GREENBUG CONTROL

BY

APPLICATION OF INSECTICIDES

TO THE SOIL

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By

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VITA

James Austin Baker was born in Omaha, Nebraska, January 3, 1917. He moved to Covina, California in 1920 with his parents.

His education began at the Covina grammar school. He attended Covina Union High School for two years, then transferred to the Monrovia-Arcadia-Duarte High School located in Monrovia, California, where he graduated in June, 1935. Before enrolling in Chaffey Junior College, Ontario, California in the fall of 1935, he spent a summer working for Los Angeles County Department of Agriculture as a fruit and vegetable inspector.

In 1936 he was employed by the Wilcox Nurseries, Montebello, California, as an assistant entomologist. There, he made frequent inspections of nursery stock and applied insecticides and fungicides to the nursery stock for the control of horticultural insects and diseases.

He left the nursery after the completion of one year to accept a position with the California State Department of Agriculture. The Department assigned him to insect eradication work connected with the Bureau of Pest Control. This included surveying areas to determine the amount of infestation and the application of insecticides to infested trees and shrubs.

In January, 1942, he enlisted in the United States Army Air Force. His army service continued until December, 1945. After his discharge from the Army, he enrolled at Oklahoma A. and M. College where he majored in Entomology in the school of Agriculture. He received the Bachelor of Science degree in May, 1948.

PREFACE

During June, 1948, this problem concerning the translocation of insecticides was selected as the basis of my research for graduate work directed toward the degree, Master of Science in Entomology, at the Oklahoma A. and M. College.

The physiological effects of some of the newer organic insecticides upon the plant growth as well as the insecticidal action upon insects feeding on plants grown on soil which previously had had the insecticide applied to it have been problems of interest to the writer for some time. It had been previously shown that certain insecticides are absorbed through the roots and translocated throughout parts of the plants, and it has been my desire to carry out similar experiments using some of the newer insecticides.

I am greatly indebted to Dr. R. G. Dahms, the supervisor of these experiments, for his profitable suggestions concerning the techniques used in this research, for the use of his equipment and greenhouse space, and his most constructive criticisms concerning the work performed in the experiments and in the writing of the thesis.

I am also indebted to Dr. Donald Ashdown and Dr. Charles H. Brett for their constructive criticisms on the work done on the problem and the preparation of this thesis.

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INTRODUCTION

Indirect insect control by the application of insecticides to the soil in which plants are to be grown is a relatively new phase of entomology.

The application of sodium selenate to the soil in 1936 (2)[1] was probably the first recorded instance of insect control by this method. This procedure and insecticide, even though it was the earliest approach by soil application to preventing insect damage, is still in use today.

Recently, several new organic insecticides have been developed, a few of which, if applied to the soil, are absorbed through the roots of plants and are translocated to parts of these plants.

The author became interested in the effect insecticides added to the soil had upon the germination of seeds and subsequent growth and vigor of plants grown in such treated soils.

Experiments were carried out to determine whether certain insecticides applied to the soil were absorbed and translocated in sufficient quantities to control insects feeding on the leaves of the plants.

The greenbug, Toxoptera graminum Rondani, was used in these tests. This aphid is of major economic importance on small-grains in Oklahoma. Ward barley (C.I. 6007) was used as the host plant. This is a variety which is very susceptible to greenbug damage and is one of the leading winter barleys grown in Oklahoma.

Preliminary experiments were undertaken to determine which of the newer insecticides were translocated; which, if added to the soil, did not result in injury to the plant; and which possessed insecticidal properties.

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[1] Numbers in parenthesis refer to Literature cited, page 28.

The first portion of "Results" will indicate the data obtained from the preliminary experiments.

The remaining portion involves the use of one insecticide, parathion, o, o-diethyl o-p-nitrophenyl thophosphate.[2]

REVIEW OF LITERATURE

The first report of insect control by the application of chemical compounds to the soil in which plants are to be grown appeared approximately thirteen years ago.

In 1936 Hurd-Karrar and Poos (2) grew wheat plants in soil containing three p.p.m. of selenium as sodium selenate (Na_2SeO_4). The apple-grain aphid, Rhopalosiphum prunifoliae, Fitch, and the red spider, Tetranychus telarius (L), died within a few days when allowed to feed upon these plants.

Granger and Leiby (1) applied parathion as a fifteen per cent wettable powder to the soil at the rates of 0.25, 0.50, 1.00, and 2.00 grams of the material per 500 grams of air-dried soil. Nasturtium, bean, squash, and potato seedlings were used as test plants and the black bean aphid, Aphis rumicus Linn: Mexican bean beetle larvae, Epilachna vervestis (Muls), two-spotted spider mite, Tetranychus bimaculatus (Harvey), the melon aphid, Aphis gossypii Glov., and the potato aphid, Macrosiphum solanifolii (Ashm), were used as the test insects.

In these tests, seeds were planted in soil in which parathion had been thoroughly mixed. Two weeks later, different parts of these plants were infested with certain of the insects to find if parathion had been absorbed

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[2]For simplification in reading this report, Parathion, the selected common name of the compound o, o-diethyl, o-p-nitrophenyl thiophosphate, which has been approved by the Bureau of Entomology and Plant Quarantine will be used.

by the roots and translocated throughout the plant. Other tests were made at two-week intervals.

At the time of the tests, the various parts of the plants to be tested were removed and placed in vials of water and kept at a constant room temperature of 26°C.

Measurements of the plants were taken at regular time intervals to determine the effect of the toxicant upon the growth of the plants.

Their results were as follows:

They found that black bean aphids that had been placed on nasturtium plants which were grown in soil containing two grams of the material showed one hundred per cent mortality at the end of forty-eight hours. After eleven weeks the toxicity appeared to have dropped below insecticidal levels.

According to their experiments, the per cent of mortality of insects feeding on the plants decreased as the amount of parathion per pot was decreased. Also, the workers stated that reproduction was maintained by the aphids that had been feeding on plants which had been growing in soil containing 0.25 gram of the parathion. Germination of the nasturtium seedlings was apparently not hindered. The germination of bean seeds was reduced when they were placed in soil containing one and two grams of parathion. Retardation of growth was apparent in all cases and was especially noticeable where two grams of parathion were mixed with the soil. The maximum toxic effect to the aphids was six weeks after the seeds were planted.

These workers placed bean beetle larvae in covered petri dishes and fed them bean seedlings grown in parathion-treated soil. No kill of the larvae was obtained on plants grown in soil containing 0.25 gram of parathion, but as the amount of parathion in the soil was increased, there was a corresponding increase in the mortality of the bean beetle larvae fed on plants grown

in such soil. The range of the per cent of kill of the larvae was thirty to one hundred per cent. The highest mortality was found three weeks after planting the seeds.

No control of the two-spotted mite was indicated at any time when the mites fed on bean plants grown in parathion-treated soil.

They obtained some dwarfing and rosette-like growth on potato plants when parathion was applied to the soil. Potato aphids feeding on potatoes grown in soil containing two grams of material showed a mortality of one hundred per cent at four, five, and six weeks after planting. At the 0.50 gram level, one hundred per cent mortality resulted only after six weeks. Twenty per cent kill resulted at the end of six weeks when aphids were placed on plants which had been growing in soil containing 0.25 gram of parathion.

Granger and Leiby obtained one hundred per cent kill of the melon aphids when they were caged on young squash plants that were grown in soil containing two grams per pot. This kill was obtained on eight-week-old plants. The maximum of seventy per cent mortality was indicated when aphids fed on plants that were grown in soil containing one gram of parathion per pot. None of the aphids died when fed on plants grown in soil containing 0.25 gram of material per pot.

A test was conducted by Granger and Leiby to determine if any change in the parathion was taking place when the material was applied to the soil three, seven, and ten days before the seeds were planted. Their preliminary tests indicated that deterioration of the insecticide might take place.

Questel and Connin (4) obtained ninety-four to one hundred per cent control of the European corn borer, Pyrausta nubilalis Hbn., when this insect was allowed to feed on corn plants which were grown in soil mixed with parathion at the rate of two grams per five-inch flower pot. The material was absorbed by the roots and translocated to other parts of the plant.

These authors applied a water suspension of parathion, containing two grams of the material, to each pot which contained plants that had been grown in soil free from parathion. At the end of one week, five per cent control of European corn borer larvae was obtained when the larvae fed on leaves from these plants. Ninety-six per cent of the larvae that fed on portions of corn stalks were dead at the end of one week. At the end of two weeks, one hundred per cent mortality was obtained when both leaves and stalks were fed to the larvae.

Smith and Clifford[3] applied parathion spray containing two pounds of twenty-five per cent wettable powder per one hundred gallons, to twelve vegetables under greenhouse conditions. The edible portions of the plants, in all cases, were protected from receiving deposits of the toxicant at the time of application. Subsequent analyses were run on these portions to determine the amount of parathion that had been translocated to such edible portions.

> "Analyses of the protected portions showed no parathion in shelled lima beans or tomato; less than 0.01 p.p.m. in cucumber, pepper and radish; 0.01 to 0.04 p.p.m. in snap beans and beets; and 0.13 to 0.34 p.p.m. in cabbage, collards, and kale also showed a reading of 0.15 p.p.m. for parathion. Unbagged lima beans showed 11.2 p.p.m. of parathion whereas bagged beans from the same plant showed none."

Field tests indicated that snap beans sprayed during the blossoming period did not contain any parathion at time of harvesting. By spraying when the beans were 1.5 inches long, parathion was present at 0.02 p.p.m. when harvested eight days later. After two applications, 0.02 p.p.m. of

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[3] Floyd F. Smith and Paul A. Clifford, "Translocation of Parathion from Foliage Application." Unpublished paper that was given at the December meeting of Amer. Assoc. of Econ. Ent. in New York. 1948.

parathion was present in bagged cucumbers and summer squashes. Shelled peas showed no traces of parathion at harvest time which was fourteen days after treatment, whereas 0.05 p.p.m. parathion was present in the vine.

Strawberries sprayed with parathion in the blossom and young fruit stage showed 0.02 p.p.m. of parathion at time of harvest. Applying the insecticide eight days before harvest, 0.23 p.p.m. parathion was recovered from the berries and 3.05 p.p.m. from the stems and caps.

Four days after parathion had been applied as an aerosol (one gram per one hundred cubic feet), rose leaves contained a maximum of 33.0 p.p.m. Nine days later no parathion was found in the rose leaves. A fumigating action was noticed upon two-spotted spider mites present on the leaves. Seventy days after applying the insecticide to the soil 15.1 p.p.m. of parathion was obtained from the leaves of wild cherry. Parathion was applied at the rate of five hundred pounds per acre.

Wolfenbarger (5) used potato plants to compare the action of the newer phosphatic insecticides with a phosphoric acid formulation upon plant growth and yields when the materials were applied to the soil.

The results indicated that the actions of both materials are similar. Stimulated growth reactions were obtained in each test and corresponding yields were in response to nutritional ingredients of the phosphatic materials that were applied to the soil in which the plants were grown.

MATERIALS USED

Four insecticidal materials, benzene hexachloride, chlorinated camphene, chlorinated naphthene, and parathion, were used in the experiments.

The benzene hexachloride (hexachlorocyclohexane) used was a wettable powder containing twelve per cent gamma isomer, formulated by Thompson-Hayward Chemical Company, Kansas City, Missouri.

The toxaphene used was a twenty-five per cent wettable powder of chlorinated camphene and was obtained from the Hercules Powder Company, Wilmington, Delaware.

The chlorinated naphthene ("Compound 118") used was a twenty-five per cent wettable powder manufactured by Julius Hyman and Company, Denver, Colorado.

The parathion was manufactured by the American Cyanamid Company, New York, N. Y. Formulations containing a twenty-five per cent wettable powder and five per cent dust were used in these tests. Parathion was used very extensively and will be discussed in more detail than the other insecticides.

Parathion was first formulated in I. G. Farben research laboratory at Elberfeld, Germany. The material was originally known under the code name, "E 605." Allied scientific teams scouring the defeated enemy country took this formulation out of Germany and brought it to the United States. The American Cyanamid Company, New York, N. Y., formulated the chemical compound as it is today. For a short period of time the material manufactured by this company went under the code number "3422," the trade mark "parathion" being accepted generally in December, 1947. It is an ester of thiophosphoric acid. Its properties are as follows: boiling point, 375°C. at 760 mm. pressure; specific gravity, 1.26; weight per gallon, approximately 10.5 pounds; vapor pressure, 0.0006 mm. of mercury at 24°C.; can be purified to approximately 95 per cent; is very slightly soluble in water (20 p.p.m.); completely miscible in many organic solvents such as esters, alcohols, ketones, ethers, aromatic or alkylated aromatic hydrocarbons, and animal and vegetable oils; is rather insoluble in such parafinic hydrocarbons such as petroleum ether, kerosene, and refined spray oils (about two per cent) unless a mutual solvent is used; is stable to hydrolysis in distilled water, hard water containing

as much as 650 p.p.m. of dissolved solids, and in acid solution. It is readily hydrolyzed in the presence of alkaline material such as lime, sodium hydroxide, lime sulfur, and Bordeaux mixture, and calcium arsenate containing an excess of lime.

METHOD OF PROCEDURE

For the preliminary test, forty six-inch flower pots were filled two-thirds full with a finely screened Reinach sandy loam soil. This soil was fairly high in organic matter and possessed properties very suitable for greenhouse work. A weighed amount of insecticide was uniformly distributed on top of the soil and then covered with approximately one-fourth to one-half inch of soil. Twenty seeds were then placed on top of this layer and covered with soil. Care was taken to prevent any of the insecticide from directly contacting the seeds. The pots were then placed in a metal pan containing three inches of water. The soil in the pots absorbed the water quite readily.[4]

The procedure for the second test was the same as for the preliminary experiment. Six different concentrations of parathion were used in this experiment: 0.06, 0.13, 0.25, 0.50, 0.75, and 1.00 gram of actual toxicant per pot. Ten pots were prepared for each amount. Ten other pots containing only soil were used as untreated checks.

A third test was set up to determine if there was a difference in the results when parathion was mixed thoroughly with the soil rather than applied as a layer. At this same time, experiments were made to determine if parathion dust formulations would give results comparable to those obtained where wettable powders were used.

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[4]The term "layering method" will appear throughout the remainder of this paper and will refer to the method of procedure just described.

Two dosage levels of parathion, 0.25 and 0.03 gram of actual toxicant per pot, obtained by adding 5.0 and 0.625 grams of five per cent dust, were applied to the soil by the layer and mixing techniques. Two replicates (one pot each) were used for the four treatments.

Three dosage levels of parathion, 0.25, 0.06, and 0.03 gram of actual toxicant per pot, obtained by adding 1.00, 0.25, and 0.125 grams of twenty-five per cent wettable powder, were applied to the soil by the layer technique and by mixing with the soil. Two or four pots per treatment were used at each concentration and for each method of application. Twenty seeds of Ward barley were planted in each pot in all cases.

Experiments using a seed treatment method were set up in the following manner. Ten cc of barley seeds, approximately 185 seeds, were mixed thoroughly with five grams of twenty-five per cent wettable powder. Calculations, after weighing excess insecticide, showed that each seed received approximately 0.41 mg of actual toxicant. Seeds were then selected at random, and twenty seeds were planted in each of two pots containing non-treated soil. The same procedure was repeated, using five grams of a five per cent dust. Each of these seeds received approximately 0.08 mg of actual toxicant.

To determine if any of the toxic materials from the insecticide had been translocated to the leaves and stems of the plants, greenbugs were placed on seedling plants that had been cut off a short distance above the soil level two weeks after the seeds were planted in the soil.

The equipment used for this experiment consisted of a transparent celluloid tube about seventy mm long and twenty-six mm in diameter, over one end of which a piece of cloth had been glued. Seedling plants that had been grown in the treated soil were cut off a short distance above the soil level and placed between the halves of a slit rubber stopper with the cut end of the

stem below and the leaves above. The small end of the stopper was then inserted in a shell vial containing water so that the cut end of the stems were immersed, while the larger end was fitted tightly into the open end of the celluloid tube so the leaves on which the greenbugs were to feed were on the inside. (Figure 1)

One plant from each pot was used for each test. Thirty ten-day-old adult greenbugs were placed on each plant. Records of greenbug mortality and number of nymphs produced were taken at twenty-four, forty-eight, and ninety-six-hour intervals.

Phytotoxic observations to determine any effects on the growth of the seedlings when parathion was added to the soil, height measurements, and the physical condition of the plants were noted at intervals and compared with those of similar plants grown in pots of soil not treated with parathion.

Tests were conducted to study the feeding preference of greenbugs for barley plants grown in parathion-treated or untreated soil. Plants that had been growing in treated soil (0.50 gram of actual toxicant, obtained by applying in the layering method two grams of twenty-five per cent wettable parathion powder) and untreated soil were cut off at the soil level and transferred to six-inch pots filled with moist sand. Plants of each treatment were arranged alternately in a circular pattern in the pot of moist sand. A celluloid cage, approximately eighteen inches in height and five inches in diameter, was then placed over the plants of each pot. Approximately seventy winged adult greenbugs were caged on these plants and the number of greenbugs on each was recorded at certain intervals.

Figure I. Laboratory cage used to determine the rate of reproduction and mortality of greenbugs.

RESULTS

Benzene Hexachloride, Chlorinated Camphene, Chlorinated Naphthene, and Parathion.

The results of the preliminary test, in which Ward barley plants were grown in soil containing different insecticides, are shown in table I. Parathion added to the soil stimulated plant growth, while benzene hexachloride showed a definite detrimental effect as indicated by the number of leaves on the plant and the height of the plants twenty-one days after the seeds were planted. Parathion-treated plants were approximately an inch taller than the check plants while those treated with benzene hexachloride averaged more than three inches shorter. Plants grown in soil containing chlorinated camphene and chlorinated naphthene compared favorably with those grown in non-treated soil, having the same number of leaves and only a slight variation in the average height of the plants.

Table I. Effect of various insecticides when applied to soil on growth of Ward barley plants when grown under greenhouse conditions.

	Actual amount of toxicant.		Measurements 21 days after treatment.	
Insecticide	Grams per pot	Pounds per acre	Average number of leaves	Average height of plants per cm
Benzene hexachloride	0.96	837.5	2.0	3.2
Chlorinated camphene	1.00	837.5	2.7	6.9
Chlorinated naphthene	1.00	837.5	2.7	6.4
Parathion	1.00	837.5	3.0	7.5
Check	0.00	000.0	2.7	6.6

Plants grown in soil treated with these insecticides varied considerably in color. The plants grown in soil containing chlorinated naphthene and those grown in untreated soil were very similar in appearance. They

were of a bright green hue and appeared to be in a healthy condition. Chlorinated camphene-treated plants were of a slightly darker shade of green than the checks, while the plants treated with parathion were still darker. All of these plants appeared to be healthy. The plants growing in benzene hexachloride-treated soil had a grayish appearance and were somewhat shriveled and dry.

Adult greenbugs placed in cages containing plants which had grown in soil treated with parathion usually died before they reproduced nymphs. (Table II) Some greenbugs died when caged on plants grown in soil treated with chlorinated naphthene, chlorinated camphene, and benzene hexachloride, but the mortality was relatively low, especially as the plants became older. The rate of reproduction of greenbugs feeding on these plants was about equal to that on the checks except that on thirty-five-day-old plants grown in benzene hexachloride-treated soil. A lower reproduction rate in this case was probably due to the poor condition of the plants.

Table II. Rate of reproduction and mortality, at indicated time intervals, of 30 adult greenbugs that were allowed to feed on Ward barley plants grown in soil treated with various insecticides.

	14 Days		21 Days		35 Days**		49 Days	
Insecticide	Adult mortality 96 hrs.	Number nymphs per adult per day	Adult mortality 96 hrs.	Number nymphs per adult per day	Adult mortality 96 hrs.	Number nymphs per adult per day	Adult mortality 96 hrs.	Number nymphs per adult per day
Benzene-hexachloride	9	1.50	11	0.04	1	0.28	--	----
Chlorinated camphene	8	1.41	2	0.06	1	0.39	--	----
Chlorinated naphthene	8	1.54	4	0.11	4	0.46	--	----
Parathion	30*	0.00	30	0.00	28	0.10	22	0.42
Check	4	1.55	0	0.02	0	0.50	0	0.00

* All dead at end of 48 hours.

** Experiments discontinued at end of 35 days except for plants grown in parathion-treated soil.

Since the greenbug mortality was rather low on plants that had grown in soil treated with chlorinated naphthene, chlorinated camphene, and benzene hexachloride, the tests on these plants were discontinued after thirty-five days. However, plants grown in parathion-treated soil were tested and compared with checks at given intervals for twenty weeks. (Table III)

Table III. Rate of reproduction and mortality, at indicated time intervals, of 30 adult greenbugs that were allowed to feed on Ward barley plants grown in soil treated with parathion.

	78 Days		86 Days		105 Days		140 Days	
Insecticide	Adult mortality 96 hrs.	Number nymphs per adult per day	Adult mortality 96 hrs.	Number nymphs per adult per day	Adult mortality 96 hrs.	Number nymphs per adult per day	Adult mortality 96 hrs.	Number nymphs per adult per day
Parathion	30	0.00	25	0.51	30	0.27	28	0.69
Check	0	0.59	0	0.69	0	0.71	0	0.19

The plants grown in non-treated soil failed to kill any adult greenbugs caged on them after the plants were two weeks of age. However, plants grown in soil treated with parathion killed a rather high percentage of greenbugs caged on them every time they were tested throughout the twenty weeks. In fifty per cent of the tests which were run on the parathion-treated plants, one hundred per cent mortality of the adult greenbugs was obtained in ninety-six hours.

The few greenbugs which survived on the treated plants reproduced nymphs at rates varying from 0.10 to 0.69 nymphs per adult day. The rate of reproduction increased on the older plants indicating that these plants were less toxic than at the earlier stages of growth.

Parathion.

After completing the series of preliminary tests, two tests were run to determine if possible the lowest amount of parathion that could be added to the soil and still produce plants that were toxic to greenbugs.

In the first series of tests, enough twenty-five per cent wettable parathion powder was added to the soil to supply from 1.00 gram to 0.06 gram of actual toxicant per pot.

The second series was run on soil that received twenty-five per cent wettable parathion powder in amounts to supply from 0.25 to 0.03 gram of actual toxicant per pot. Similar tests were also run in which the same amounts of parathion were supplied in the form of a five per cent dust. Each amount of toxicant was applied also by two different methods. One method was to mix the parathion thoroughly with the soil; the other was to apply the toxicant by the layering method previously described. Along with this series of tests, plants grown from seeds treated with parathion were also tested. Seeds were treated both with twenty-five per cent wettable powder and five per cent dust.

The first series of tests was continued for six weeks, at which time all tests of plants except those receiving 0.06 gram actual toxicant were discontinued as results showed that larger amounts of the insecticide were unnecessary. (Table IV) The tests were continued on these plants for nine weeks. At this time, the plants which had been growing in soil which received 0.06 gram of parathion per pot continued to give one hundred per cent kill of adult greenbugs and the insects had at no time reproduced at a rate of more than 0.87 nymph per adult per day. At nine weeks, the number of nymphs reproduced was only 0.09 for an adult in a day.

Table IV. Rate of reproduction and mortality, at indicated time intervals, of 30 adult greenbugs that were allowed to feed on Ward barley plants grown in soil treated with different amounts of parathion.

Grams of actual toxicant applied per pot	20 Days		27 Days		43 Days****		62 Days	
	Adult mortality 96 hrs.	Number nymphs per adult per day	Adult mortality 96 hrs.	Number nymphs per adult per day	Adult mortality 96 hrs.	Number nymphs per adult per day	Adult mortality 96 hrs.	Number nymphs per adult per day
1.00	30*	0.11	30	0.00	30	0.00	--	----
0.75	30*	0.16	23	0.00	***	***	--	----
0.50	30*	0.00	30	0.01	29	0.24	--	----
0.25	30*	1.16	30	0.00	30	0.50	--	----
0.13	30*	0.25	30	0.00	30	0.77	--	----
0.06	30*	0.75	30	0.22	29	0.87	30	0.09
Check	1**	0.52	0	0.59	0	0.69	0	0.72

* All greenbugs dead at end of 48 hours.
** Caught in cotton holding plant.
*** No plants available, caused by poor germination.
**** Experiments discontinued on all plants except those grown in soil containing 0.06 gram parathion.

One hundred per cent of the insects feeding on the plants were killed within ninety-six hours except in three cases. The first was on plants which were four weeks old and had been grown in soil receiving 0.75 gram of actual toxicant. The mortality of the adults feeding on these plants was seventy-seven per cent, and the insects did not produce any nymphs.

A second series of tests was run to determine if parathion applied at rates below 0.60 gram per pot would be successful in such tests. These tests resulted in one hundred per cent mortality of greenbugs feeding on plants grown in soil treated with 0.25 and 0.06 gram of actual toxicant per pot (both wettable powder and dust) which was applied by both methods (layering method and mixing with the soil) except in one case in which ninety-two per cent mortality resulted. (Table V) This occurred from three-week-old plants which had grown in soil treated with 0.06 gram of actual toxicant (twenty-five per cent wettable powder), applied by mixing thoroughly with the soil. In

eighty-three per cent of the tests in which 0.25 gram of parathion was added to the soil, there were no nymphs reproduced by the adults feeding on the plants. The remaining tests showed only 0.12 and 0.25 nymphs reproduced per adult per day. In half of the tests, no nymphs were produced by the adults when feeding on plants grown in soil treated with 0.06 gram of insecticide. In the remaining cases, a smaller number of nymphs was reproduced on plants where parathion had been applied by the layering method. Considering all tests, the highest rate of reproduction for greenbugs feeding on plants grown in soil containing 0.06 gram of parathion per pot was 0.61 nymph per adult per day, whereas the highest rate of reproduction for those feeding on plants grown in non-treated soil was 2.05 nymphs per adult day.

Table V. Rate of reproduction and mortality, at indicated time intervals, of 12 adult greenbugs that were allowed to feed on Ward barley plants grown in soil treated with different amounts of parathion.

Grams of insecticide applied per pot		20 Days		29 Days		35 Days	
		Adult mortality 96 hrs.	Number nymphs per adult per day	Adult mortality 96 hrs.	Number nymphs per adult per day	Adult mortality 96 hrs.	Number nymphs per adult per day
Check		0	0.83	0	0.60	0	2.05
Layering Method:							
0.25	W	12	0.12	12	0.25	12	0.00
0.06	W	6*	0.00	6*	0.13	6*	0.00
0.03	W	7	0.39	12	0.29	11	0.31
0.25	D	12	0.00	12	0.00	12	0.00
0.03	D	5	0.06	8	0.37	9	0.10
Toxicant mixed with soil:							
0.25	W	12	0.00	12	0.00	12	0.00
0.06	W	11	0.00	12	0.61	12	0.35
0.03	W	6	0.22	2	0.75	5	0.44
0.25	D	12	0.00	12	0.00	12	0.00
0.03	D	3	0.79	1	0.47	4	0.17
Seed Treatment:							
0.0082	W	0	0.82	5	0.60	1	1.04
0.0016	D	2	0.43	3	0.10	3	1.00

W -Twenty-five per cent wettable powder.
D -Five per cent dust.
* Only six greenbugs available.

The mortality of greenbugs feeding on plants grown in soil treated with 0.03 gram of parathion was somewhat lower than when feeding on plants grown in soil treated with 0.25 and 0.06 gram of insecticide. However, the mortality was higher in all cases than when the insects fed on the plants grown in untreated soil. The highest percentage of greenbug mortality was found on plants which had grown in soil where twenty-five per cent wettable powder was applied by the layering method. Greenbugs feeding on plants grown in soil treated with 0.03 gram insecticide per pot reproduced at a lower rate in all but two tests than those feeding on plants grown in non-treated soil. In all cases there was some mortality of the adult greenbugs when feeding on plants grown in treated soil; however, there was no mortality of insects in any test when feeding on plants grown in non-treated soil.

Greenbug mortality in all cases was less than fifty per cent when the insects were confined to plants which were grown from the seeds treated with twenty-five per cent wettable parathion powder and five per cent parathion dust. The rate of reproduction of the adult insects feeding on these same plants varied from 0.10 to 1.04 nymphs per adult per day.

In connection with the foregoing experiments, three other tests were made. One was made to determine if the greenbugs showed a preference for feeding on plants grown in soil treated with parathion over those grown in untreated soil. In another test the effect of parathion on the rate of growth of the barley plants was investigated. A third experiment was to determine what effect parathion applied to the soil would have upon germination of the barley seeds grown at given temperatures.

Preference Test of Greenbugs.

The preference test (table VI) was initiated after it was noticed that the greenbugs seemed to live longer on plants grown in a high concentration of parathion than on lower concentrations. Upon closer observation, it was noted that greenbugs confined to plants grown in soil containing the higher dosages of parathion remained off the plants for a longer period of time than those caged on plants grown in soil containing lower amounts.

Table VI. Preference shown by greenbugs feeding on plants grown in non-treated soil compared to those grown in soil treated with 0.50 gram actual toxicant per six-inch pot.

Time after insect admitted to cage	First Test		Second Test	
	Number insects on treated plant	Number insects on untreated plant	Number insects on treated plant	Number insects on untreated plant
10 minutes	24	37	37	40
1 hour	29	30	34	35
3 hours	24	36	16	30
4 hours	27	42	27	38
6.5 hours	25	34	28	39
22 hours	5	58	1	43
24 hours	5	60	2	40
27 hours	3	47	4	41
76 hours	10	15	1	20

For the preference tests, stems of plants grown in soil containing 0.50 gram of parathion per pot and plants from non-treated soil were placed in alternating positions around the outer edge of a pot of moist sand. Approximately seventy greenbugs were caged on these plants and counts made at certain intervals. For the first six and one-half hours, slightly more insects were found on the plants grown in non-treated soil. However, there was soon a decided reduction of the number of insects on the plants grown in treated soil and an increase in the number of aphids appearing on the plants grown in

non-treated soil. Similar results were obtained when this same test was performed at a later date.

Effect of Parathion on Growth Rate of Plants.

Measurements of the rate of growth of plants growing in parathion-treated soil were made over a period of four weeks. (Table VII) These plants were grown in soil containing various amounts of parathion and the two methods of applications were used.

Table VII. The rate of growth of Ward barley plants grown in soil treated with various amounts of parathion (Layering method) as compared to plants grown in soil in which various amounts of parathion were mixed thoroughly in the soil and plants grown in non-treated soil.

Grams of actual toxicant per pot	Number of days after seed was planted			
	8 Days	15 Days	23 Days	29 Days
	centimeters	centimeters	centimeters	centimeters
Check	5.90	13.00	19.00	21.00
Layering Method:				
0.25 W	7.40	16.50	27.50	28.50
0.06 W	4.70	12.50	18.00	20.00
0.03 W	4.50	12.85	19.00	20.00
0.25 D	4.60	12.50	20.50	22.00
0.03 D	5.25	13.50	21.50	22.50
Toxicant mixed with soil:				
0.25 W	5.90	13.00	21.00	23.00
0.06 W	6.20	14.00	22.00	23.50
0.03 W	6.30	15.25	22.75	27.25
0.25 D	5.30	10.80	18.50	21.00
0.03 D	5.90	15.00	24.50	27.00
Seed Treatment:				
0.0082 W	6.60	14.20	22.30	24.70
0.0016 D	6.30	15.10	23.60	24.90

W -Wettable Powder
D -Dust

When parathion was mixed with the soil, plant growth was equal to or greater than the growth of the checks except where 0.25 gram of actual toxicant (five per cent dust) per pot was applied. In this instance, the plants were shorter until the fourth week, at which time they averaged the same as the non-treated plants.

When parathion was applied by the layering method, twenty-five per cent wettable powder applied at the rate of 0.25 gram of actual toxicant per pot caused the greatest rate of plant growth. (Figure II) The twenty-five per cent wettable powder, applied at rates of 0.06 and 0.03 gram of actual insecticide, resulted in plants having a slightly lower rate of growth than plants growing in untreated soil. Plants grown in soil containing 0.25 and 0.03 gram of actual toxicant per pot supplied as five per cent dust were taller at the conclusion of the experiment, but they were smaller for the first two weeks than plants growing in non-treated soil.

Plants grown from seeds which had been treated with parathion were consistently taller than the check plants.

Effect of Parathion on Germination of Barley Seeds.

The final experiment was conducted to determine the effect of parathion on germination of barley seeds. (Table VIII) Varying amounts of twenty-five per cent wettable powder and five per cent dust were used and seeds treated with wettable powder and dust were also included. At 80.4°F. barley seeds planted in soil mixed with 0.03 to 0.25 gram of parathion per pot germinated better than those planted in non-treated soil. When the test was conducted at 71°F., and the insecticide was applied by the layering method, the germination of the seeds was lower than the check in all cases. Under similar treatments seed germination appeared to be better at 80.4°F. than

at 71°F. In all but two cases at 80.4°F., seeds germinated better in treated than in non-treated soil. Seeds which had been treated with twenty-five per cent wettable powder germinated better than in any other test, and the seeds treated with five per cent dust germinated fifteen per cent better than those planted in non-treated soil.

Table VIII. Effect of parathion, applied at various rates by mixing with the soil and by applying it in a layer, on the germination of Ward barley seeds, at two different temperatures.

Grams of actual toxicant per pot		Per Cent Germination	
		71°F.	80.4°F.
Check		63	60
Layering Method:			
0.03	W	—	78
0.04	W	60	—
0.06	W	—	55
0.12	W	37	—
0.25	W	59	65
0.50	W	18	—
0.75	W	6	—
1.00	W	15	—
0.25	D	—	53
0.03	D	—	78
Toxicant mixed with soil:			
0.03	W	—	70
0.06	W	—	74
0.25	W	—	70
0.03	D	—	78
0.06	D	—	73
Seed treatment:			
0.0082	W	—	83
0.0016	D	—	75

W -Twenty-five per cent wettable parathion powder
D -Five per cent parathion dust

DISCUSSION

Since the release of the newer organic insecticides, research workers have been working ardently to obtain information of their possibilities in the field of entomology. Parathion has become one of the most promising of these many insecticides.

The experiments, described in this paper, have indicated that parathion, if applied to the soil, is absorbed by the roots of barley plants and translocated throughout the plant. Results also indicate that under certain conditions the rate of growth of some plants may be increased. Much more fundamental research must be done to answer the many problems that might arise. The actual toxic material that is translocated has not been determined, and it is not known what physiological conditions are responsible for the increase in vigor of plants grown in the parathion-treated soil. It has been suggested that the action of phosphorus in parathion acts as a fertilizer, but this would not explain the toxic effect on insects. Therefore, there must be something in addition to phosphorus that is absorbed from the soil and translocated to the stems and leaves. The inhibitory effect of parathion on growth at higher concentrations could be caused by an over-accumulation of phosphorus compounds which would speed up the metabolic activity in plants in excess of the normal rate of activity which in turn would either stunt growth or cause injury to the plant tissues. Closer cooperation among entomologists, plant physiologists, horticulturalists, chemists, et. al, must be attained in order to increase our knowledge of the action of chemical compounds upon tissues of plants and animals if these problems are solved.

Variations on the effect of parathion on plants have been found thereby showing a distinct correlation between the chemical compound and the plant

Figure II. Effect of parathion on the growth of barley plants. Left plants grown in non-treated soil; right plants grown in soil containing 0.25 gram of parathion.

tissues. The effect of this material upon the greenbug indicates its extreme toxicity to insect life. It was also noted that greenbugs placed on plants which had been growing in the higher concentrations of parathion took a longer time to die. This suggested the possibility of the insects being repelled by the material in the plant as was indicated by the results of the preference test shown in table VI. This suggests that through some chemical reaction the repelling factor may be associated with transpiration in plants. It is possible that through transpiration, the material might volatilize and escape into the surrounding atmosphere.

The results of the tests in this thesis indicate that amounts as low as 0.06 gram of actual toxicant (parathion) per six-inch pot are effective in controlling the greenbug. If applied on a comparative basis with the procedure of the tests described, parathion would have to be applied at the rate of approximately fifty pounds of actual material per acre. The cost of applying this insecticide to the soil at this rate in most cases would be prohibitive. However, under certain greenhouse conditions, where plants are grown for experimental purposes, treating the soil might be practical.

SUMMARY

Preliminary tests begun in October, 1948, indicated that Ward barley, which is very susceptible to the greenbug, Toxoptera graminum, Rondani, was able to absorb the toxic ingredient of parathion (o, o-diethyl o-p-nitrophenyl thiophosphate) from the soil and translocate it in sufficient quantities to kill greenbugs feeding on the plant leaves.

Barley plants grown in soil treated with three other insecticides showed little or no toxicity to greenbugs that were allowed to feed on them.

Tests were run to determine the minimum amount of parathion that could be added to the soil which would produce barley plants toxic to greenbugs. One series ranged from dosages of 1.0 gram to 0.06 gram of actual toxicant per six-inch pot. One hundred per cent of the greenbugs were killed when confined on plants grown in soil that received 0.06 gram of actual toxicant per pot. In the third test, the amount of parathion used per pot varied from 0.25 gram to 0.03 gram and application was made by both the layering and mixing methods. In this test, parathion supplied as a twenty-five per cent wettable powder and a five per cent dust were compared.

Results showed that one hundred per cent of the greenbugs were killed when caged on barley plants that had grown in soil containing 0.06 gram of parathion per pot, supplied as a twenty-five per cent wettable powder applied by either method. Greenbug reproduction was greater when the insects were feeding on plants grown in soil that contained 0.06 gram than 0.25 gram per pot. A comparison of plants grown in twenty-five per cent wettable parathion powder and five per cent dust showed little difference in the per cent of mortality of greenbugs on the plants.

When greenbugs fed on barley plants grown from seed that had been treated with a twenty-five per cent wettable powder and five per cent parathion dust, their mortality was low and rate of reproduction high.

While observing the different tests, it was noted that as the concentration of parathion in the soil became higher, a longer time was required to kill the insects on the plants growing in the soil. To determine if greenbugs were repelled from barley plants that had grown in high concentrations of parathion, the preference of this insect for plants grown in soil which contained 0.50 gram of parathion and those grown in untreated soil was compared. These results indicated that there was little difference in the

numbers of greenbugs on the plants the first six hours. However, at the end of twenty-two hours, there were many more adults on plants grown in non-treated soil than on those grown in treated soil.

A test to determine the effect of parathion on plant vigor at the various dosage rates indicated that plant growth in treated soil was almost equal to or greater than that of non-treated soil. Barley plants grown in 0.06 gram of parathion per pot showed the greatest growth gain.

In most cases, barley seeds planted in soil mixed with parathion germinated better than those planted in non-treated soil. However, a higher germination occurred when the seeds were treated with the insecticide and planted in non-treated soil. Seeds planted in soil in which the toxicant was layered showed inconsistent results.

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