THE EFFECTS OF AERIALLY APPLIED ULTRA-LOW VOLUME MALATHION ON HONEY BEES (*Apis mellifera* L.).

A Thesis

Submitted to the Faculty

of

Graduate Studies

by

Tanya Pankiw

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

Department of Entomology

ISBN 0-315-51673-9

THE EFFECTS OF AERIALLY APPLIED ULTRA-LOW VOLUME MALATHION ON HONEY BEES (*Apis mellifera* L.)

BY

TANYA PANKIW

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. S.C. Jay, for his helpful advice, guidance and, encouragement throughout this study. The helpful criticisms of this study made by Dr. R.A. Brust and, Dr. A.K. Storgaard, were also appreciated.

I would like to thank the following for their generosity in allowing the use of their property: Mr. Art Enns & Sons, Mr. Peter M. Friesen, Mr. Harold Janzen, Mr. Leroy Johnson, Mr. George Manchulenko, Mr. Maurice Maynard, Mr. R. Clarence McVicar and, Mr. Louis Saurette.

I would like to thank the following for funding this study: Manitoba Department of Agriculture, Manitoba Beekeepers Association, Red River Apiarists and the City of Winnipeg for supplying the malathion. Additionally I would like to thank Mr. Don Dixon for the generous availability of his staff, Murray's Air Service of Carman and, the staff of the pesticide laboratory at the W.M Ward Provincial Laboratory of Manitoba.

The magnitude of this study could not have been realized without the help of my family and so, my sincerest thanks and gratitude go to my family. I would especially like to thank my father, Roman Pankiw, for the design of the dead bee trap used here and for his constant encouragement, guidance and advice.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF TABLES

ABSTRACT

The effects of aerially applied ultra-low volume malathion on honey bees (Apis mellifera L.).

Colonies of honey bees were treated with aerially applied ultra-low volume malathion at a rate of 210 ml/ha from 3 meters above ground level. The application was made during Brassica napus L. (Westar canola cv.) nectar flows in the mid to late part of July.

This thesis examines: (1) the relationship between colony weight gain and adult numbers, and the effects of a ULV malathion application on colony weight gain, adult numbers, and adult mortality at the hive; (2) the effect of a ULV malathion application on honey bee foraging activity including pollen trap weights, the number of foragers entering colonies and, the number of foragers actively working on blossoms in field plots and; (3) caged bee and mosquito mortality, the application rate of malathion at the caged bee and mosquito locations within the treated area, bee mortality downwind from the treated area, and malathion residues.

Treated colonies gained significantly ($p<0.05$) less weight than control colonies for up to 3 days after treatment. Treated colonies had significantly ($p<0.0001$) lower adult numbers than control colonies for up to 86 days after treatment. Dead bee trap counts were significantly ($p<0.05$) higher in treated colonies than control colonies for three to four days after treatment.

Forager bees in treated colonies collected significantly ($p<0.05$) less pollen than forager bees in control colonies for two to three days after treatment. The number of foragers entering treated colonies was significantly ($p<0.05$) less than control colonies for one to two days after treatment.

There was a strong positive correlation between caged bee and mosquito mortality and application rate; $r=0.900$, $p<0.0001$ and $r=0.920$, $p<0.0001$ respectively. Caged bee mortality was determined up to one kilometer downwind and depended on the meteorological conditions at the time of treatment.

Residue analysis of pollen, blossoms, hive pollen, honey, wax, and a contact residue exposure estimate indicated that contact with sub-lethal doses an hour or more after treatment would be more likely than contact with lethal doses for foraging honey bees.

CHAPTER I

GENERAL INTRODUCTION

The purpose of this study was to examine the effects of an ultra-low volume (ULV) malathion application on honey bees. In 1983, from July 23 to August 16, ULV malathion was used to control adult mosquitoes vectoring Western Equine Encephalitis in Manitoba. During this period the Manitoba Department of Agriculture's Apiculture section monitored the effects of such sprayings on honey bees by visiting and recording observations from treated apiaries and by recording caged bee mortality (Dixon 1983). In 1984, the Province paid beekeepers, whose colonies were in the spray zone, a total of $800,000 as compensation for losses as a result of the ULV malathion applications. The lack of data on the effects of ULV malathion applications on honey bees, apart from impromptu monitoring studies and the various reports of honey bee losses, prompted this study.

Ultra-low volume is not only the application of small volumes of a concentrated pesticide but an application method that produces sufficient spray droplets, within a specified size range, to cover the desired area. In Europe, controlled drop application (CDA) is the standard name but it includes all applications where the droplet size is purposely controlled. In North America ULV means that droplets are usually less than 150 microns in diameter and that application rates are less than 1 liter of liquid formulation per hectare.

Ultra-low volume malathion is applied as a technical formulation which means that nothing has been added to

malathion after synthesis nor has anything been added to the formulation before application. The technical malathion used in this study, Cythion, was produced by American Cyanamid. It has a guaranteed minimum purity of 91 % malathion and a maximum of 30 ppm methyl mercaptan. Insecticidal impurities in technical malathion include, isomalathion, O,S,S-trimethylphosphorothioate, O,O,S-trimethylphosphorothioate, and O,O,S-trimethyl-phosphorodithioate (Lin et al. 1984).

Malathion is an organophosphate insecticide that acts as an acetylcholinesterase inhibitor (Metcalf and March 1949; O'Brien 1956; Mengle and Casida 1958; Mayer et al. 1959; Stegwee 1959; O'Brien 1967; Smallman 1968; Yu and Terriere 1971; Wilkinson 1973; Eto 1974; Welling 1977; Matsumura 1980; Yu et al. 1984). Malathion must pass through several different types of biological barriers before it can act on the nervous system of the honey bee. Integument penetration depends on a hydrophilic-lipophilic balance (Olson and O'Brien 1963). The penetration rate of malathion follows first order kinetics where the time required for penetration is dependent on concentration (Matsumura 1963; Olson and O'Brien 1963; Nobel-Nesbit 1970; Wilkinson 1973; Hartley and Graham-Bryce 1980). Once past the integument malathion must move through the generally aqueous environment of intercellular spaces and lipophilic barriers of cell walls (Eldefrawi and O'Brien 1966; Eldefrawi and O'Brien 1967; Toppazoda and O'Brien

1967; Conner et al. 1978). Honey bees have enzymes that will detoxify organophosphate insecticides (Gilbert and Wilkinson 1974; Yu et al. 1984) however, honey bees lack microsomal oxidase activity in the stomach (Gilbert and Wilkinson 1974) and do not contain a large amount of fat in the abdomen. The former are important in detoxification and the latter in storage and detoxification of insecticides (Yu et al. 1984). These features make honey bees particularly susceptible to orally ingested insecticides.

Symptoms of honey bee poisoning by an organophosphate insecticide include; regurgitation (with an extended proboscis), disorientation, lassitude, distended abdomen, erratic cleaning attempts, stumbling gait, and unhinged wings (Atkins 1978). Older foraging bees are more susceptible to poisoning than are younger bees (Mayland and Burkhardt 1970; Nazer et al. 1974) because older forager bees have less brain acetylcholinesterase than younger bees (Nazer et al. 1974).

Studies of insecticidal poisoning have provided many examples of differential toxicity due to the differences in insecticide formulations. In general the order of hazard is; dusts > wettable powders > flowables > emulsifiable concentrates and soluble powders of liquid solution > granular formulations (Johansen and Kleinschmidt 1972). Ultra-low volume insecticides applied with small droplets (20 to 40 microns in diameter) are easily picked up by insect hair (Spillman 1976). The particularly hairy bodies

of honey bees and, their habit of repeated floral visitation for pollen and nectar gathering (Wahl and Ulm 1983) make ULV formulations just as hazardous and sometimes even more hazardous, than dust formulations (Atkins et al. 1975; Caron 1979).

This study was divided into three different areas to examine the effects of ULV malathion on honey bees. These include: (1) The effects of ULV malathion on honey bee colony weight gain, adult numbers and observed adult mortality,; (2) The effects ULV malathion on honey bee foraging activity and,; (3) i. the relationship between ULV malathion application rate and mortality of caged bees and mosquitoes; ii. ULV malathion drift and caged bee mortality; iii. Malathion residues inside and outside the hive.

CHAPTER II

GENERAL METHODS

One kilogram packages of yellow strain bees were hived in 9 frame brood chambers on May 3, 1984 and on April 27, 1985. With each pretreatment apiary check the colony populations were equalized by redistributing brood frames. Colony populations were permitted to increase in size as they would in a commercial beekeeping operation so that high populations would coincide with nectar flows. All colonies were kept in a parent apiary until they were chosen randomly to be moved two days before the ULV malathion treatment to either control or treatment apiaries. Hives in control and treatment apiaries were arranged as shown in Figure 1 in 1984 and in Figure 2 in 1985.

A total of nine 32 hectare fields were used, for the control and treated apiary locations (Figure 3). Forage for control and treated apiaries in 1984 and 1985 consisted of fields of Brassica napus (L.) Westar (a canola quality cultivar; low erucic acid, 0.2% and low glucosinolate, 15 umol g meal^{-1}, Klassen et al. 1987), located at least 8 km away from each other. All of the fields were located in the Red River Valley of Southern Manitoba which is flat and open; the only obstructions are windbreaks and power lines.

Five replications were made on five different fields on five different apiaries on the follwing dates and times (C.D.T.): in 1984; (1) July 17, 1000 h and, (2) July 26, 0800 h; in 1985; (3) July 16, 0800 h, and July 27, (4) 1900 h and, (5) 2100 h.

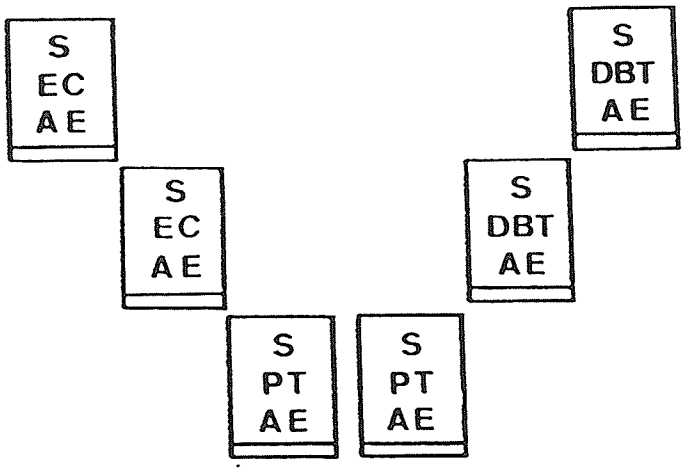
All treatments were made with a Piper Cub aircraft

at a ground speed of 160 kph and approximately 3 meters above ground level. The Cythion treatments were applied at a rate of 210 ml/ha (registered rate for aerially applied control of adult mosquitoes) with TeeJet 800050 cone nozzles and at 200 kPa pressure.

Weather data were collected with hygrothermographs in Stevenson screens and wind speed was measured with an anemometer 2.7 m above ground level. Wind direction was approximated with the use of a wind sock. Weather conditions at the times of treatment are summarized in Table 1. Weather conditions during the period of time when daily measurements were taken are summarized in Appendix 1.

Figure 1. Apiary layout in 1984; (S) indicates balance beam scale, (EC) indicates number of foragers entering the colony were counted, (AE) indicates the number of adults in the colony were estimated, (PT) indicates an O.A.C. pollen trap was used and, (DBT) indicates a dead bee trap was used.

Figure 2. Apiary layout in 1985; (S) indicates balance beam scale, (EC) indicates number of foragers entering the colony were counted, (AE) indicates the number of adults in the colony were estimated, (PT) indicates an O.A.C. pollen trap was used, (DBT) indicates a dead bee trap was used.


S
EC
AE
S
EC
AE
S
PT
AE
S
PT
AE
S
DBT
AE
S
DBT
AE

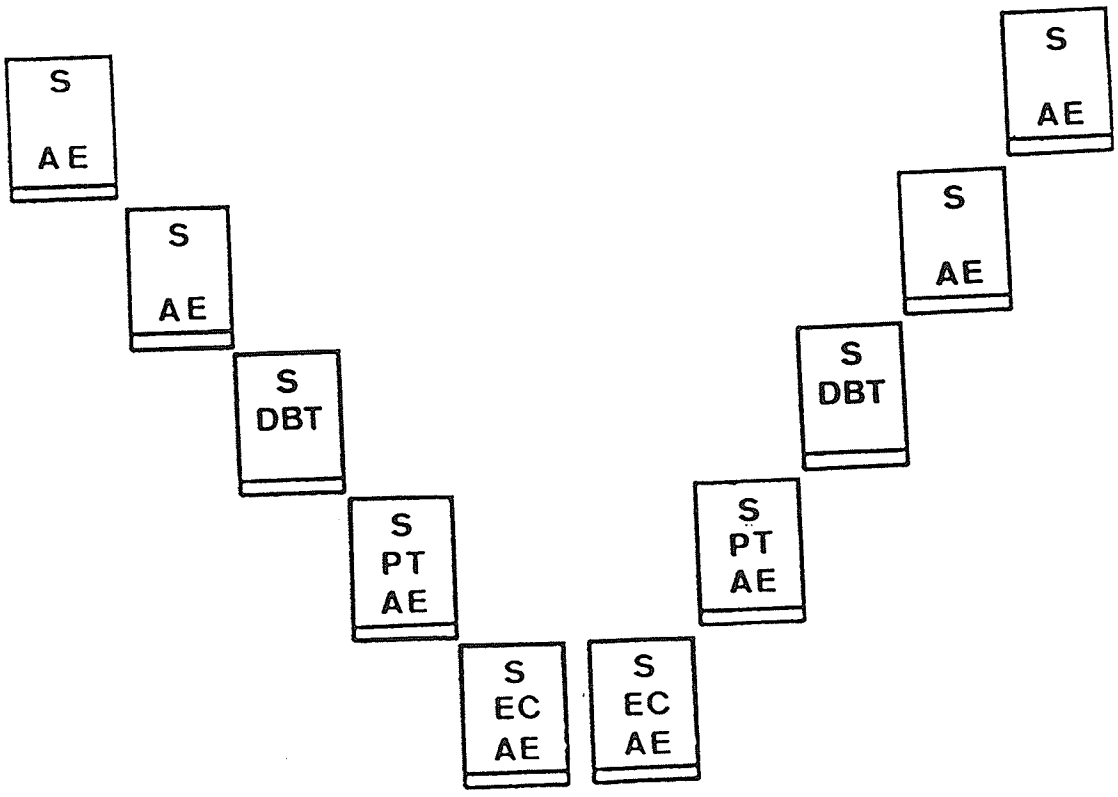

S
AE
S
AE
S
DBT
S
PT
AE
S
EC
AE
S
EC
AE
S
PT
AE
S
DBT
S
AE
S
AE

Figure 3. Treated and control fields and dates and times of treatment; (✱) indicates apiary location, ( ) indicated wind direction, (→) indicates flight path of airplane.

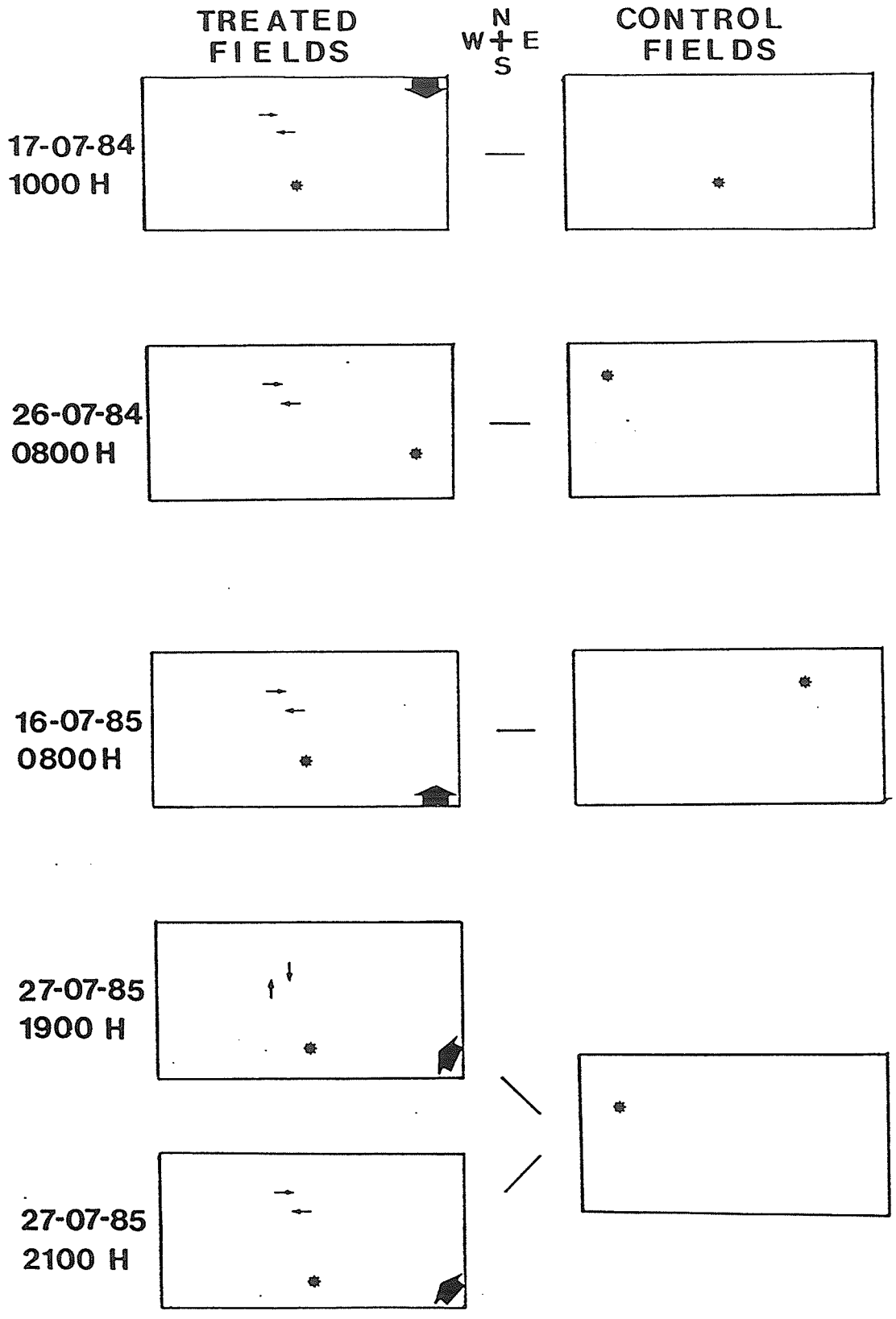

TREATED FIELDS
N
W E
S
CONTROL FIELDS
17-07-84
1000 H
26-07-84
0800 H
16-07-85
0800 H
27-07-85
1900 H
27-07-85
2100 H

TABLE 1. Weather conditions at the times of treatment.

DATE	TIME	WIND VELOCITY (KM/H) AND DIRECTION	TEMPERATURE (^{o}C)
17 July, 1984	1000 h	6 N	15.6
26 July, 1984	0800 h	0	19.1
16 July, 1985	0800 h	3 S	15.4
27 July, 1985	1900 h	4 ESE	23.0
27 July, 1985	2100 h	3 SE	17.5

CHAPTER III

THE EFFECTS OF ULV MALATHION ON HONEY BEE COLONY WEIGHT GAIN, ADULT NUMBERS AND, ADULT MORTALITY.

INTRODUCTION

The effect of an application of ULV malathion on honey bee colonies is highly variable depending on the application rate used, time of application, method of application and methods used to measure effects.

When ULV malathion was used to control grasshoppers, rates from 712 to 562 ml/ha were applied by aircraft with observed losses of honey bees reported as very high (Anderson and Atkins 1966; Hitchcock et al. 1966; Levin 1966; Levin et al. 1968). Rates used for mosquito abatement were from 281 to 219 ml/ha (Caron 1979; Dixon 1983; Herbert and Shimanuki 1983). Only the effects reported by Dixon (1983) were from applications made aerially for mosquito abatement; the others were from applications made from truck sprayers. Caron (1979) and Herbert and Shimanuki (1983) reported losses to be moderate while Dixon (1983) reported losses to be high.

The effect of exposure to ULV malathion on colony weight after has been reported by Dixon (1983) and, Herbert and Shimanuki (1983). Herbert and Shimanuki exposed 12 colonies to ULV malathion at 1300 h with a truck sprayer at a rate of 112 ml/minute. Colony weight gain for treated and control colonies over a four week period before application was approximately equal, i.e. 8.9 kg and 8.8 kg respectively. In a two week period after application control colonies lost an average of 2.6 kg and treated

colonies gained an average of 1.8 kg. The control colonies were moved two days before the malathion application and this was considered to be more disruptive than the malathion application itself (Herbert and Shimanuki 1983).

Dixon (1983) reported colony weight loss for two to three days after application. Initial weight losses were attributed to a loss of foragers (observed at hive entrances) while later weight loss was attributed to a reduction in honey production.

No previous studies examined the effect of a ULV malathion application on adult population in colonies except to measure mortality with the use of dead bee traps (Levin et al. 1968; Herbert and Shimanuki 1983) and to observe colony strength after application (Hitchcock et al. 1966; Levin 1966).

Levin et al. (1968), using a dead bee trap, reports losses of over 3,000 bees per colony over a five day period. The colonies were treated indirectly as part of a large aerial grasshopper control program at a rate of 562 ml/ha of ULV malathion. When colonies were treated directly with ULV malathion at a rate of 562 ml/ha, dead bee trap counts over a five day period ranged from 9,000 to 7,500 (Anderson and Atkins 1966). With truck sprayer applications at mosquito abatement rates of about 246 ml/ha, dead bee trap counts were lower and more variable ranging from 8-157 dead bees per colony four days after application (Herbert and Shimanuki 1983).

The objectives of this study were to determine the effects aerial ULV malathion treatments on: (1) honey bee colony weight gain; (2) adult numbers in the colony and; (3) adult mortality at the hive. Also the objective of this study was: (4) to determine the relationships between; (i) weight gain and the increase in adult numbers in a colony, (ii) between weight gain and adult numbers in a colony and, (iii) between adult numbers and the increase in adult numbers in a colony.

MATERIALS AND METHODS

Colony weight gain

Colony weight was measured with balance beam scales to the nearest 0.5 kg. Measurements were taken in the morning (before 0800 h). Daily colony weight measurements began at least one day before treatment and continued for seven days after the treatment. Colony weight was measured at 7 day intervals for 21-28 days after application. Six colonies in 1984 and 10 colonies in 1985 were weighed from each control and each treated apiary.

Estimate of the number of adults in colonies

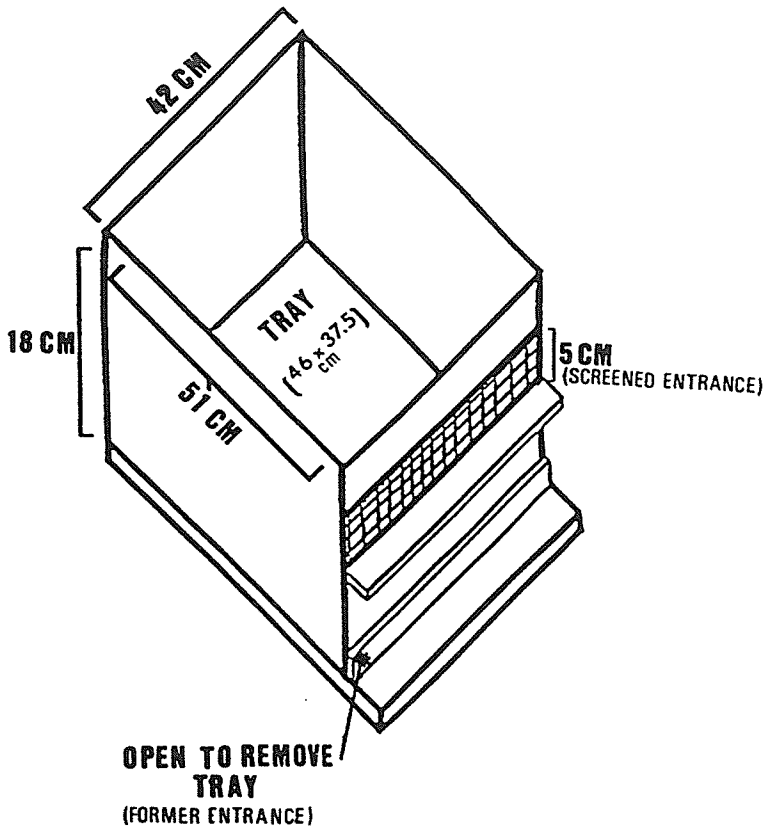
Adult numbers were estimated in the morning (before 0800 h) after a method developed by Nelson and Jay (1972[a]). Adult numbers were estimated two days before treatment, two days after treatment, and then at 7 day intervals for up

to 86 days after treatment. Adult numbers from six colonies in 1984 and six colonies in 1985 were estimated in each control and treated apiary.

Adult mortality at the hive

Colony adult mortality at the hive was measured with dead bee traps (Figure 4). The dead bee traps were constructed from shallow supers (18 cm in depth, 42 cm wide and, 51 cm long) by removing a 5 cm horizontal strip from one side of the shallow super and screening off the strip with 6 mm mesh hardware cloth. The dead bee trap entrance was provided with a landing board of the same size as all the other hives, fixed below the screened off area. The dead bee trap was mounted on a bottom board with sufficient space for a removable metal tray (46 cm X 37.5 cm) where the dead bees collected and could be removed from the hive for counting. The bottom board entrance was blocked off with a piece of wood and a latch to facilitate easy access and closure. Within one day all of the colonies, with dead bee traps, used the screened entrances readily. The traps were tested against dead bee removal by introducing 10 dead, marked bees into the colonies. After being left in the colonies for 12 hours the traps were checked for the marked bees. All marked bees were recovered from the dead bee traps. The accuracy of the dead bee traps was determined every second day by placing 10 marked, dead bees into each colony with a dead bee trap at 0800 h and retrieving them

Figure 4. Dead bee trap.


42 CM
18 CM
51 CM
TRAY
(46 x 37.5) cm
5 CM
(SCREENED ENTRANCE)
OPEN TO REMOVE
TRAY
(FORMER ENTRANCE)

from the traps at 2000 h. In 1985 the trap was modified because in larger colonies groups of house bees were observed to be removing dead bees through the wire mesh. At various times groups of two to six bees were observed to be pushing and pulling dead bees through the wire mesh. Usually two bees from inside the trap would position the dead bee so that it would approach an opening in the mesh and two bees on the outside of the trap would grasp any part of the dead bee that emerged through the mesh. With a combination of pushing and pulling from inside and outside the dead bee would be removed from the trap. A second piece of wire mesh was added to the trap about one cm from the first piece and the mesh offset. House bees were then no longer able to remove dead bees from the trap.

Daily dead bee trap counts began at least one day before treatment and continued for seven days after treatment. In 1984 dead bee trap counts were taken every two hours beginning at 0800 h and ending at 2000 h, in 1985 dead bee trap counts were taken every 4 hours. Dead bees were counted from two colonies in 1984 and two colonies in 1985 from each control and each treated apiary.

Colony weight and adult numbers relationships

A total of 32 control colonies were used to determine the relationships between weight gain and the increase in adult population in a colony, between weight gain and number of adults in a colony and, between the number of adults and

the increase in the number of adults in a colony. The 14 day nectar flow periods were as follows; (1) 17 to 31 July, 1984, (2) 26 July to 09 August, 1984, (3) 16 to 30 July, 1985 and, (4) 27 July to 10 August, 1985.

Statistical analysis

The data were analyzed by t-Tests for significance for two treatment mean comparisons (Little and Hills 1978). Correlation analysis was used to determine the relationship between colony weight gain and colony adult population over the two week periods (Snedecor and Cochran 1980).

RESULTS

Colony weight gain

In 1984 colonies treated at 1000 h gained less weight ($p<0.05$) than control colonies for up to 21 days after treatment. There was an exeception on the third day after treatment where there was no difference ($p>0.05$) between the control and treated mean weight gain (Table 2). One day after treatment treated colonies lost weight (-0.16 ± 0.09 kg). The pre-treatment means were not different ($p>0.05$).

Colonies treated at 0800 h in 1984 on 26 July exhibited the same pattern of weight gain ($p<0.05$) for up to 21 days after treatment. There was an exception on the third day after treatment where there was no difference ($p>0.05$) between the control and treated mean weight gains (Table 2).

TABLE 2. Mean (± standard error) colony weight gains (kg) in 1984.

DAYS AFTER TREATMENT	17 JULY, 1984 CONTROL	TREATED 1000 h	26 JULY,1984 CONTROL	TREATED 0800 h
-3	2.56±0.39	3.03±0.38	-	-
-2	1.91±0.97	0.95±0.28	4.31±0.45	3.98±0.31
-1	1.19±0.97	0.95±0.28	2.88±0.48	3.50±0.19
0	0.60±0.21	0.02±0.18	2.90±0.24	3.36±0.08
+1	4.38±0.89	-0.16±0.09*	2.70±0.39	-0.30±0.10*
+2	2.33±0.43	0.26±0.19*	2.28±0.17	0.50±0.43*
+3	1.73±1.54	2.20±0.35	3.31±0.77	2.00±0.53
+4	2.28±0.18	0.76±0.09*	3.21±0.24	1.63±0.33*
+5	1.53±0.18	0.76±0.13*	2.98±0.18	1.41±0.21*
+6	1.10±0.23	0.96±0.14	3.35±0.38	1.66±0.41*
+7	1.38±0.17	0.75±0.08*	3.21±0.22	1.26±0.21*
+14	11.48±0.89	5.41±1.07*	21.86±0.98	4.51±1.09*
+21	27.05±2.04	11.05±1.53*	45.61±2.44	12.55±2.51*

*Treated means are significantly different from control means $p<0.05$.

The pre-treatment means were not different ($p>0.05$).

In 1985 colonies treated on 16 July at 0800 h gained less weight ($p<0.05$) than control colonies for up to 14 days after treatment. There was an exception on the third day after treatment where there was no difference ($p>0.05$) between the treated and control weight gain means (Table 3). Treated colonies lost weight on the fourth day after treatment (-0.41 ± 0.41 kg). By the twenty-first day after treatment there was no difference between treated and control mean weight gains ($p>0.05$). By the twenty-eighth day after treatment treated colonies gained less ($p<0.05$) weight than control colonies. Pre-treatment weight gains were not different ($p>0.05$).

Colonies treated on 27 July, 1985 at 1900 h gained less weight ($p<0.05$) for four days after treatment (Table 3). On the fifth and on the fourteenth day after treatment there was no difference ($p>0.05$) in weight gain between the 1900 h treatment and the control colonies. On the seventh day and on the twenty-first day after treatment treated colonies gained less ($p<0.05$) than control colonies. Colonies treated at 2100 h on the same date gained less weight ($p<0.05$) for up to one day after treatment (Table 3). There was no difference ($p>0.05$) in weight gain for all remaining days except the fourth, the seventh and, the twenty-first day after treatment. Pre-treatment weight gains were not different ($p>0.05$).

Although treated colonies did not gain the same amount

TABLE 3. Mean (± standard error) colony weight gains (kg) in 1985.

DAYS AFTER TREATMENT	16 JULY, 1985 CONTROL	16 JULY, 1985 TREATED 0800 h	27 JULY, 1985 CONTROL	27 JULY, 1985 TREATED 1900 h	27 JULY, 1985 TREATED 2100 h
-2	-	-	0.51±0.08	0.60±0.13	0.60±0.05
-1	1.36±0.10	1.48±0.24	1.53±0.14	1.58±0.13	1.21±0.07
0	1.71±0.33	1.88±0.26	3.56±0.20	2.20±1.23*	1.86±0.26*
+1	1.60±0.11	0.51±0.13*	2.46±0.42	0.36±0.08*	0.70±0.18*
+2	1.91±0.11	1.81±0.09*	1.41±0.19	0.73±0.20*	0.79±0.32*
+3	1.75±0.13	1.31±0.17	2.08±0.35	1.16±0.18*	1.40±0.19
+4	1.34±0.98	-0.41±0.41*	2.86±0.38	1.76±0.24*	1.71±0.16*
+5	1.70±0.09	0.36±0.06*	3.11±0.41	2.26±0.27	2.25±0.21
+6	1.98±0.06	0.36±0.05*	2.50±0.34	1.55±0.39	1.55±0.32
+7	2.03±0.03	0.43±0.06*	3.11±0.38	1.60±0.26*	1.68±0.21*
+14	14.80±0.32	4.85±0.65*	33.83±2.55	43.36±3.86	38.73±1.64
+21	39.27±0.67	40.16±0.46	5.61±0.45	-0.40±0.74*	-0.10±0.22*
+28	3.06±0.05	0.11±0.55*	-	-	-

*Treated means are significantly different from control means $p<0.05$.

of weight as control colonies, the same daily weight gain patterns were exhibited by both groups except for colonies treated on 17 July, 1984 (Figure 5). That is, the treated colonies of 17, July 1984 did not, consistently, have an increase in weight gain when control colonies increased in weight gain and, did not have lower weight gain when control colonies had lower weight gain as all other replicates demonstrated.

Adult numbers in colonies

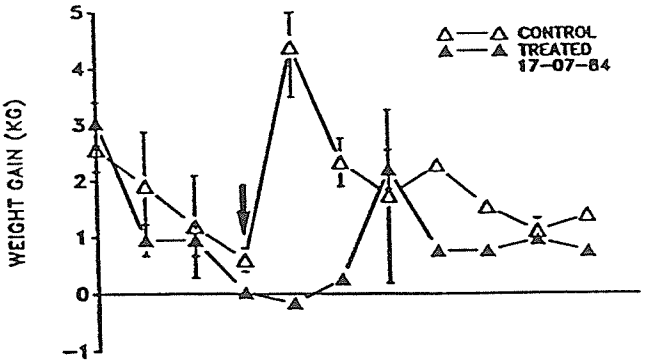
Since there were differences ($p<0.05$) between treatments within years and between years, adult numbers mean comparisons were made only between single treatments. All treatments showed the same pattern of no difference ($p>0.05$) in pre-treatment means and significantly lower ($p<.0001$) mean adult numbers from up to 2 days after treatment to up to 86 days after treatment (Tables 4 and 5).

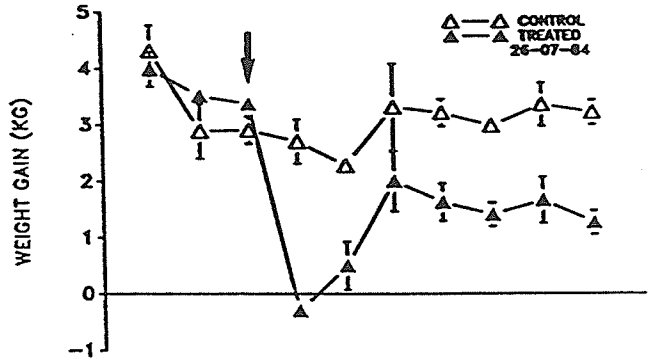
Adult mortality at the hive

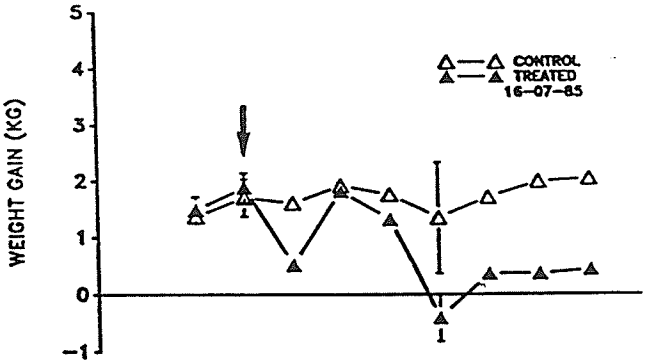
Colonies treated in 1984 on 17 July had higher ($p<0.05$) dead bee trap counts than control colonies for up to three days after treatment (Table 6). However, higher ($p<0.05$) dead bee trap counts were observed on population count days (-2 and +2 days after treatment), than on other pre-treatment days.

The same high ($p<0.05$) dead bee counts were observed on population count days (-2 and +2 days after treatment) for

Figure 5. Colony weight gain patterns in 1984 and 1985. Arrow indicates application date.


CONTROL
TREATED
17-07-84
WEIGHT GAIN (KG)
5
4
3
2
1
0
-1


CONTROL
TREATED
26-07-84
WEIGHT GAIN (KG)
5
4
3
2
1
0
-1


CONTROL
TREATED
16-07-85
WEIGHT GAIN (KG)
5
4
3
2
1
0
-1

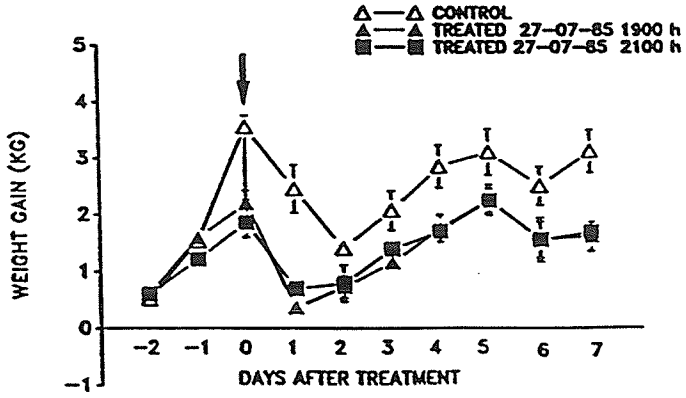

CONTROL
TREATED 27-07-85 1900 h
TREATED 27-07-85 2100 h
WEIGHT GAIN (KG)
5
4
3
2
1
0
-1
-2 -1 0 1 2 3 4 5 6 7
DAYS AFTER TREATMENT

TABLE 4. Mean (± standard error) number of adult bees in 1984.

DAYS AFTER TREATMENT	17 JULY, 1984 CONTROL	17 JULY, 1984 TREATED 1000 h	26 JULY, 1984 CONTROL	26 JULY, 1984 TREATED 0800 h
-2	24,525±99.7	24,558±95.2	26,125± 38.1	26,033± 55.7
+2	24,850±99.1	21,408±103.6*	26,450± 50.0	22,983± 57.3*
+14	26,833±107.7	23,475±137.1*	28,400± 44.7	24,641±194.6*
+28	28,825± 80.3	25,650±215.6*	30,375± 64.2	26,733±188.3*
+72	30,508±127.7	27,633±245.8*	31,725±148.7	28,641±210.3*
+86	31,750± 75.0	28,491±228.9	32,358±184.1	29,675±201.9*

*Treated means are significantly different from control means $p<0.0001$.

TABLE 5. Mean (± standard error) number of adult bees in 1985.

DAYS AFTER TREATMENT	16 JULY, 1985 CONTROL	TREATED 0800 h	CONTROL	27 JULY, 1985 TREATED 1900 h	TREATED 2100 h
-2	50,045± 42.4	49,891± 76.8	50,975± 49.5	50,991± 47.3	51,041± 59.7
+2	50,433± 24.4	46,984± 85.0*	51,508±102.8	48,083±165.6*	48,283±110.1*
+14	51,508±122.9	47,566±122.2*	52,300±130.4	48,658±160.9*	48,933±144.7*
+28	52,545±140.1	48,241±139.8*	52,725±186.1	49,291±147.9*	49,633±182.4*
+42	53,470±170.9	48,791±195.1*	53,075±122.3	50,116±163.3*	50,450±215.6*
+86	53,762±162.9	49,266±156.3*	53,460± 48.4	50,808±258.9*	51,258±196.4*

*Treated means are significantly different from control means $p<0.0001$.

TABLE 6. Mean (± standard error) dead bee trap counts in 1984.

DAYS AFTER TREATMENT	17 JULY, 1984 CONTROL	TREATED 1000 h	26 JULY, 1984 CONTROL	TREATED 0800 h
-2	113.5± 5.5	119.5±8.5	129.0±8.0	121.0± 5.0
-1	11.5± 1.5	9.5± 0.5	41.5±2.5	21.0±6.0
0	15.5± 2.5	553.0± 9.0*	21.5± 6.0	572.5±89.0*
+1	19.5± 4.5	60.0± 8.0*	30.5± 0.5	136.0±6.0*
+2	182.5± 3.5	95.0±11.0*	200.0± 3.0	205.0± 3.5
+3	12.0±7.0	81.5±2.5*	23.0± 4.0	104.0±3.0*
+4	15.5± 2.5	20.5± 1.5	19.0±2.0	99.5± 1.5*
+5	20.0±0.0	17.5± 0.5	20.5± 3.5	26.0±1.0
+6	19.0± 0.0	19.5±0.5	21.0± 2.0	24.0± 1.0
+7	19.5±0.5	20.5±2.5	24.0± 2.0	28.5± 1.5

*Treated means are significantly different from control means $p<0.05$.

the treatment date of 26 July, 1984 (Table 6). Treated colonies had higher ($p<0.05$) dead bee trap counts than control colonies for up to four days after treatment.

In 1985 colonies treated on 16 July had higher ($p<0.05$) dead bee trap counts for up to one day after treatment (Table 7). By the second day after treatment the treated mean dead bee trap counts were not different ($p>0.05$) from the pre-treatment mean dead bee trap counts and also not significantly different from the control mean dead bee trap counts.

Colonies treated at 1900 h on 27 July, 1985 had higher ($p<0.05$) dead trap bee counts than control colonies for up to three days after treatment (Table 7). The largest number of dead bees were recovered on the day of treatment. Colonies treated at 2100 h had higher ($p<0.05$) dead bee trap counts for up to five days after treatment (Table 7). For up to two days after treatment dead bee trap counts were higher ($p<0.05$) in colonies treated at 1900 h than in colonies treated at 2100 h. However, from the third to the fifth day after treatment the colonies treated at 2100 h had higher ($p<0.05$) dead bee trap counts than the colonies treated at 1900 h.

Colony weight and colony adult numbers relationships

There was a significant ($p<0.0001$) negative correlation ($r=-0.848$) between colony adult numbers increase and colony weight gain over a 14 day period for colonies in 1984 and

TABLE 7. Mean (± standard error) dead bee trap counts in 1985.

DAYS AFTER TREATMENT	16 JULY, 1985 CONTROL	16 JULY, 1985 TREATED 0800 h	27 JULY, 1985 CONTROL	27 JULY, 1985 TREATED 1900 h	27 JULY, 1985 TREATED 2100 h
-2	-	-	25.0±2.0	23.0±4.0	17.5±0.5
-1	21.0±0.7	17.0±4.0	17.0±7.0	17.0±2.0	23.5±1.5
0	23.3±1.3	146.5±3.5*	17.5±1.5	1018.0±185.0*	152.0±4.0**
+1	21.0±0.9	57.5±6.5*	17.0±4.0	49.5±3.5*	31.0±1.0**
+2	23.7±1.6	19.5±3.5	23.0±1.5	63.0±1.5*	54.0±4.0*
+3	24.5±2.1	27.0±0.0	16.0±1.0	32.0±1.5*	81.5±0.5**
+4	23.2±2.9	12.5±0.5	12.5±3.5	18.5±0.5	55.0±2.5**
+5	25.0±1.4	21.5±0.5	15.0±1.0	15.0±1.0	44.5±3.5**
+6	23.5±2.1	25.5±0.5	12.5±2.0	15.5±2.5	22.5±2.5
+7	22.2±0.4	23.5±0.5	17.5±0.5	23.0±4.0	25.0±2.0

* Treated means are significantly different from control means $p<0.05$.
** Treated means are significantly different from treated and control means $p<0.05$.

1985 (Figure 6). That is, those colonies with greater increases in adult numbers had less weight gain than those colonies that had lower increases in adult numbers. There was a significant ($p<0.0001$) positive correlation ($r=0.877$) between colony adult numbers and colony weight gain (Figure 7). Larger colonies gained more weight than smaller colonies. There was a significant ($p<0.0001$) negative correlation ($r=-0.978$) between colony adult numbers and colony increase in adult numbers (Figure 8). Larger colonies had adult number increases that were lower than smaller colonies.

DISCUSSION

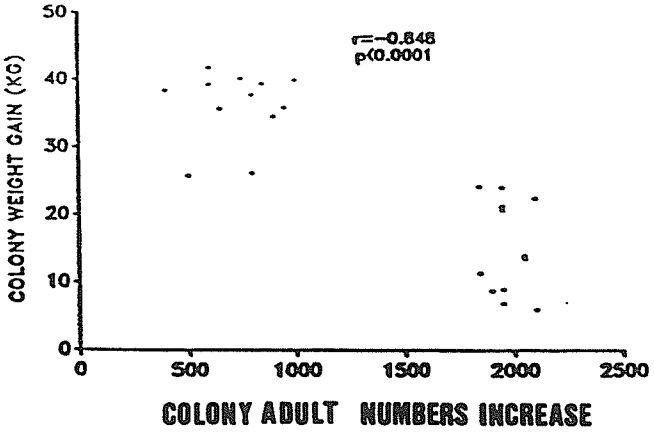
Colony weight gain

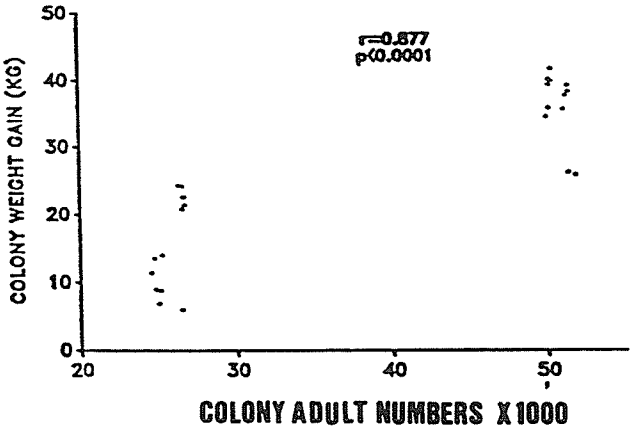
Treatments of ULV malathion affected colony weight gain whether the treatments were applied in the morning or the evening (Tables 2 and 3). Treated colonies exhibited a pattern of significantly less ($p<0.05$) weight gain for up to 3 days after treatment. On the third day after treatment the treated colonies gained the same ($p>0.05$) amount of weight as control colonies. This was possibly due to an increase in the amount of "wet" honey that was brought into treated colonies as foraging resumed after treatment. However, after the third day the treated colonies continued to gain less weight than control colonies. Some of the colony weight lost may be attributed to bee losses.

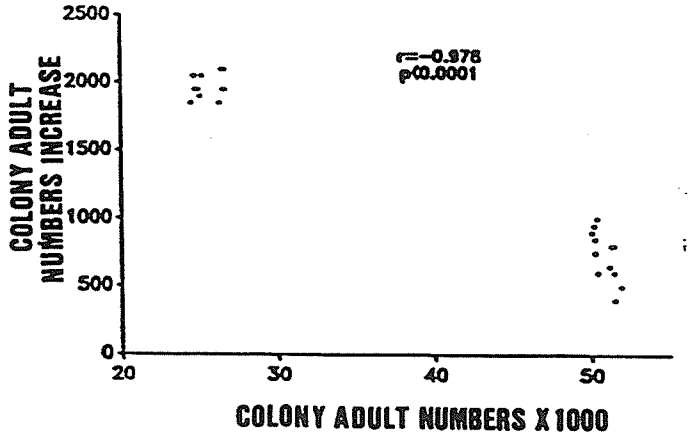
Figure 6. Correlation analysis is colony adult number increase and colony weight gain (kg) in a 14 day period during a canola nectar flow.

Figure 7. Correlation analysis of colony adult number and colony weight gain (kg) in a 14 day period during a canola nectar flow.

Figure 8. Correlation analysis of colony adult number and colony adult number increase in a 14 day period during a canola nectar flow.


r=-0.848
p<0.0001
COLONY WEIGHT GAIN (KG)
50
40
30
20
10
0
0
500
1000
1500
2000
2500
COLONY ADULT NUMBERS INCREASE


r=0.877
p<0.0001
COLONY WEIGHT GAIN (KG)
50
40
30
20
10
0
20
30
40
50
COLONY ADULT NUMBERS X1000


r=-0.976
p<0.0001
COLONY ADULT NUMBERS INCREASE
2500
2000
1500
1000
500
0
20
30
40
50
COLONY ADULT NUMBERS X1000

However, number of bees in the dead bee traps were not sufficient to account for the weight loss alone. A worker bee weighs from 80-129.5 mg (Mitchell 1970; Combs 1972; Otis 1982); the largest cumulative loss over the three day period was 1089 bees, equal to about 0.12 kg. As indicated by the population estimates, more bees were lost than were observed in the dead bee traps. Therefore the majority of the bees must have been lost in the field.

The nectar collecting behavior of honey bees is influenced by temperature, wind speed, sunshine, and the nectar source species (Free 1970). There is a positive, signifcant correlation between honey bee flight activity and colony weight gain (Szabo 1980). Honey bees foraging on one nectar source species on the same day and in approximately the same environmental conditions would be expected to forage in similar patterns throughout the day. In this study treated colonies did not gain the same amount of weight as control colonies but, treated colonies began to gain weight in the same daily weight gain patterns as control colonies, 3 to four days after treatment (Figure 5). Thus, monitoring treated and control colony daily weight gains for colonies foraging in similar conditions can be used as a rough indicator as to when colonies resume regular colony foraging patterns. Simply comparing colony weight gains would only indicate the nectar collecting capacity of colonies rather than a rough estimate of foraging activity.

The colonies treated on 17 July, 1984 did not follow

the same pattern of weight gain, and had lower colony populations. This may be due to the fact that some of colonies were infected with American Foul Brood (A.F.B.) and European Foul Brood (E.F.B.). Morse and Gunnison (1967) observed that colonies infected with E.F.B. and sac brood had increased levels of the diseases after insecticide exposure. Two of the treated colonies became queenless after the treatment. Queen losses have been observed from bees fed sub-lethal doses of carbofuran (Stoner et al. 1982) however, with these treated colonies it is believed that the combination of the insecticide treatment and the foul brood diseases caused the colonies to supersede their queens. The smaller 1984 treated colonies did not at any time gain enough weight to be comparable to the control colonies. However, the larger treated colonies of 1985 did gain the same amount or more weight than control colonies.

In 1985 cool wet weather may have been just as detrimental to colony weight gain as the ULV malathion treatment (Appendix 1). The amount of honey and pollen (as colony weight) a colony consumes in the summer has not been measured but Seeley (1985) estimates that an average colony could consume about 2.4 kg/week of honey during inclement weather. The amount of consumption and needs of the colony would vary according to the size of the colony and the brood area. So, an extended period of inclement weather may have the same effect as a treatment of ULV malathion as far as colony weight gain is concerned.

Colony adult number estimate

In 1984 the number of dead bees increased dramatically on days (-2 and +2) when colony adult number estimates were made. It appears this may be due to the disruption of the colonies on these days. The colony adult number estimation method therefore masked the effect that the ULV malathion treatments had on the dead bee trap counts. The number of dead bees recovered from the dead bee traps on the colony adult number estimation days represented less than 1% of the total adult number estimated on that day however, the counts were significantly ($p<0.05$) higher than on other pre-treatment days and all other control colony days. The percentage of bees that forage remains constant (Farrar 1931; Woodrow 1934) at about 4% (Danka et al. 1986). So, an increased dead bee trap count due to disruptive practices will only mask actual losses of foragers due to malathion applications. The difference in colony adult numbers between control and treated colonies for the treated date, 17, July 1984, is believed to be the result of a combination of the brood diseases and the ULV malathion treatments.

In 1985 colony adult number estimates were not done on colonies with dead bee traps. The number of adults in treated colonies remained significantly lower than control colonies throughout the measurement period. Possibly other factors were affecting the colony adult numbers than the simple loss of foragers. Barker and Waller (1978) found that colonies exposed to sub-lethal doses of parathion (an

organophosphate) produced less brood, honey and, wax. Nunamaker et al. (1984) believed that colonies demonstrated aberrant behavior that was not readily evident when fed sub-lethal doses of fenthion (an organophosphate). Although no dead brood was observed in the dead bee traps the lower adult populations may have occurred from brood cannibalism for a period of time after the treatments.

Adult mortality at the hive

Dead bee counts in treated colonies were greater than control colonies for three to four days after treatment. The colonies treated on 27 July, 1985 at 1900 h had the highest dead bee counts apparently because more foraging activity was occurring during this treatment than during any of the other treatments. The temperature was warm (Table 1) so bees were clustered on the outside of the colonies. Thus a large proportion of the bees were exposed on the outside of the hive.

The colonies treated in the evening exhibited the same pattern of mortality as the morning treated colonies except for those treated at 2100 h. Unfortunately, on the third day after treatment a neighboring sunflower field was sprayed with carbofuran (a carbamate) and these colonies experienced two additional days of higher dead bee trap counts than the control and the 1900 h treatment colonies. However, this additional amount of bee loss is not evident in the daily colony weight gain. It seems that there is a

mortality level at which weight gain is not affected.

Colony weight gain and colony adult numbers relationships

Smaller colonies (21,000 to 27,000 bees) did not gain as much weight as larger colonies (45,000 to 53,000 bees) during 14 day periods of nectar flow (Figure 7). Smaller colonies were increasing in numbers more than larger colonies were during the 14 day periods (Figure 8) however, the colonies with greater increases in numbers were gaining less weight than colonies with lesser increases in numbers (Figure 6).

The relationship between colony adult numbers and weight gain (considered as honey production) have weak to negative correlations (Farrar 1937; Woyke 1984). However, Woyke (1984) admits that the method of measuring total honey production over the entire summer may not be representative of the relationship between honey production and colony adult numbers during a honey flow period. Ratnieks (1986) shows that larger colonies produce more honey than small colonies during periods of nectar flow. In this study during the periods of two week nectar flows large colonies gained more weight than small colonies. The colonies used in this study were of two distinctive size groups and the smaller colonies were increasing in size at a faster rate than the larger colonies. Felton et al. (1986) recommend that colonies should contain at least 10,000 to 15,000 bees, 5 to 6 frames of brood and that estimates should be made of

the number of bees on frames and brood "status" on frames when testing for insecticide toxicity in the field.

In examining the effect of a ULV malathion treatment on colony weight gain (considered as honey production) the size of the colonies used, colony condition (queen state, health, etc.) and the type and amount of available forage are important in interpreting the colony weight gain results. Since the number of adults in a colony and change in the number of adults effects colony weight gains, studies examining the weight gains of colonies involving insecticide applications should include not only sizes of the colonies used but also the change in size during the time weight gains are being measured. Additionally, I believe that brood measurements should also be included along with adult numbers measurements and colony weight gain in order to further understand the effects of an insecticide treatment in a field situation during a nectar flow, and to determine possible interactions between adult forager losses and brood area, brood area changes, adult numbers and changes in adult numbers and, colony weight gain.

CHAPTER IV

EFFECTS OF ULV MALATHION ON THE FORAGING ACTIVITY OF HONEY BEES

INTRODUCTION

Insecticide applications disrupt honey bee foraging activity (Anderson and Atkins 1968), for some time after treatment depending on the residual activity of the insecticide (Johansen 1966; Johansen 1972) and the extent of mortality at the hive (Atkins 1978).

In spite of this general conclusion regarding insecticide hazards, the effects of a ULV malathion application on the foraging activity of honey bee colonies is not well known. Anderson and Atkins (1958) counted the numbers of bees actively foraging on alfalfa blossoms in five $18m^2$ areas in a 2 minute period before and after treatment with 560 ml/ha of ULV malathion. They found that within minutes after the application the numbers of bees observed in the field dropped to zero. Then began to reappear in six hours and the, numbers returned to pre-treatment levels by 24 hours.

The purpose of this study was to determine the effects of a ULV malathion treatment at 210 ml/h on: (1) amount of pollen collected by honey bees; (2) number of foragers entering hives, the proportion of pollen and non-pollen foragers to the total number of foragers entering hives in control and treated apiaries and in colonies with different populations; and, (3) field foraging activity in control and treated fields in which small and large plots were designated.

MATERIALS AND METHODS

Pollen collection

From two colonies in each control and treated apiary pollen was collected by the use of O.A.C. (Ontario Agriculture College, Guelph Ontario) pollen traps every two hours in 1984 and every 4 hours in 1985, beginning at 0800 h and ending at 2000 h. This was done from one day before treatment to up to seven days after treatment. Pollen was sorted according to color and weighed (wet weight) with an electronic balance to within $\pm$0.005 grams. The pollen was not dried because pollen from treated colonies was also used for residue analysis.

Number of foragers entering hives

The number of foragers entering hives was determined by counting the number of foragers entering two colonies in each treated and control apiary for three 30 second intervals every 2 hours in 1984 and every 4 hours in 1985. All foragers entering the colony were counted with a counter held in one hand and all foragers carrying pollen loads were counted with a counter held in the other hand. Any disturbance caused by the observer was minimized by the use of a "blind" placed near the colonies.

The number of foragers entering hives was also examined at four different times of the day, with respect to the

proportions of incoming pollen foragers and non-pollen foragers to the total number of incoming foragers for the day. The colony proportions were grouped by treatment and the treatment means compared. The same comparisons were made with colonies grouped according to population sizes and proportion means compared between population sizes.

Field forager numbers

In 1984 the number of field foragers was determined done by counting bees in three 9 m^2 areas in each of the control and treated fields. Due to low counts in 1984 the field foraging activity observation area was expanded in 1985. One area of 250m X 3m (750 m^2), was staked out approximately one third of the way into each field . The number of foragers actively working blossoms were counted, as opposed to those flying in the plot or resting on leaves. For both 9 m^2 and 750 m^2 plots, the counter slowly walked along the plot counting only those bees within the plot that could be viewed directly beside the counter, that is not ahead or hehind. The counter also was careful to walk on that side of the plots so as to avoid casting a shadow on the plots to ensure minimal disturbance of foragers. Counts were taken every 4 hours beginning at 0800 h and ending at 2000 h for one day before treatment to seven days after treatment.

Statistical analysis

Assumptions for analysis of variance (ANOVA) were

tested by using "Bartlett's test for homogeneity of variance" (Little and Hills 1978). For more than two treatment means comparisons ANOVA was used to test the assumptions for Tukey's HSD test (Little and Hills 1978). For only two treatment means comparisons the T-Test was used. Correlation analysis was used to determine the relationship between forager entrance activity and field forager activity (Snedecor and Cochran 1980).

RESULTS

Pollen collection

Bees in the treated colonies collected less pollen ($p<0.05$) than in the control colonies for up to 2 to 3 days (Tables 8 and 9) after treatment. By the fourth day after treatment bees in the treated colonies were collecting as much as ($p>0.05$) or more pollen as in the control colonies. Only the control colonies for the 26 July, 1984 treatment collected pollen that appeared to be from a source other than canola. Otherwise all other colonies collected yellow colored pollen that was assumed to be canola, since no other crops attractive to honey bees were present in the area.

Number of foragers entering hives

The total number of foragers entering the treated colonies are shown in Tables 10 and 11. The total number of foragers entering treated colonies was less ($p<0.0001$) than the total number of foragers entering control colonies on

TABLE 8. Mean (± standard error) daily pollen collected (grams) in 1984.

DAYS AFTER TREATMENT	17 JULY, 1984 CONTROL	TREATED 1000 h	26 JULY, 1984 CONTROL	TREATED 0800 h
-1	11.65±1.81	11.94±1.83	10.10±2.32	12.67±3.78
0	13.08±2.21	8.32±2.40	12.87±3.08	6.40±1.49*
+1	7.75±1.76	2.75±1.03*	15.42±4.57	7.66±2.21*
+2	12.98±3.00	4.58±1.20*	22.16±2.87	13.85±2.29*
+3	0.18±0.07	0.02±0.01	25.71±3.47	17.48±2.31
+4	15.17±3.08	15.95±1.36	30.50±3.86	30.35±1.96
+5	18.15±3.53	17.62±1.72	36.07±3.30	37.70±2.53
+6	18.09±3.53	10.09±1.85	39.85±4.82	34.00±3.11
+7	17.19±3.44	11.29±2.14	44.85±5.89	45.21±2.89

*Treated means are significantly different from control means $p<0.05$.

TABLE 9. Mean (± standard error) daily pollen collected (grams) in 1985.

DAYS AFTER TREATMENT	16 JULY, 1985 CONTROL	TREATED 0800 h	CONTROL	27 JULY, 1985 TREATED 1900 h	TREATED 2100 h
-1	62.50±3.18	56.78±8.62	38.70±0.40	32.55±2.95	32.75±2.05
0	96.15±4.50	10.22±0.47*	68.05±2.65	39.10±0.70*	63.40±4.40
+1	57.75±1.64	17.02±1.27*	65.85±0.25	36.00±0.40*	26.45±0.15*
+2	56.90±0.78	46.85±1.05	32.00±1.00	3.2±0.10*	5.6±0.10*
+3	51.65±0.87	30.65±0.25*	55.30±3.70	26.55±2.85*	42.65±1.25*
+4	54.15±15.8	56.40±3.10	104.90±7.00	169.85±0.25	136.00±8.00
+5	48.60±1.63	46.55±1.95	49.10±1.70	41.15±1.15	59.40±0.40*
+6	70.95±1.03	74.65±4.05	46.00±0.80	49.25±0.45	41.25±0.47*
+7	59.07±1.51	52.07±0.07	52.55±1.25	47.65±0.75	44.55±1.85

* Treated means are significantly different from control means $p<0.05$.

TABLE 10. Mean (± standard error) daily forager entrance totals in 1984.

DAYS AFTER TREATMENT	17 JULY,1984 CONTROL	17 JULY,1984 TREATED 1000 h	26 JULY,1984 CONTROL	26 JULY,1984 TREATED 0800 h
-1	235.0±4.53	228.5±0.18	176.5±1.01	171.0±3.78
0	190.0±1.51	70.0±3.22*	230.5±0.94	59.5±5.48*
+1	111.0±0.37	92.5±1.10	118.5±2.83	124.0±2.26
+2	108.5±0.18	104.3±1.26	148.0±0.20	118.7±1.32
+3	5.5±0.56	5.3±.056	163.5±8.50	106.3±0.18*
+4	95.4±6.6	104.5±3.60	178.0±0.23	171.0±0.23
+5	155.0±4.36	161.0±3.02	188.0±0.37	185.4±0.18
+6	130.7±0.75	180.5±0.91	162.5±1.32	150.9±0.37
+7	117.5±2.07	115.0±3.02	194.5±0.56	181.3±0.94

* Mean is significantly different from control ($p<0.0001$).

TABLE 11. Mean (± standard error) daily forager entrance totals in 1985.

DAYS AFTER TREATMENT	16 JULY, 1985 CONTROL	16 JULY, 1985 TREATED 0800 h	27 JULY, 1985 CONTROL	27 JULY, 1985 TREATED 1900 h	27 JULY, 1985 TREATED 2100 h
-1	145.3±0.87	147.2±1.02	134.5±0.94	143.5±4.72	137.5±3.21
0	139.4±1.32	67.4±0.89*	205.5±4.34	112.0±1.31*	164.0±1.51
+1	125.8±2.01	98.3±0.59*	6.0±0.37	1.0±0.00*	1.3±0.01*
+2	110.5±0.73	107.3±0.71	169.5±5.85	166.0±9.07	174.0±0.75
+3	149.2±1.24	131.6±0.98	157.0±8.31	106.5±3.96*	88.0±6.80*
+4	98.7±0.02	91.3±0.17	153.5±2.83	141.0±3.40	113.0±1.13*
+5	132.8±0.19	141.9±1.77	158.5±2.45	154.2±2.83	168.5±0.94
+6	145.3±2.21	139.3±0.73	147.3±3.71	137.7±2.50	141.3±1.21
+7	153.4±1.13	151.3±0.93	121.5±1.70	119.2±2.10	123.3±1.51

* Mean is significantly different from control ($p<0.0001$).

the day of treatment except for the 2100 h treatment where the treatment took place after the final day's count (Table 11). Colonies treated in 1984 on 26 July, at 0800 h were the least affected. The number of foragers entering the hive in these colonies was lower ($p<0.0001$) on the day of treatment but by the second day after treatment the number of foragers entering the hive was not significantly different ($p>0.05$) but was greater than in control colonies. The number of foragers entering hives was significantly ($p<0.0001$) lower in treated colonies than in control colonies for 12 hours to three days after treatment for all the treatments.

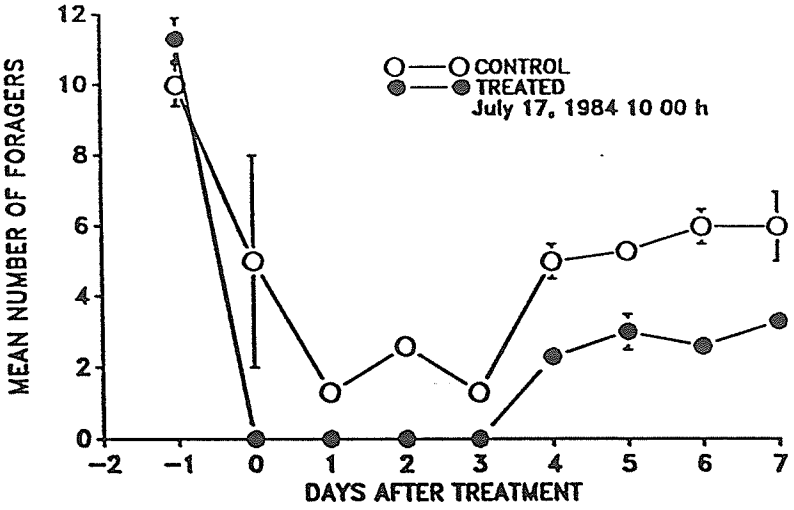
Field forager numbers

Field forager numbers in 1984 was less ($p<0.05$) in treated fields than control fields for seven days after treatment (Figures 9 and 10). In 1985 the number of foragers in the field was lower for two days after treatment for the 0800 h treatment (Figure 11). However, the field forager numbers in the 2100 h treated field did not differ from that in the control field (Figures 12).

The number of bees counted in the three 9 m^2 areas did not appear to be representative of the great deal of activity observed across the fields in 1984. There were significant correlations ($p<0.0001$) between the number of foragers observed in the field and the total number of bees entering colonies (Figures 13 and 14). However, the

Figure 9. Comparison of the number of foragers in treated and control plots for the treatment applied on 17 July, 1984 at 1000 h.

Figure 10. Comparison of the number of foragers in treated and control plots for the treatment applied on 26 July, 1984 at 0800 h.


MEAN NUMBER OF FORAGERS
DAYS AFTER TREATMENT
CONTROL
TREATED
July 17, 1984 10 00 h
0
2
4
6
8
10
12
−2
−1
0
1
2
3
4
5
6
7

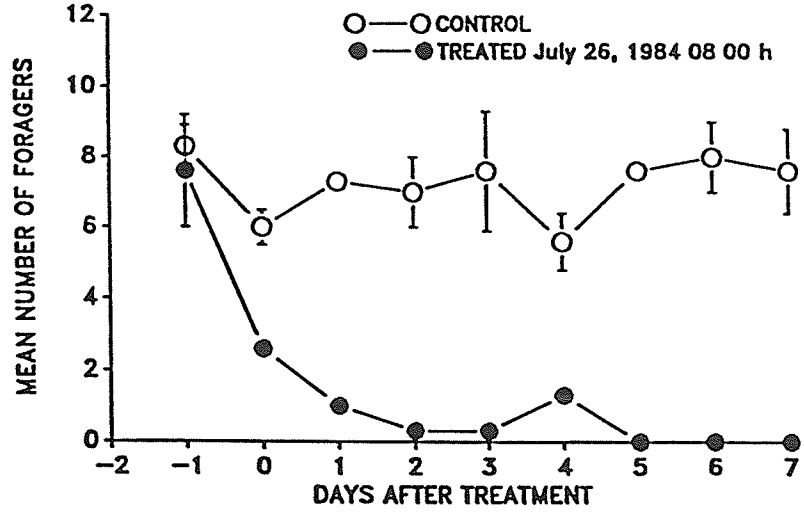
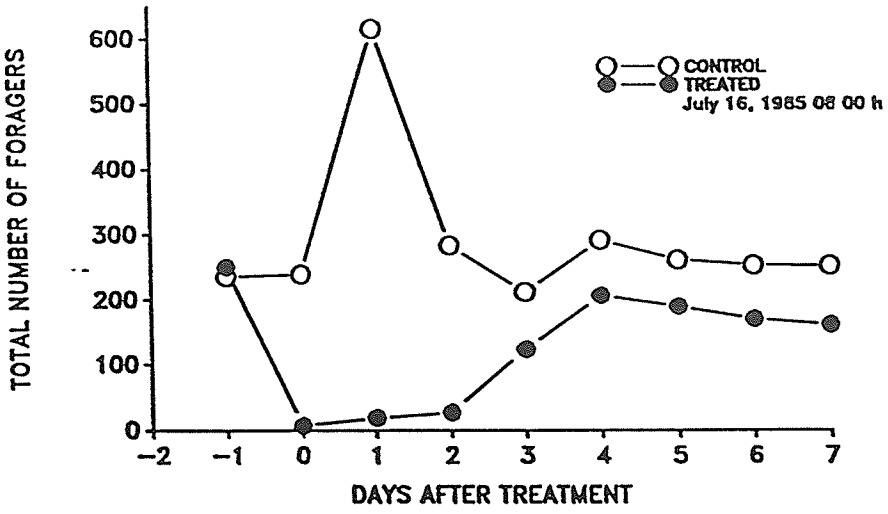

MEAN NUMBER OF FORAGERS
DAYS AFTER TREATMENT
CONTROL
TREATED July 26, 1984 08 00 h
0
2
4
6
8
10
12
−2
−1
0
1
2
3
4
5
6
7

Figure 11. Comparison of the number of foragers in treated and control plots for the treatment applied on 16 July, 1985 at 0800 h.

Figure 12. Comparison of the number of foragers in treated and control plots for the treatment applied on 27 July, 1985 at 1900 h and 2100 h.


TOTAL NUMBER OF FORAGERS
600
500
400
300
200
100
0
−2
−1
0
1
2
3
4
5
6
7
DAYS AFTER TREATMENT
CONTROL
TREATED
July 16, 1985 08 00 h

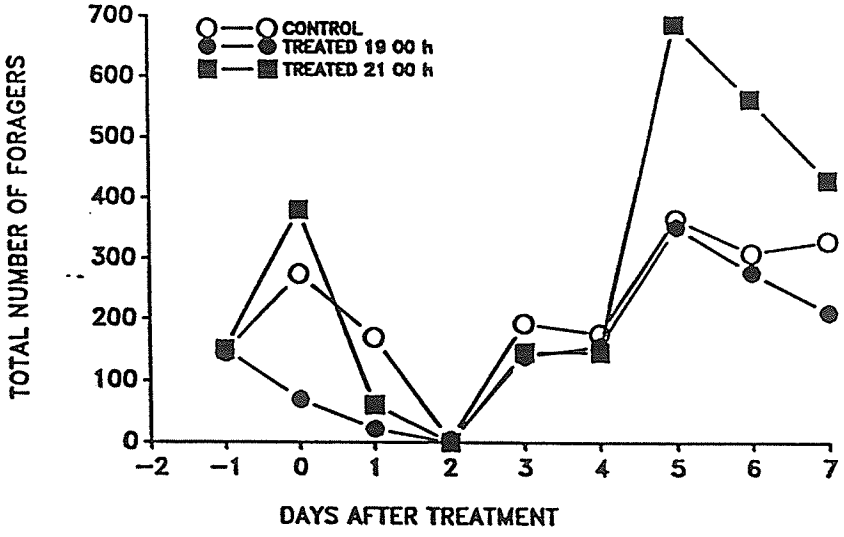
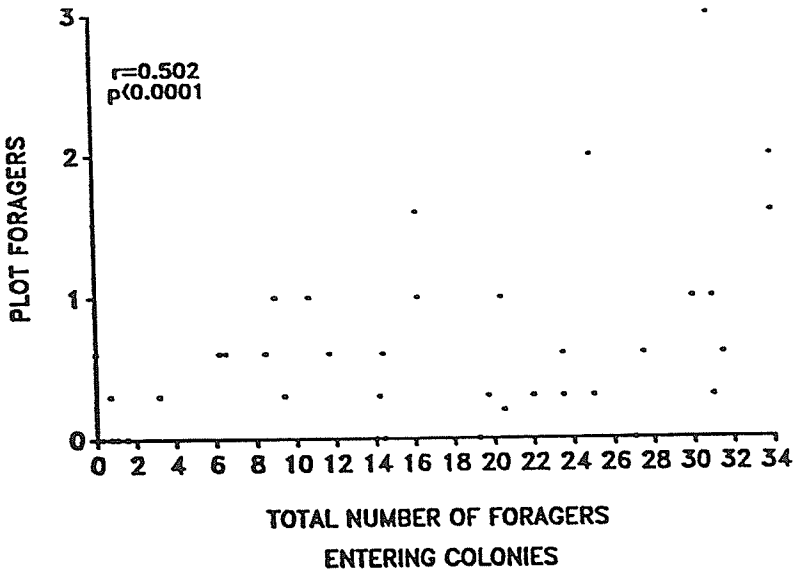
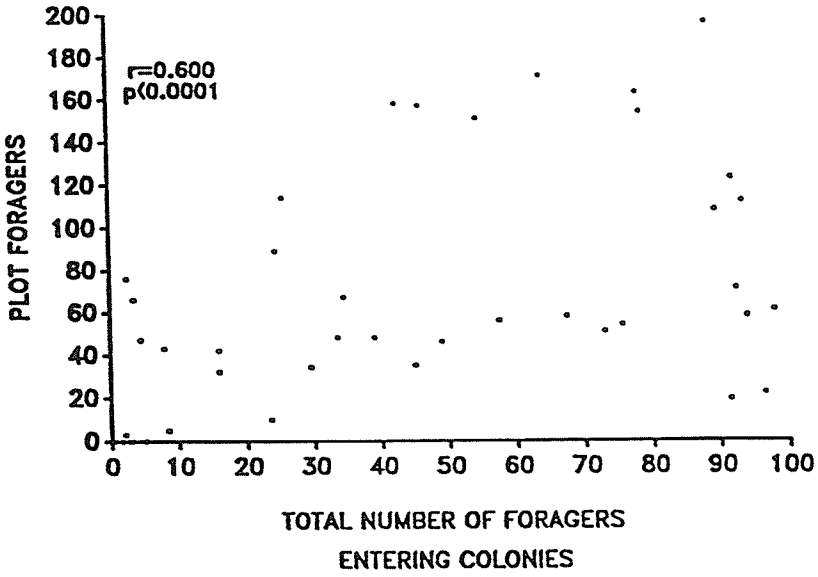

TOTAL NUMBER OF FORAGERS
700
600
500
400
300
200
100
0
−2
−1
0
1
2
3
4
5
6
7
DAYS AFTER TREATMENT
CONTROL
TREATED 19 00 h
TREATED 21 00 h

Figure 13. Correlation analysis of the total number of foragers entering colonies and the total number of foragers observed in plots in 1984.

Figure 14. Correlation analysis of the total number of foragers entering colonies and the total number of foragers observed in plots in 1985.


r=0.502
p<0.0001
PLOT FORAGERS
0
1
2
3
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34
TOTAL NUMBER OF FORAGERS
ENTERING COLONIES


r=0.600
p<0.0001
PLOT FORAGERS
0
20
40
60
80
100
120
140
160
180
200
0 10 20 30 40 50 60 70 80 90 100
TOTAL NUMBER OF FORAGERS
ENTERING COLONIES

correlation coefficient was small (r=0.502). In 1985 when field foraging activity area was expanded to 750 m^2, the correlation was significant ($p<0.0001$) and the correlation coefficient was larger (r=0.600).

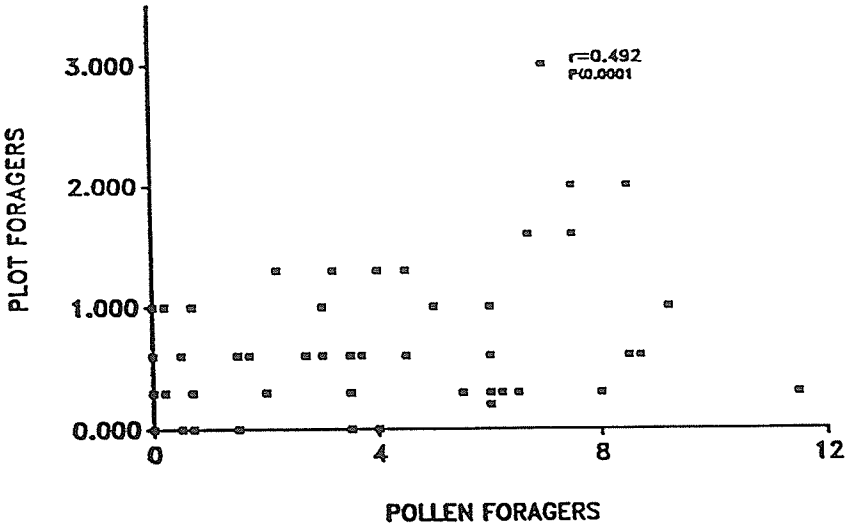
A further examination of the relationships between number of foragers in the field, the number of foragers entering the hives and, pollen collected showed significant correlations ($p<0.0001$) (Figures 15 to 20). However, the correlation coefficients for all were small (Figures 15 to 19) except for the number of field foragers and pollen collected in the O.A.C. pollen trap (r=0.623, $p<0.0001$) (Figure 20).

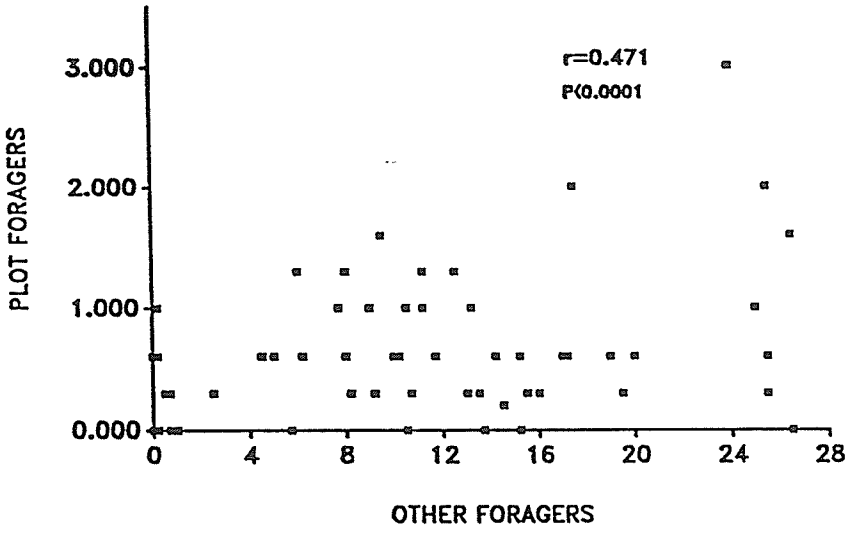
There were no differences ($p>0.05$) between the proportion of total incoming pollen foragers and non-pollen foragers over the total number of incoming foragers between treated and control colonies for all treatment dates and times (Figures 21 to 28). There were no differences ($p>0.05$) between the proportion of incoming pollen and non-pollen forager over the total number of incoming foragers across times and colony adult numbers compared except that a higher proportion ($p<0.05$) of pollen was collected by the smallest colony group (24,500 adult bees) than the largest colony group (51,000 adult bees) (Figures 29 and 30).

Figure 15. Correlation analysis of pollen foragers entering colonies and field forager activity in 9 m^2 plots in 1984.

Figure 16. Correlation analysis of non-pollen foragers entering colonies and field forager activity in 9 m^2 plots in 1984.

Figure 17. Correlation analysis of pollen collected from colonies and forager activity in 9 m^2 plots in 1984.


r=0.492
P<0.0001
PLOT FORAGERS
3.000
2.000
1.000
0.000
0
4
8
12
POLLEN FORAGERS


r=0.471
P<0.0001
PLOT FORAGERS
3.000
2.000
1.000
0.000
0
4
8
12
16
20
24
28
OTHER FORAGERS

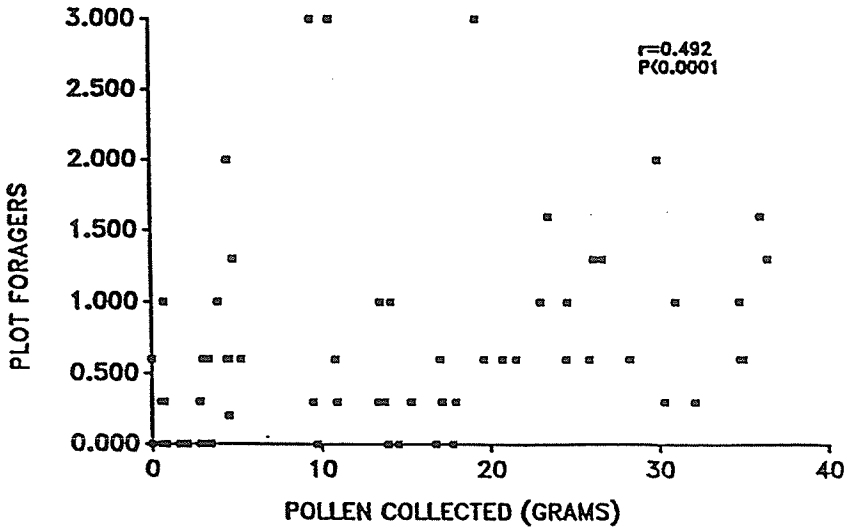
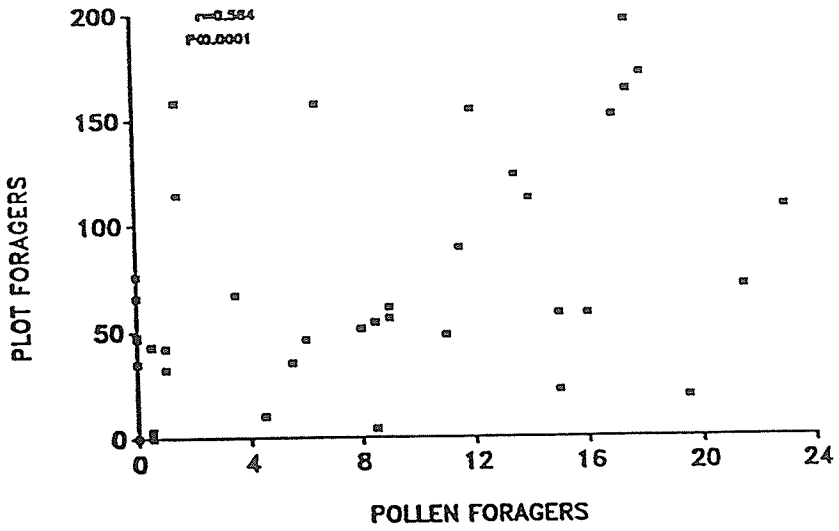
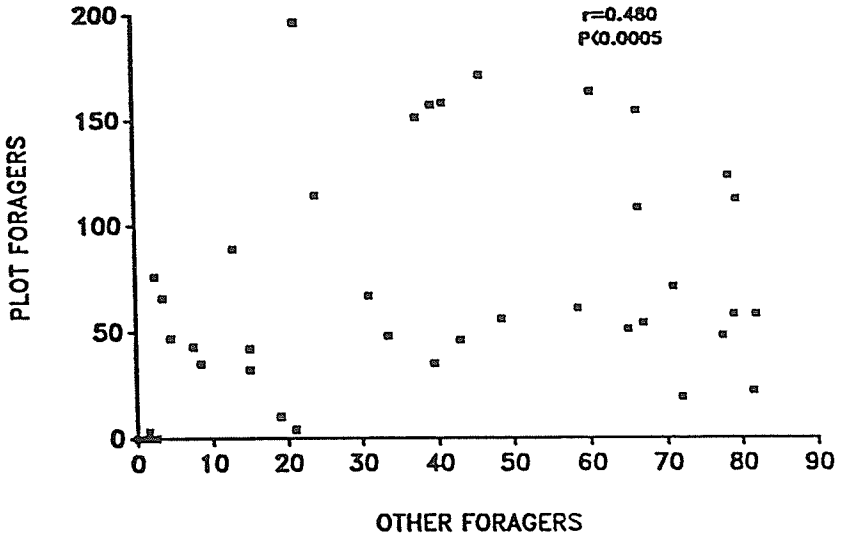

r=0.492
P<0.0001
PLOT FORAGERS
3.000
2.500
2.000
1.500
1.000
0.500
0.000
0
10
20
30
40
POLLEN COLLECTED (GRAMS)

Figure 18. Correlation analysis of pollen foragers entering colonies and field forager activity in 750 m^2 plots in 1985.

Figure 19. Correlation analysis of non-pollen foragers entering colonies and field forager activity in 750 m^2 plots in 1985.

Figure 20. Correlation analysis of pollen collected from colonies and field forager activity in 750 m^2 plots in 1985.


r=0.584
P<0.0001
PLOT FORAGERS
POLLEN FORAGERS
0
50
100
150
200
0
4
8
12
16
20
24


r=0.480
P<0.0005
PLOT FORAGERS
OTHER FORAGERS
0
50
100
150
200
0
10
20
30
40
50
60
70
80
90

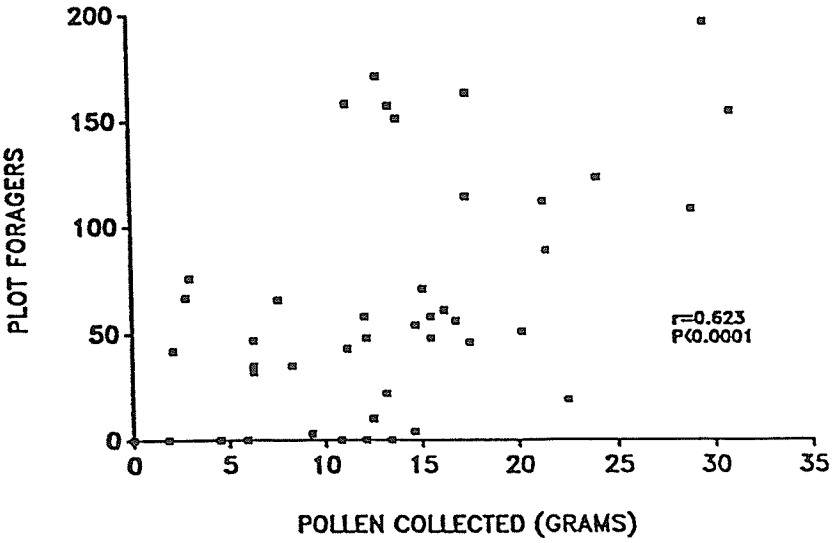
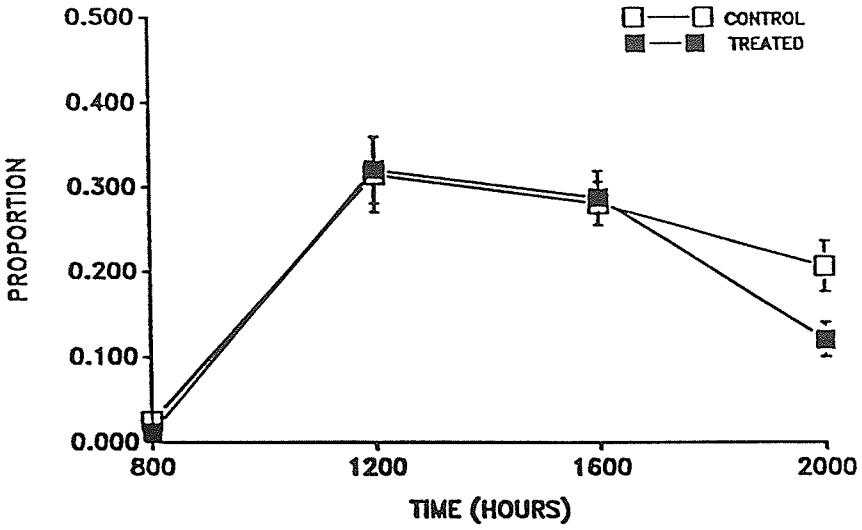

r=0.623
P<0.0001
PLOT FORAGERS
POLLEN COLLECTED (GRAMS)
0
50
100
150
200
0
5
10
15
20
25
30
35

Figure 21. Proportion of non-pollen foragers to total number of foragers entering control and treated colonies for the treatment applied on 17 July, 1984 at 1000 h.

Figure 22. Proportion of non-pollen foragers to total number of foragers entering control and treated colonies for the treatment applied on 26 July, 1984 at 0800 h.


CONTROL
TREATED
0.500
0.400
0.300
0.200
0.100
0.000
PROPORTION
800
1200
1600
2000
TIME (HOURS)

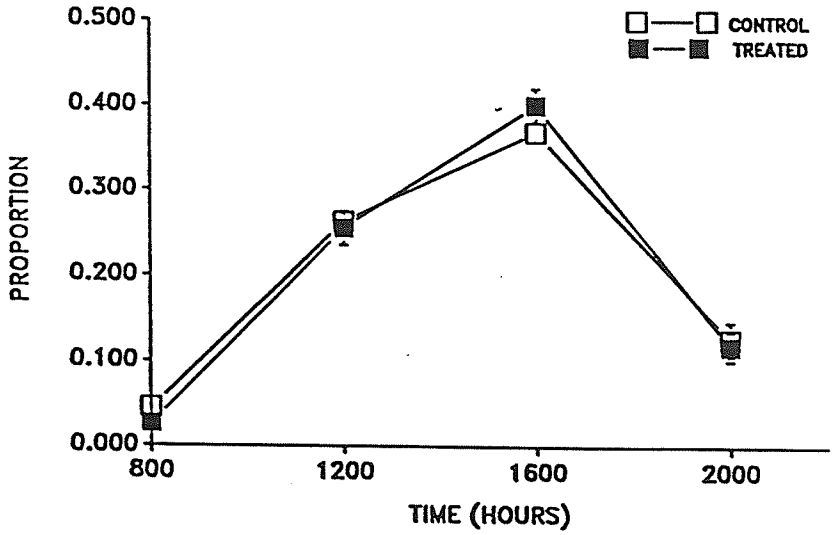
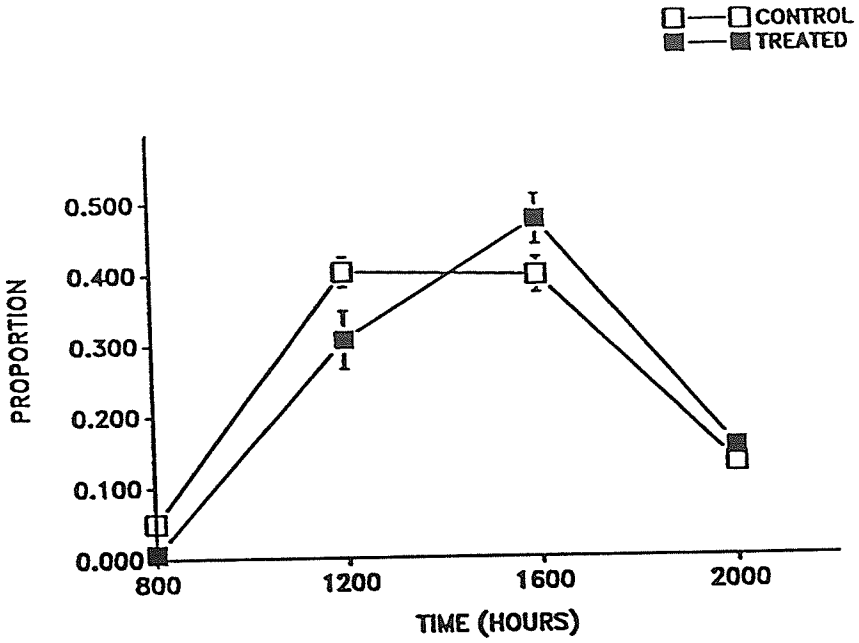

CONTROL
TREATED
0.500
0.400
0.300
0.200
0.100
0.000
PROPORTION
800
1200
1600
2000
TIME (HOURS)

Figure 23. Proportion of non-pollen foragers to total number of foragers entering control and treated colonies for the treatment applied on 16 July, 1985 at 0800 h.

Figure 24. Proportion of non-pollen foragers to total number of foragers entering control and treated colonies for the treatments applied on 27 July, 1985 at 1900 h and 2100 h.


CONTROL
TREATED
PROPORTION
0.500
0.400
0.300
0.200
0.100
0.000
800
1200
1600
2000
TIME (HOURS)

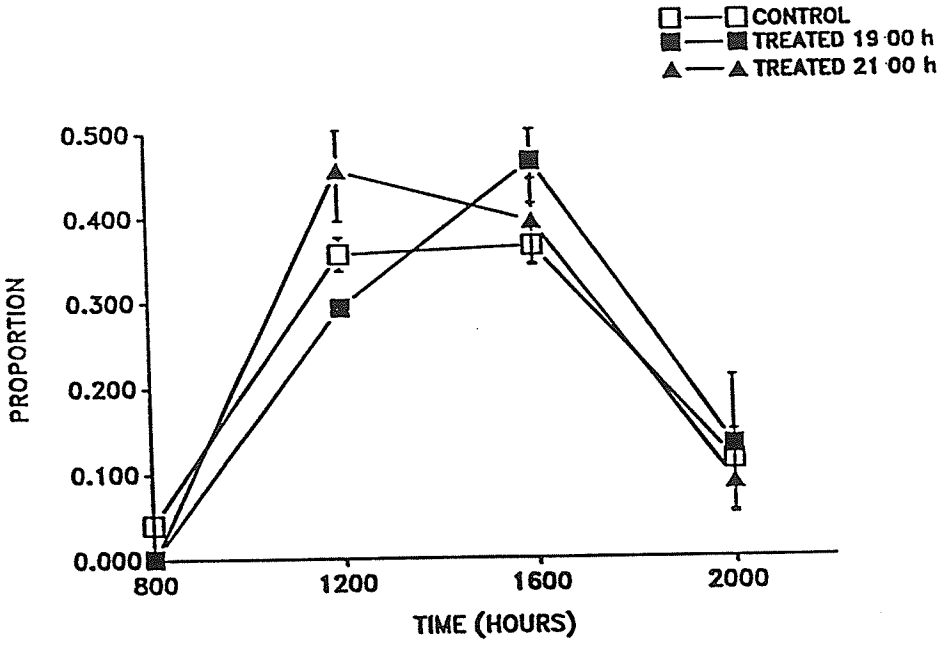
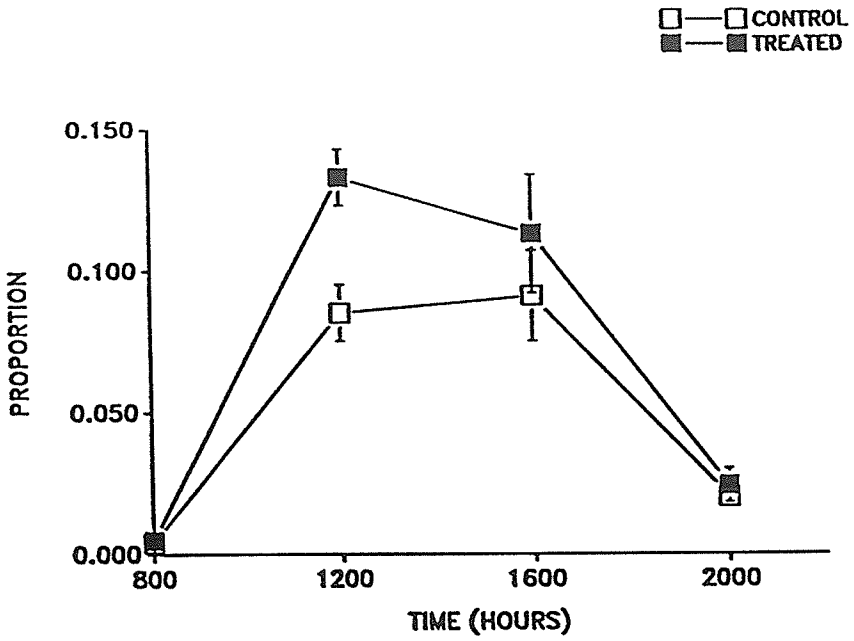

CONTROL
TREATED 19 00 h
TREATED 21 00 h
PROPORTION
0.500
0.400
0.300
0.200
0.100
0.000
800
1200
1600
2000
TIME (HOURS)

Figure 25. Proportion of pollen foragers to total number of foragers entering control and treated colonies for the treatment applied on 17 July, 1984 at 1000 h.

Figure 26. Proportion of pollen foragers to total number of foragers entering control and treated colonies for the treatment applied on 26 July, 1984 at 0800 h.


CONTROL
TREATED
0.150
0.100
0.050
0.000
PROPORTION
800
1200
1600
2000
TIME (HOURS)

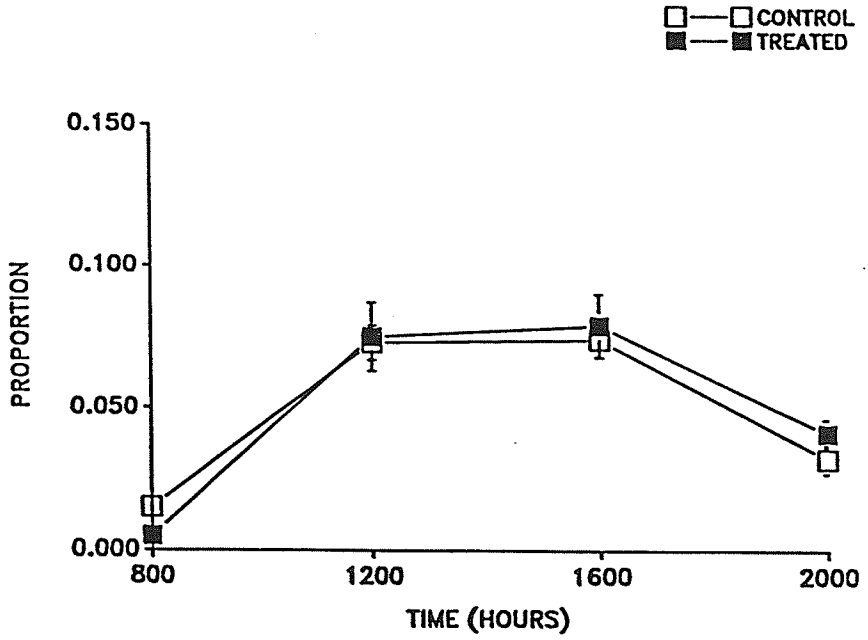
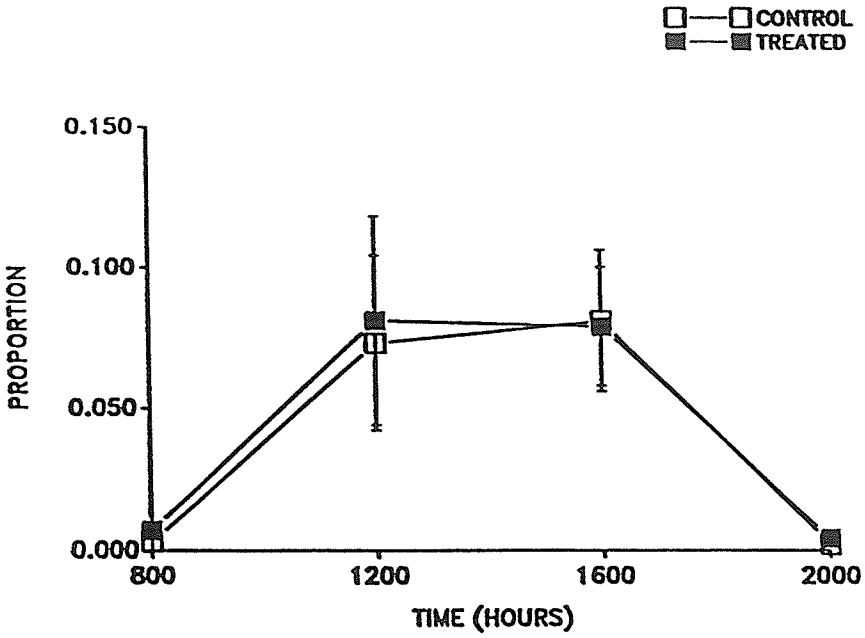

CONTROL
TREATED
0.150
0.100
0.050
0.000
PROPORTION
800
1200
1600
2000
TIME (HOURS)

Figure 27. Proportion of pollen foragers to total number of foragers entering control and treated colonies for the treatment applied on 16 July, 1985 at 0800 h.

Figure 28. Proportion of pollen foragers to total number of foragers entering control and treated colonies for the treatments applied on 27 July, 1985 at 1900 h and 2100 h.


CONTROL
TREATED
0.150
0.100
0.050
0.000
PROPORTION
800
1200
1600
2000
TIME (HOURS)

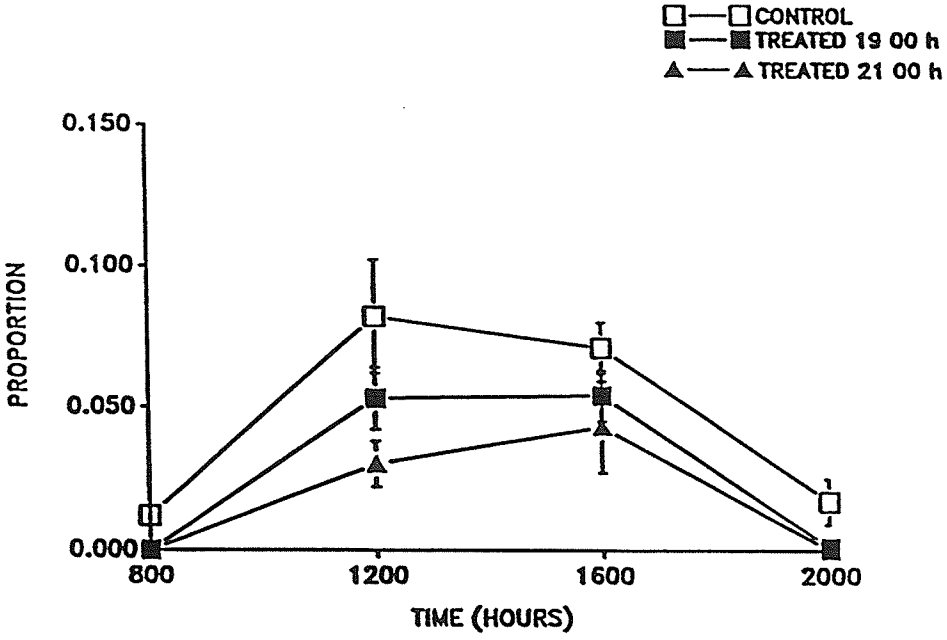
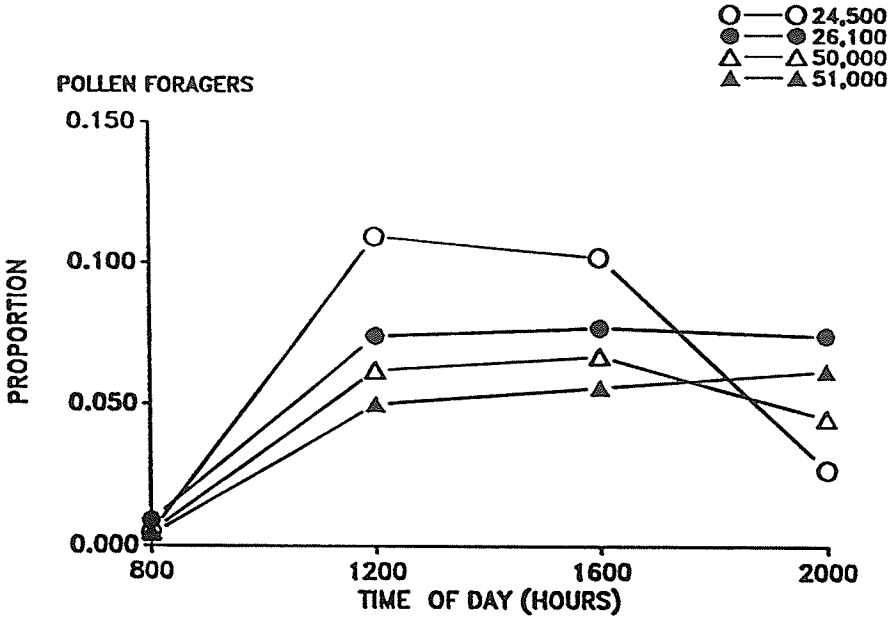
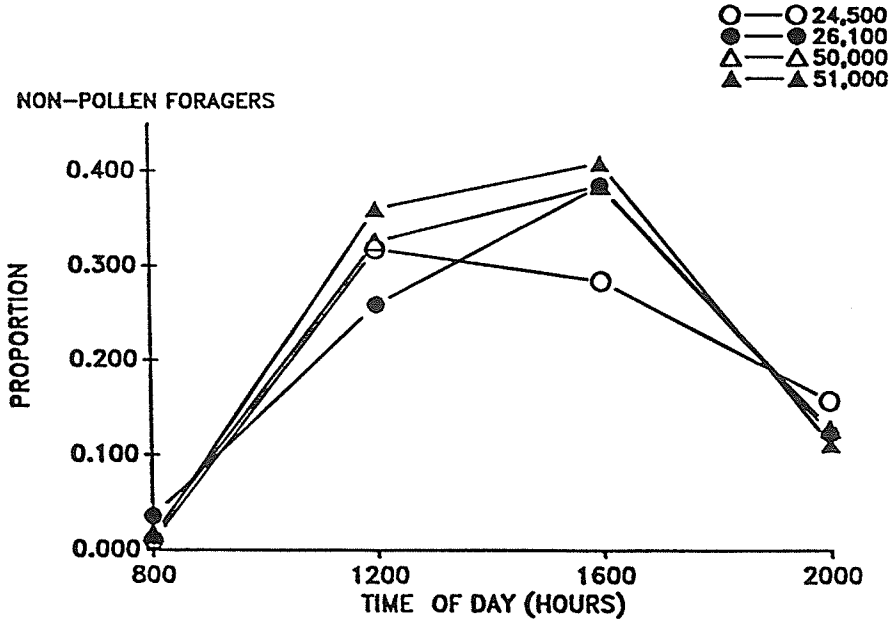

CONTROL
TREATED 19 00 h
TREATED 21 00 h
0.150
0.100
0.050
0.000
PROPORTION
800
1200
1600
2000
TIME (HOURS)

Figure 29. Comparison of the proportion of pollen foragers at various times of the day and colony adult numbers.

Figure 30. Comparison of the proportion of non-pollen, other, foragers at various times of the day and colony adult numbers.


24,500
26,100
50,000
51,000
POLLEN FORAGERS
0.150
0.100
0.050
0.000
PROPORTION
800
1200
1600
2000
TIME OF DAY (HOURS)


24,500
26,100
50,000
51,000
NON-POLLEN FORAGERS
0.400
0.300
0.200
0.100
0.000
PROPORTION
800
1200
1600
2000
TIME OF DAY (HOURS)

DISCUSSION

The foraging behavior of honey bees is affected by extra-colony factors such as weather (Szabo 1980; Burrill and Dietz 1981), and intra-colony factors such as queen state, amount of brood, and available stores (Jay 1986). All measurements used in this study indicated that the foraging activity (includes the number of foragers entering colonies, pollen collected and number of foragers working plots) of honey bees was reduced for two to three days after treatment. Malathion has no residual fumigant activity in the laboratory (Clinch 1967) nor in the field (Metcalfe 1967). Most of the methyl mercaptan (maximum 30 ppm) has been removed from Cythion so it is not expected to be a repellent. The apparent lower forager activity would appear to be due to the initial loss of foragers by direct contact mortality and by residual contact mortality (Johansen et al. 1983). Foraging activity of treated colonies reached and exceeded pre-treatment and control levels by the fourth day after treatment for all treatments. The low ($p<0.0001$) number of foragers entering hives observed on the fourth day after treatment for colonies treated on 27 July, 1985 at 2100 h was due to a carbaryl application on a neighboring sunflower field on the evening of the third day after treatment.

The caste structure of a colony is influenced by the relative number of caste members in the colony population (Wilson 1971). Generally the task performed by an

individual is determined by its age. Initially duties include brood cell cleaning, comb building, brood care and queen care, grooming, feeding nest mates, ventilating and shaping comb, receiving nectar, packing pollen and storing nectar and finally, foraging (Wilson 1971). However, in colonies where the majority of worker bees are removed from the hive, workers begin foraging at younger ages (Lindauer 1961; Winston and Ferguson 1985). It is not known how quickly individuals react to changes in the relative number of members within a caste or by what mechanism recognition of the changes occur. However, within the practical terms of this study the colony response to the loss of the foraging caste took about four days after treatment.

The proportions of pollen foragers and non-pollen foragers to total foragers was not different ($p>0.05$) between treated and control colonies (Figures 21 to 28). The proportions of pollen foragers and non-pollen foragers to total foragers was not different ($p>0.05$) with different adult populations except that the smallest population group (24,500 adult bees) collected proportionately more pollen than the largest population group (51,000 adult bees). It has been established that the amount of pollen gathered increases with the amount of brood in a colony (Free 1967; Al-Tikrity et al. 1972; Todd and Reed 1970). Nelson and Jay (1972^b) determined that the amount of sealed brood in a colony reaches a maximum about 75 days after package hiving and that the ratio of sealed brood to adult population

declines during the same period due to the large number of adult bees in the colony. Smirl and Jay (1972) suggest that nectar flows stimulate brood rearing. The small population group (24,500 adult bees) in this study collected more pollen than the large population group (51,000 adult bees) possibly because there was a greater ratio of brood to adult bees in the small population group than the large population group. Since both groups had been hived at about the same time of the year and had reached, or were near, their maximum brood production. All experiments were conducted at a time when the canola was expected to have its highest nectar flow. The greater proportion of pollen foragers from the smaller population group may also be attributed to an increase in brood rearing stimulated by the nectar flow.

The significant positive correlation between the number of foragers entering hives and the number of foragers counted in plots indicated that the field forager counting method could be used to assess the impact of an insecticide treatment on foraging within specific crops, especially if the insecticide is repellent. Counting the number of foragers entering the hive may be deceptive since untreated, attractive crops may be within foraging distances and thereby attracting foragers away from the target crop.

CHAPTER V

THE EFFECTS OF ULV MALATHION ON HONEY BEES: (1) APPLICATION RATE AND CAGED BEE MORTALITY, (2) DRIFT, AND (3) MALATHION RESIDUES.

INTRODUCTION

The evaluation of contact mortality on honey bees treated with ULV malathion is most often done by exposing caged bees, inside and outside the treated area, at rates greater than 210 ml/ha (Anderson and Atkins 1958; Metcalfe 1967; Anderson and Atkins 1968; Levin et al. 1968). Higher rates of ULV malathion cause greater rates of mortality than lower rates (Atkins et al. 1975). Womeldorf (1974) observed that the highest mortalities of caged bees were found at or near the center lines of aircraft passes where higher application rates were expected. Dixon (1983) reported a "strong correlation", without statistical analysis, between observed application rate and caged bee mortality. The laboratory contact LD_{50} of malathion for honey bees is 0.709 ug/bee (Atkins et al. 1975, 1981).

Downwind deposition (drift) of aerially applied pesticides depends on droplet size, release height, meteorological conditions, and topography (Matthews 1982). Small droplets drift further than large droplets, droplets disperse more and travel further when released from greater heights, increased wind speeds and updrafts contribute to droplet drift, and uneven topography contributes to the formation of eddies, gusts of wind that will increase drift, and surface friction that will decrease drift. As the distance from the target area increases caged bee mortality decreases (Metcalfe 1967; Moffet and Stith 1972). These studies were done with conventional aerial application

systems that produce droplets from 200 to 250 microns MMD (Matthews 1982). Small droplets travel further than large droplets because they remain airborne for longer periods of time (Crabbe et al. 1980; Hartley and Graham-Bryce 1980) and, smaller droplets have a greater tendency to impinge on sharply curved small surfaces, such as antennae, than on large surfaces, thus decreasing the dilution factor expected as small droplets disperse (Spillman 1976; Johnstone 1985). Levin et al. (1968) reported detectable levels of malathion residues in dead bees collected from dead bee traps located 5.6 to 6.4 km away from the treated area after treatment with 560 ml/ha ULV malathion. Dixon (1983) reported highly variable mortality up to 2.4 km away from the treated area after treatment with 210 ml/ha ULV malathion from high altitudes (100 meters).

Malathion residues have been detected from alfalfa, pollen from pollen traps and, live and dead bees exposed to 560 ml/ha ULV malathion (Levin et al. 1968). The highest malathion residue levels were found on alfalfa and pollen. Ultra-low volume malathion at 560 ml/ha applied to alfalfa required 131 hours of field weathering to reduce bee mortality to 25% after confined exposure to the treated alfalfa (Johansen et al. 1983).

The purposes of this study were to determine: (1) the relationship between application rate and caged bee mortality; (2) caged bee mortality from low altitude (3 meters) ULV malathion applications of 210 ml/ha up to 1 km

from the target area (drift); (3) malathion residues inside and outside the hive and to estimate a lethal contact area (cm^2) using teflon covered slides.

MATERIALS AND METHODS

Part 1

Caged bee and mosquito mortality

Caged bees were placed at monitoring stations, described below, spaced 25 m apart across the treated area perpendicular to the flight path of the aircraft. The cages were made of 3.5 mm wire mesh, in the shape of a cylinder, 6 cm in diameter and 21.6 cm long. Each cage contained thirty worker bees that were captured from the blocked entrances of colonies. The bees were caught in the late afternoon and stored in cages in their colony until they were used that evening or the next morning. The colonies that caged bees were captured from were maintained in an apiary composed of colonies made up from excess brood and bees of the parent apiary (nucleus colonies).

The caged bees were held in their own colony so their nest mates could attend to their needs, this ensured 100% caged bee survival before the cages were placed. For each treatment there were six control cages handled in an identical manner as the treated cages.

One hour after treatment the cages were collected, labeled and placed in a cool dark place on top of sugar syrup dampened paper toweling. Mortality counts were taken

1, 6, and 12 hours after treatment. Only those individuals that showed no movement, after prodding, were counted as dead.

Since the registered application rate of ULV malathion used was to control adult mosquitoes, adult mosquitoes were used to determine the efficacy of the treatment. Caged mosquitoes were provided by the mosquito laboratory of the Department of Entomology (University of Manitoba). Twenty newly emerged female Aedes sp. were placed into each 15.2 cm X 2.5 cm X 2.5 cm cage. Four sides of the six sided cage were made of clear plastic and the two remaining sides were made of 1 mm plastic mesh. The cages were positioned so the plastic mesh would be exposed to the spray. For each treated apiary there were 3 control cages. One hour after treatment the cages were collected, labeled and kept in a cool dark place on water dampened paper toweling. Mortality counts were made at 1, 12, and 24 hours after treatment. Only those individuals that showed no movement, after prodding, were counted as dead.

Monitoring stations

Determination of the relationship between application rate and caged bee and mosquito mortality were conducted at monitoring stations consisting of a bee cage (1 meter above ground level (a.g.l.)), a mosquito cage (0.5 m a.g.l.) , a teflon coated microscope slide and a Gelman Type A-E glass fiber filter mat (1 m a.g.l.). The monitoring stations were placed every 25 m across the treated areas perpendicular to

the flight path of the aircraft. The aircraft flew perpendicular to the direction of the wind progressing upwind except for the 27 July, 1985 1900 h treatment. Because of a power line and shifting wind conditions the aircraft was required to make passes that had the aircraft heading with the wind and into the wind during half of the treatment. Each treated area contained 16 monitoring stations.

Rate (ml/ha) of malathion measurement

The amount of malathion on glass fiber filter mats was measured by capturing the deposited spray on Gelman Type A-E glass fiber filter mats with a minimum particle capture of 0.45 microns. The mats were 10 cm X 10 cm and pre-rinsed with 3 washes of acetone and 3 washes of hexane (solvent rinsed) then wrapped in solvent rinsed aluminum foil paper until they were used. The mats were handled with solvent rinsed stainless steel forceps. The mats were stationed 1 m a.g.l. on a 10 cm X 10 cm platform covered with solvent rinsed aluminum foil paper. After application the glass fiber filter mats were left for one hour then collected, wrapped in solvent rinsed aluminum foil paper, labeled and stored at $-18^{0}C$ till extraction.

Soxhlet extraction was used. The glass fiber filter mats were placed in the inner tube of the Soxhlet apparatus and a condenser was fitted to the top of the apparatus. A round bottom flask containing 200 ml of ethyl acetate as the solvent was fitted to the bottom of the apparatus. The

filters were extracted for six hours and then the extracts were concentrated to 5 ml at 38^{0}C and vacuum pressure. The solvent was exchanged to 2,2,4-trimethylpentane (TMP), diluted to 10 ml and stored in glass amber injection tubes with teflon lined lids at 4^{0}C till injection.

Extracts were injected into a Varian Model 3700 gas chromatographer with a phosphorous flame photometric detector (Tracer 560/700A Hal Elec Cono Detector) and a Hewlett-Packard 3390A Integrator Digital Printout. The column temperature was maintained at 200^{0}C (isothermal), injector temperature at 220^{0}C and the detector temperature at 250^{0}C. The results were converted from parts per million (ppm) malathion to ml per hectare malathion. The lower detection limit was 0.05 ppm. A total of 48 glass fiber filter mats were analysed.

Part 2

Downwind drift mortality

Caged bees were placed 1m a.g.l., 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 meters away from the edge of the treated area in a downwind direction. Mortality counts were taken at 1, 6, and 12 hours after treatment. Only those that showed no movement after prodding were counted as dead.

Part 3

Malathion residue detection

Samples collected for malathion residue detection were

dead bees from dead bee traps, pollen from pollen traps, hive pollen, hive raw honey, hive wax, and canola blossoms. All samples were placed in solvent rinsed jars with solvent rinsed aluminum foil lined lids and stored at -18^{0}C till extraction. Where necessary samples were handled with solvent rinsed stainless steel forceps. The same extraction method was used for all sample types.

The majority of sample sizes were 10 grams. Any samples less than 10 grams required individual treatment with respect to extraction dilution and conversion to ppm malathion. All equipment used was rinsed 3 times with acetone and 3 times with hexane. Samples were weighed, labeled and placed into Polytron centrifuge glass jars with 100 ml of ethyl acetate. The samples were homogenized for at least 3 minutes to up to 5 minutes. The samples were then centrifuged at 3000 rpm for 15 minutes. The centrifuged samples were decanted over muffled sodium sulfate in fretted funnels and the extract was caught in 500 ml round bottom flasks. The extract was condensed to 1 ml with a flash evaporator at 38^{0}C under vacuum pressure. Three rinses of TMP were added to the extract. Then the extract was centrifuged once more for 5 minutes at 1500 rpm, decanted into glass amber injection tubes and more TMP was added, diluting to 10 ml. The extract was stored at 4^{0}C till injection. Injection equipment and specifications were the same as those used for the glass fiber filter mat extractions.

Some samples such as honey and wax proved to be particularly difficult to homogenize. Since all preliminary work showed that residues were below the detectable level for these samples further sampling was abandoned to avoid any damage to the equipment.

A total of 63 canola blossom samples, 42 pollen samples, 30 dead bee samples, 3 honey samples, 3 wax samples, and 3 hive pollen samples were analysed.

Quality control

To test the residue detection methods for percent recovery, untreated materials (bees, pollen, blossoms, solvent carrier only etc.) were 'spiked' with a standard 10 ul of 0.151 ng/ul of malathion provided by Cyanamid. Spiked materials were tested initially, and then once injection of treated materials was being conducted spiked solvent extract was injected for every treated material extract. Throughout the course of residue analysis percent recovery ranged from 88% to 113% .

Droplet size

Teflon coated microscope slides were used to determine spray droplet size. Cyanamid recommends that droplets ranging from 40-60 microns be used with aerial applications, however smaller droplets can be used and are just as efficacious (Mount and Lofgren 1967; Mount et al. 1970). The recommendation is directed toward applications made from 30 to 4.6 m at speeds of 242 km/h. Lower speeds and

altitudes may be used with smaller droplets (Mount 1970). The slides were left for one hour after application and then collected and labeled. Determination of spray droplet size was done according to the method outlined by Cyanamid for Cythion (see Appendix 2) within 36 hours after collection. A total of 66 teflon slides were counted.

Estimate of a lethal contact area (cm^2)

The method used was adapted from a method outlined by Hadaway et al. (1978). In 1985 10 teflon coated slides were chosen randomly from the three treatments (3 from 16 July, 0800 h, 4 from 27 July, 1900 h, 3 from 27 July, 2100 h) and the total number of drops per cm^2 were counted, as well as the MMD determined for each slide. An average droplet size and and average total number of drops per cm^2 was determined from the sample. From this nanograms of active ingredient per drop were estimated as well as the area that a bee would be required to expose herself to in order to come in contact with a lethal dose of residual malathion.

Statistical analysis

Correlation analysis was used to determine the relationship between application rate and mortality of caged bees and caged mosquitoes (Finney 1964; Snedecor and Cochran 1980). All other data is presented as means and standard errors.

RESULTS

Part 1

Relationship between application rate and mortality of caged bees and mosquitoes

In 1984 a very limited amount of data were collected. Droplet size data and residue analysis data were combined with 1985 results.

Six hours after treatment all treated caged bees were dead. Data were corrected for control caged bee mortality (0.7%). Caged mosquito results were based on 12 hour counts and, corrected for control caged mosquito mortality (3.7%).

The correlation was significant ($p<0.0001$) for caged bee mortality and application rate ($r=0.900$) (Figure 31).

The correlation was significant ($p<0.0001$) for caged mosquito mortality and application rate ($r=0.920$) (Figure 32).

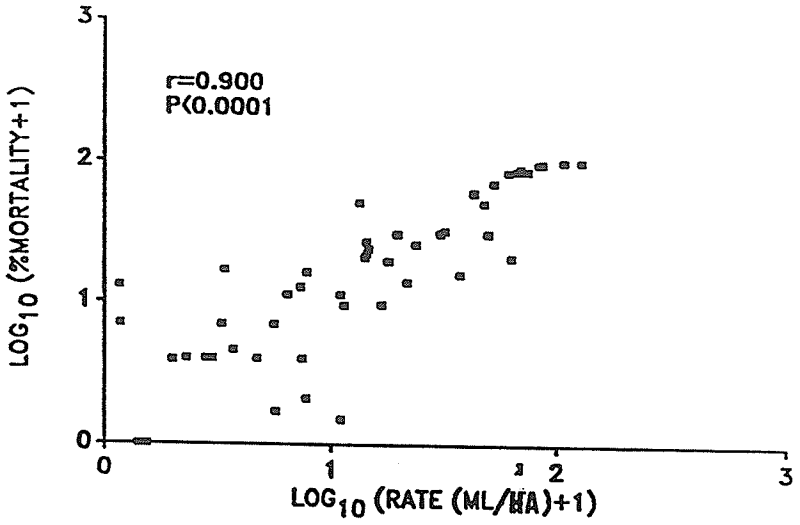
Part 2

Downwind drift mortality

The downwind drift mortality of caged bees outside the treated area for all three 1985 treatments are shown in Figures 33, 34 and 35. The 1900 h treatment had the highest mortality and most fluctuating mortality pattern (Figure 34). The 0800 h and the 2100 h treatments had mortality ranging from 29.5% to 1% mortality up to 600 m from the treated areas. From 600 m to 1000 m mortality ranged from 10.2% to 0% (Figures 33 to 35).

Figure 31. Correlation analysis of ULV malathion application rate and one hour mortality of caged honey bees.

Figure 32. Correlation analysis of ULV malathion application rate and 12 hour mortality of caged mosquitoes.


r=0.900
P<0.0001
LOG10 (%MORTALITY+1)
LOG10 (RATE (ML/HA)+1)
0
1
2
3

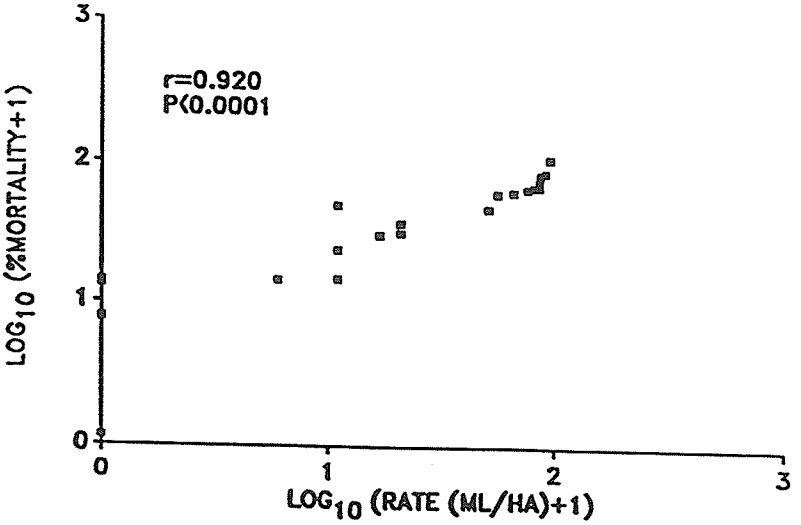
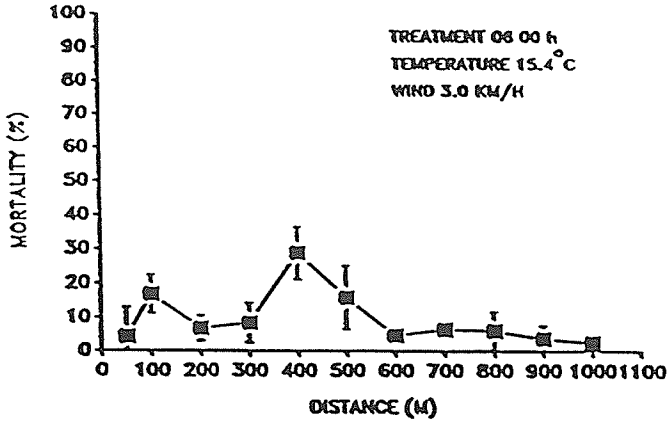
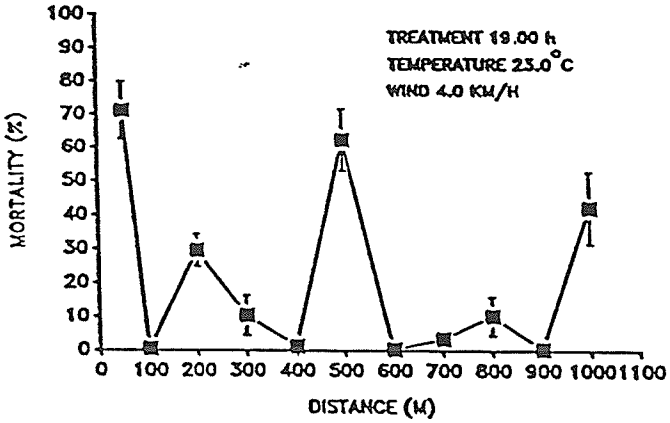

r=0.920
P<0.0001
LOG10 (%MORTALITY+1)
LOG10 (RATE (ML/HA)+1)
0
1
2
3

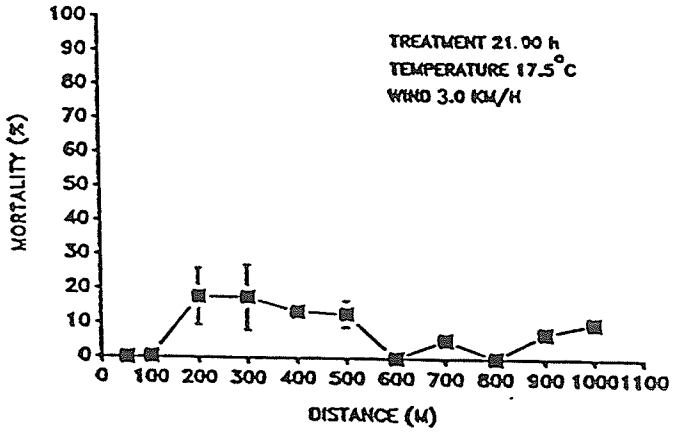
Figure 33. Caged bee mortality (± standard error) at various distances downwind from the treatment applied on 16 July, 1985 at 0800 h.

Figure 34. Caged bee mortality (± standard error) at various distances downwind from the treatment applied on 27 July, 1985 at 1900 h.

Figure 35. Caged bee mortality (± standard error) at various distances downwind from the treatment applied on 27 July, 1985 at 2100 h.


TREATMENT 06.00 h
TEMPERATURE 15.4°C
WIND 3.0 KM/H
MORTALITY (%)
DISTANCE (M)


TREATMENT 19.00 h
TEMPERATURE 23.0°C
WIND 4.0 KM/H
MORTALITY (%)
DISTANCE (M)


TREATMENT 21.00 h
TEMPERATURE 17.5°C
WIND 3.0 KM/H
MORTALITY (%)
DISTANCE (M)

Part 3

Malathion residues

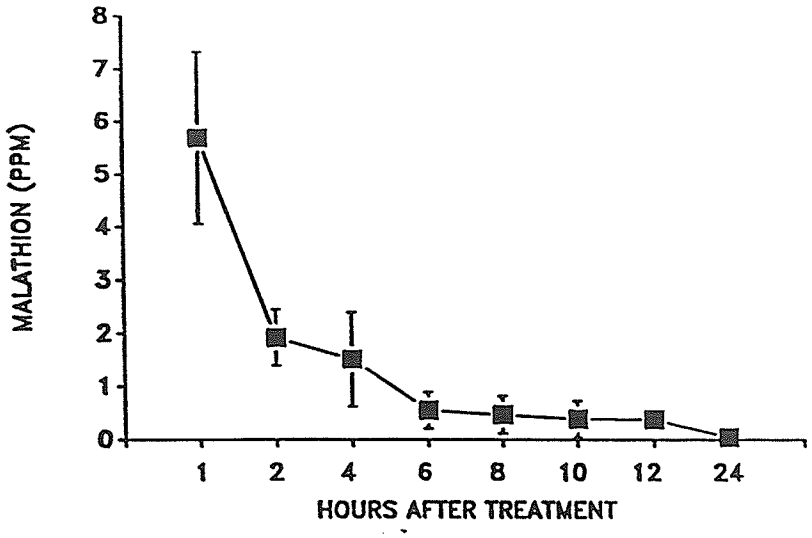
Malathion residue levels, detected in pollen from pollen traps are shown in Figure 36. Twenty-four hours after treatment the malathion residues were below detectable levels.

Malathion residue levels, detected in canola blossoms are shown in Figure 37. Twenty-four hours after treatment the malathion residues were below detectable levels.

Residue levels for all other materials tested are shown in Table 12. Bees used here were from cage studies conducted within the treated area. Bees caught in the dead bee traps were used for residue detection; material was analysed from their honey stomachs because they would have been foraging. Due to the great number of bees required to collect a sample size sufficient for extraction, only three samples of about 400 bees each were tested. All of the honey stomachs were collected from bees treated on 27 July, 1985 at 1900 h. A very low level of malathion was detected in the honey stomachs (0.09 ± 0.03 ppm). Raw honey and wax were very difficult to work with. The samples were very cold and thus resisted homogenization and rather coagulated into hard masses that constantly jammed the Polytron and overheated the motor. No sample had residues above the detectable limit. Pollen collected from within the hive also had no residues above the detectable limit.

Figure 36. Malathion residues (± standard error) at various times after treatment on pollen collected from pollen traps.

Figure 37. Malathion residues (± standard error) at various times after treatment on canola blossoms.


MALATHION (PPM)
8
7
6
5
4
3
2
1
0
1
2
4
6
8
10
12
24
HOURS AFTER TREATMENT

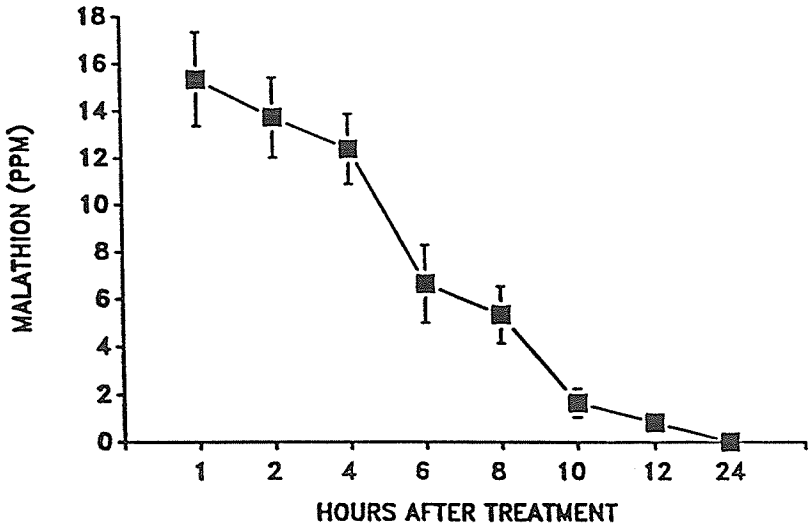

MALATHION (PPM)
18
16
14
12
10
8
6
4
2
0
1
2
4
6
8
10
12
24
HOURS AFTER TREATMENT

TABLE 12. Malathion residues detected from dead bees, hive pollen, honey, wax, and honey stomachs, one to three hours after treatment.

SAMPLE	NUMBER OF SAMPLES	TIME AFTER TREATMENT (HOURS)	MEAN PPM MALATHION (±STD.DEV.)
DEAD BEES	30	1	< 0.05
HIVE POLLEN	3	3	< 0.05
HONEY	3	3	< 0.05
WAX	3	3	< 0.05
HONEY STOMACHS	3	1	0.09±0.03

Droplet size

The average droplet size produced by the application system used was 43.3±12.5 microns for both 1984 and 1985 (Table 13).

Estimate of a lethal contact area (cm^2)

Results of the contact residue exposure estimate are shown in Table 14.

DISCUSSION

Part 1

Relationship between application rate and mortality of caged bees and mosquitoes

The laboratory contact LD_{50} for malathion is 0.709 ug/bee (Atkins et al. 1975, 1981). Atkins et al. (1981) have a prediction method of field toxicity based on laboratory contact LD_{50} and a field applied rate. However the method cannot be used for predictions with ULV formulations since the method is based primarily on formulations that have relatively low active ingredient rates and high formulated product application rates per hectare compared to ULV malathion.

No other work reports the relationship between application rate and mortality of caged bees. However, some work suggests that a positive relationship exists (Clinch 1967, 1969: Clinch and Ross 1970; Atkins 1975; Johansen 1977; Dixon 1983; Johansen et al. 1983).

A field LD_{50} for malathion or any other insecticide,

TABLE 13. Spray droplet size (MMD) and size range for all treatments.

TREATMENT	TIME	MEAN	RANGE	N
JULY 17, 1984	1000	48	5-73	6
JULY 26, 1984	0800	33	1-105	6
JULY 16, 1985	0800	39	3-89	20
JULY 27, 1985	1900	47	1-96	20
JULY 27, 1985	2100	51	1-103	20
TOTAL		43.3±12.5		72

TABLE 14. Contact residue exposure estimate.

Drop size in microns	30.0±11.7
Number of drops per cm^2 at 210 mL/ha	449.5±59.8 (450)
Ng a.i. per drop at 95% active ingredient	0.359
Contact area for exposure to a lethal dose (based on a contact LD_{50} of 0.709 ug/bee Atkins et al. 1975)	43 cm^2

that is expressed in the same units as the application method, would assist insecticide applicators and beekeepers in assessing the expected potency of the rates being applied. Presently, applicators and beekeepers have available to them the residual activity time of an insecticide and the laboratory LD_{50}, usually expressed in ug/bee. This information helps the beekeeper and insecticide applicator to compare the relative potency of one insecticide to another but does not help in determining the expected potency of the rate that is to be applied.

It is difficult to determine a field LD_{50} since there is little control over the amount of malathion that will be deposited in any one place. Therefore, a good range of application rates, necessary for probit analysis, is difficult to achieve in a field situation.

Part 2

Downwind drift mortality

The mortality for the treatment applied on 27 July, 1985 at 1900 h showed the greatest variation in drift mortality from the treatment area. The drift cages were placed in the middle of a 300 m tilled border between two wheat fields. The aircraft was flying passes that required it to head into and with the wind and to approach the swath so that the wind and the vortices would be pushing the spray directly into the border where the cages were placed. A combination of wind, aircraft vortices, and possible turbulence (as a result of warm air and warm soil

conditions) within the border may have contributed to the variable downwind dispersal of the spray droplets and resultant variable caged bee mortality.

An aircraft produces vortices generated from a combination of airflow over the wings and the propeller. The vortices radiate primarily from the tips of the aircraft wings circulating counter clockwise and rolling inward but expanding as the distance from the aircraft increases (Matthews 1982). The centrifugal force within the vortex pulls droplets to the outside and sends them in whatever direction that portion the vortex happens to be heading in (Lawson and Uk 1980; Matthews 1982). A small droplet does not reach that area within an aircraft vortex where the airflow direction is downward but, instead remains airborne and is influenced by an "upwash" of the vortex (Lawson and Uk 1980). The downwind dispersal from experimental sprays show that the total volume of the spray is still airborne 120 m from the release point and 13 m above ground level when droplets are less than 64 microns in diameter (Lawson and Uk 1980). Other factors that influence downwind dispersal besides droplet diameter are release height, air turbulence (upward and downward) and various environmental factors (Jegatheeswaran 1978; Matthews 1980).

The drift mortality, in this study, 1 km downwind from the treatment areas was expected given the droplet size (see Ghassemi et al. 1982; Matthews 1982; Ware et al. 1984) and the efficacy of small droplets (Himel 1969; Spillman 1976;

Hartley and Graham-Bryce 1980). However, there did not appear to be a direct relationship between the distance from the treatment area and drift mortality as reported by Moffet and Stith (1971). Instead, the drift mortality fluctuated with no apparent relationship to the distance from the treatment area. Dixon (1983) observed similar fluctuations that appeared to be more closely related to drift quantity as measured on teflon coated slides rather than distance from the treatment area. Atkins's (1981) model although based on different insecticide formulations recognizes that increased application rates results in increased expected mortality. It appears that the downwind deposition of the spray droplets causes greater and lesser amounts to be deposited at various distances from the treatment areas.

Part 3

Malathion residues and estimate of a lethal contact area

The level of residual malathion that is harmful to honey bees depends on the amount of residue the honey bee comes into contact with (LD_{50}=0.709 ug/bee) and whether the residue is available for insecticidal activity. The toxicity of residual insecticides is usually determined by confined exposure studies (Clinch 1967; Johansen 1972). However, bees do not rest for long periods of time on treated blossoms or treated leaf matter but they actively collect pollen and nectar from the floral sources that are available. In the case of Brassica napus the majority of foragers collect nectar intentionally and pollen

incidentally; very few bees collect only pollen (Langridge and Goodman 1975; Free and Ferguson 1983). Foragers may approach the flower from the side and thus do not come into contact with the stigma or anthers or, they may approach the flower from the top and come into contact with the stigma and anthers and clasp onto the petals with their tarsi (Free and Ferguson 1983). The number of flowers a forager will visit ranges from 10 to 14 per minute and from 4.1 to 6 seconds are spent visiting each flower (Free 1970; Benedek and Prenner 1972; Langridge and Goodman 1982). Those foragers that approach the flower from the top must alight onto the blossom in order to insert the tongue into nectaries at the base of the corolla. However, those bees that approach from the side of the flower may have limited (or no) contact with the flower as they can remain hovering while foraging.

The amount of contact a forager will have with a treated plant will vary and be cumulative as a result of the number of visits made to flowers. Exposure is not continuous as in the confined exposure studies where bees are placed in containers with treated materials and continuously exposed to the treated material, for long periods of time. A source of extended contact can result from the pollen load a bee carries while foraging on canola. Approximately 10,000 grains of pollen can be found on a forager in canola (Williams 1980). The forager will either discard pollen grains from her body or pack the pollen into her corbiculae

(Free 1970). Brassica napus pollen grains are 41 to 47 microns in diameter (Nair and Sharma 1976), about the same size of the droplets used in this study. Gratwick (1957), using lipophilic dye to measure the uptake of insects walking on residual deposits found that during the first few steps the amount of residue picked up was similar for worker wasps (Vespula vulgaris(L.)), ground beetles (Feronia madida (F.)), solier beetles (Rhagonycha fulva (Scop.)), cotton stainers (Dysdercus fasciatus (Sign.)), and plant bugs (Notostira erratuca (L.)). The smaller insects picked up more particles per unit weight, the hind legs for all species picking up the same or more particles than all the other legs combined. Insects with higher densities of setae were protected from the particles because the particles were held away from the main surface of the cuticle. However, the cleaning actions of insects, with dense setae, offset any protection the setae provide.

A forager would have to come in contact with 19,750 droplets of 30±11.7 microns in diameter (MMD) with 0.0359 ng active ingredient per droplet to be exposed to a contact LD_{50} dose. Even if the droplets that fall onto pollen grains are smaller (and as a result carry less active ingredient of malathion) a forager will become covered several times a day with a total of more than 19,750 pollen grains. The frequent cleaning activity of the honey bee would bring the residue into contact the bee's body. Assuming that a honey bee weighs about 128 mg the contact

LD_{50} based on a laboratory LD_{50} of 0.709 ug/bee is 5.53 ppm. The residual malathion recovered from pollen in pollen traps one hour after treatment was 5.7±1.6 ppm, sufficient to produce a lethal reaction.

The blossom residual malathion level was 15.3±2.0 ppm one hour after treatment. The estimated area a forager would have to be exposed to, to come into contact with a lethal dose (based on a laboratory contact LD_{50}=0.709 ug/bee), was approximately 44 cm^2. Because of the bee's foraging behavior of on Brassica napus they would have the greatest contact with blossoms when they approach flowers from the top and/or when they actually land on the blossom and contact the floral parts. A combination of being covered with pollen grains and repeatedly contacting floral parts would probably be the most hazardous form of foraging.

The oral LD_{50} of malathion is 0.38 ug/bee (Atkins et al. 1975, 1981). One hour after treatment the malathion residue level detected in pollen from pollen traps was 5.7 ppm. In order to consume a lethal dose a bee weighing 128 mg would be required to consume 0.52 grams of pollen from the pollen trap one hour after treatment. It is not known how much pollen a bee will consume at one time but it is unlikely an individual bee could consume four times its weight in pollen during one feeding. Pollen loads are about 15 mg and range from 7 to 20 mg (Park 1922; Fukuda et al. 1969). Even if a bee were to eat her entire pollen load she would not consume a lethal dose.

The results of this study indicated that initially (the first 12 hours after treatment) foragers could accumulate lethal doses of malathion and that subsequently foragers could accumulate sub-lethal doses of malathion. The duration and extent of effects of a sub-lethal dose of malathion on honey bee individuals and colonies is not known. More work is needed in this area.

CHAPTER VI

GENERAL DISCUSSION

Colonies of honey bees were treated with aerially applied ultra-low volume malathion at a rate of 210 ml/ha from 3 meters above ground level during Brassica napus L. (Westar canola cv.) nectar flows in the mid to latter part of July in 1984 and 1985.

Treated colonies gained significantly ($p<0.05$) less weight, on a daily basis, than control colonies for 3 to 7 days after treatment. Small treated colonies, with brood diseases, gained significantly ($p<0.05$) less weight than small control colonies with brood diseases, for up to 21 days after treatment (Table 2). Large, healthy, treated colonies gained the same as or more weight than large, healthy control colonies for 14 days after treatment. Cool, wet, inclement weather resulted in low weight gains for control colonies and low to negative weight gains for treated colonies (Table 3). Treated and control colonies followed the same patterns of daily weight gain (Figure 4).

Some of the colony weight lost may be attributed to forager losses. However, the number of bees in the dead bee traps was not sufficient to account for the weight loss alone. The majority of the bees that were lost did not return to the hive and thus, were assumed to be lost in the field. There is a positive, significant correlation between honey bee flight activity and colony weight gain (Szabo 1980). Honey bees foraging on one nectar source species on the same day and in approximately the same environmental condition would be expected to forage in

similar patterns throughout the day. In this study treated colonies did not gain the same amount of weight as control colonies but, treated colonies began to gain weight in the same daily weight gain patterns as control colonies. Thus, daily monitoring for one week, treated and control colony weight gains for colonies foraging in similar conditions could be used as a rough indicator as to when treated colonies resume control colony foraging patterns. Simply comparing colony weekly weight gains would only indicate the nectar collecting capacity of colonies rather than a rough estimate of the foraging activity.

Colony adult numbers were estimated by estimating the number of bees on all the frames in each hive (Nelson and Jay 1972[a]). Adult numbers for treated colonies were significantly lower ($p<0.0001$) than control colonies for 86 days after treatment (Tables 4 and 5). Colonies with greater increases in numbers gained less weight than colonies with lesser increases in numbers ($r=-0.820$ $p<0.0001$) (Figure 6). Colonies with large numbers gained more weight than colonies with small numbers ($r=0.909$ $p<0.0001$) (Figure 7). Large colonies increased in numbers less than small colonies ($r=-0.921$ $p<0.0001$)(Figure 8).

Sub-lethal doses of organophosphates cause colonies to produce less brood, honey, and wax (Baker and Waller 1978) and, to demonstrate aberrant behaviour (Numamaker et al. 1984). The significantly lower treated colony numbers may be due to brood cannibalism for a period of time after

treatment. Since colony adult numbers and number changes affect colony weight gain, studies examining the weight gains of colonies involving insecticide applications should include not only colony sizes but, also the change in number during the time weight gains are being measured. Additionally, brood measurements should also be included to determine effects on brood.

Dead bee trap counts were significantly ($p<0.05$) higher in treated colonies than control colonies for 3 to 4 days after treatment (Tables 6 and 7). The method used to estimate adult numbers in colonies was disruptive and resulted in high dead bee trap counts. Dead bee traps measure only those bees that die at the hive. The majority of the foragers that were in the field at the time of treatment were probably unable to return to their hives and died in the field.

Treated colonies collected significantly ($p<0.05$) less pollen than control colonies for 2 to 3 days after treatment (Tables 8 and 9). The number of foragers entering colonies was significantly ($p<0.0001$) lower in treated colonies than in control colonies for 1 to 2 days after treatment (Tables 10 and 11). The number of foragers in treated field plots was lower for 2 to 3 days after treatment (Figures 9 to 12). Malathion is not a repellent so the significantly lower foraging by treated colonies was due to the loss of foragers.

The correlation between the number of foragers

entering colonies and the field forager activity was significant ($p<0.0001$) in both small and large plots. The correlation coefficient was larger for the larger plots ($r=0.600$) than for the small plots ($r=0.502$) (Figures 13 and 14). Correlations of pollen foragers, non-pollen foragers and pollen collected between field forager activity in small and large plots was significant but the correlation coefficients were low ($r=0.471$ to 0.623) (Figures 15 to 20).

The significant positive correlation between the number of foragers entering colonies and the number of foragers in plots indicated that counting the number of foragers in field plots could be used to assess the impact of an insecticide treatment on foraging activity. Larger plots had higher correlation coefficients and appeared to be more representative of the foraging activity in the treated area than small plots. Forager entrance activity may be deceptive since, untreated, attractive crops may be within foraging distances and thereby attracting foragers away from the target crop.

The number of pollen and non-pollen foragers entering colonies were counted to determine the proportion of pollen and non-pollen foragers between treated and control colonies and, between small (24,500 bees) and large (51,000 bees) colonies. The proportion of pollen foragers and non-pollen foragers was the same ($p>0.05$) for treated and control colonies (Figures 21 to 28). There was no significant difference in the proportion of pollen and non-pollen

foragers between colonies of different sizes except small colonies (24,500 bees) had proportionately more ($p<0.05$) pollen foragers than large colonies (51,000 bees) (Figures 29 and 30). The small colonies may have had more pollen foragers than the large colonies because there may have been a greater ratio of brood to adult bees in the small colonies than in the large colonies.

Caged bees and mosquitoes were placed in the treated areas next to 10 cm X 10 cm glass fiber filter mats (to capture the malathion spray). Caged bee and mosquito mortality was counted one and twelve hours after treatment respectively. The glass fiber filter mats were analysed for malathion and, the amount of malathion per mat was converted to ml/ha of malathion. The relationship between application rate and mortality was determined by correlationanalysis. There was a significant ($p<0.0001$) positive correlation ($r=0.900$) between application rate and caged bee mortality (Figure 31). There was a significant ($p<0.0001$) positive correlation ($r=0.920$) between application rate and caged bee mortality (Figure 31).

The results of the caged bee mortality studies indicated that most of the bees foraging in the field that would come into direct contact with the ULV malathion spray died within 6 hours. Those that were in contact with higher rates of malathion died more quickly since malathion penetration follows first order kinetics (Matsumura 1963; Olson and O'Brien 1963; Nobel-Nesbit 1970; Wilkinson 1973;

Hartley and Graham-Bryce 1980). The foragers in the field that were in contact with high rates of malathion probably died in the field. Since organophosphates affect the ability of honey bees to communicate the location of food sources correctly (Schricker and Stephen 1970), organophosphates may also affect the orientation ability of sub-lethally dosed honey bees so that they would become lost while attempting to return to their hives. Dead bee trap counts represented only a small percentage of the number of bees that died according to the adult number estimates. The difference between the +2 and -2 days after treatment adult number estimate not accounted for in dead bee trap counts was due to bees dying in the field.

Caged bee downwind mortality was variable ranging from 1% to 41% 1 km downwind from the treatment area (Figures 33 to 35). Downwind mortality of caged bees depended on the meteorological conditions at the time of application; influenced spray droplet deposition. Honey bees foraging one kilometer from their hives or apiaries located one kilometer from a ULV malathion application could be affected the application.

Malathion residues in pollen degraded from a high of 5.7±1.6 ppm one hour after treatment to lower than the detectable limit in 24 hours (Figure 36). Malathion residues on canola blossoms degraded from a high of 15.3±2.0 ppm one hour after treatment to lower than the detectable limit in 24 hours (Figure 37). All other

material tested for malathion residues had very low levels or levels below the detectable limit (Table 12). The average spray droplet size (MMD), for the spraying system used was 43.3±12.5 (Figure 13). The contact residue exposure estimate to an LD_{50} dose for honey bees, on a teflon treated surface was approximately 448 cm^2 (Table 14).

The malathion residues in pollen and in blossoms reduced to levels below the detectable limit in 24 hours. However, the measurements taken on treated colonies indicated that colonies were affected from 2 to 4 days, at the least and, up to 86 days after treatment with respect to adult population estimates. A combination of being covered with pollen grains and repeatedly contacting floral parts would probably be the most hazardous form of foraging because bees would be contacting the greatest surface area with this method of foraging. After 24 hours, it is unlikely that a foraging honey bee, no matter how industrious an individual, would accumulate a lethal dose of malathion. Therefore, other factors are involved in affecting colonies rather than simple direct contact and short term residual contact lethal exposure. Various responses to sub-lethal doses of organophosphates have been observed (Schricker and Stephen 1970; Barker and Waller 1978; and, Nunamaker et al. 1984). The effects of sub-lethal doses of organophosphates on honey bees are not well known. A combination of sub-lethal doses and the loss of the foraging population may be the cause of the

measurable effects beyond the time when malathion is detectable in pollen and on blossoms.

CHAPTER VII

SUMMARY AND CONCLUSIONS

SUMMARY

Colonies of honey bees were treated with aerially applied ultra-low volume malathion at a rate of 210 ml/ha from 3 meters above ground level during Brassica napus L. (Westar canola cv.) nectar flows in the mid to latter part of July in 1984 and 1985 in the Red River Valley of Southern Manitoba. Colony weight gain, adult numbers, adult mortality at the hive, foraging activity (entrance counts and field counts), caged bee mortality, caged mosquito mortality, the application rate of malathion at the caged bee and mosquito locations, caged bee mortality downwind from the treated area, and malathion residues inside and outside the hive were examined in this study.

Treated colonies gained significantly ($p<0.05$) less weight than control colonies for up to 3 days after treatment. Small treated colonies continued to gain significantly ($p<0.05$) less weight than small control colonies for 21 days after treatment. Strong treated colonies gained the same amount or more weight than strong control colonies for up to 21 days after treatment. Treated and control colonies began to follow the same daily weight gain pattern 3 to 4 days after treatment. Colony weight gains in this study were used to compare the nectar collecting capacity and also used as a rough estimate of foraging activity. Inclement weather conditions had the same effect on weight gain as the ULV malathion treatments.

Treated colonies had significantly ($p<0.0001$) lower

adult numbers than control colonies for up to 86 days after treatment. There was a significant, negative correlation between the increase in adult numbers and colony weight gain, and between colony adult numbers and colony adult number increase. There was a significant, positive correlation between colony adult numbers and colony weight gain.

A dead bee trap was developed with two, offset, 6 mm wire mesh barriers to prevent hive bees from removing dead bees from the hive. Hive bees did not remove marked, dead bees from the traps during 12 hour test periods. Dead bee trap counts were significantly ($p<0.05$) higher in treated colonies than control colonies for three to four days after treatment. A comparison of pre-treatment and post-treatment population estimates to the dead bee counts indicated that the majority of the forager bees died in the field and not in the hive.

Treated colonies collected significantly ($p<0.05$) less pollen than control colonies for two to three days after treatment. The number of foragers entering treated colonies was significantly ($p<0.05$) less than control colonies for one to two days after treatment. There was no change in the proportion of pollen foragers or non-pollen foragers entering colonies as a result of the treatments. Foragers were not repelled from the treatment areas and foraging returned to control levels in three to four days.

There was a significant ($p<0.0001$), positive correlation ($r=0.900$) between application rate and caged bee

mortality. There was a significant ($p<0.0001$), positive correlation ($r=0.920$) between application rate and caged mosquito mortality.

Caged bee mortality was variable ranging from 1% to 41% one kilometer downwind from the treatment area. Downwind mortality depended on the meteorological conditions at the time of the application which influenced spray droplet deposition. Malathion residues in pollen degraded from a high of 5.7 ± 1.6 ppm one hour after treatment to lower than the detectable limit in 24 hours. Malathion residues in canola blossoms degraded from a high of 15.3 ± 2.0 ppm one hour after treatment to lower than the detectable limit in 24 hours. All other materials tested for malathion residues had very low levels or levels below the detectable limit at times ranging from one to three hours after treatment. An estimate of the total area a bee would have to come into contact with in order to be exposed to a residual lethal dose of malathion was determined using treated teflon slides. The estimated area was approximately 44 cm^2. A combination of being covered with pollen grains and repeatedly contacting floral parts would probably be the most hazardous form of foraging because bees would be contacting the greatest surface area. Sub-lethal doses probably were responsible for some of the effects that were measurable beyond the day of treatment.

CONCLUSIONS

1. Comparing only weight gain differences between

treated and control colonies compares the nectar collecting capacity of colonies. Treated colonies would be expected to gain less weight than control colonies since forager numbers would be lower. However, when treated colonies begin to gain weight in a similar pattern as control colonies, this indicates that normal foraging patterns have resumed in the treated colonies. In order to observe a resumption in normal foraging patterns 'true' controls are necessary. This includes colonies foraging on an identical crop under similar meteorological conditions.

2. Colony size and increase in size are important in interpreting the colony weight gain. Understanding the effect of colony size and increase in size on colony weight gain will assist in ascertaining the effect of a ULV malathion application on weight gain as opposed to colony size effects.

3. Lower forager activity was due to the loss of foragers but, by the third to fourth day after treatment, the foraging activity measurements used here, returned to near control levels. It is believed that in treated colonies workers required three to four days to respond to the loss of forager bees within the colonies.

4. There was a significant, positive correlation between application rate and mortality of caged bees and mosquitoes. Obtaining a field LD_{50} for any insecticide is difficult since it is difficult to control the application rate at any one place in the field. However, it is believed

that a field LD_{50}, expressed in ml/ha, would be extremely helpful in assisting beekeepers and insecticide applicators in assessing the expected potency of insecticide applications.

5. The results indicated that foraging bees and apiaries located up to one kilometer from a low level ULV malathion application may be effected. The results from this study examining the relationship between application rate and mortality demonstated that the application rate will determine the mortality of caged bees. Mortality downwind from the target area, may not necessarily be dependant on the distance of caged bees from the target area but, upon the amount of malathion deposited at various locations from the target area. Droplet deposition is dependant on release height, droplet size, meterological conditions and, topography.

6. Results from pollen and blossom malathion residue analysis as well as from estimating a lethal contact area indicated that, foragers could accumulate a lethal dose of malathion within the first 12 hours and possibly a sub-lethal dose beyond that time by, foraging on blossoms and collecting pollen incidentally or intentionally.

CHAPTER VIII

LITERATURE CITED

Al-Tikrity, W.S., A.W. Benton, R.C. Hillman, and W.W. Clarke, Jr. 1972. The relationship between the amount of unsealed brood in honeybee colonies and their pollen collection. J. Apic. Res. 11:9-12.

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. J. Econ. Entomol. 51:103-108.

Anderson, L.D., and E.L. Atkins, Jr. 1966. 1965 Research on the effect of pesticides on honey bees. Am. Bee J. 106:206-208.

Anderson, L.D., and E.L. Atkins, Jr. 1968. Pesticide usage in relation to beekeeping. Ann. Rev. Entomol. 13:213-238.

Atkins, E.L., Jr. 1978. The hive and the honey bee. Am. Bee J. Hamilton, Illinois. 740 pp.

Atkins, E.L., Jr., E.A. Greywood, and R.L. Macdonald. 1975. Toxicity of pesticides and other agricultural chemicals to honey bees. Division of Agricultural Sciences, University of California. Leaflet 2287. 38 pp.

Atkins, E.L., Jr., D. Kellum, and K.W. Atkins. 1981. Reducing pesticide hazards to honey bees: Mortality prediction techniques and integrated management strategies. Division of Agricultural Sciences, University of California. Leaflet 2883. 23 pp.

Barker, R.J., and G.D. Waller. 1978. Sublethal effects of parathion, methyl parathion, or formulated methoprene fed to colonies of honey bees. Environ. Entomol. 7:569-571.

Benedek, P., and J. Prenner. 1972. Effect of temperature and pollinating efficiency of honeybees on winter rape flowers. Z. Ang. Ent. 71:120-124.

Burrill, R.M., and A. Deitz. 1981. The response of honey bees to variations in soloar radiation and temperature. Apidologie 12:319-328.

Caron, D.M. 1979. Effects os some ULV mosquito abatement insecticides on honey bees. J. Econ. Enotomol. 72:148-151.

Clinch, P.G. 1967. The residual contact toxicity to honey bees of insecticide sprays on to white clover (Trifolium repens L.) in the laboratory. New Zealand J. Agric. Res. 10:289-300.

Clinch, P.G. 1969. Laboratory determination of the residual fumigant toxicity to honey bees on insecticide sprays on white clover (Trifolium repens L.). New Zealand J. Agric. Res. 12:162-170.

Clinch, P.G., and J.G.M. Ross. 1970. Laboratory assessment of the speed of action on honey bees of orally dosed insecticides. New Zealand J. Agric. Res. 13:717-725.

Colburn, R.B., and G.S. Langford. 1970. Field evaluation of some mosquito adulticides with observations on toxicity to honey bees and house flies. Mosquito News 30:518-522.

Conner, W.E., C.F. Wilkinson, and R.A. Morse. 1978. Penetration of insecticides through the foregut of the honey bee (Apis mellifera L.). Pestic. Biochem. and Physiol. 9:131-139.

Crabbe, R., L. Elias, M. Krymien, and S. Davie. 1980. New Brunswick forestry spray operations: Field study of the effects of atmospheric stability of long range pesticide drift. National Research Council of Canada Report LTR-UA-52.

Danka, R.G., T.E. Rinderer, L. Hellmich II, and A.M. Collins. 1986. Foraging population sizes of Africanized and European honey bee (Apis mellifera L.) colonies. Apidologie 17:193-202.

Dixon, D.P. 1983. Preliminary report on the effects of the 1983 Manitoba Emergency Mosquito Control Program on Bees. Canadian Association of Professional Apiculturists Proceedings. 1983: 63-81.

Eldefrawi, M.E., and R.D. O'Brien. 1966. Permeability of the abdominal nerve cord of the American cockroach to fatty acid. J. Insect Physiol. 12:1133-1142.

Eldefrawi, M.E., and R.D. O'Brien. 1967. Permeability of the abdominal nerve cord of the American cockroach Periplaneta americana L. to aliphatic alcohols. J. Insect Physiol. 13:691-198.

Eto, M. 1974. Organophosphorus pesticides: Organic and biological chemistry. CRC Press, Inc. Cleveland, Ohio. U.S.A. 387 pp.

Farrar, C.L. 1931. The evaluation of bees for pollination. J. Econ. Entomol. 24:622-627.

Farrar, C.L. 1937. The influence of colony populations on honey production. J. Agric. Res. 12:945-954.

Felton, J.C., P.A. Oomen, and J.H Stevenson. 1986. Toxicity and hazard of pesticides to honeybees: harmonization of test methods. Bee World 67:114-124.

Finney, D.J. 1964. Statistical Methods in Biological Assay, Second Edition. Griffin Press. London. 438-490 pp.

Free, J.B. 1967. Factors determining the collection of pollen by honeybee foragers. Anim. Behav. 15:134-144.

Free, J.B. 1970. Insect pollination of crops. Academic Press Inc. New York, New York. U.S.A. 544 pp.

Free, J.B., and A.W. Ferguson. 1983. Foraging behaviour of honeybees on oilseed rape. Bee World 64:22-24.

Fukuda, H., K. Moriga, and A. Sekiguchi. 1969. The weight of crop contents in foraging honeybee workers. Annot. Zool. Japoneses 42:80-90.

Ghassemi, M., P. Painter, M. Powers, N.B. Adesson, and M. Dellarco. 1982. Estimation drift and exposure due to aerial applications of insecticides in forests. Environ. Sci. Technol. 16:510-514.

Gilbert, M.D., and C.F. Wilkinson. 1974. Microsomal oxidases in the honey bee, (Apis mellifers L.). Pestic. Biochem. Physiol. 4:56-66.

Gratwick, M. 1957. The contamination of insects of different species exposed to dust deposits. Bull. Entomol. Res. 48:741-753.

Hadaway, A.B., F. Barlow, C.R. Turner, and L.S. Flower. 1978. Contact toxicity to tsetse flies of deposits with different drop size characteristics. British Crop Protection Council Monograph No. 22:219-230.

Hartley, G.S., and I.J. Graham-Bryce. 1980. Physical principles of pesticide behaviour. Volume 2. Academic Press Inc. New York, New York. U.S.A. 1024 pp.

Herbert, E.W., Jr., and H. Shimanuki. 1983. Impact on honey bees of ULV malathion application to control mosquitoes in Maryland. Am. Bee J. 123:26-28.

Himel, C.M. 1969. The optimum size for insecticide spray droplets. J. Econ. Entomol. 62:919-925.

Hitchcock, J.D., J.R. Elliot, and D.A. George. 1966. Effects on honey bees of aerial application of technical malathion. Am. Bee J. 106:294-295.

Jay, S.C. 1986. Spacial management of honey bees on crops. Bee World 67:98-113.

Jegatheeswaran, P. 1978. Factors concerning the penetration and distribution of drops in low growing crops. British Crop Protection Council Monograph No. 22:91-99.

Johansen, C.A. 1966. Digest on bee poisoning, its effects and prevention. Bee World 47:9-21.

Johansen, C.A. 1972. Toxicity of field-weathered insecticide residues to four kinds of bees. Environ. Entmol. 1:393-394.

Johansen, C.A. 1977. Pesticides and pollinators. Ann. Rev. Entomol. 22:177-192.

Johansen, C.A., and M.G. Kleinschmidt. 1972. Insecticide formulations and their toxicity to honeybees. J. Apic. Res. 11:59-62.

Johansen, C.A., D.F. Mayer, J.D. Eves, and C.W. Kious. 1983. Pesticides and bees. Environ. Entomol. 12:1513-1518.

Johnstone, D.R. 1985. Pesticide application: Principles and practice. Oxford University Press. New York, New York. U.S.A. 494 pp.

Klassen, A.J., R.K. Downey, and J.J. Capcara. 1987. Westar summer rape. Can. J. Plant Sci. 67:491-493.

Langridge, D.F., and R.D. Goodman. 1975. A study of oilseed rape (Brassica campestis). Aust. J. Exp. Agri. Anim. Husb. 15:285-288.

Lawson, T., and S. Uk. 1980. The influence of aircraft wake on the downwind dispersal of ULV sprays. Agric. Aviation 21:59-67.

Levin, M.D. 1966. The impact on honey bees of large-scale malathion application for grasshopper control. Am. Bee J. 106:174-175.

Levin, M.D., W.B. Forsyth, G.L. Fairbrother, and F.B. Skinner. 1968. Impact on colonies of honey bees of ultra-low-volume (undiluted) malathion applied for control of grasshoppers. J. Econ. Entomol. 61:58-62.

Lindauer, M. 1961. Communication of social bees. Cambridge, MA, U.S.A. Harvard University Press. 161 pp.

Little, T.M., and F.T. Hills. 1978. Agricultural Experimentation. John Wiley and Sons. New York, New York, U.S.A. 350 pp.

Matsumura, F. 1963. The permeability of the cuticle of Periplaneta americana (L.) to malathion. J. Insect Physiol. 9:207-221.

Matsumura, F. 1980. Toxicology of insecticides. Plenum Press. New York, New York. U.S.A. 503 pp.

Matthews, G.A. 1982. Pesticide application methods. Longman Inc. New York, New York. U.S.A. 336 pp.

Mayer, S., R.P. Maikel, and B.B. Brodie. 1959. Kinetics of penetration of drugs and other foreign compounds into cerebrospinal fluid and brain. J. Pharmalcol. Exptl. Therapy 27:205-211.

Mayland, P.G., and C.C. Burkhardt. 1970. Honey bee mortality as related to insecticide treated surfaces and bee age. J. Econ. Entomol. 63:1437-1439.

Mengle, D.C., and J.E. Casida. 1958. Inhibition and recovery of brain cholinesterase activity in houseflies poisoned with organophosphate and carbamate compounds. J. Econ. Entomol. 51:750-757.

Metcalf, R.L., and R.B. March. 1949. Studies on the mode of action of parathion and its derivaties and their toxicity to insects. J. Econ. Entomol. 42:721-728.

Metcalfe, J.R. 1967. Experiments to assess the extent of honey bee poisoning by malathion in Jamaica. J. Apric. Res. 6:45-58.

Moffet, J.O., and L.S. Stith. 1972. Bee losses from parathion decreased as distance from sprayed field increased. Am. Bee. J. 112:174-175.

Morse, R.A., and A.F. Gunnison. 1967. Honey bee insecticide loss - an unusual case. J. Econ. Entomol. 60:1196-1198.

Mount, G.A., and C.S. Lofgren. 1967. Ultra-low volume and conventional aerial sprays for control of adult salt-marsh mosquitoes, Aedes sollicitans (Walker) and Aedes taeniorhynchus (Wiedeman), in Florida. Mosquito News 27:473-477.

Mount, G.A., C.S. Lofgren, N.W. Pierce, K.F. Baldwin, H.R. Ford, and C.T. Adams. 1970. Droplet size, density, distribution, and effectiveness in ultra-low volume aerial sprays dispersed with TEEJET nozzels. Mosquito

News 30:589-599.

Nair, P.K.K., and R.K. Sharma. 1976. Pollen morphology of cultivated Brassica. New Botanist 3:115-153.

Nazer, I.K., T.E. Archer, N.E. Gary, and J. Marston. 1974. Honeybee pesticide mortality: intoxication versus acetylcholinesterase concentration. J. Apic. Res. 13:55-60.

Nelson, D.L., and S.C. Jay. 1972[a]. Estimating numbers of adult honey bees on Langstroth frames. Manitoba Entomol. 6:5-8.

Nelson, D.L., and S.C. Jay. 1972[b]. Population growth and honey yield studies of package bee colonies in Manitoba. II. Colonies initiated with four package sizes on one date. Manitoba Entomol. 6:17-22.

Nobel-Nesbit, J. 1970. Structural aspects of penetration through insect cuticles. Pest. Sci. 1:204:208.

Nunamker, R.A., A.J. Harvey, and W.T. Wilson. 1984. Inability of honey bee colonies to rear queens following exposure to fenthion. Am. Bee J. 24:308-309.

O'Brien, R.D. 1956. The inhibition of cholinesterase and succinoxidase by malathion and its isomer. J. Econ. Entomol. 49:484-490.

O'Brien, R.D. 1967. Insecticides: action and metabolism. Academic Press. New York, New York. U.S.A. 332 pp.

Olson, W.P., and R.D. O'Brien. 1963. The relation between physical properties and penetration of solutes into cockroach cuticle. J. Insect Physiol. 9:777-786.

Otis, G.W. 1982. Weights of worker honeybees in swarms. J. Apic. Res. 21:88-92.

Park, O.W. 1922. Time and labour factors involved in gathering pollen and nectar. Am. Bee J. 62:254-255.

Ratnieks, F.L.W. 1986. [Effect of colony population size on the efficiency of nectar collection and honey production in honey bee (Apis mellifera) colonies.] Cornell University M.Sc. Thesis 88 pp. (Apic. Abs. 582/87)

Schricher, B., and W.P. Stephen. 1970. The effect of sublethal doses of parathion on honeybee behaviour. I. Oral administration and the communication dance. J. Apic. Res. 9:141-153.

Seeley, T.D. 1985. Honeybee ecology. A study of adaptation in social life. Princeton University Press. Princeton, New Jersey. U.S.A. 201 pp.

Smallman, B.N. 1968. The cholinergic system in insect development. Ann. Rev. Entomol. 13:387-408.

Smirl, C.B., and S.C. Jay. 1972. Population growth and honey yield studies of package bee colonies in Manitoba. I. Colonies initiated with two package sizes on three dates. Manitoba Entomol. 6:9-16.

Snedecor, G.W., and W.G. Cochran. 1980. Statistical methods. The Iowa State University Press. Ames, Iowa. U.S.A. 507 pp.

Spillman, J.J. 1976. Optimum droplet sizes for spraying against flying targets. Agric. Aviation 17:29-32.

Stegwee, D. 1959. Esterase inhibition and organophosphorus poisoning in the housefly. Nature 184:1253-1254.

Szabo, T.I. 1980. Effect of weather factors on honeybee flight activity and colony weight gain. J. Apic. Res. 19:164-171.

Todd, F.E., and C.B. Reed. 1970. Brood measurement as a valid index to the value of honey bees as pollinators. J. Econ. Entomol. 63:148-149.

Toppazoda, R., and R.D. O'Brien. 1967. Permeability of the willow aphid Tuberolachus salignus to organic ions. J. Insect Physiol. 13:941-954.

Ware, G.W., N.A. Buck, and B.J. Estesen. 1984. Deposit and drift losses from aerial ultra-low-volume and emulsion sprays in Arizona. J. Econ. Entomol. 77:298-303.

Wahl, O., and K. Ulm. 1983. Influence of pollen feeding and physiological condition on pesticide sensitivity of the honeybee Apis mellifera carnica. Oecologia 59:106-128.

Welling, W. 1977. Dynamic aspects of insect-insecticide interactions. Ann. Rev. Entomol. 22:53-78.

Wilkinson, C.F. 1973. Pesticide formulations. M. Dekker Inc. New York, New York. U.S.A. 481 pp.

Williams, I.H. 1980. Oil-seed rape and beekeeping, particularily in Britain. Bee World 61:141-153.

Wilson, E.O. 1971. The insect societies. The Belknap Press of Harvard University Press. Cambridge, Massachusettes. U.S.A. 548 pp.

Winston, M.L., and L.A. Ferguson. 1985. The effect of worker loss on temporal casts structure in colonies of the honeybee (Apis mellifera L.). Can. J. Zool. 63:777-780.

Womeldorf, D.J., E.L. Atkins, P.A. Gilles. 1974. Honey bee hazards associated with some mosquito abatement aerial spray applications. Vector Views 21:51-55.

Woodrow, A.W. 1934. The effect of colony size on the flight rates of honey-bees during the period of fruit blossom. J. Econ. Entomol. 27:624-629.

Woyke, J. 1984. Correlations and interactions between population, length of worker life and honey production by honeybees in a temperate region. J. Apic. Res. 23:148-156.

Yu, S.J., F.A. Robinson, and J.L. Nation. 1984. Detoxification capacity in the honey bee, Apis mellifera L. Pestic. Biochem. Physiol. 22:360-368.

Yu, S.J., and L.C. Terriere. 1971. Induction of microsomal oxidases in the housefly and action inhibitors and stress factors. Pestic. Biochem. Physiol. 1:173-167.

CHAPTER XI

APPENDICES

APPENDIX 1

Weather conditions during the time daily measurements were taken in 1984 and 1985.

Date	Temperature	General Conditions
1984	(^{0}C)	
July 14	18.2	sunny, evening showers
15	18.0	sunny
16	16.3	partly cloudy
* 17	16.0	sunny, light wind
18	19.6	morning sunny, afternoon cloudy
19	16.0	sunny
20	16.2	morning cloudy, afternoon sunny
21	22.5	cloudy, showers overnight
22	18.7	mostly cloudy
23	17.1	sunny
24	16.0	sunny
25	18.9	partly cloudy, showers 17:00 h
* 26	17.2	sunny
27	19.3	sunny
28	21.5	sunny
29	19.8	partly cloudy, showers 12:00 h
30	20.6	cloudy and showers
31	20.1	fog, cloudy, showers 15:00 h
Aug. 01	21.7	partly cloudy, showers 16:00 h
02	20.3	sunny
1985		
July 14	16.3	cloudy, showers
15	16.1	morning showers, afternoon sunny
* 16	18.6	sunny, showers 17:00 h
17	16.1	morning fog, sunny afternoon
18	17.4	cloudy, intermittent rain
19	17.5	heavy morning dew, showers 20:00 h
20	13.7	cloudy, cool
21	12.6	sunny, windy and cool
22	15.2	cloudy, showers 20:00 h
23	17.4	cloudy, showers 10:30 h
24	13.9	cloudy
25	14.9	cloudy
26	14.4	sunny, showers 19:00 h
* 27	18.7	cloudy, sunny afternoon
28	13.3	sunny, partly cloudy evening
29	14.7	cloudy, showers 15:00 h
30	17.8	cloudy
31	17.5	sunny to cloudy, showers 15:30 h
Aug. 01	18.1	partly sunny
02	20.7	cloudy
03	19.7	cloudy, intermittent showers

APPENDIX 1 cont'd

In 1984 the average temperature during the period from July 14 to August 02 was 18.7 ^{0}C, with 5 days of rain out of 20 days. In 1985 the average temperature during the period from July 14 to August 03 was 16.4 ^{0}C, with 9 days of rain out of 21 days.

APPENDIX 2

Cythion Droplet Size Determination Method

1. The eyepiece micrometer was calibrated at 600 X magnification with a slide micrometer.

2. One eyepiece division equalled 1.74 microns. Cythion spread factor is 0.69. The conversion factor equalled 1.74 micron X 0.69 = 1.2 microns.

3. The first 200 droplets are measured, and recorded in a table like the one shown (Table 15).

4. Accumulative percentages and corresponding eyepiece divisions are plotted on a probability graph.

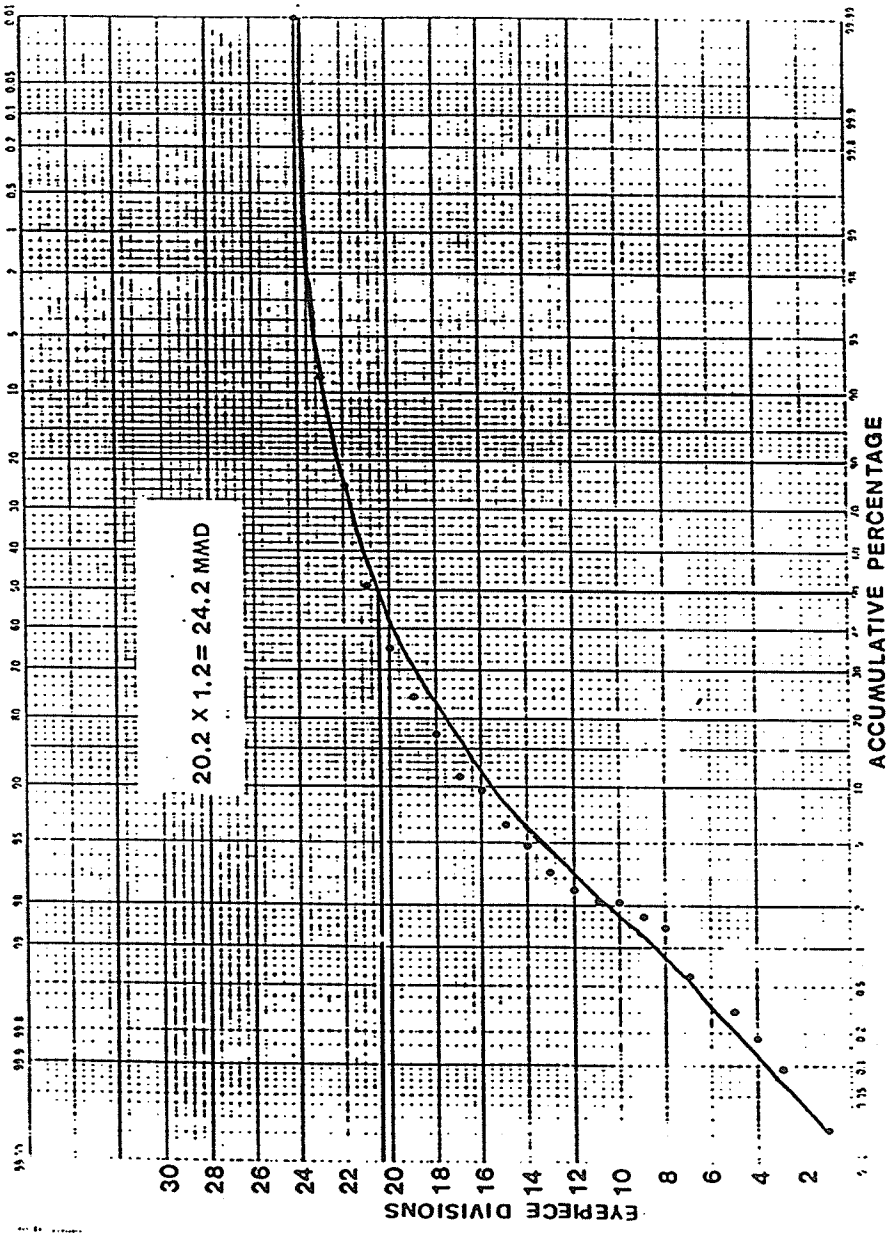
5. Mass median diameter (MMD) is equal to the eyepiece division at the 50 percentile mark multiplied by the conversion factor (Figure 37).

TABLE 15. Tabular determination of spray droplet eyepiece division accumulative percentage.

APPENDIX 2 cont'd

EYEPIECE DIVISION (D)	NUMBER OF DROPLETS (N)	D X N	% OF TOTAL D X N	ACCUMULATIVE PERCENTAGE
1	1	1	0.024	0.024
2				
3	1	3	0.072	0.096
4	1	4	0.096	0.192
5	1	5	0.120	0.312
6				
7	2	14	0.330	1.410
8	4	32	0.768	2.780
9	1	9	0.210	2.380
10	2	20	0.480	2.860
11	1	11	0.260	3.120
12	1	12	0.280	3.400
13	2	26	0.620	4.200
14	5	70	1.680	5.700
15	3	45	1.080	6.780
16	9	144	3.450	10.230
17	4	68	1.630	11.860
18	14	252	6.040	17.900
19	16	304	7.290	25.190
20	24	480	11.520	36.710
21	30	630	15.120	51.830
22	47	1034	24.820	76.650
23	30	690	16.560	93.210
24	13	312	7.7100	99.990
25				
26				
27				
28				
29				
30				
TOTALS	212	4166		

Figure 38. Plotting of eyepiece divisions and accumulative percentages for the determination of spray droplet mass median diameter (MMD).


20.2 X 1.2= 24.2 MMD
EYEPIECE DIVISIONS
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
ACCUMULATIVE PERCENTAGE