TABLE 1

Mean Number of Larvae per Sweep of an Insect Net in an Alfalfa Field Treated with DDT and HETP

Date surveyed	Time since treatment	Treatment											
		Wettable DDT			DDT in oil			HETP, 50%					
								One quart per acre			One pint per acre		
		Large larvae	Small larvae	Total larvae	Large larvae	Small larvae	Total larvae	Large larvae	Small larvae	Total larvae	Large larvae	Small larvae	Total larvae
July 23	Before	13.0	21.0	34.0	16.6	20.3	36.9	18.7	26.9	45.6	18.7	26.9	45.6
July 25	11 hours	2.1	1.6	3.7	1.6	0.6	2.2	3.9	2.3	6.2	2.2	2.2	4.4
July 28	3 days	0.0	0.0	0.0	0.2	0.2	0.4	2.8	1.1	3.9	5.0	2.3	7.3
July 30	5 days	0.0	0.0	0.0	0.2	0.1	0.3	2.6	1.0	3.6	4.0	1.8	5.8
Aug. 1	7 days	0.0	0.0	0.0	0.4	0.1	0.5	2.1	0.8	2.9	3.4	3.2	6.6
Aug. 7	13 days	0.0	0.0	0.0	0.1	0.1	0.2	0.9	1.1	2.0	1.7	3.9	5.6

chlorosis and small light necrotic areas (usually circular) surrounded by darker rings. Sometimes much of the peripheral area of the leaf was necrotic. Three days after treatment this injury was general but not serious where the one-quart dosage was applied, and hardly noticeable in the one-pint dosage. A week after treatment, it was much less apparent and by cutting time, thirteen days after treatment, no injury could be detected. Undoubtedly, the dropping of the seriously injured leaves accounts for the absence of apparent injury at harvest time. Although very evident, the injury was at no time serious.

The results of this very preliminary test offer some promise for the use of HETP as a control for the alfalfa butterfly. Further work is needed on dosages, methods of application and their probable effect upon the plants. Although injury was slight in this case, caution must be used wherever HETP in oil is applied to alfalfa. The results of other studies on the control of the alfalfa butterfly will be published elsewhere. This report is not to be construed as a recommendation for the use of these materials on alfalfa but as a recording of experimental results.

Tests on Bees

J. E. Eckert

Apiculturist in the Experiment Station

THE PRODUCTION OF A MAJORITY of our food crops is influenced not only by the control of the injurious pests but also by the presence of sufficient pollinators to effect maximum yields. Many of our orchard and field crops either are dependent on or are greatly benefited by pollinating insects. Any pest control program which materially reduces the number of pollinating insects is quite likely to adversely affect the production of those crops that are benefited by the visits of pollinators.

In recent years, the increased use of chemicals in insect and weed control has caused the loss of thousands of colonies of honeybees. Poisons which are injurious to honeybees must necessarily kill the solitary bees as well. The beekeeping industry performs the important function of providing honeybee colonies for the use of agriculture and a substantial loss of colonies of bees is not only a monetary loss for their owners but may mean a greater loss to the growers of those crops serviced by the honeybees. The honeybee is the only insect that can be propagated and moved from place to place as needed for pollination services. As the solitary bees are reduced in numbers by the application of chemicals, the honeybee becomes still more important as the remaining source of essential pollinating services. Since the beekeeping industry must depend largely on the income from the sale of the byproducts of the hive, the honey, beeswax, queens and swarms, rather than on the rentals received for the pollination services, conditions must be favorable for honey production in the areas in which bees are needed to enable the beekeeper to maintain his colonies. While hives of bees can be moved from one place to another, at certain times of the year, it is physically impossible for a beekeeper to move his hives on short notice. When an operator owns from one thousand to several thousand colonies, the movement of his hives to new locations is a major operation that involves the use of considerable manpower as well as heavy trucks. The hives must be loaded in late afternoon and moved at night. The movement of a large number of colonies from any given area results in the removal of millions of pollinators, and the result from the viewpoint of pollinization is about as positive for that area as if the bees had been killed.

For these reasons, it is important for those using or recommending the use of chemicals in the control of crop pests to know how the application of any chemical will effect the balance of pollinators in the region in which the poisons are applied. Honeybees may fly a distance of from two to five miles in quest of nectar and pollen although the average distance probably, is not more than two miles. Poisonous dusts may drift in substantial quantities for two miles or more beyond the fields treated, so the danger area of poisoning also must be considered. When poisons are confined to the fields treated, the chemical hazards to pollinating insects are materially reduced. The ideal pest control program would be one in which the poisons could be confined to the fields treated and applied at a time or in such a manner as to cause no appreciable injury to pollinating insects. One of the factors to consider in devising a pest control program, is to know the probable toxicity to the pollinators of the chemicals employed. Another factor is to use chemicals that are least toxic to beneficial insects.

The production in recent years of many new chemical compounds for use as insecticides and herbacides has made it necessary for a large amount of work to be done in determining the toxicity of the various chemicals to bees and to other beneficial insects. The phosphates are probably the newest addition to a long list of useful chemicals. They have not been on the market long enough or in sufficient quantity for tests to be made on a large acreage basis. Consequently, their probable injury under field conditions will have to be deduced from various laboratory tests.

The bees used in our toxicity tests were taken from the top story of hives, usually in the morning, without smoking the colony, and placed either in individual 2- or 3-hole queen cages or 4 × 5 × ¾ inch cages. The larger cages have a wood frame with a screen fastened to the bottom and a sliding screen on top. Approximately 30 to 40 bees were run into the larger cages for each test. The individual cages were fitted with queen cell cups to hold sugar sirup while the bees in the 4 × 5 cages were fed sirup through the top screen. From a micropipette the individual bees were fed known quantities of each chemical dissolved or suspended in 20% sugar sirup. Groups of 10 or more bees were fed .01, .015, .02 or .025 ml. at each feeding, and each lot of bees was fed only once. Soon after the bees were fed, the queen cell cups were filled with 20% sugar sirup and the cages placed on a laboratory table with other cages of check bees in a room where the temperature generally varied from 70 to 80° F., although in summer the temperatures were higher. Bees in the 4 × 5 cages were fed known concentrations of the chemicals in 20% sugar sirup from inverted vials covered with parafined paper with a small hole in each center.

The study of the reaction of the bees to contact with a chemical was conducted in different ways. The bees were placed in a cage and either sprayed or dusted; or the bees were forced to walk over a sprayed or dusted surface; or bees in a nucleus or hive were sprayed or dusted and then permitted to fly.

In testing the reaction of bees to the fumigant action of a chemical bees were placed above a sprayed or dusted area or were placed in a cage or room which had been sprayed with a known concentration of the chemical. A combination of fumigant and contact action was obtained by exposing a comb to the fumes of a poison and the reaction of bees observed when they were confined on the comb.

In addition to the laboratory tests, observations were made when possible on colonies located in the vicinity of fields to which dusts or sprays were applied.

Laboratory tests are not entirely reliable because some bees obviously recover from the effects of certain poisons when they would surely die if affected in a like manner in the field and exposed to the sun, dust, and other natural conditions. Cage tests may indicate that certain chemicals shorten the natural life of bees as well as be more toxic to them within a two- or three-day period. The toxicity of a chemical is generally based on the reaction of various lots of bees within two- or three-day intervals.

Methods of making dilutions: As a general rule, each chemical was dissolved in either distilled water, acetone or 95% alcohol. The solutions were handled at room temperatures without allowances for the slight changes that occur in volume due to changes in room temperature. Solutions were made to contain not more than 2% acetone or alcohol as bees are tolerant to this concentration for three day periods. Solutions or suspensions were made just before they were fed to the bees, except in the determination of the loss of toxicity due to hydrolysis.

When caged bees were dusted with a dust containing 3% of HETP—hexaethyl tetraphosphate—31% of the bees were killed within 48 hours. The bees became greatly agitated and rapidly cleaned their bodies with their feet and fanned the dust from the cage.

A sample of 50% HETP (Vapatone) diluted 1 to 12,800 parts of sugar sirup, killed 71% of the bees within 48 hours. A dilution of 1 to 25,600 killed only 25% of caged bees within 48 hours.

More extensive tests were run with two samples of 100% HETP. The L/D-50 fell between .24 micrograms and .34 micrograms per bee. However, under field conditions this L/D-50 would be somewhat lower because all of the bees that survived were definitely incapacitated for some time after feeding and certainly would have died had they not been given the protection of the cage as well as sugar sirup. A dilution of 1–200,000 killed 65% of the caged bees within 48 hours.

The more dilute concentrations lost their toxicity more rapidly than the concentrated solutions. A concentration of 1 part of HETP to 800 of sugar sirup in distilled water killed 64% of caged bees after the solution was 72 hours old, whereas it killed all bees within two hours when freshly made. A solution of 1–48,000 killed all bees within eight hours but only 50% of the bees after standing 24 hours. A dilution of 1–200,000 killed 65% of caged bees during the first 24 hours but none of a fresh lot of bees was killed after the solution had stood for 24 hours.

A series of dilutions of TEPP, Tetraethyl pyrophosphate from 1–1,000 to 1–2,000,000 in sugar sirup were made with distilled water, and fed to caged bees while individual bees were fed known amounts of the chemical by means of a micropipette. All dilutions were in 20% sugar sirup. The L/D–50 was .075 micrograms per bee. A dilution of 1–1,000,000 killed 90% of caged bees within 48 hours, a dilution of 1–1,500,000 killed 46% in 48 hours, and 1–2,000,000 killed 37% of the bees in 48 hours.

TEPP solutions exhibited considerable toxicity to bees as a contact poison for a period of two days at a concentration of 1–800. In checking this factor, a 4 × 5 × ¾ inch cage was sprayed with an aqueous solution of TEPP, 1–800, and allowed to dry. Bees died when inserted in this cage over a period of 48 hours.

The TEPP solutions lost their toxicity in a similar manner to the HETP solutions. A dilution of 1–512,000 killed 100% of the bees when newly made but 24 hours later only 10% of a fresh lot of bees died when fed on this solution.

The behavior of the bees when fed solutions containing as little as one part of TEPP to 2,000,000 parts of sugar sirup indicated that the mortality of bees getting this amount in the field would be greater than the 37% indicated above and that the L/D–50 would also be less than 0.075 microgram per bee.

Parathion appears to be about the most toxic compound to bees that the writer has observed. When bees were placed in a cage so that they had to walk on a piece of waxed paper lightly covered with a dust containing 2% of parathion, the bees ran wildly, brushed their tongues and bodies immediately and all were moribund within 25 to 30 minutes.

When a 4 × 5 × ¾ cage of bees was placed over a similar cage containing a paper lightly dusted with a 2% parathion dust, but not in contact with the lower cage, the bees began to run wildly within one hour and all were moribund within three hours. Since it was probable that the bees may have fanned some of the dust onto their bodies, a third cage was sprayed with a 1% solution of parathion in 95% alcohol and allowed to dry in the sun for three hours. This cage was sprayed on November 25 and at intervals between then and January 2, all bees placed in the cage died within 20 minutes to two hours. Bees that were left in the sprayed cage for one minute and then transferred to an unsprayed cage, became moribund within an hour. Bees placed in 4 × 5 cages, ¼ to 1 inch above this sprayed cage, but not in contact with it became moribund within an hour during the first week after the cage had been sprayed. New lots of bees were placed in cages above the same cage during the following weeks. On January 2, bees placed in a cage above the sprayed cage became incapacitated within three hours and all were dead within 18 hours.

When parathion was dissolved in 95% alcohol and then diluted with 20% sugar sirup, the L/D–50 was approximately .07 microgram per bee. A feeding of .063 microgram per bee killed 25% of the bees while .078 microgram per bee killed 95% within three days. A majority of the bees were killed within 12 hours and of those that survived, most of them lived for a week or more. As with the other phosphates, the effect of the median lethal dose was such that many of the bees which survived in the cages would have died under natural conditions.

The results of the above preliminary tests would indicate that the phosphates, including HETP, TEPP, and parathion are toxic to bees in minute quantities in

sirup or nectar and will incapacitate bees for a few hours in quantities that are even less than the indicated L/D–50 amounts. Tetraethyl pyrophosphate and parathion in particular are highly toxic to bees when the chemicals are ingested. All three phosphates killed bees through contact with dusts or treated surfaces, indicating that, if bees come in contact with the dusts or treated surfaces, while working on plants, loss of bees may occur. Furthermore, surfaces covered with dusts or sprays containing parathion will kill bees through fumigant action when bees do not come into contact with the treated surfaces. This action is quick enough and lasts over a sufficiently long period to make this compound a potential hazard to pollinating insects if it is applied when plants are in bloom. The residual action of parathion apparently is longer than that of TEPP and, consequently, has greater killing power.

Aqueous solutions of hexaethyl tetraphosphate and tetraethyl pyrophosphate lose their toxicity within one to three days, depending on their concentrations, and in this regard will create a far less hazardous situation for bees than other insecticides with longer residual action.

Since it appears that the phosphates can be used effectively at very low concentrations, as compared with many of the older insecticides, and their residual action is of shorter duration, the potential hazards to beekeeping will not be serious if the phosphates are applied prior to or after the blooming period of plants which are attractive to bees. Only large-scale applications of the phosphates can reveal their actual relation to beekeeping and to pollinating insects in general.

Mosquito Larvae

Richard M. Bohart

Assistant Entomologist in the Experiment Station

SINCE THE DISCOVERY that DDT was a phenomenal mosquito larvicide it has been rapidly replacing the other two formerly used materials, oil and paris green. Many other new insecticides have been suggested for use on mosquito larvae but they have not been definitely proven superior to DDT and at present they are at a disadvantage on a cost basis. Since February, 1947 laboratory larvicide tests have been conducted to observe the mode of action of various insecticides and to compare the susceptibility of different species of mosquitoes. Materials used have included DDT, DDD, chlorinated camphene—Toxaphene—chlordan, benzene hexachloride, and parathion. All of these were extremely toxic to mosquito larvae but the only one definitely of a higher order than DDT was parathion.

Early fourth instar larvae were transferred on a net-covered wire loop from rearing pans to heavy china cups. The cups were of two sizes, holding 100 cc and 200 cc but with liquid the same depth in each type. Ten larvae were used in the smaller cups and 20 in the larger. The two species used in the tests with parathion were *Culiseta incidens* (Thomson), reared in the laboratory from egg rafts, and *Aedes squamiger* (Coquillett), collected as early instars in brackish water and reared in 0.5% NaCl—sodium chloride—solution. While being reared, the larvae were fed a mixture of ground dog biscuit and yeast, and appeared to thrive on this diet. The test period was 48 hours without food, the *C. incidens* in distilled water and the *A. squamiger* in 0.5% NaCl solution. Dilutions of DDT were prepared from a stock mixture of 20 grams of technical grade made up to 100 cc in xylene containing 10% Triton X-100. A graduated 1 cc pipette was used to transfer the DDT to the test cups from dilutions of 1 to 10,000 and 1 to 1,000,000 by weight in distilled water (disregarding weight of the solvent and emulsifier). Parathion dilutions were prepared in the same manner except that the stock solution contained 1 gram of insecticide (96% to 98% pure) made up to 100 cc in xylene and Triton X-100.

TABLE 1—Toxicity of Parathion and DDT Emulsions to C. Incidens (Thomson); Water Temperature About 23° F.; Length of Tests 48 Hours

Larvicide and dilution by weight in water	Number of tests	Total number of larvae	Per cent mortality
Parathion			
1–30 million	3	60	100.0
1–50 million	5	100	100.0
1–100 million	5	100	91.0
1–200 million	3	50	26.0
1–400 million	1	20	5.0
DDT			
1–10 million	2	40	100.0
1–30 million	5	100	88.0
1–50 million	5	100	44.0
1–100 million	5	100	8.0
1–200 million	2	40	2.5
1–400 million	1	20	0
Checks	5	100	0

TABLE 2—Toxicity of Parathion and DDT Emulsions to A. Squamiger (Coquillett); Water Temperatures About 23° F.; Length of Test Period 48 Hours

Larvicide and dilution by weight in water	Number of tests	Total number of larvae	Per cent mortality
Parathion			
1–50 million	5	50	100.0
1–100 million	10	100	98.0
1–200 million	10	100	17.0
1–400 million	10	100	5.0
1–800 million	2	20	5.0
DDT			
1–1 million	2	20	100.0
1–10 million	10	100	80.0
1–30 million	10	100	65.0
1–50 million	10	100	47.0
1–100 million	10	100	5.0
1–200 million	2	20	0
Checks	10	100	1.0