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Effects of direct application of selected pesticides upon honey bee colonies

James Alvin Keener

To the Graduate Council:

I am submitting herewith a thesis written by James Alvin Keener entitled "Effects of direct application of selected pesticides upon honey bee colonies." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agricultural Biology.

S.E. Bennett, Major Professor

We have read this thesis and recommend its acceptance:

A.C. Cole Jr, C.D. Pless

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

August 21, 1973

To the Graduate Council:

I am submitting herewith a thesis written by James Alvin Keener entitled "Effects of Direct Application of Selected Pesticides Upon Honey Bee Colonies." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agricultural Biology.

Stelmon E. Bennett
Major Professor

We have read this thesis and recommend its acceptance:

Charles D. Pless

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Accepted for the Council:

Hilton A. Smith
Vice Chancellor for
Graduate Studies and Research

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EFFECTS OF DIRECT APPLICATION OF SELECTED PESTICIDES

UPON HONEY BEE COLONIES

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

James Alvin Keener

December 1973

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ABSTRACT

The purpose of this investigation was to determine the effects of seven selected pesticides upon colonies of honey bees.

Tests were conducted in a specially constructed building on The University of Tennessee Agriculture Campus. Healthy frames from strong hives were placed in glass observation hives. These frames were then treated by direct application with one of the seven pesticides to be tested: carbaryl, malathion, diazinon, carbofuran, Kelthane, alkanolamine salts of 2,4-D, and low-volatile ester formulation of 2,4-D.

The results indicated that carbofuran, diazinon, and malathion are highly toxic to bees when the hive is contaminated by direct application. Carbaryl caused only light mortality. Kelthane is nontoxic, as were both formulations of the herbicide 2,4-D.

An attempt was made to construct a "dead bee trap" which would be functional on an observation hive of the type used in this experiment. All attempts were unsuccessful.

A laboratory experiments, with the greater wax moth *Galleria mellonella* (L.), was conducted during the winter months. Different media were tested and the number of larval instars studied.

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CHAPTER I

INTRODUCTION

The honey bee, *Apis mellifera*, is a social honey-producing bee that is native to Europe. The most ancient records indicated through fossil preservation that the honey bee appeared on earth before man and existed in much the same form as it does today (Grout, 1946). There are five known species of the genus *Apis*: *A. dorsata*, *A. indica*, *A. florea*, *A. cerana*, and *A. mellifera* - the common honey bee (James and Harwood, 1969). *A. mellifera*, the only species represented in America, was brought over in early colonial times (Metcalf et al., 1967). The honey bee is now found in every state in the United States (Grout, 1946).

The honey bee is the world's most beneficial insect due to its work as a pollinator and producer of honey and beeswax. It is the only insect under man's control which is suited and adapted for pollination, and is important as a pollinator of at least fifty of our agricultural crops (Grout, 1946). As result of its pollinating ability, it comes into contact with many chemicals man applies to protect his crops. The application of these pesticides to control pests and diseases of plants is an ever-increasing occurrence. All too often, in the control of a detrimental pest, a beneficial insect such as the honey bee will be destroyed. Therefore, it is of major importance to determine the effects of pesticides upon honeybees, especially those chemicals that are most commonly used in and around areas where bees are kept.

In this study, seven pesticides were tested to determine their toxicity to honey bees when hives become contaminated by direct contact.

The pesticides used were Kelthane, a specific miticide; carbofuran, diazinon, carbaryl, and malathion, insecticides; and a herbicide, 2,4-D, using both the alkanolamine salts formulation and a low-volatile ester formulation.

CHAPTER II

REVIEW OF LITERATURE

The most common symptom of bee poisoning is an excessive number of dead bees in front of the hive (Johansen, 1963). Among one of the earliest references of bee losses blamed on pesticide applications was a report by Thompson (1881) that he killed many bees when he applied Paris green to a pear tree in bloom.

Recently, much work has been done with honey bees and pesticides, especially in establishing oral and contact LD_{50} values. Anderson and Tuft (1952) found that 1% malathion dust gave 100% mortality within five hours to honey bees confined in cages with bouquets of flowers treated with the dusts. They also observed 100% mortality within twenty minutes by direct application of 1% malathion dust to honey bees.

Johansen (1963) found malathion to be very highly toxic in his laboratory study. Toxicity was moderate to high with a residual effect of two hours to two days when he made a field application. He also made the statement that he did not consider malathion hazardous in field applications if applied when bees are not foraging, as in late evenings.

O'Brien (1956) poisoned cockroaches with malathion and observed their reactions. The poisoned cockroaches at first went into a stage of hyperexcitability which was followed by complete ataxia with tremors. At first these tremors involved the whole body but later only the limbs. The onset of the ataxia was relatively rapid, one half to one hour, yet extraordinarily prolonged, up to five days after injection; however, none of the severely affected cockroaches recovered. Similar reactions were observed in honey bees treated with 1% malathion.

Palmer-Jones (1950) stated that 2,4-D did not cause bee losses at normally encountered field doses. Later, he (Palmer-Jones, 1964) reported a 20% loss in field force and loss in honey crop from colonies in New Zealand when receiving an aerial application of a dust mixture containing superphosphate and sodium salt of 2,4-D. The New Zealand Agricultural Chemical Board (1961) also stated that large-scale field applications of 2,4-D were causing bee losses.

The ester formulation of 2,4-D was found to be very toxic to bees by direct contact when applied at concentrations that would normally be used in areas where bees forage. The amine salt formulations were found to be relatively nontoxic at normal dosages when applied directly to bees. A test was also conducted to determine the toxicity of 2,4-D upon honey bees by direct colony feeding. It was concluded that 2,4-D does not present a threat to the colony as a whole when fed concentrations as great as 250 parts per million (King, 1964).

Leppik (1951) stated that 2,4-D will slowly poison bees, extensive poisoning taking place if the herbicide 2,4-D is used for treating blossoming weeds. Johansen (1963) states that the alkanolamine salts and isopropylesters formulations of 2,4-D are toxic.

Eckert (1949), Hocking (1950), and King (1964) reported that most herbicides including 2,4-D were more hazardous to bees through foliage reduction and elimination of bee pastures than through actual toxicity. Plants injured by the chemical action of 2,4-D soon become unattractive to bees. The destruction of dandelions, mustards, thistles, and other broad-leafed weeds reduce the available pasture of many important nectar and pollen producing plants. It is through this action that 2,4-D most strongly affects honey bees.

Eide (1947), Eckert (1949), Palmer-Jones (1950), King (1961), Anderson and Atkins (1968), Anderson et al. (1971), and Moffett and Morton (1971) categorized 2,4-D as a relatively nontoxic pesticide and safe for use around bees in usual field doses. Moffett and Morton (1971) observed colonies of honey bees for at least two months after spraying with 2,4-D; 2,4,5-T; and a cotton desiccant, and found no abnormal behavior or brood development.

Georghiou and Atkins (1964) found carbaryl to be more toxic to honey bees at lower temperatures. Contact LD_{50} values at 15.6° C were 0.230-0.295 micrograms of toxicant per bee, compared to 1.110-1.336 micrograms per bee at 26.7° C (Georghiou and Metcalf, 1962), (Georghiou and Atkins, 1964), (Atkins and Anderson, 1967), (O'Brien, 1967). Stevenson (1968) found the oral LD_{50} value of carbaryl to be 0.11-0.14 micrograms per bee at 26.7° C. Alvarez et al. (1970) determined the oral LD_{50} value of carbaryl to be 0.178 micrograms per bee at 32° C. According to the rating used by Anderson et al. (1971), these LD_{50} toxicity values fall well within the category of the highly toxic group.

In 1961, carbaryl was being applied as a control procedure for the gypsy moth, Porthetria dispar (L.). Morse (1961) set up bee colonies in the gypsy moth control area, and found that direct application to the hive is not as harmful as contamination to plants and the field area being visited by bees. Contaminated pollen is gathered by field bees and returned to the hive where it is stored, and may kill bees that consume it for up to three weeks afterwards (Morse, 1961).

In 1964, Morse conducted a series of experiments to determine the importance of hive contamination. Some U.S.D.A. Publications suggested covering hives with burlap when hazardous pesticides are being used. Morse questioned the value of this for carbaryl. He concluded from his experiments that hive contamination from carbaryl showed no significant increase in bee mortality. Therefore, the covering of hives to prevent external contamination is of little value. Preventing bees from foraging after flight is another matter (Morse, 1964).

From his experiments in 1968, Morse (1972) concluded that colonies removed from an area to be treated with carbaryl, and then returned one week later, would not suffer significant losses. Johansen (1963) found carbaryl to be more toxic, having a residual effect of seven to twelve days, and hazardous to bees at any time.

Johansen (1963) and Anderson et al. (1971) found diazinon and malathion to be highly toxic and Kelthane, a specific miticide, and 2,4-D a herbicide, to be relatively nontoxic and not hazardous to bees at any time. Carbofuran, a more recent insecticide, was found to be very toxic to bees by Anderson et al. (1971).

Anderson et al. (1971) categorized pesticides into one of three groups in relation to their toxicity to honey bees. Only a selected few of the most commonly used pesticides will be mentioned here.

GROUP I <u>Highly toxic</u>

LD_{50} contact toxicity values of less than two micrograms of toxicant per bee. Those pesticides listed here will cause severe bee losses if applied when bees are present at treatment time or within a day thereafter.

DIAZINON MALATHION

CARBOFURAN CARBARYL

GROUP 2 Moderately toxic

LD_{50} values are from two to ten micrograms of toxicant per bee. If dosage, timing, and method of application are correct, these can be used around bees, but should not be applied directly to bees in the field or to the hive. None of the pesticides from this group were used in this experiment, and only a few of the more common ones are mentioned.

CHLORDANE	DI-SYSTON	SYSTOX
CIODRIN	ENDRIN	
DDT	MIREX	

GROUP 3 Relatively non-toxic

LD_{50} values above ten micrograms of toxicant per bee. These pesticides are safe to use around bees with only a minimun of injury.

2,4-D KELTHANE

CHAPTER III

MATERIALS AND METHODS

1. Description of Building

In order to conduct the experiment, a temporary building (Figure 1) was constructed from a shipping crate donated to the Agricultural Biology Department by Dr. E. A. Heinrichs when he returned from the US-AID Program in Bangalore, India. The exterior was covered with alumnium siding on all four sides and on top. The outside dimensions were eight feet long by six feet wide and six and one-half feet high at center. Inside, the building measured seven and one-half feet long, five and one-half feet wide by six feet high at the center.

Inside two shelves were constructed along the east wall. These were constructed of one-by-twelve-inch shelving boards and extended the inside length of the building. The bottom shelf was 25 inches from the floor and the top shelf 25 inches above that. Eight holes were bored through the east wall to accommodate the plastic entrance tubes from the hives which were placed on the shelves. The hives were spaced a distance of one foot, seven inches apart.

The building was then wired with 120-volt electrical current. This enabled the use of portable lights for better observation, and also the use of a heater for maintaining a constant temperature during the winter. The east and west walls on the inside of the building were also lined with alumnium siding for better light reflection.

Figure 1. Bee lab on the Agriculture Campus of The University of Tennessee, Knoxville.

2. Description of Hives

The hives (Figure 2) used for the experiment were of the small observation type rather than the large conventional hives as used by beekeepers. A limited supply of bees and the need for close and continuous observation after treatment indicated the practicality of the small hives. The hives were purchased from the Walter T. Kelly Company, Clarkson, Kentucky, for five dollars each.

The hive dimensions, as given in a catalog by Walter T. Kelly, were as follows:

1. The bottom panel is 4 3/4" by 21 1/2".
2. The end panels are 3 1/4" wide by 11" long. The plowed out end goes up and the top bar slips down through this onto the rabbet, and has saw cuts for ventilation, and one end has a 1" hole near the bottom board.
3. The two top rails measure 1 7/16" wide by 19 7/8" and are rabbeted out at the edges.
4. The cover measures 3 5/8" by 19 1/2" and is rabbeted on the four edges of one side and has six saw cuts for ventilation.
5. The two glass sides are 10" by 19" and are double-strength glass.

3. Description of Pesticides

In selecting chemicals for the study, it seemed desirable to use those chemicals both insecticidal and herbicidal which are in common use today and could serve as sources of bee poisoning or hive contamination. Those chemicals are described at greater length below.

<u>DIAZINON - Organic Phosphate - Thiophosphoric Acid Prototype</u>

DIAZINON is also known as BASUDIN, DIPOFENE, NEOCID, NEOCIDOL,

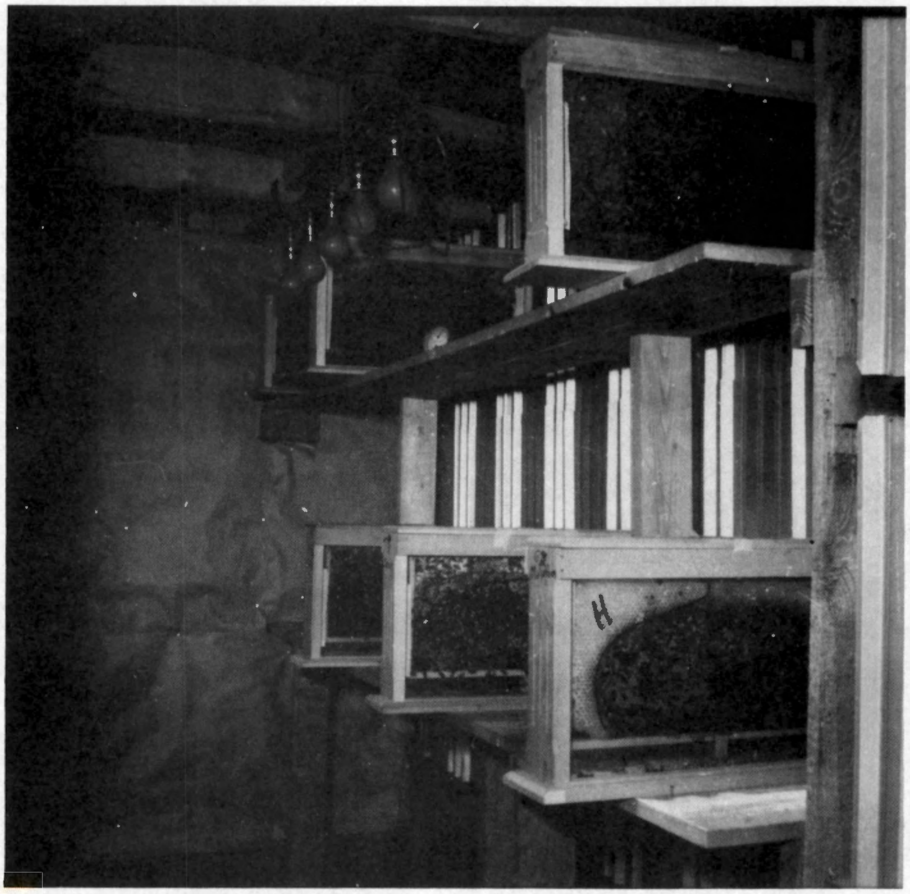

Figure 2. Observation hives in bee lab.

and SPECTRACIDE. The chemical formula is 0,0-diethyl-0-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate. Diazinon had its origin in 1958 with Geigy Chemical Company. It is an organic phosphate insecticide-acaricide with contact and stomach poison activity.

Some of the important pests controlled by diazinon are: cockroaches, mosquitoes, mites, lice, codling moths, chiggers, aphids, fleas, scale, silverfish, leafhoppers, ticks, pear psylla, ants, corn earworms, and houseflies. It can by sprayed on most garden crops, nut and fruit crops, and grains and grasses without injury. It is compatible with fungicides and other insecticides (Thomson, 1972).

The formulation used in this study was 25% emulsifiable concentrate (E. C.). The acute contact toxicity is 0.22 micrograms per honey bee and the acute oral toxicity is 0.20 micrograms per honey bee (Stevenson, 1968). Anderson et al. (1971) place diazinon in the highly toxic group.

MALATHION - Organic Phosphate - Dithiophosphate Prototype

MALATHION is also know as MALAPHOS, MALATHON, MALPHOS, CYTHION, EMNATOS, CARBOPHOS, and MERCAPTOTHION. The chemical formula is 0,0-dimethyl dithiophosphate of diethyl mercaptosuccinate. It, like diazinon, is an organic phosphate insecticide-acaricide.

Malathion was first produced in 1950 with American Cyanamid. It is used for the control of aphids, mosquitoes, mites, spiders, scale, ants, flies, ticks, leafhoppers, lice, leafminers, bollworms, bollweevils, thrips, armyworms, loopers, grasshoppers, mealybugs, chinchbugs, Japanese beetles, corn earworms, and spittle bugs. It is safe to use on most garden crops, nut and fruit crops, grains and grasses, and livestock.

Malathion is compatible with most insecticides and fungicides; however, mixtures with alkaline materials show a decreased residual toxicity (Thomson, 1972).

The formulation used in this study was 55% E. C.; five pounds active per gallon. Stevenson (1968) gives the acute contact toxicity of 0.22 to 0.27 micrograms of malathion per bee, and the acute oral toxicity of 0.20 micrograms per bee. Anderson et al. (1971) places malathion in the group of insecticides highly toxic for honey bees.

Malathion in pure form is a liquid, yellow to dark brown in color, the technical grade being 85% to 95% pure. The boiling point is 155-157° C. Malathion tends to crystallize at low temperatures, the melting point being -7° C. It is stable between pH 5 and pH 7; above pH 7 it hydrolyzes. The solubility in water is 145 ppm, it is miscible in most organic solvents but in petroleum oils its solubility is limited (De Ong, 1956; Frear, 1955).

According to O'Brien (1967), organophosphates such as diazinon and malathion kill animals "both vertebrate and invertebrate, by inhibiting cholinesterase with consequent disruption of nervous activity caused by accumulation of acetylcholine at nerve ending." This allows continuous impulses to pass along the nerve causing a twitching condition that results in paralysis and eventually leads to death (Kearns, 1956; Spencer and O'Brien, 1957; Fukuto, 1961).

Symptoms of poisoning by organophosphates are first excitability, followed by tremors especially in the extremities, then paralysis and death (O'Brien, 1967). Occasionally there is regurgitation from the digestive track during the hyperexcitability stage (Johansen, 1963).

KELTHANE - Diphenyl Compound

This miticide is also known as DICOFOL and ACARIN. The chemical formula is 1,1-Bis(p-Chlorophenyl)-2,2,2 trichloroethanol. Kelthane is a chlorinated hydrocarbon acaricide.

Kelthane had its origin in 1955 with Rohm and Haas Company. It has no insecticidal activities, and is used only for the control of mites. It is safe to use on most garden crops, nut and fruit crops and ornamentals, but shows phytotoxicity cn eggplant and avocado. It is compatible with other commonly used pesticides (Thomson, 1972).

The Kelthane used in this experiment was 18.5% emulsifiable concentrate. Anderson et al. (1971) place Kelthane in the relatively nontoxic group. The LD_{50} value is greater than ten micrograms of toxicant per bee.

CARBOFURAN - Carbamate

FURADAN is another name for this insecticide. The chemical formula is 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate.

Carbofuran is a carbamate compound used as a contact and stomach poison insecticide. Its use was initiated in 1968 with Niagara Chemical Division of FMC Corporation. It is used to control such pests as aphids, bollworms, bollweevils, alfalfa weevils, mosquitoes, codling moth, corn rootworm and scale. Carbofuran, at recommended rates, is nonphytotoxic and possesses long residual activity. It does not mix with alkaline pesticides, but is compatible with other pesticides.

An experimental formulation, 4 Flowable (4F), was used in this study and found to be very toxic to bees. Anderson et al. (1971) classified carbofuran as highly toxic to honey bees.

CARBARYL - Carbamate

CARBARYL is also know as SEVIN and RAYVON. Its chemical formula is 1-Naphthyl methylcarbamate. Like carbofuran, it is a carbamate insecticide, possessing both contact and stomach poison action.

Carbaryl had its origin in 1957 with Union Carbide Chemical Corporation, and is labeled to control pests such as: aphids, cucumber beetles, codling moth, gypsy moth, plum curculio, stink bugs, grasshoppers, scale, leafhoppers, thrips, bollworms, armyworms, bollweevils, Japanese beetles, ticks, and fleas. However, it will not control spider mites. It has a widespread use on most agricultural crops, nuts, berries, ornamentals and forest plants. Carbaryl is noncompatible with alkaline materials (Thomson, 1972).

The formulation used in this study was 50% wettable powder (WP). Alvarez et al. (1970), Georghiou and Atkins (1964), Georghiou and Metcalf (1962), Atkins et al. (1970), O'Brien (1967), Stevenson (1968), Anderson et al. (1971), and Thomson (1972) listed carbaryl as highly toxic toward bees, and having an LD_{50} value of less than two micrograms of toxicant per bee. The mammalian acute oral LD_{50} value is 500 milligrams per kilogram of body weight; therefore, it is considered a safe insecticide for man to use.

Carbamates such as carbaryl and carbofuran are considered to kill insects entirely by cholinesterase inhibition (O'Brien, 1967). Whole colonies killed, or dead brood in or in front of the hive, or a slowed-down appearance, as if they had been chilled, are symptoms of carbaryl poisoning (Johansen, 1963).

2,4-D - Phenoxy Compounds

Other names for 2,4-D are AQUA KLEEN, DED WEED, DMA-4, DACAMINE, BUTYL-400, FERNESTA, FERNIMIRE, EMULSAMINE E-3, EMULSAVERT-D, WEED-AG-BAR, TRIBUTON, SALVO, ESTERON-99, FORMULA 40, VERTON, CROTILIN, HEDONOL, PENNAMINE-D, PHENOX, KROTILINE, WEEDAR-64, CHLOROXONE, WEED-B-BON, WEEDONE-170, WEEDONE-638, WEEDONE LV4, AMOXORE, WEEDONE 48, and WEEDONE-AERO-CONCENTRATE. The chemical formula is 2,4-Dichlorophenoxyacetic acid.

The chemical 2,4-D is used mainly in post-emergency applications and is a selective, translocated phenoxy herbicide. It was originated in 1942 by the Amchem Products Inc.

There are various formulations such as sodium and ammonium salts, amine salts, high-volatile esters, and low-volatile esters. In this experiment two formulations were used. The first was alkanolamine salts of ethanol and isopropanol series; the second was propylene glycol butyl ether ester, a low-volatile ester. This herbicide controls most broad-leaf weeds such as: chickweed, cocklebur, mustards, ivy, goldenrod, plantain, bindweed, thistle, willow, sunflower, and purslane. Many desirable plants such as cotton, tomato, grape, fruit trees, ornamentals, and several grasses are susceptible to 2,4-D. Caution should be used when treating areas close to these plants. Rapidly growing plants are the most susceptible and usually become deformed before they die (Thomson, 1973).

The ester form of 2,4-D penetrates foliage more readily than do the salts. The earlier types of esters were the high-volatile types made with low-boiling alcohols methyl, ethyl, and propyl. These gave off volatile fumes in large quantities that would injure nearby susceptible plants. Later, high-boiling alcohols such as propylene glycol were used to produce low-volatile ester formulations (De Ong, 1956).

The ester formulations are combined with a wetting agent, thus yielding a milky emulsion when mixed with water. The amine salts are completely soluble in water and are less active, less volatile, and therefore less hazardous than the ester formulations. Concentrations are expressed in terms of "acid equivalent," which is defined as the actual amount of the active ingredient (2,4-D) present per unit volume or weight of the compounded material (King, 1961).

The chemical, 2,4-Dichlorophenoxyacetic acid, is a white solid having a melthing point of 138° C. It is practically insoluble in water and oils, but soluble in alcohols and alkaline solutions. It exhibits corrosive action and reacts with metals and bases (Frear, 1955). Anderson et al. (1971) place 2,4-D in the group of relatively nontoxic herbicides.

<u>Dilution and Application of Pesticides</u>

All pesticides were diluted to approximately 1%, using dilution tables by Williams (1968). This concentration is similar to that of actual field doses. After dilution, pesticides were put into individual 32-ounce hand sprayers. Each sprayer had an adjustable nozzle for fine spray, coarse spray, and jet stream. The spray was ejected by means of a trigger action located under the nozzle. The amount of spray delivered per trigger squeeze was then determined by use of a triple beam balance. A container was weighed on the scales and the weight recorded. Ten squeezes of the trigger were directed into the container and the weight was taken again. The difference in weight was the amount ejected from the sprayer. This procedure was repeated twice and the three values were averaged. This number was then divided by ten, which gave the average amount of spray ejected per trigger squeeze. Each sprayer was calibrated in the above manner and adjusted to deliver approximately one gram per trigger squeeze. Nozzles were then taped to prevent movement out of adjustment.

After dilution and calibration, the pesticides were applied to the hives (Figure 3). Each hive was sprayed with four shots from a selected pesticide. The spray was directed into the four groups of saw cuts (Figure 4) used for ventilation, one squeeze in each end and two on the top. This application represented heavy external and internal contamination which might be encountered by the apiarist.

4. Problems

Drifting of Bees

One of the initial problems encountered in September of 1972 was bees drifting from one hive to another. Drifting was probably due to overcrowding in the bee barn, since all eight hives were active and the similarity of entrance tubes led to confusion. The problem was solved by rotating weak hives with strong hives to maintain a population balance. Drift was not a problem during the summer of 1973, as no more than five hives of bees, and generally just four, were in the building at one time. The hives were more widely separated and similarity at the point of entrance varied with the different "dead bee traps."

Queens

Another problem encountered in 1972 was maintaining a queen in a small hive. The queen would suddenly disappear. Whether she was

Figure 3. Application of a pesticide to an observation hive.

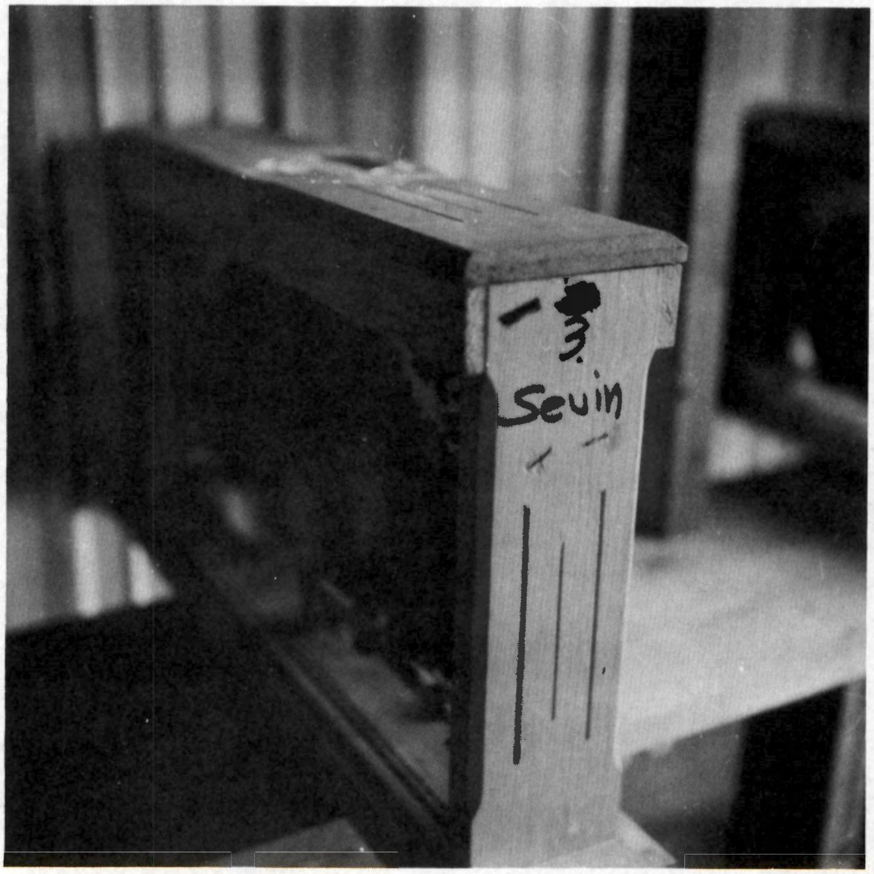

Figure 4. Observation hive showing saw cuts.

killed and removed by the workers, or if she left the hive in search for more egg-laying sites and was unable to return, was never determined.

A queen is capable of laying an average rate of 1500 to 2000 eggs per day during the height of brood rearing. Egg deposition by the queen shows seasonal variation. During the spring and summer months she lays the most eggs (Grout, 1946). The high reproductive potential could possibly be a source of frustration to a productive queen placed upon a single frame with approximately seven to eight thousand cells. Many of these cells are filled with either honey or pollen, thus reducing to only two or three thousand the cells available for egg laying.

In the summer of 1973, it was decided not to use queens, as the bees will remain at the hive without a queen. The duration of each test lasted three weeks or less and no difficulty was noticed due to lack of a queen.

Robber Bees

Robber bees were a problem the second year, occurring in hives weakened or killed by poisoning. This would be a problem in a standard hive accidentally contaminated by a lethal poison. Robbers entered, uncapped the honey and removed the contents; however, brood, dead bees, pollen, and comb were not disturbed. Upon removal of the honey, the robbers ceased to return to the hive.

Dead Bee Traps

The "dead bee traps" were a problem of the second year. Three traps were designed: a) the "offset tube," b) the "open top," and c) the "intermediate." The "offset tube" dead bee trap (Figure 5) proved

Figure 5. Offset tube dead bee trap.

too difficult for the bees to master, and large numbers died in the trap. The screened vents were covered with paper to avoid the confusion with the exit tube, which was further restricted with nails driven through it resembling a queen excluder in a standard hive. Bees could enter and leave but with great difficulty, and survival of the hive was endangered. The "open top" dead bee trap (Figure 6) was too much in contrast with the "offset tube" type and failed to serve its purpose of collecting dead bees. Adults were observed flying out with the dead bees. The "intermediate" dead bee trap (Figure 7) was designed as the intermediate between the first two. While this was not as confining as the "offset tube" type, it was too open for efficiency. With some difficulty bees were able to crawl out with a dead bee and fly off. The "intermediate" trap would probably be more functional if the top opening was reduced in size and a heavy layer of fresh petroleum jelly maintained around it at all times.

All traps were constructed from inverted one gallon plastic mayonnaise jars. The bottom of the trap (the lid of the jar) provided easy removal of dead bees and an efficient counting tray. The lid was punctured with nail holes to allow drainage of water during rains.

Wax Moths

Weakened hives and stored combs are often damaged by the greater wax moth, Galleria mellonella (L.). Wilson (1965) reported the greater wax moth as a serious pest in warmer regions of the United States. Milum (1952b) gives the greater wax moth the credit for being the "most universal despoiler of combs of the honeybee." He attributes this to their technique of laying eggs, up to 1800 by a single female, in cracks and

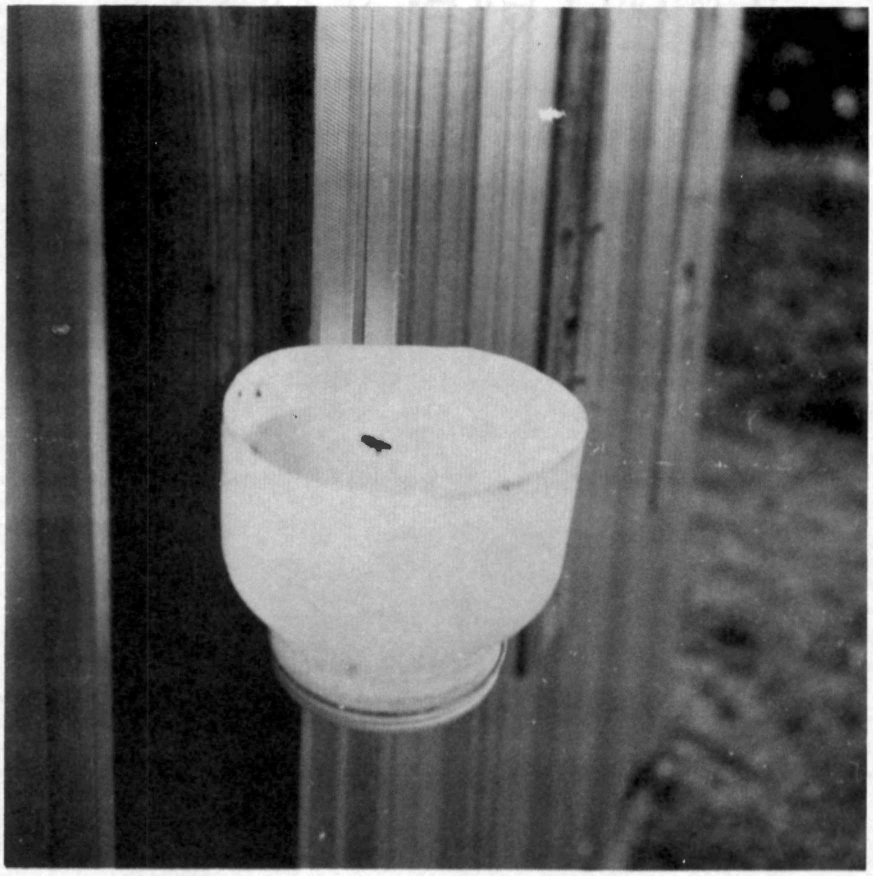

Figure 6. Open top dead bee trap.

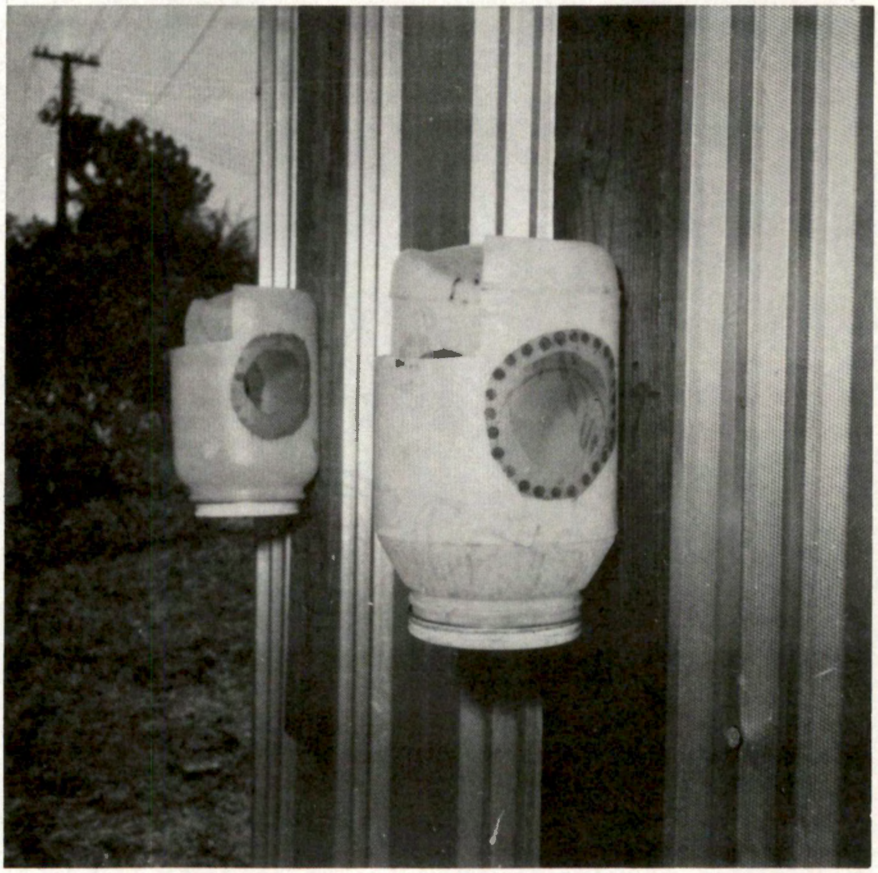

Figure 7. Intermediate dead bee trap.

crevices where they are protected before hatching. Milum and Geuther (1935) reported that an individual female lays an average of 750 eggs.

Below is a lengthy description of the greater wax moth, as presented by V. G. Milum.

> The length of adults from front of head to tip of wings when folded varies from 5/16 to 3/4 inch. The wing span is 1 1/4 inches as measured from tip to tip of expanded front wing. The outer wings are mottled, brown to purplish; silver gray with fraying; underwings creamy white. Front two-thirds of outer wings are folded at a sharp angle downward giving a boat-shaped appearance. Head is light gray to light brown. Female has two short prominent pointed palps on front of head. Outer margins of forewings of male are deeply scalloped and fringed (Milum, 1952b).
>
> The younger larvae of this species are grayish white, extremely active, rapid running with thoracic legs prominent. Older larvae are of solid dirty gray color, up to 1 1/8 inches in length . . . . Newly hatched larvae may feed at first on isolated portions of comb, on or beneath surface, forming silken tunnels, with added frass and bits of comb, gradually assembling in a mass of webbing, with tunnels extending through the remainder of combs in search of food consisting of meconium, cast larval skins, and pupal cases of the bees lining the cells, in brood combs either with or without pollen. Development dependent upon temperature and food available, with a reported average larval period of 28.85 days at 35 degrees C. (95°F.).
>
> In pupating the larva usually crawls upward then grooves out a shallow place in the wood of frames or other portions of the hive such as the inner cover or side wall of the hive. The cocoons often in rows or tier, side by side, varying with larval size, up to 1 1/8 inches, usually white, but sometimes are covered with bits of frass. Each larva, before actually changing to a pupae, cuts three flap-like slits in an exposed end of its cocoon to facilitate the future adults emergence.(Milum, 1952a).

Much experimentation in the control of wax moths has occurred over the years. Harmatiuk (1936) found that by shining a light near the hives at night he could catch the moths with his "quick hand." Wilson (1965) stated that freezing weather lasting several days would kill all stages of this moth, thus limiting its range in the colder regions of the country. Johansen (1962) used Bacillus thuringiensis in an experimental attempt to control the greater wax moth, but was able to conclude nothing.

Krebs (1957) conducted the first experiment using ethylene dibromide as a fumigant. Previous to this time carbon disulphide, gasoline, sulfur, cyanide, paradichlorobenzene, methyl bromide and other fumigants had been used. There were several drawbacks to these controls. The first three are fire hazards. Cyanide, carbon disulphids, paradichlorobenzene, and sulfur do not kill the egg stage without requiring several treatments. Methyl bromide and cyanide are extremely toxic to the handler and have a rapid rate of evaporation, necessitating the use of an air-tight room. Ethylene dibromide, on the other hand, is nonexplosive and will not burn. It is considerably less toxic to humans than cyanide or methyl bromide and is effective in killing all stages of the wax moth. It does not leave an offensive odor like that of the paradichlorobenzene which is absorbed in the honey. There is no residue and it does not require air tight rooms, as it has a slow rate of evaporation and is heavier than air. It is nontoxic to bees, but direct application causes bees to temporarily lose their ability to fly (Krebs, 1957).

Wilson (1965) recommended either ethylene dibromide or paradichlorobenzene for empty comb fumigation. Lehnert and Shimanuki (1967) showed that 0.02 ml. of ethylene dibromide per liter will kill all stages of the greater wax moth. In 1968 they found ethylene oxide to be a suitable fumigant against the wax moth. The best control, however, against the wax moth in the field is a strong colony of bees (Wilson, 1965).

During the winter months of 1972-73, wax moths were reared successfully in the laboratory, using old infested brood combs as a growth medium. The infested combs were maintained in screened cages and covered with a glass top to prohibit moths from escaping. Experiments were attempted to rear the larvae on a pure beeswax diet and on a diet of honey and beeswax, both without brood cells or pollen. The first instar larvae transferred to the pure beeswax showed no signs of feeding and apparently died shortly after transfer. First instar larvae placed on honey and beeswax progressed further, but not normally. One larva had reached the pupal stage after a long developmental period, but was considerably smaller than a normal wax moth pupa.

A search through the literature revealed nothing as to the number of larval instars of this moth so an attempt was made to determine that number using "Dyars Law" as a guideline. Dyars Law states that "the widths of the head of a larvae in its successive stages follow a regular geometrical progression." He selected the head as "the part not subject to growth during the stage, and its width as the most convenient measurement to take." (Dyar, 1890). Unfortunately there are exceptions and irregularities to this law, such as those listed by Forbes (1934). Frequently one sex may have one or more instars than the other. This happens when one sex is much larger in the adult stage. Another variation is that "one of the regular stages should be omitted, giving a double ratio for one molt and a total number of stages one less; or in contrast there may be an additional stage interpolated, giving half ratio for two successive molts. Finally the law may be less rigidly carried out, and we get a variable head size, depending on the food supply or some other unknown factor." Gaines and Campbell (1935) and Ludwig and

Abercrombie (1940) found that the progression factor often changed in successive instars. They were working with the corn ear worm, Heliothis obsoleta (Fab.) and the Japanese beetle, Popilla japonica, respectively. Quaintance and Brues (1905) found that highly nutritious food and excessive care caused less than normal number of molts in the cotton bollworm, Heliothis obsoleta (Fab.), while insufficient or disagreeable food and neglect caused molts in excess of normal.

In an attempt to determine the number of larval instars a total of 335 larval, were collected from the rearing cages and head capsule measurements were plotted on a graph (Figure 8). The measurements were made in millimeters and a range from 0.17 mm to 2.40 mm was observed. There was no definite pattern of peaks and the number of larval instars could not be determined. This might possibly be a result of a limited food supply at the time the sample was taken. Several generations had already been reared on the frames which were reduced to a mass of silk, larvae, and frass (Figure 9).

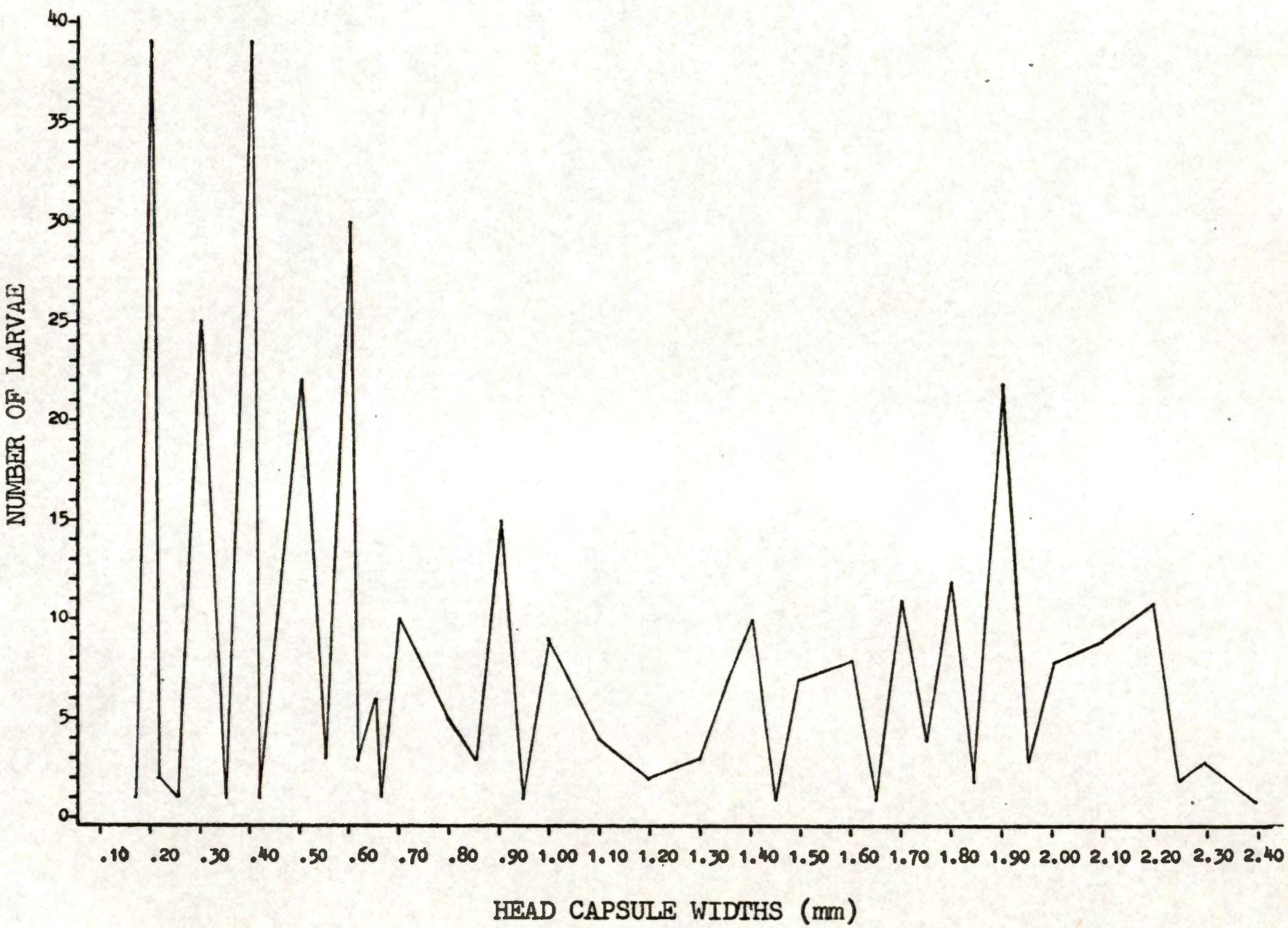

Figure 8. Head capsule measurements of the greater wax moth, Galleria mellonella (L.).

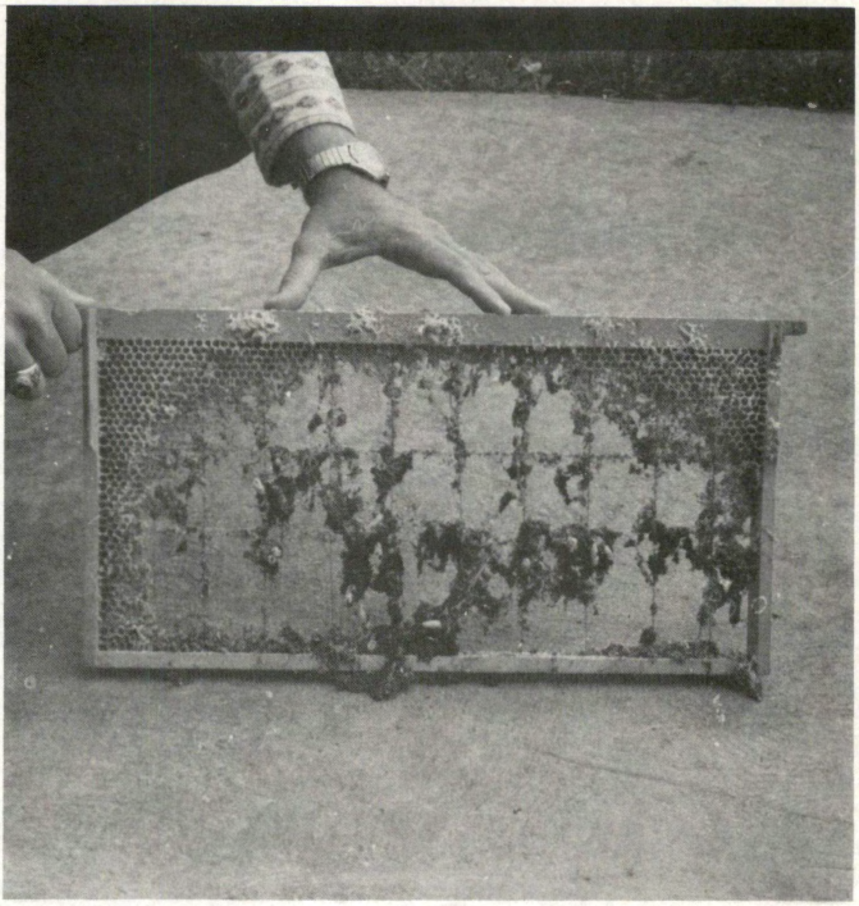

Figure 9. Frame damaged by the greater wax moth, Galleria mellonella (L.).

CHAPTER IV

RESULTS AND DISCUSSION

Eight hives were introduced into the bee lab in September of 1972. These hives were observed until June 1973, when they were removed and cleaned. They were heavily contaminated with wax moths, and all the bees were dead and the combs destroyed. The hives were fumigated with ethylene dibromide as recommended by Krebs (1957), Wilson (1965), and Lehnert and Shimanuki (1967).

Hive Number 1 - Check

New frames of bees were introduced into the bee lab on July 2, 1973, and poisoning of hives began on the following day. Hive Number 1 served as a control and averaged approximately 475 adults throughout the first test, which lasted until July 19. There was no abnormal behavior or high mortality observed in the check hive during the first test.

Hive Number 1 became a target for robber bees because of its location near Hive Number 2, where the resident bees had been killed off and the hive was being heavily raided by robbers. On the second day robbers began entering the check hive and continued for eight more days, with as many as 20 dead robbers observed on the floor of the hive. The heaviest fighting took place the first four days with rapid decrease in robber bees after that. Very few robbers were able to escape from Hive Number 1 and their honey supply was not damaged.

The first test ended on July 19, and the bees were exchanged for a new colony from one of the hives at the Plant Science Farm. Several other frames were secured to test other pesticides. These remained closed up overnight in a storage laboratory and several bees died during the night. The bees were still in an excited condition the following morning when the hive was opened to remove the dead bees. The check hive, with approximately 800 bees, was taken to the bee lab and the bees released to become accustomed to a new hive, dead bee trap, and new location. The bees in the check hive remained extremely excited for some time, and that evening 55 dead bees were counted. Twenty-four hours later 72 additional dead bees were counted. Worker bees began removing the dead bees on the third and fourth day after introduction. The hive population began to increase and, by termination of the second test, there were an estimated 1000 workers in the hive. The same hive remained as the check for the third test which began on July 30, by which time an estimated 1400 workers were present.

Hive Number 2 - Diazinon

Hive Number 2, containing an estimated 900 adult bees, was sprayed with a 1% emulsion of diazinon on July 2, 1973. The bees became very excited and began dying within fifteen minutes. In one hour 75% were dead. After two and one-half hours no bees were alive, and a count revealed 1050 dead bees.

Late the same evening robber bees were attracted to the hive and several died as a result of being poisoned by the diazinon. The honey was completely removed by the fourth day, but the residual action of the diazinon was effective, since many robbers were still dying. The robbers took only honey, leaving the pollen and wax in the hive. The hive was opened on July 10 to check pupal cells, which were found to be in good condition. The entrance was closed off with screen to prevent robbers from entering and to determine if adults were able to emerge from the remaining 90 pupal cells. Four days later the emergence of two adults confirmed the viability of the pupae. They appeared healthy and normal, but had no food source. The bees were given sugar water through the feeder cap at top of the hive. As additional bees emerged, the hive was opened to allow for cleansing flights. By the end of the test 65 bees had emerged and died, leaving 25 capped pupae. Those were opened and found to be dead also.

Shortly after the adult bees were killed, the larvae began crawling from the cells and falling to the bottom board. Two days after treatment, a total of 201 larvae were removed from the floor of the hive. Larvae had not become discolored or otherwise abnormal, apparently not affected by the diazinon. Similar behavior was listed by Johansen (1963) as a symptom characteristic of poisoning by carbaryl. This behavior was checked to determine if the response was a result of poisoning or other factors. A check frame, heavy with larvae but without adult bees, was placed in an observation hive. Within four hours larvae were protruding from their cells and falling to floor. Twenty hours later, 45 larvae had fallen to the floor and the frame was reinstalled in its original hive. The behavior exhibited by the larvae was therefore assumed not a response to the diazinon (or to carbaryl as suggested by Johansen, 1963), but rather to some other factor, such as lack of attention from nurse bees, or a change in temperature due to absence of bees.

Larvae crawling out of cells would be a characteristic symptom of bee poisoning when a complete adult bee kill has occurred. The writer is not familiar with any diseases of bees, either adult or larval, which are rapid enough to produce the same larval behavior. In all three of the major brood diseases: American Foulbrood (AFB), European Foulbrood (EFB), and Sac Brood, the larvae remain within the cell, or, in the case of Sac Brood, the workers remove the dead larvae which are in a stretched out position. In no instance does the larva crawl out on its own accord. Also, the pearly white color of these "wandering larvae" distinguishes them from diseased larvae. Larvae killed by AFB will change in color to coffee brown. They become distorted, shrunken, and lie on the bottom of the cell. Larvae killed by EFB change from pearly white color to slightly yellow, then to a brownish yellow. They remain in the cell as a greasy mass in various positions. Even under such conditions as chilled, starved, and overheated brood the larvae become a dark gray color. The larvae in Hive Number 2 would have eventually changed to a dark gray color because of starvation, but in actual cases of starvation the larvae remain in their cell and are removed by the worker bees when they die (Little and Wallace, 1972).

Hive Number 3 - Carbaryl

Hive Number 3, before treatment with carbaryl, contained approximately 1100 adult bees. Within five minutes after spraying with a 1% suspension of carbaryl the bees became hyperexcited, with the affected bees going into a very rapid spin. All their motions became jerky. Unaffected bees followed the sick bees very closely and touched them with their antennae in much the same manner they follow a bee returning from the field as it performs a "round" or "waggle" dance (Frisch, 1955).

Several bees were dead within fifteen minutes and hyperexcitement lasted for two hours. By late afternoon workers began carrying dead bees out of the hive and into the dead bee trap. An estimated 15% kill occurred, with approximately 100 to 200 bees dead. The third day after treatment, all activity was back to normal with approximately the same number of bees as before treatment. Apparently no significant damage had resulted from hive contamination with carbaryl.

The test was concluded on July 19, and a new frame of bees was put into the hive. Bees were subjected to the same conditions as described for Hive Number 1, July 19. The bees, approximately 500, were in a highly excited condition before and after application of 1% carbaryl. Reactions were the same as observed for the first application of carbaryl: spinning, turning, and running. There was, however, higher bee kill from this application, namely approximately 40% as compared to 15% for the first treatment. The hive was heavy with larvae, and workers continually feeding and capping the larvae. Removal of dead bees was therefore delayed until three days after treatment and continued the remaining four days of the experiment.

It was noted prior to termination of the experiment that many young adults were unable to emerge from their pupal cells. The inmates would struggle for approximately thirty hours before dying of exhaustion. Workers would then begin excavating around dead bees and remove the dead bodies. Milum (1952b) noted this same situation and upon investigation found the bees to be "anchored to the bases of their cells by the webbing of greater wax moth larvae tunneling next to the comb midrib." Mature greater wax moth larvae were observed lying in the dead bee trap after excavation around imprisoned bees began.

On July 27, 1973, the test was concluded, and there were approximately 600 adults on the frame. Although a 40% kill occurred on July 20, the hive was able to recover to the original state within one week.

A new frame of bees was introduced into the bee lab and released. The bees had two days to become accustomed to their new surrounding before treatment on July 30. Prior to treatment with 1% carbaryl there were an estimated 900 bees in the hive, and no signs of excitement were observed. In minutes after spraying, the bees became highly excited and exhibited symptoms similar to those of the first two sprayings with carbaryl; that is, running rapidly, spinning in circles, bouncing, and even walking backwards (not observed during the first two sprayings). Again the excitement lasted only a couple of hours and then stopped. Excitement started again an hour later and lasted approximately fifteen minutes, after which no further excitement was detected.

A total of 150 dead bees was counted after the third treatment with carbaryl, which gave an approximately 15% kill, as it had in the first test. Workers began removing dead bodies the day after treatment and completed the job within two days. The test lasted one week, during which time the hive strength was recovered. The hive contained an estimated 900 adult bees at the start of the test. In one week after treatment, it contained approximately 1100 bees.

Hive Number 4 - Alkanolamine Salts of 2,4-D

Hive Number 4 contained an estimated 1000 bees prior to spraying and approximately 1700 bees at termination of test two and one-half weeks later. The number of bees increased daily with an average number of 1250 adults in the hive. On July 3, the hive received a direct application of 1% solution of alkanolamine salts of 2,4-D. There was no excitement or abnormal behavior other than a large number of bees ventilating the hive. A total of five dead bees was counted the following morning. This was insignificant, as there were eleven dead bees counted in the check hive. Therefore, this was assumed to be natural mortality. According to Little and Wallace (1972), the average daily death rate is 1200 bees in a hive of approximately 50,000 workers. The hive remained strong throughout the test, and no robber bees were able to enter. The test was terminated on July 19.

A new frame was put into the observation hive and retained in a storage laboratory, as was previously discussed. This particular hive exhibited the greatest excitement and approximately 300 bees were lost. While still excited, the hive was sprayed with 1% solution of alkanolamine salts of 2,4-D. Bees were then installed in the bee lab and opened to new surroundings. As with the first test, there was no reaction from application of 2,4-D.

On the following morning, 90 dead bees of the estimated 900 were counted on the floor of the hive. However, it was felt that this was not a result of spraying with 2,4-D since the check hive, which had gone through the same conditions, had a count of 72 dead bees. The removal of dead bees began on the second day. The hive increased in strength and, by the end of the test on July 27 there were an estimated 1000 adults in the hive.

<u>Hive Number 5 - Kelthane</u>

This hive was introduced into the bee lab on July 19 under the same conditions discussed earlier. The bees, approximately 500, were in a very excited condition immediately before direct application of 1% Kelthane. No added excitement was observed following treatment. Fifty dead bees were observed in the hive late the same evening. The deaths were attributed to causes other than pesticide poisoning, since the check hive experienced approximately the same mortality rate. Removal of dead bees was postponed until the third and fourth day after treatment, because priority was given to care of the larvae. When the test was terminated on July 27, the hive was in good condition and contained an estimated 600 adults.

A new frame containing an estimated 600 workers was put into the observation hive. Bees were allowed an orientation period of two days before direct application of 1% emulsion of Kelthane. The following morning there were ten dead bees among an estimated 700 living workers. Apparently no bees died as a result of the Kelthane and only natural mortality occurred throughout the remainder of the test. The hive contained an estimated 900 adults at the end of the test on August 6.

Hive Number 6 - Malathion

Hive Number 6 was subjected to the same treatment as hives number 1,3,4, and 5, which were introduced on July 19. There were an estimated 500 worker bees prior to treatment with 1% malathion. Within thirty minutes they went into a stage of hyperexcitability, exhibiting the following reactions: loud buzzing, appearance of fighting, discharge of intestinal contents, rubbing feet together, running aimlessly on hive, falling and forming a writhing mass of bees on the floor of the hive. The hyperexcitement lasted two to three hours, during which time ataxia set in. Within six hours all bees were considered dead, even though slight tremors were observed the following morning. As with O'Brien's (1956) work with cockroachs, none of the affected bees recuperated. Because adult bees were not present on the frame, the larvae began protruding from cells and dropping to the floor within five hours after treatment. By late evening a few young adults had emerged from pupal cells; however, most of these were dead by the following morning. A total of 561 dead bees were removed from the hive.

Several workers continued emerging throughout the remainder of the test. An average of 35 bees a day was maintained until the end of test on August 2. A total of 65 dead bees were counted the last day of the test. A few bees were unable to emerge from the pupae cells, and, when removed, they were observed to be entangled in the webbing of the greater wax moth. Robber bees removed the honey on the second and third day after treatment.

Hive Number 7 - Carbofuran

Hive Number 7 was introduced into the bee lab on Friday, July 27, and an application of 1% carbofuran was postponed until the following Monday morning. Within five minutes after application of carbofuran the bees entered a state of hyperexcitement. Their reactions included: running over comb with wings outstretched, regurgitation, stumbling, and dropping off the frame to the floor, and exhibiting uncontrolled leg movements for a short time before dying. A few bees were observed crawling on the ground outside the hive unable to fly. All bees in the hive were dead within one and one-half hours after treatment. Dead bees were removed and a total of 1241 bees were counted. The larvae, as noticed in other complete kills, began falling to the floor.

Very few robbers entered this hive and most of these died. Lack of robbers may be for several reasons: a) very little capped honey in the hive, b) possible repellency of carbofuran, c) robbers dying before returning to their colony, d) rainy conditions during test, or e) robbers working a more desirable location.

Hive Number 8 - Low Volatile Ester Formulation of 2,4-D

Hive Number 8, with an estimated 800 bees, was established in the bee lab on July 27 and sprayed with a 1% emulsion of low-volatile ester formulation of 2,4-D on July 30. There was no excitement or unusual behavior and within twenty-four hours only ten bees had died. The dead bees were assumed to be a result of natural mortality due to the fact that within the same twenty-four hours only ten bees had died in the check hive. The test ended one week later; a total of 25 bees had died. All deaths were attributed to natural causes.

A second hive received an application of the low-volatile ester formulation of 2,4-D on August 2. Again, no unusual behavior was observed. Only five bees out of an estimated 1300, died in the first twenty-four hours after treatment. When the test was completed there were an estimated 1500 bees and no harmful effects from treatment had been observed.

Summary of Pesticide Application

Diazinon, malathion, and carbofuran proved to be highly toxic to bees upon receiving a direct application to the hive (Figure 10). This was in agreement with the findings of Stevenson (1968) and Anderson et al. (1971). The time required for mortality varied with the different

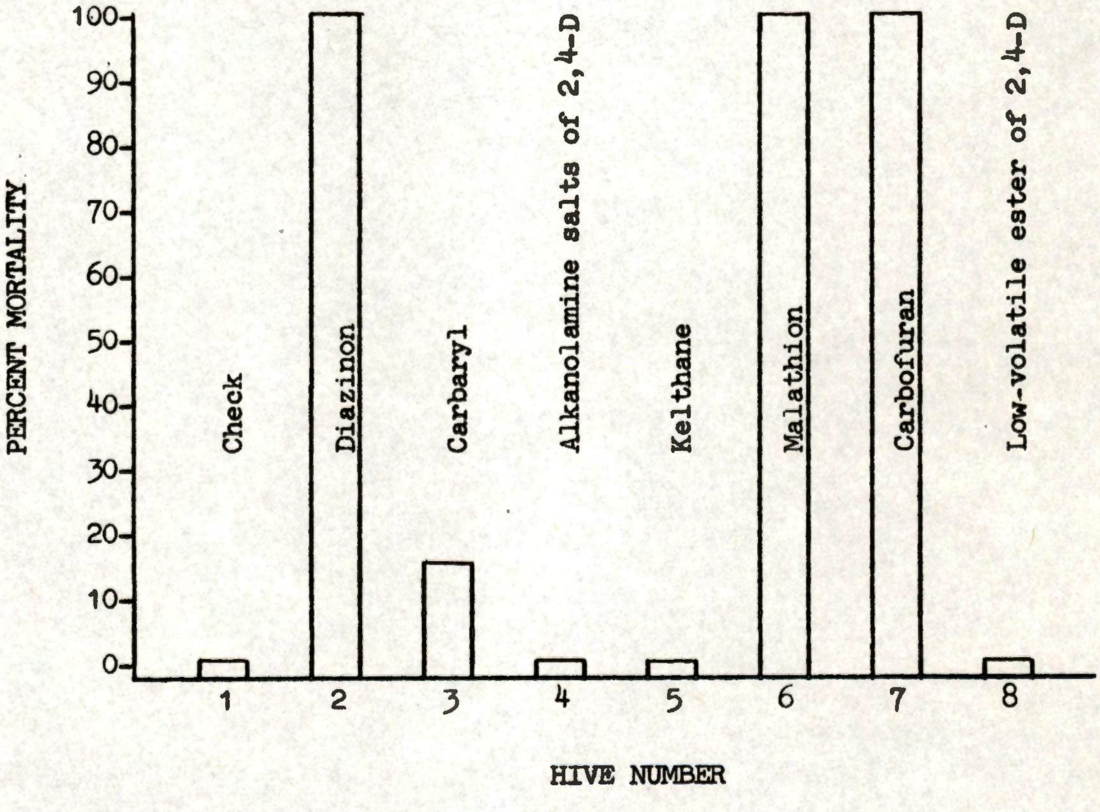

Figure 10. Percent mortality caused by each pesticide.

insecticides (Figure 11), diazinon and carbofuran being very rapid in their kill, while malathion had a slower action.

Carbaryl was only moderately toxic upon direct application with as estimated 15% kill (Figure 10). (The second replication was not included in this graph as the high mortality, 40%, resulted from a combination of factors, and the percent mortality from carbaryl could not be distinguished from the total mortality.) These findings were in agreement with Morse (1964) but differ with those of Anderson et al. (1971), who placed carbaryl in the highly toxic group. It is not the intent of the writer to suggest that carbaryl is nontoxic to honey bees; many experiments have proven otherwise. The writer is only saying that contamination of hives by direct application of 1% carbaryl causes very little increase in bee mortality.

Kelthane, a specific miticide, and both formulations of 2,4-D (alkanolamine salts and low-volatile ester formulations) proved to be relatively nontoxic to bees upon direct application to the hive. King (1964) found the ester formulation of 2,4-D to be highly toxic upon direct application to individual bees; however, he did not specify whether this was the low-volatile or high-volatile ester formulation.

Because of some unknown factor, all frames taken from the Plant Science Farm on July 19 experienced a higher than average mortality. By comparing the number of dead bees within each hive the day following treatment, a 10% kill had taken place. Kelthane, a hive of approximately 500 bees, lost 50; 2,4-D, a hive of approximately 900 bees, lost 90; and the check hive, with approximately 800 bees, lost 72, all in the twenty-four hour post-treatment period. In other replications of Kelthane and 2,4-D, there was no mortality observed.

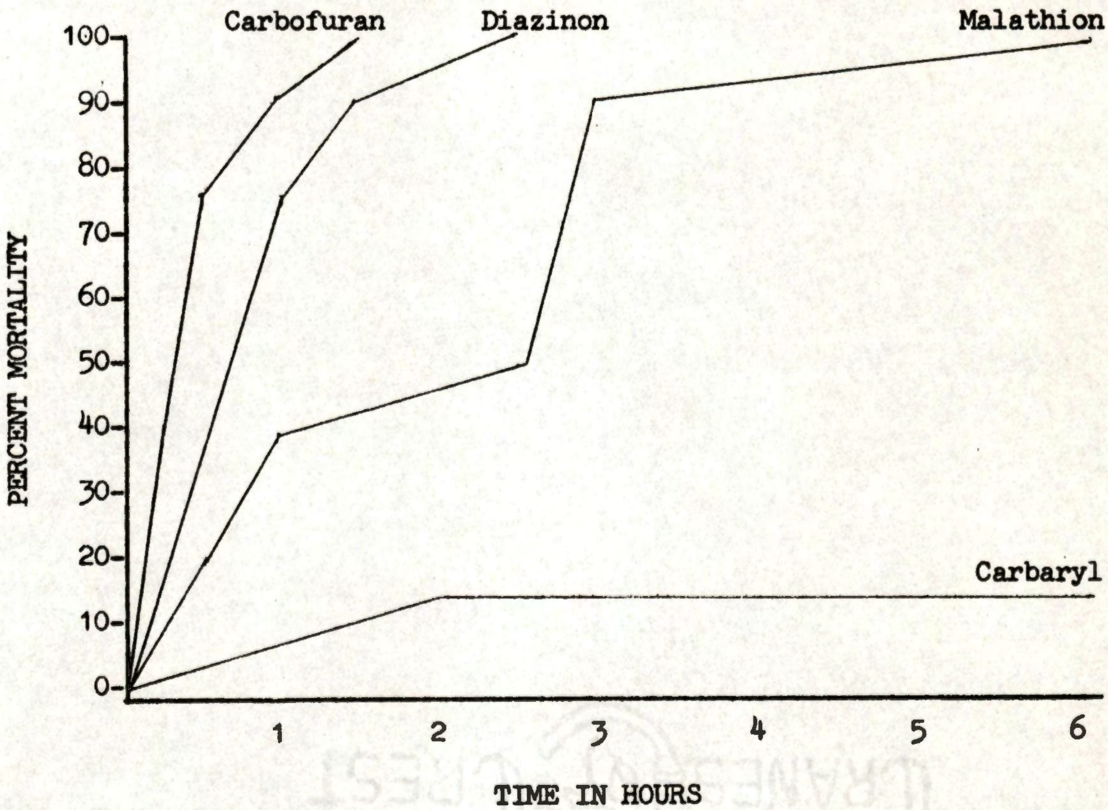

Figure 11. Percent mortality in relation to time.

CHAPTER V

SUMMARY AND CONCLUSIONS

This study on the effects of selected pesticides upon honey bees was conducted over a period of twelve months, from September 1972 through August 1973. It consisted of building construction, construction of dead bee traps, laboratory studies with the greater wax moth Galleria mellonella (L.) and application of pesticides to hives.

Three dead bee traps were designed and tested; none were efficient enough to be recommended for further use. Rearing of wax moths was attempted on two diets, neither of which was successful. Also, head capsule measurements were made in an attempt to determine the number of larval instars, but these did not follow a set pattern as indicated by Dyar (1890).

The following conclusions were made concerning the application of pesticides.

1. Contamination of hives by direct application of a 1% concentration of diazinon, malathion, or carbofuran was considered highly toxic to honey bees. Mortality of 100% can be expected in a few hours after hive contamination.

2. Contamination of hives by direct application of a 1% suspension of carbaryl was considered to be only moderately toxic. A 15% kill would be expected in the first couple of hours, after which recovery is very rapid.

3. Hive contamination from Kelthane or either of the formulations of 2,4-D (alkanolamine salts of low-volatile ester formulations) was

considered nontoxic to honey bees and may be used around hives, in recommended dosages, without danger to bees.

4. Healthy larvae of a pearly white color crawling out of cells would be a characteristic symptom of bee poisoning when a complete adult bee kill has occurred.

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VITA

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