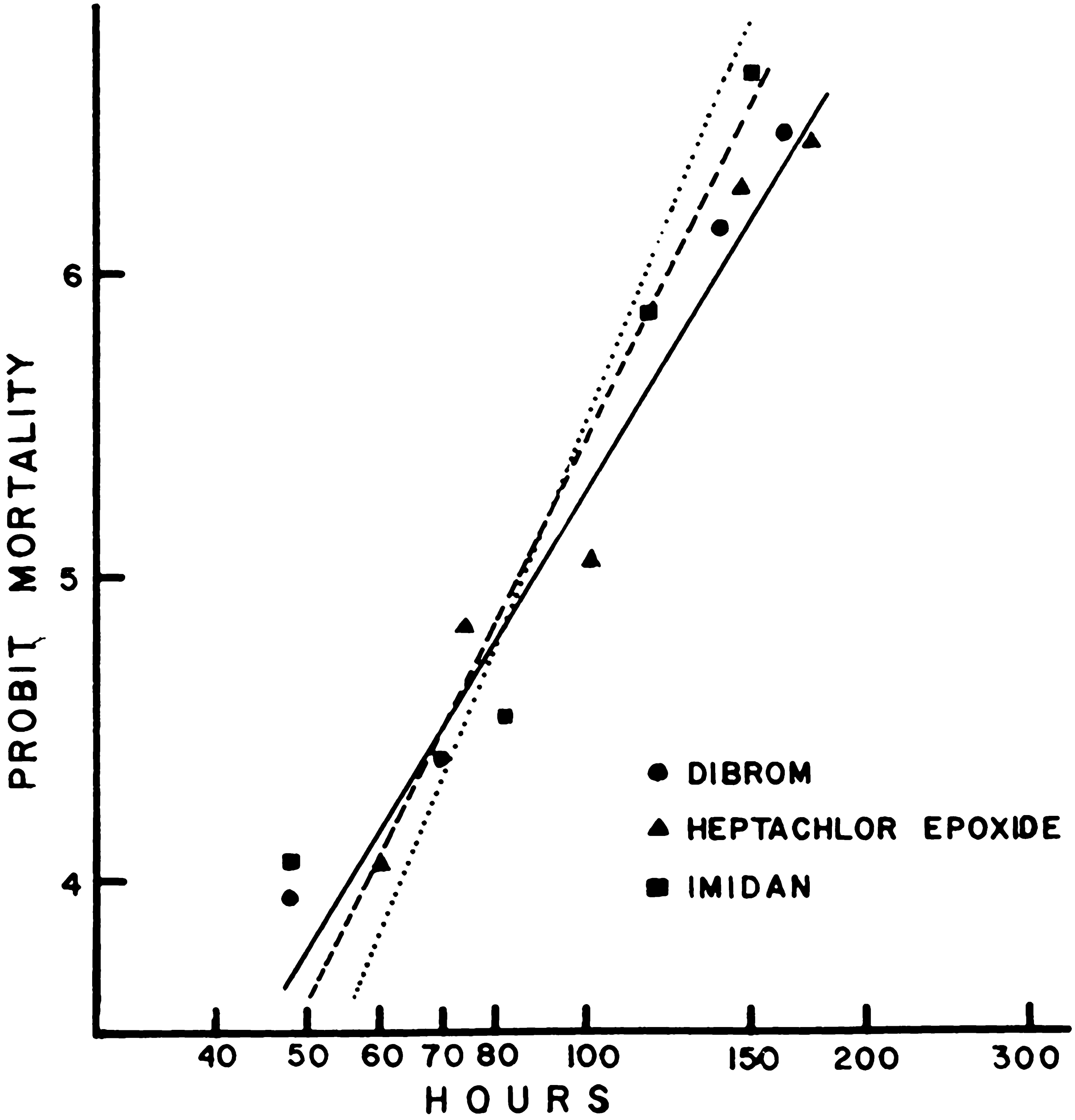
PROBIT MORTALITY
6
5
4
40
50
60
70
80
100
150
200
300
HOURS
● DIBROM
▲ HEPTACHLOR EPOXIDE
■ IMIDAN

Plate VI. Log time-probit mortality lines for cocooned pupae of the alfalfa weevil tested against 100mg/cm^2 of each of the following insecticides:

A. ———————— Dibrom

B. – – – – – – – – Heptachlor epoxide

C. .... Imidan

PLATE VI

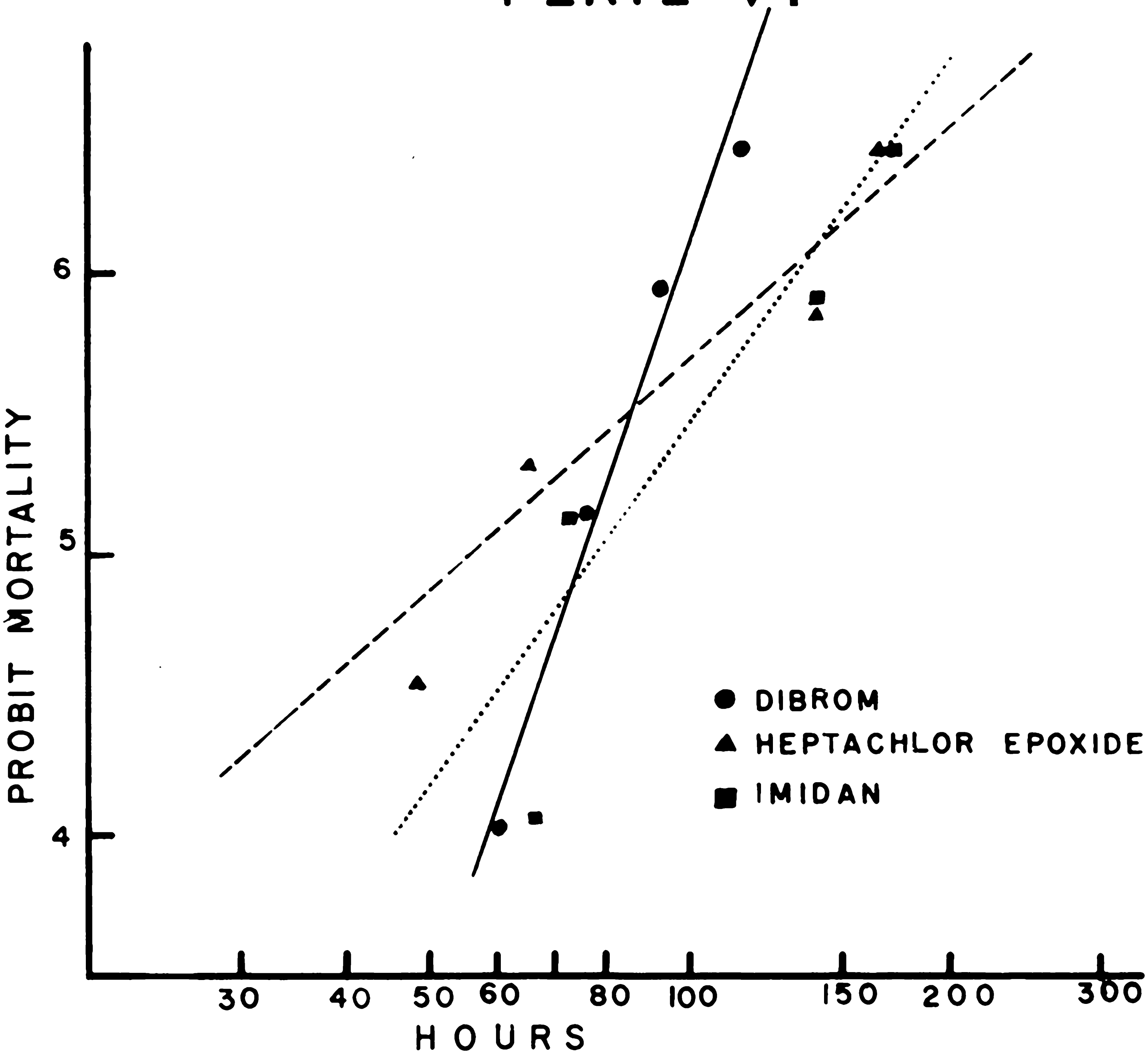
PROBIT MORTALITY
6
5
4
30
40
50
60
80
100
150
200
300
HOURS
● DIBROM
▲ HEPTACHLOR EPOXIDE
■ IMIDAN

Plate VII. Log time-probit mortality lines for the adult alfalfa weevil tested against 100mg/cm^2 of each of the following insecticides:

A. ———————— Dibrom

B. — — — — — — — — Heptachlor epoxide

C. .... Imidan

PLATE VII

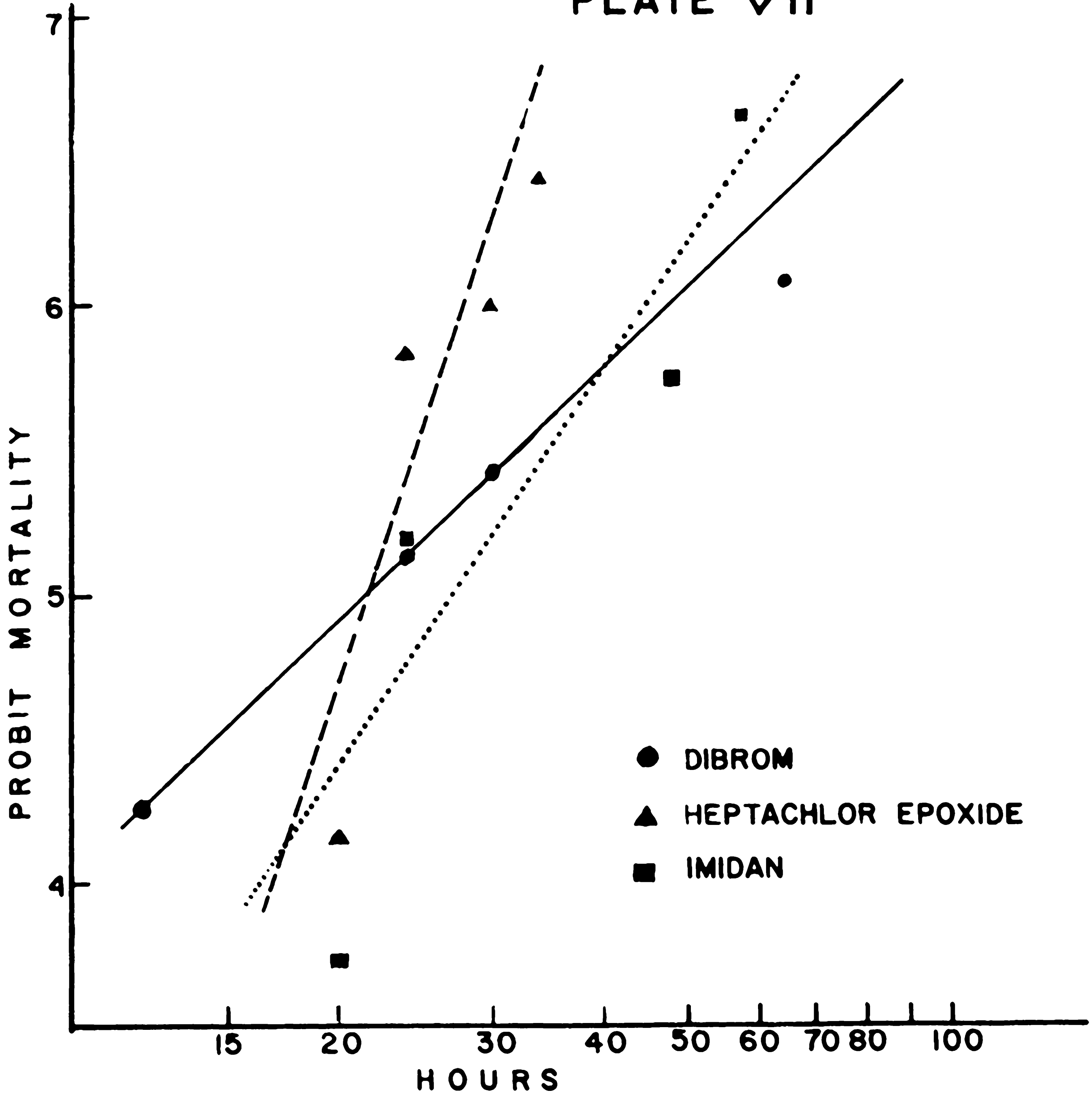
PROBIT MORTALITY
7
6
5
4
15
20
30
40
50
60
70
80
100
HOURS
● DIBROM
▲ HEPTACHLOR EPOXIDE
■ IMIDAN

Table 1. Calculated values for LT$_{50}$, LT$_{90}$[a] and beta for each life stage of the alfalfa weevil tested against Dibrom, Heptachlor epoxide, and Imidan. Blacksburg, Va., 1961-62.

Dibrom	1st	2nd	3rd	4th	Pupae (n)	Pupae (c)	Adults
LT50	5.6	13.6	23.5	50.5	89.0	75.0	21.5
LT90	11.0	31.0	45.2	100.0	160.0	104.0	60.0
beta	4.36	3.55	4.40	4.53	5.11	8.83	2.89
Heptachlor epoxide							
LT50	6.3	17.0	26.5	42.5	86.0	56.0	21.5
LT90	13.9	32.0	50.2	65.1	141.0	170.0	30.0
beta	3.6	2.83	4.44	6.62	5.29	2.72	9.09
Imidan							
LT50	5.4	12.9	23.0	80.0	86.0	76.0	26.6
LT90	16.5	35.0	50.1	140.0	137.0	158.0	51.0
beta	2.4	2.90	3.69	3.97	7.55	4.11	4.66

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[a] Hours

A word of explanation is needed to clarify the apparent anomaly of cocooned pupae being killed sooner than naked pupae. In this case, the cocooned pupae were not in contact with the residues as were the naked pupae, and further development occurred which permitted young adults to emerge. These adults died almost immediately upon contact with the insecticide residues. On the other hand, the naked pupae were in contact with these insecticide residues and did not develop into adult weevils. They were able, in all cases, to remain alive for long periods of time (Plate V). By contrast, cocooned pupae mortality was dependent upon length of time required to emerge from their cocoon. It is probable that newly-emerged adults were not fully sclerotized and this, along with physiological changes, accounted for their rapid mortality.

Each of the three insecticides dissolved parts of the insect exoskeleton upon contact. This was especially noticeable around the mouthparts. Dibrom caused considerable breakdown of the exoskeleton immediately upon contact. Heptachlor epoxide was somewhat slower but, in time, was almost as destructive to the exoskeleton as Dibrom. Imidan was not as harmful to the insect in this manner as the other two insecticides.

In every case the three insecticides inhibited feeding, molting, or maturation to another life stage. The one exception to inhibition of maturation was that of cocooned pupae developing into adults. However, as stated previously, these young adults succumbed almost immediately to the insecticides.

SUMMARY AND CONCLUSIONS

Dibrom, Heptachlor epoxide, and Imidan were tested against various life stages of the alfalfa weevil. These three insecticides were chosen because of the differences in their chemistry and activity. Dibrom, a volatile organophosphate, could be expected to act fast but have a relatively limited residual life. Heptachlor epoxide as a cyclodiene compound should have exhibited the characteristic latent period of from two to six hours before symptoms of its activity appeared. The residual activity should be great. Imidan, a more stable organophosphate than Dibrom, would be expected to be somewhat intermediate between the other two in speed of action and residual activity.

Each insecticide was impregnated in filter paper at the dosage of 100 micrograms per square centimeter of filter paper surface. The insects were introduced on to the filter paper for continuous exposure to the insecticide residue. Mortality and certain other effects were recorded according to time of exposure.

In general, responses of the alfalfa weevil to the three insecticides were comparable. One insecticide may have been quicker acting than the other two against a particular life stage, but the differences in the effects of the insecticides were not great.

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ABSTRACT

The purpose of this investigation was to test three insecticides on all the life stages of the alfalfa weevil.

The objectives were to obtain information on the toxicity of certain insecticides on the alfalfa weevil in the laboratory, to establish standard susceptibility curves of the life stages of the alfalfa weevil to the insecticides tested, to establish a basis for insect resistance, to compare the speed of action of certain classes of insecticides and to observe the effects of certain insecticides on molting, feeding and other processes that may be affected.

Dibrom, heptachlor epoxide, and Imidan were tested against various life stages of the alfalfa weevil. These three insecticides were chosen because of the differences in their chemistry and activity. Dibrom, a volatile organophosphate, could be expected to act fast but have relatively limited residual life. Heptachlor epoxide as a cyclodiene compound should have exhibited the characteristic latent period of from two to six hours before symptoms of its activity appeared. The residual activity should be great. Imidan, a more stable organophosphate than Dibrom, would be expected to be somewhat intermediate between the other two in speed of action and residual activity.

Each insecticide was impregnated in filter paper at the dosage of 100 micrograms per square centimeter of filter paper surface. The insects were introduced on to the filter paper for continuous exposure to the insecticide residue. Mortality and certain other effects were recorded according to time of exposure.

In general, responses of the alfalfa weevil to the three insecticides were comparable. One insecticide may have been quicker acting than the other two against a particular life stage, but the differences in the effects of the insecticides were not great.

In every case, the three insecticides inhibited feeding, molting, or maturation to another life stage.