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The effects of Sevin : alone and in fungicidal combinations, and DDT on the honey bee, Apis mellifera L.

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THE EFFECTS OF SEVIN, ALONE AND IN FUNGICIDAL COMBINATIONS, AND DDT ON THE HONEY BEE, APIS MELLIFERA L.

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The Effects Of Sevin, Alone and in Fungicidal Combinations, and DDT on the Honey Bee, *Apis mellifera* L.

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INTRODUCTION

New and intensive methods of agriculture have greatly lessened the number of wild pollinating agents. In order to obtain satisfactory crops, it is now more apparent that the farmer must rely on the honey bee, *Apis mellifera* L., for good yields.

For many years, mortality of honey bees has resulted from injudicious use of pesticides toxic to them. Each year new pesticides are introduced to the commercial market. Their effects on honey bees should be determined so that if toxic, they will not be applied in a manner harmful to bees.

Sevin, 1-Naphthyl *N*-methylcarbamate, a pesticide recently introduced, has shown considerable promise for orchard and forest pest control. The purpose of this investigation was to determine the effects on the honey bee of Sevin, alone and in combination with orchard fungicides.

REVIEW OF LITERATURE

Shaw in 1941 and Sutherland in 1957 made thorough reviews of the literature on bee poisoning. These are their conclusions about the following compounds:

Arsenicals:

Arsenicals were found to be toxic as stomach poisons in laboratory tests and under field conditions. The principal source of danger was from poisoned pollen. Investigations indicate arsenicals should be applied only when necessary, in minimum concentrations, and never during pre-bloom and bloom stages.

Fluorine:

Sodium fluosilicate and cryolite were quite toxic to bees. Fluorine compounds were less toxic than the arsenicals, but must be used with caution to avoid bee poisoning.

Sulfur:

Sulfur was sometimes toxic to bees as a stomach poison and caused high mortality as a contact poison in laboratory tests. In field tests, sulfur was repellent to bees but caused little mortality. Lime sulfur was non-toxic as a stomach poison in laboratory tests and repellent to bees in the field. Sulfur compounds do not present a serious threat to bees in the field.

Copper compounds:

Copper compounds as generally used did not appear to be responsible for bee poisoning.

Thallium compounds:

Thallium sulfate used in sweetened baits, was quite toxic to bees. Since it is used almost exclusively indoors, it does not pose a serious threat to bees.

Nicotine compounds:

Nicotine compounds were toxic to bees as stomach and direct contact action poisons in laboratory and field tests. They may be used safely during bloom when applied at dusk as a rapid loss of toxicity occurs. A repellent action was noted.

Pyrethrum compounds:

Pyrethrum compounds were toxic in the laboratory as contact poisons, but caused little mortality in field tests. they lost their toxic effect quickly and were strongly repellent to bees. Applications at dusk were considered safe.

Rotenone compounds:

Rotenone compounds were toxic as stomach and contact poisons in the laboratory. Direct contact action was shown in field tests, Applications at dusk were considered safe due to their rapid break-down. In general rotenone compounds did not seem to pose any serious problem to bees in the field.

Sabadilla:

Sabadilla was found to be toxic as a stomach and contact poison. Conclusions were the same as for rotenone compounds.

Ryania and quassia:

Ryania and quassia were found slightly or non-toxic to bees in laboratory tests. Applications at dusk were considered safe.

Phenothiazine:

Phenothiazine, thiodiphenylamine, was found to be slightly toxic as a stomach, contact, and residual poison in laboratory tests. It was non-toxic in field tests. Its substitution for arsenicals would greatly reduce bee mortality.

DDT:

Laboratory reports indicated that DDT, 1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane, was a toxic contact, stomach, and residual poison. Varying degrees of toxicity were shown in field tests. It should only be applied in necessary quantities and not during the bloom period.

Analogues of DDT:

Methoxychlor, 1,1,1-trichloro-2,2-bis(p-methoxyphenyl)-ethane, and DDD, 1,1-dichloro-2,2-bis(p-chlorophenyl)-ethane, were generally less toxic than DDT in laboratory tests. In field tests they were practically non-toxic. They can be used safely if bees are not flying and bloom is not present during application.

BHC and lindane:

BHC, 1,2,3,4,5,6-hexachlorocyclohexane, and lindane, the gamma isomer of BHC, were highly toxic as stomach, contact, and residual poisons in laboratory and field tests. They should not be applied where bees would come in contact with them.

Chlordane and heptachlor:

Chlordane, 1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene, and heptachlor, 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene, were highly toxic as stomach, contact, and residual poisons in laboratory and field tests. They should not be applied where bees would come in contact with them.

Aldrin, dieldrin, endrin, and isodrin:

Aldrin, 1,2,3,4,10,10,-hexachloro-1,4,4a,5,8,8a,-hexahydro-1,4,5,8-dimethanonaphthalene, and dieldrin, 1,2,3,4,10,10,-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,5,8-dimethanonaphthalene, were highly toxic as stomach, contact, and residual poisons in laboratory tests, and were found to be generally toxic in field tests. Endrin, 1,2,3,4,10,10,-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a,-octahydro-1,4-_endo_-_endo_-5,8-dimethanonaphthalene, and isodrin, 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-_endo_-_endo_-dimethanonaphthalene, were generally less toxic than aldrin and dieldrin in laboratory tests. Aldrin and dieldrin should not be applied to crops in bloom. No general statement may be issued on endrin or isodrin until further testing is done.

Toxaphene:

Toxaphene, chlorinated camphene containing 67 to 69% chlorine, varied from non-toxic to very toxic in laboratory tests. It caused little or no mortality to bees under field conditions regardless of the time of application. Applications during bloom when bees are actively foraging seem safe, but should be avoided if possible.

EPN:

EPN, O-ethyl O-p-nitrophenyl benzene thiophosphonate, was very toxic as a stomach, contact, and residual poison in laboratory tests. No field tests have been conducted with EPN but its toxicity indicates that it should not be used on plants in bloom.

HETP and TEPP:

HETP, hexaethyl tetraphosphate, and TEPP, tetraethyl pyrophosphate, were quite toxic as contact, residual, and stomach poisons in laboratory and field tests. TEPP showed a rapid break-down under field conditions. It may be applied at dusk during bloom. HETP should not be applied to crops during bloom.

Parathion, para-oxon, methyl-parathion:

Parathion, O,O-diethyl O-p-nitrophenyl thiophosphate, para-oxon, diethyl p-nitrophenyl phosphate, and methyl-parathion, O,O-dimethyl O-p-nitrophenyl thiophosphate, were very toxic as stomach, contact, and residual poisons under laboratory and field conditions. They should never be used as pre-bloom and bloom applications.

Malathion:

Malathion, O,O-dimethyl dithiophosphate of diethyl mercaptosuccinate, was very toxic as a contact and residual poison in laboratory and field tests. It should not be applied to crops near or in bloom.

Diazinon:

Laboratory and field tests indicate that Diazinon, O,O-diethyl O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorodithioate, is a highly toxic stomach, contact, and residual poison. It should not be applied to crops during bloom.

Systox, schradan:

Systox, O,O-diethyl (2-ethylmercaptoethyl) thiophosphate, a mixture of thiono and thiol isomers, is quite toxic to bees and should not be applied as bloom and pre-bloom treatment. In laboratory and field tests, schradan, octamethylpyrophosphoramide, proved to be relatively non-toxic to bees and can be used as a pre-bloom and bloom application.

Elgetol and DN-111:

Elgetol, dinitro-o-cresol, DN-111, 2,4-dinitro-6-cyclohexylphenol, and other dinitro compounds were toxic to bees as stomach, contact, and residual poisons in laboratory tests. Field tests showed varying results indicating that dusk applications of these materials would be safest for bees.

Phenoxyacetic acid compounds; 2,4-D, 2,4,5-T

2,4-D, 2,4-dichlorophenoxy acetic acid, in acid,base, salt, and ester forms, 2,4,5-T, 2,4,5-trichlorophenoxy acetic acid and others were slightly or not at all toxic to bees in laboratory tests. Field tests showed variable results, but indicated danger of bee poisoning with indiscriminate use of these compounds. They should not be used on plants in bloom or applied unnecessarily to plants from which bees obtain the greater part of their nourishment.

The newer organic fungicides:

Most organic fungicides may be concluded to be relatively safe for bees when properly applied. In sufficient quantities, they may have a harmful effect. Further testing of some of these compounds is necessary before any general statement is issued about them.

Aramite, Ovotran, and Sulphenone:

Aramite, 2-(p-*tert*-butylphenoxy)-isopropyl-2-chloroethyl sulfite, Ovotran,*p*-chlorophenyl-*p*-chlorobenzene sulfonate, and other organic sulfur compounds were non-toxic as dusts in laboratory tests. Sulphenone, *p*-chlorophenyl phenyl sulfone, as a dust was moderataly toxic to bees. As research is limited on these compounds, evaluation of their toxicity under field conditions is impossible.

During a literature search subsequent to the period covered by Sutherland, the following publications were discovered.

Svoboda (1958) reported extensive poisoning of honey bees due to arsenic in Czeckoslovakia in recent years. Areas within a radius of three to six kilometers from various industrial plants showed poisoning of bees from arsenic in smoke from plants burning low grade fuel coal. Pollen in hives had from 0.07 to 0.12 milligrams arsenic per gram, an amount sufficient to kill any bee eating the pollen.

It is noteworthy that bee poisoning from industrial gases containing arsenic can occur, and these gases should be periodically checked.

Burch (1955) reported that Valin and Monteira in France found that the lethal dose of fluorine was 3 to 5 micrograms per bee, and that fluorine was probably causing bee mortality in parts of Savoie.

Maurizio and Staub (1956) reported that mass poisoning of bees near Swiss aluminum factories was traced to high quantities of fluorine in waste gases. Plant, pollen and rain water contained considerable amounts of fluorine. The average fluorine content of dead bees was 15 micrograms per bee.

Guilhon (1958) conducted experiments to determine the average fluorine content per bee and found 0.29-30 micrograms in rural areas and 1.30 to 9.4 micrograms around large cities in France.

Juvin (1955) stated that lindane was non-toxic to bees and used it as a treatment during bloom to control pests of rape with no ill effects on honey bees reported.

Wiese (1957-1958) reported the median lethal dosage of 30 percent gamma-BHC in the laboratory to be 0.00885 milligramd per square centimeter, the LD50 of lindane to be 0.110 micrograms per bee,

Sachs (1957) reported an interesting case of poisoning from BHC. Trees along the edge of a woodland were dusted with BHC to control cock-chafers. As no nectar or honeydew flow was occurring, no damage to bees was expected. Seven days after the last dusting, one hundred colonies were found dead and many others suffered severe losses when attracted to a heavy honeydew flow from an extensive aphid infestation presumed to have resulted from the destruction of their natural enemies by the BHC. Laboratory tests carried out showed dusts six days old were stomach poisons but not contact poisons.

Wiese (1957-1958) determined the oral dose of chlordane necessary to give fifty per cent mortality in the South African honey bee as 1.90 micrograms per bee. The LD50 of chlordane applied in acetone on the thorax of honey bees was 7.03 micrograms per bee.

Juvin (1955) stated that dieldrin was considered non-toxic to honey bees and used it during bloom for pests of rape. No ill effects were noted.

MacCollom (1958) reported that a mixture of one pound DDT and 0.25 pound of dieldrin per acre did not lower the bee population below that needed for adequate pollination of birdsfoot trefoil. He also reported that endrin at 0.2 pound per acre did not lower the bee populatiom below that needed for adequate pollination.

Moffett (1958) reported that aldrin at 2 ounces per gallon of diesel oil per acre was sprayed by plane on 80,000 acres in Colorado for grasshopper control. He concluded that this spraying caused some loss of honeybees, but it was not disastrous. Sweet clover and lucerne were in bloom at the time of application.

Shaw (1959) reported that residues from 0.25 pound actual dieldrin per 100 gallons of spray could be highly toxic to honey bees for periods up to ninety-six hours after application.

Wiese (1957-1958) tested aldrin, dieldrin, and endrin on the South African honey bee. He foumd that the oral dose necessary to give fifty percent mortality with dieldrin and endrin were 0.153 and 1.029 micrograms per bee, respectively. The MLD of aldrin and dieldrin vapors were 0.0048 and 8.83 milligrams per square centimeter, respectively.

The LD50 for dieldrin, aldrin, and endrin applied in acetone to the thorax of bees were 0.414, 0.800, and 1.311 micrograms per bee, respectively.

The Laboratoires des Recherches Veterinaires (laboratoire apicole) de Nice (1955) reported that field tests with twenty per cent toxaphene dusts at twenty-five kilograms per hectare applied to rosemary in bloom showed no ill effects on ten hives of bees foraging in the treated fields. This report also stated that bees fed a mixture of 1 cc of a toxaphene solution (750 grams per liter) in 20 cc sugar syrup lived as long as bees fed pure syrup. Bees dusted with various concentrations of toxaphene in the laboratory, and then released lived longer than those not treated.

Weaver and Garner (1955) reported that single applications of toxaphene and Systox to hairy vetch during the pre-bloom stages reduced the population of injurious insects without apparent injury to pollinating insects.

Meyerhoff (1958) reported that toxaphene preparations sprayed from an airplane just before the bees were flying strongly caused no damage except to a few bees already flying. Bees would not work flowers wet with spray and were not affected on those where application had dried.

Juvin (1955) stated that parathion is non-toxic to bees and used as a spray against pests of rape that appear only during flowering has no apparent ill effects to bees.

Wiese (1957-1958) tested malathion on the South African honey bee and reported the LD50 applied in acetone to the thorax to be 0.094 micrograms per bee.

Wolfenbarger and Robinson (1957) reported that widespread use of malathion in Florida at a rate of 0.5 pound per acre caused colonies of honey bees to lose less weight than had been lost in previous years when no spray was applied.

Palmer-Jones, Forster, and Griffin (1957) reported that meta-Systox, a systemic insecticide which the makers stated as being harmless to honey bees except in direct contact, was extremely toxic to honey bees as a residual insecticide. Eleven acres of chou moellier were sprayed in early evening with 16 fluid ounces of meta-Systox per acre. Three days later, nearly all bees working the crop were killed and the residue remained toxic for five days. An extract of the flowers also proved to be toxic.

Wiese (1957-1958) tested Systox and schradan in the laboratory for toxicity to the South African honey bee. As a stomach poison the oral doses necessary to give 50 percent mortality were 0.681micrograms of Systox and 8.82 micrograms per bee of schradan. The LD50 of these insecticides applied in acetone to the thorax was 0.842 micrograms per bee of Systox and 46.7 micrograms per bee of schradan.

Weaver and Garner (1955) applied a mixture of toxaphene and Systox as a pre-bloom treatment on hairy vetch, and reported no ill effects on bees. Bees sprayed with Systox in the laboratory, however, showed a high mortality rate.

Shaw, Bourne, and Migliorini (1957) found that bees exposed to captan, *N*-trichloromethyl mercapto-4-cyclohexene-1,2-dicarboximide, ferbam, ferric dimethyl dithiocarbamate, glyodin, 2-heptadecyl glyoxalidine acetate, and phenyl mercury lactate in concentrations recommended for apple scab control were not affected. Mortality of caged bees treated with glyodin did not exceed controls until the concentration was eight times that recommended.

Anderson, Shaw, and Sutherland (1957) found that captan, ferbam, glyodin and phenyl mercury lactate were relatively non-toxic to bees as sprays. Glyodin was found to have residual action that caused fifty percent mortality in two days.

Shaw (1959) reported that Cyprex, dodecyl guanidine acetate, at one pound per 100 gallons water did not cause mortality in bees that differed significantly from the untreated check.

King (1959) conducted tests with Thylate, Cyprex, Ferbam WP, Dithane Z-78, Puratized Apple Spray, Coromerc, Crag Glyodin, Panogen Apple Spray, Tag, Captan 50 W, Phygon XL, Phix, and liquid lime sulfur, which indicated that Thylate, Cyprex, and Dithane Z-78 caused a rate of mortality significantly different from that of the untreated check.

It appears that most of the organic fungicides are relatively harmless to bees when properly applied. In excessive quantities, however, they may have a harmful effect. Further testing of some of these compounds is necessary before any definite conclusions can be drawn about them.

Sevin is a relatively new pesticide, recently introduced to the commercial market. Little research on its relative toxicity has been reported. It is in the carbamate class of insecticides.

According to Anderson and Atkins (1958), Sevin as a 2.5 percent dust was highly toxic to honey bees. A 400 milligram dose killed 96 percent and a 100 milligram dose killed 41 percent of the sample bees within twenty-four hours. In comparing DDT, as a standard treatment, to Sevin, it was concluded that Sevin was more toxic than DDT.

Shaw (1959) did field tests with Sevin, testing the wettable powder formulation at one pound and two pounds per 100 gallons of water and the thirty-six percent mull formulation at one pound per 100 gallons of water. He applied these materials to caged bees tied in apple trees, spraying with both an air blast sprayer and an hydraulic sprayer, using the methods of commercial growers. Residual effect was tested by exposing caged bees to the dried residues left on the trees. The results of these experiments led Shaw to state that the toxicity of

Sevin as a contact insecticide is very high. All treatments caused fifty percent mortality within six hours. Shaw contends that the method of application influences the residual effects of Sevin. The residues resulting with an air blast application produced greater toxicity than residues of sprays applied with a hydraulic sprayer. After a period of ninety-six hours, the residual toxicity of Sevin to honey bees was reduced.

Anderson and Atkins (1958) grouped all the pesticides tested by them for the past several years into the following four groups:

Group I

Highly toxic materials that should not be used when there is a possibility of poisoning bees at treatment or within a few days thereafter.

Aldrin	Dibrom	Lead arsenate
BHC	Dicapthon	Lindane
Calcium arsenate	Dieldrin	Metacide
Chlordane	DNOSBP (DN-211)	Methyl Parathion[1]
Chlorthion	EPN	Parathion
DDVP	Guthion	Sevin[1]
Diazinon	Heptachlor	

Group II

Highly toxic materials that can be used around bees when certain precautions are used.

Di-Syston	Phosdrin[1]	TEPP
Malathion[1]	Sabadilla	Thimet

Group III

Moderately toxic materials that can be used around bees if timing and dosage are correct, but should not be applied directly on bees in field or at colonies.

Chlorbenzilate	DDT[1]	Perthane
Co-Ral	Endrin[1]	Tartar emetic
Cryolite	Ethion[1]	Tedion
DDD (TDE)	Isodrin	Thiodan[1]
	Korlan	Toxaphene
		Trithion[1]

Group IV

Relatively non-toxic materials that can be used around bees.

Allethrin	Ferbam[2]	Rotenone
Aramite	Genite 923	Ryania
Bordeaux mixture	IPC[2]	Sulfur
Captan[2]	Karathane[1]	Sulphenone
Copper oxychloride sulfate	Kelthane[1]	Systox[1]
Copper sulfate	Maneb[2]	Thiram[2]
Gunilate	MCP[2]	2,4-D[2]
Cuprous oxide[2]	Methoxychlor	2,4,5-T[2]
Delnav[1]	Mitox	Zineb[2]
Dilan	Monuron	Ziram[2]
DMC	Nabam[2]	
DNOCHP	Neotran	
Dylox	Nicotine	
	OMPA	
	OVEX	
	Phostex	
	Pyrethrins	

1 These materials field and laboratory tested; all others laboratory tested only.

2 Data obtained from other research workers.

PROCEDURES AND TECHNIQUES

I. Pesticides tested.

Six pesticides recommended in the 1959 Pest Control Schedule for Apples published by the University of Massachusetts were selected for toxicity determinations. Of these, two, Sevin and DDT are insecticides, while four, captan, ferbam, glyodin, and thiram are fungicides. Sevin was used alone and in combination with captan, ferbam, glyodin, and thiram. DDT was used alone to determine its toxicity relative to that of Sevin on honey bees.

The 1959 Pest Control Schedule for Apples was used in determining the fungicides to be tested because in recent years, a fungicidal spray applied while the trees are in bloom has been recommended for the control of apple scab. To avoid possible losses of bees and crop, it is important to revise such recommendations if toxicity is found.

Sevin was also chosen for testing because of the interest in its use as a possible substitute for DDT in gypsy moth and other control programs. Any pesticide proposed for such widespread application should be thoroughly tested for its effects on honey bees. Another goal of this testing program was to determine the safety to bees of Sevin when applied immediately before bloom.

The pesticides tested are listed below:

Pesticides included in test

Chemical name	Common name
1. 1-Naphthyl N-methylcarbamate	none
2. 1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane	DDT
3. N-trichloromethyl mercapto-4-cyclohexene-1,2-dicarboximide	captan
4. ferric dimethyldithiocarbamate	ferbam
5. 2-heptadecyl glyoxalidine acetate	glyodin
6. tetramethyl thiuram disulfide	thiram

Commercial product and formulation	Recommended amount at 2X concentration per 100 gallons
1. Sevin (50% WP*)	2 pounds
2. DDT (50% WP)	4 pounds
3. Orthocide 50 (50% captan WP)	4 pounds
4. Ferberk (76% ferbam WP)	3 pounds
5. Crag Glyodin (34% glyodin)	3 pints
6. Thylate (65% thiram WP)	3 pounds

* WP = wettable powder

II. Field procedures and techniques.

A. Collection of honey bees

In these toxicity tests it seemed desirable to select young bees of uniform age and vigor to avoid mortality due to causes such as old age and thus not attributable to the effects of the treatment. Such bees are to be found in the upper brood chambers of the hives and were used in all of the field tests reported here. The manner of collection was to open a hive, remove frames from the upper brood chamber, checking to ascertain absence of the queen, and shaking the bees into a pail. Approximately 100 bees were then transferred directly into each of the cages used in the field tests (see figure I).

These cages were constructed of 8-mesh wire cloth and measured 12 inches long by 8 inches in diameter. They were closed on one end by 8-mesh wire cloth and on the other by a heavy cheesecloth sleeve.

B. Treatment procedure.

1. Direct contact tests.

As soon as the bees were caged they were taken to the orchard and kept in the shade until the time of treatment. At the start of each test, cages were hung by the sleeves to the ends of apple tree branches about six feet from the ground. Care was taken to place the cages where they would swing freely, unprotected by foliage and they were always attached to the side of the tree nearest the sprayer path.

Three replicates (cages) were used in each test.

The statistical process used with the data obtained was the chi square test of significance devised by Pearson(1899). The average number of hours necessary for a fifty percent mortality level to be reached among replicates was compared to that of an untreated check.

Immediately after the cages were in place, the pesticide was applied with an air-blast sprayer employing exactly the same technique used by commercial growers. The sprayer maintained a distance of about eight feet from the trees, spraying each for about ten seconds. Immediately after spraying the cages were removed from the trees and the bees transferred to holding cages (see figure II).

The holding cages were squat one quart ice cream containers that had the tops replaced with 8-mesh wire cloth. Holes had been cut in the bottoms with cork stoppers inserted in the holes. Dead bees were readily removed from the holding cages through these holes.

The day of the direct contact tests was seasonably warm, clear, and the orchard temperature at that time varied between 83° and 85° F. The relative humidity ranged between 31 and 44 percent.

2. Residual tests

Residual tests were conducted on the day of spraying and at five day intervals thereafter up to fifteen days. The bees used in the residual tests on the day of spraying were held in the shade at the orchard until all spray materials had thoroughly dried. Then, branches of the treated trees were inserted into the cages for a period of thirty minutes. Gentle shaking of cages at five minute intervals insured that all bees came into contact with the treated foliage. After exposure the cages were transported to the apiary where the bees were transferred to holding cages. Three replicates were used in each test. The statistical method used was the same as in the direct contact action tests.

C. Handling of bees after direct contact and residual action tests.

The bees from the direct contact and residual action tests, in their holding cages, were placed on tables in a darkened room at the apiary. Daily conditions of temperature and humidity varied between 65° and 85° F. and 40 to 60% relative humidity for the duration of the tests. The bees were fed a syrup made of one part sugar to one part water by weight in small bottles with punctured metal covers inverted on the tops of holding cages (see figure II).

The bees were observed daily at 7 A. M. to record mortality. Dead bees were removed from the holding cages every day, until a 50 percent mortality level had been reached.

D. Measures against contamination.

The trees used in these tests had not been previously treated during the year and thus were free of pesticidal residues.

To check the cleanliness of the spray equipment, three replicates of bees were sprayed with water alone before any pesticides were mixed in the tank. The tank was thoroughly rinsed and the spray lines flushed after each application.

All direct contact sleeve cages, all holding cages, and all feeder bottles were discarded after one test.

The sleeve cages used in the residual tests were tagged with the name of the pesticides with which they were originally used. These were re-used with the same pesticides for the three subsequent tests. The cages were left out of doors to expose them to the same weather conditions as the treated trees.

All tables in the holding room were covered with clean paper which was replaced after each test.

III. Laboratory procedures and techniques.

A. Collection of honey bees.

Honey bees collected in the same way as those used in the field tests were placed in holding cages.

B. Treatment procedure.

1. Stomach poisoning tests.

Three replicates of about 100 bees each were used in each of the stomach action tests. The statistical method used was the same as in the direct contact action tests. The holding cages and feeder bottles previously described were used.

Dilutions of four times the recommended concentrations of pesticides were made.

These were mixed with an equal amount of 1:1 sugar syrup. This resulted in mixtures of the pesticides in 0.5:1 sugar syrup, which simulates the sugar concentration of apple nectar. Since syrup was used for a carrier, any unusual death rate could be attributed to the toxic affects of the pesticides, not to death by starvation.

The bees, in holding cages, were put in a darkened room for one hour before being fed the pesticide mixtures. Thus the bees were hungry and would feed readily unless the mixtures offered repelled them.

After treatment the bees were observed daily at 7 A.M. for mortality.

C. Measures against contamination.

The cages and feeder bottles were discarded after one test.

RESULTS

Table I shows results of direct contact action of pesticides on honey bees.

Tables II, III, IV, and V show the results of residual action tests of the pesticides tested.

Table VI shows the results of stomach poisoning tests on bees.

Table I Direct contact action of pesticides on honey bees.

Pesticide and Formulation	Amount per 100 gallons water	Time (in hours) to produce 50% mortality	
		Ave.	Range
Sevin (50% WP)	2 lb.	Within 15 hours	
Sevin (50% WP) Orthocide 50 (50% captan WP)	2 lb. 4 lb.	"	"
Sevin (50% WP) Ferberk (76% ferbam WP)	2 lb. 3 lb.	"	
Sevin (50% WP) Crag Glyodin (34% glyodin)	2 lb. 3 pts.	"	
Sevin (50% WP) Thylate (65% thiram WP)	2 lb. 3 lb.	"	
DDT (50% WP)	4 lb.	435 hrs.*	
Untreated check		535 hrs.	519-555 hrs.
Water check		495 hrs.	459-555 hrs.

* significantly different from untreated check at 0.5% level.

The first check for mortality (table I) occurred 15 hours after treatment. As Sevin, alone and in combination with fungicides, showed greater than fifty per cent mortality when first checked, the average between replicates is given as "within 15 hours" and a range is omitted.

A range for DDT is omitted as only one replicate was used in the direct contact action test.

A difference of 64.9 hours is necessary between the untreated check and the treated replicates to reach a 0.5% level of significance. This difference is called the L.S.D.

The fifty percent mortality levels of Sevin, alone or in combination with fungicides differed significantly from that of DDT at the 0.5% level of significance, and the L.S.D. in this case would be 58.8 hours.

Table II Effects on bees of 30 minute exposures to residual of pesticides tested on day of application.

Pesticide and Formulation	Amount per 100 gallons water	Time in hours to produce 50% mortality	
		Ave.	Range
Sevin (50% WP)	2 lb.	within 15 hours*	
Sevin (50% WP) Orthocide 50 (50% captan WP)	2 lb. 4 lb.	36 hours*	15-87 hours
Sevin (50% WP Ferberk (76% ferbam WP)	2 lb. 3 lb.	within 15 hours*	
Sevin (50% WP) Crag Glyodin (34% glyodin	2 lb. 3 pts.	"	
Sevin (50% WP) Thylate (65% thiram WP)	2 lb. 3 lb.	"	
DDT (50% WP)	4 lb.	463 hours*	423-483 hours
Untreated check		535 hours	519-555 hours

* Significantly different from untreated check at 0.5% level.

In table II the explanation of averages stating "within 15 hours" and omitted ranges for the Sevin - fungicide mixtures is the same as in table I.

The L.S.D. necessary for a 0.5% level of significance when comparing treated replicate averages with that of the untreated check is 64.9 hours.

The 50% mortality levels of Sevin, alone or in combination with fungicides, differed significantly from that of DDT at the 0.5% level and the L.S.D. in this case is 60.4 hours.

Table III Effects on bees of 30 minute exposures to residual action of pesticides five days after application.

Pesticide and Formulation	Amount per 100 gallons water	Time in hours to produce 50% mortality	
		Ave.	Range
Sevin (50% WP)	2 lb.	546 hrs.	510-582 hrs.
Sevin (50% WP) Orthocide 50 (50% captan WP)	2 lb. 4 lb.	538 hrs.	534-546 hrs.
Sevin (50% WP) Ferberk (76% ferbam WP)	2 lb. 3 lb.	342 hrs.*	234-522 hrs.
Sevin (50% WP) Crag Glyodin (34% glyodin	2 lb. 3 pts.	422 hrs.*	282-510 hrs.
Sevin (50% WP) Thylate (65% thiram WP)	2 lb. 3 lb.	154 hrs.*	18-426 hrs.
DDT (50% WP)	4 lb.	598 hrs.	570-630 hrs.
Water check		558 hrs.	
Untreated check		518 hrs.	510-522 hrs.

* Significantly different from untreated check at 0.5% level.

The data in table III show that large variations occurred between the 50% mortality levels of replicates of the Sevin - ferbam, Sevin - glyodin, and Sevin - thiram mixtures. A possible reason for this was that branches of the trees received different amounts of spray when treated. Thus, bees put on them five days after treatment would show differing rates of mortality if exposed to branches with different amounts of residue on them.

Only one replicate was used as a water check so a range is omitted.

The L.S.D. necessary for a 0.5 percent level of significance when comparing treated replicates with the untreated check is 63.8 hours.

Table IV Effects on bees of 30 minute exposure to residual action of pesticides tested ten days after application.

Pesticide and Formulation	Amount per 100 gallons water	Time in hours to produce 50% mortality	
		Ave.	Range
Sevin (50% WP)	2 lb.	480 hrs.	452-500
Sevin (50% WP) Orthocide 50 (50% captan WP)	2 lb. 4 lb.	424 hrs.	368-464
Sevin (50% WP) Ferbark (76% ferbam WP)	2 lb. 3 lb.	476 hrs.	464-500
Sevin (50% WP) Crag Glyodin (34% glyodin)	2 lb. 3 pts.	460 hrs.	416-512
Sevin (50% WP) Thylate (65% thiram WP)	2 lb. 3 lb.	468 hrs.	464-476
DDT (50% WP)	4 lb.	452 hrs.	428-464
Water check		458 hrs.	452-464
Untreated check		468 hrs.	440-488

No significant difference between treated and check bees.

Table V Effects on bees of 30 minute exposure to residual action of the pesticides tested fifteen days after application.

Pesticide and Formulation	Amount per 100 gallons water	Time in hours to produce 50% mortality	
		Ave.	Range
Sevin (50% WP)	2 lb.	377	366-390
Sevin (50% WP) Orthocide 50 (50% captan WP)	2 lb. 4 lb.	434	378-474
Sevin (50% WP) Ferberk (76% ferbam WP)	2 lb. 3 lb.	458	390-522
Sevin (50% WP) Crag Glyodin (34% glyodin	2 lb. 3 pts.	346	318-366
Sevin (50% WP Thylate (65% thiram WP)	2 lb. 3 lb.	366	318-414
DDT (50% WP)	4 lb.	446	438-462
Water check		336	306-366
Untreated check		345	330-390

No significant difference between treated and check bees.

The data in tables IV and V show that the number of hours necessary to reach a 50 percent mortality level among replicates 10 and 15 days varied considerably. The probable reason for this is that the bees used on the different days were taken from different hives. The average life span of bees from varying hives can differ greatly due to hive vigor, morale and other reasons, so that differences of this sort can be expected. Also, weather conditions varied at the times of treatment and this could also cause variation in the results.

No treatments showed mortality rates significantly greater than those of the un treated checks in tables IV and V.

Table VI Results obtained by feeding honey bees sugar syrup-pesticide mixtures.

Pesticide and Formulation	Amount per 100 gal.water-syrup	Time in hours to produce 50% mortality	
		Ave.	Range
Sevin (50% WP)	2 lb.	within 19 hours*	
Sevin (50% WP) Orthocide 50 (50% captan WP)	2 lb. 4 lb.	43*	
Sevin (50% WP) Ferberk (76% ferbam WP)	2 lb. 3 lb.	43*	
Sevin (50% WP) Crag Glyodin (34% glyodin)	2 lb. 3 pts.	43*	
Sevin (50% WP) Thylate (65% thiram WP)	2 lb. 3 lb.	43*	
DDT (50% WP)	4 lb.	91*	79-103
Syrup check		535	519-555

* Significantly different from syrup check at 0.5% level.

The bees were first checked for mortality 19 hours after treatment (table VI). At this time greater than fifty percent mortality had occurred only in the replicates fed the Sevin and sugar mixture so the average is stated as "within 19 hours," so a range is impossible.

Twenty-four hours later all replicates of the Sevin and fungicide mixtures showed greater than fifty percent mortality. As few bees were dead at 43 hours, a range between replicates was impossible.

The L.S.D. necessary for a 0.5 percent level of significance when comparing treated replicates with the untreated check is 64.9 hours.

DISCUSSION OF RESULTS

Field results.

Field tests show that Sevin, alone or in combination with fungicides is very toxic to honey bees as a direct contact poison. Although DDT showed some direct contact toxicity, Sevin was much more toxic to honey bees.

Sevin, alone or in combination with fungicides was very toxic to honey bees as a residual poison on the day of application. Five days later, only the residues of Sevin and ferbam, Sevin and glyodin, and Sevin and thiram still showed toxicity. Ten days after spraying, no residual toxicity was found under the conditions of this test.

DDT showed some residual toxicity on the day of application, but none five days later.

Laboratory results.

The stomach poisoning tests showed that Sevin, alone or in combination with the fungicides used, and DDT when fed at the recommended concentrations in 0.5:1 sugar syrup were very toxic to honey bees. Possible repellent action of the Sevin and fungicide mixtures existed. The bees readily accepted a mixture of Sevin and syrup, but Sevin and fungicide mixtures were not eaten until the bees were forced, probably by hunger, to eat them.

SUMMARY AND CONCLUSIONS

Tests were conducted to determine the contact and residual toxicity of Sevin, alone and in combination with captan, ferbam, glyodin and thiram, and DDT to honey bees.

For field tests, young bees of uniform age and vigor were selected from upper brood chambers of hives.

Direct contact toxicity was determined by hanging cages of bees in apple trees, and spraying them with an air blast sprayer using commercial spray methods.

Bees in cages were exposed to residues of the pesticides for thirty minutes on the day of application, and at five day intervals thereafter until fifteen days had elapsed.

Laboratory tests were conducted to determine the stomach action toxicity of the pesticides. Honey bees were fed concentrations of the pesticides in 0.5:1 sugar syrup. The dosages employed were at 2 X the concentrations recommended in the "1959 Pest Control Schedule for Apples" published by the University of Massachusetts.

Sevin, alone or in combination with fungicides was very toxic to honey bees as a direct contact poison. DDT showed less toxicity in these tests.

Residues of Sevin, alone or in combination with fungicides, as well as DDT were very toxic to honey bees on the day of application. Five days after application, only the residues of Sevin and ferbam, Sevin and glyodin, and Sevin and thiram still showed toxicity. Ten days after application no residual toxicity was shown.

Sevin, alone or in combination with fungicides, and DDT were very toxic when fed to bees. However, the toxicity of Sevin was greater than that of DDT.

A possible repellent action of Sevin in combination with the fungicides was noted in feeding tests.

According to these tests, Sevin should not be applied to plants approaching or in bloom. It should be applied carefully and spray drift should not come in contact with honey bees or plants in bloom.

Figure I
Treatment cage used in direct contact and field tests.

Figure II
Holding cage and feeder bottle used in tests.

REFERENCES CITED

Anderson, E.J., F. R. Shaw and D. L. Sutherland.
1957. The effects of certain fungicides on honey bees. Jour. Econ. Ent. 50: 570-573

Anderson, L. D., and E. L. Atkins, Jr.
1958 Toxicity of pesticides to honey bees in laboratory and field tests in Southern California, 1955-1956. Jour. Econ. Ent. 51: 103-108

Anderson, L. D., and E. L. Atkins, Jr.
1958. Effects of pesticides on bees. Cal. Agric. 12(12) :3-4

Burch, D. V.
1955. Fluorine poisoning. Rev. franc. Apic. 3(109): 1188 Abs. Bee World 37: 72

Guilhon, J.
1958. Fluor et apiculteur. XVII Int. Beekp. Cong. Abs. Bee World 39: 335

Juvin, P.
1955. Les traitements avec certian produits insecticides non toxiques pour les abeilles sort efficaces sur les parasites du colza. Apiculteur 99 (11) Sect. sci. 72-78 Abs. Bee World 38: 110-111

King, C. C.
1959. The effects of fungicides. Glean. Bee Cult. 87: 678:681

Krueger, H. Rand, and J. E. Casida.
1957. Toxicity of 15 organophosphorus insecticides to several insect species and rats. Jour. Econ. Ent. 50: 356-368

Laboratoires des Recherches Veterinaires (Laboratoire apicole) de Nice.
1955. Quelques experiences en vue de controler l'effet du toxaphene sur les abeilles. Rev. franc. Apic. 3(113): 1322-1324 Abs. Bee World 37: 62-63

MacCollum, G. B.
1958. Control of insects affecting the birdsfoot trefoil seed production in Vermont.
Jour. Econ. Ent. 51: 492-494

Maurizio, A., and M. Staub.
1956. Bienenvergiftungen mit fluorhaltigen Industrieabgasen in der Schweiz.
Schweiz Bienenztg. 79: 476-486
Abs. Bee World 38: 321

Meyerhoff, G.
1958. Die Wirkung des Spruh und Nebelvefohrems auf die Honigbiene.
XVII Int. Beekp. Cong.
Abs. Bee World 39: 334

Moffett, J.
1958. Grasshopper spraying and honey bees.
Amer. Bee Jour. 98: 441

Palmer-Jones, T., I. W. Forster, and L.A.M. Griffin.
1957. Effects on honey bees of metaSystox applied from the air as a spray to chou moellier.
N. Z. Jour. Sci. Tech. Sect. A 38 (7): 752-769
Abs. Bee World 39: 219

Pearson, K.
1899. Philosophical Magazine Series 5 50: 157

Sachs, H.
1957. Eine Honigtauvergiftung bei Bienvolkern als Folge einer Maikafer-Bekampfungsaction.
Z. Bienforsch. 3 (9): 205-212
Abs. Bee World 40: 51

Shaw. F. R.
1941. Bee poisoning...a review of the more important literature.
Jour. Econ. Ent. 34: 16-21

1959. The effects of field applications of some of the newer pesticides on honey bees.
Jour. Econ. Ent. 52: 549-550

Shaw, F. R., A. I. Bourne, and R. Migliorini.
1957. Newer fungicides and their effects on honey bees.
Amer. Bee Jour. 97: 437-438

Sutherland, D. J.
1957. The effect of certain modern pesticides on *Apis mellifera* and *Bombus* spp. Unpublished Univ. of Mass. thesis.

Svoboda, J.
1958. Industrielk Vergiftungen der Bienen. XVII Int. Beekpg. Cong. Abs. Bee World 39: 355

Weaver N. and C. F. Garner.
1955. Control of insects on hairy vetch. Jour. Econ. Ent. 48: 625-626

Wiese, I. H.
1957. The toxicity of modern insecticides to the South African honey bee. S. Afr. Bee Jour. 32(2): 7, 9-10; (3) 6,7;9-10; (4) 5-7; (5) 9-11

1958. The toxicity of modern pesticides to the South African honey bee. S. Afr. Bee Jour. 33(6): 10-11

Wolfenbarger, D. O. and F. A. Robinson.
1957. Honey bee weight colony changes in relation to malathion bait spray applications. Jour. Econ. Ent. 50: 694-695

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