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Varroa Mite-Honeybee Interactions under Subtropical Conditions

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TABLE OF CONTENTS

A. Israel

Abstract.....	I
Objectives.....	II
Report.....	1-12
Appendix 1: Control of Varroa...	
Appendix 2: The effect of fluvalinate...	
Appendix 3: Monitoring of pesticide resistance...	

B. U.S.

Abstract.....	I
Objectives.....	II
Report.....	1-11
Publications	

Description of Cooperation.....	III
Evaluation and Benefits to Agriculture.....	IV

ABSTRACT

Israel

To evaluate infestation of bee colonies by Varroa, six methods of detection were examined and the most promising one was: fumigation with amitraz or fluvalinate using bottom board inserts, which was later adapted by our collaborators in the U.S.

Fluctuations of Varroa populations in the Jordan Rift and Coastal regions indicate a possibility of selectively controlling highly infested colonies in order to reduce the incidence of fluvalinate treatments.

The use of fluvalinate inserts twice a year resulted in a satisfactory control of Varroa in Israel and in the U.S. Application of fluvalinate inserts or strips for periods longer than 4-6 weeks resulted in accumulation of residues in wax and honey and reduced honey yields. Placement of fluvalinate inserts at hive entrances versus brood-nest did not cause any contamination of combs and required less labor and time.

Monitoring for pesticide resistance of Varroa based on levels of esterases and glutathione-S-transferase has been assayed.

Several non-chemical control methods have been evaluated and Origanum oil was found to control Varroa.

The effect of body weight, volume and surface of worker and drone 5th stage larvae on the attraction of adult female Varroa mites was studied. The results indicate that the attractiveness of larvae of both sexes to the mite is similar if their weight, volume and surface are resembling or equal. Ethanol, Hexane, Hexane-Ethanol, Petroleum ether but not water, can be used to extract kairomonal components attractive to the mite from larval cuticle. EtOH cuticular extracts were used to attract the mites in bioassays and to characterise their composition by GC-MS. Most of the compounds of worker and drone cuticular extracts were branched alkanes.

OBJECTIVES

Israel

To study the population fluctuations and intestation levels of Varroa in honeybee colonies and to determine the appropriate timing for chemical control.

To determine the most suitable detection method for monitoring mite populations and to minimize the incidence of chemical treatments.

To evaluate the efficacy of several methods of fluvalinate application on the mortality of Varroa, the effect on bees, honey yields and the accumulation of residues in wax and honey.

To explore the effect of non-chemical agents on Varroa.

To learn whether the mite is attracted to volatiles emitted by the honey bee brood or brood cell contents.

A. ISRAELI RESEARCH

a. Specific Objective: Evaluation of six methods to detect Varroa mites in bee colonies.

(1) Natural mortality of Varroa. Floor inserts covered with a wooden frame containing a metal screen (3 mm mesh) were placed under brood chambers. The debris was removed at different intervals and the dead mites were counted. Even after smearing the basis of the hives with grease some ants succeeded to penetrate into the inserts and to collect dead mites. Therefore, daily examination of the debris may provide more accurate results than a weekly one. The number of dead mites does not represent well low infestation levels ranging from 0 to 200 mites per adult worker bee population of a colony. Since no chemicals are used this method does not effect the mite population levels.

(2) Samples of adult bees-Ethanol. Adult bees were shaken from brood combs into a 200 ml beaker and then transferred into a container with 80% Ethanol, placed in a shaker for 5-10 min. The mites detached from the bees fell down to the bottom and the workers were floating on the surface. The number of bees and mites was established and the percentage of infestation per sample calculated. The method is both time consuming and not very accurate.

(3) Samples of adult bees-fumigation with Amitraz. Adult workers were shaken from brood combs into a 200 ml beaker and transferred into wooden cages (30x30x30 cm) with a metal screen (3 mm mesh) installed 1 cm above the white bottom insert. A paper strip impregnated with 3 drops of amitraz was lit. After 30 min of fumigation the fallen mites were counted upon the inserts. Since the results were similar to those obtained with method 2, we did not use it in our observations.

(4) Varroutest-Varroa attractant (Sanders, Probel Biotechnology, Louvain-la-Neuve, Belgium). According to the producer Varroutest is an efficient attractant of Varroa and can be used for detection and trapping of the mite. We bioassayed the attractiveness of this

product in the laboratory in four repetitions. Five 5th stage worker larvae, five filter paper strips (3x7 mm) soaked with several dilutions of Varoutest and five untreated filter paper strips (controls) were placed diagonally 5 cm from the center of a Petri dish (14 cm diam). Twenty adult female mites were deposited in the center of the dish and 15 min thereafter they were found on the larvae, but not on the Varoutest strips.

(5) Brood infestation. Sections of sealed brood (6x12 cm) were cut out from brood combs. Infestation levels ranging from 0 to 16 mites per developing bee were detected. This method is a reliable one for the study of population levels of various stages of the mite and also provides a good indication of the infestation level of the brood. We used it as a supplementary method to colony fumigation with amitraz or fluvalinate smoke strips (bottom insert board). It is time consuming and reflects the infestation level of the sample, but not of the colony. Therefore, it is not practical for routine monitoring of the Varroa mite in commercial beeyards.

(6) Bottom board inserts. White painted boards with a greased surface to stick the mites were placed above the hive bottom board. Colonies were fumigated with either Amitraz™ or Mavrik™ smoke strips which affect only Varroa mites which are present on adult bees, but not those feeding on developing bees inside sealed cells. We fumigated the colonies with filter paper smoke strips impregnated with 15% KNO₃ aqueous solution and 1-2 drops of either amitraz (12.5%, FBC, England) or Mavrik™ (Zoecon, Palo Alto, CA), which were set afire, inserted through hive entrance and deposited on the hive bottom board. During the fumigation, and thereafter, the hive entrance was left open. Later, the mites, which fell down on boards placed above the bottom board, were counted. After 1 hour of fumigation with amitraz strips the mortality of Varroa is about 94% (Lupo and Garling, 1987). The result is both accurate and rapid. To inspect 30 colonies for infestation level of Varroa it takes: (a) 15 min to place the boards inside the colonies and to set afire filter

paper strips, (b) 30 min fumigation, and (c) about 30 min to remove the boards and to count the fallen mites, i.e. 45 min per inspection of 30 colonies. Of all the methods tested, this is the most accurate, practical and rapid. It is used by commercial beekeepers, extensionists and for research purposes.

b. Specific Objective: Seasonal fluctuations of Varroa populations in honeybee colonies in Upper Galilee, Jordan Valley, Northern and Southern Coastal Area.

The study of Varroa populations in honeybee colonies was carried out in several climatic zones of Israel: at Dan, Kinneret, Kerem Maharal and Rehovot (see Appendix 2).

c. Specific Objective: Monthly fluctuations of Varroa in bee colonies.

Monthly level of Varroa in bee colonies indicates a possible way of selectively controlling highly infested colonies, in view to reduce the incidence of fluvalinate treatments of all bee colonies in a bee-yard (see Appendix 2).

d. Specific Objective: Monitoring for Varroa to reduce the incidence of fluvalinate applications.

The results of monitoring Varroa for 3 years indicate the possibility of reducing the number of fluvalinate treatments per year (see Appendix 2).

e. Specific Objective: Control of Varroa with fluvalinate - Mavrik™ (Zoecon, Palo Alto, CA).

The control of Varroa between 1984 and 1987 was based in Israel on frequent fumigation of bee colonies with amitraz at 3-4 day intervals, totalling in 50 extra labor days per year in an apiary of about 500 colonies. In spite of the fumigations in 1986 about 40% of bee colonies perished and the surviving ones neither regained the normal bee population nor honey yield levels.

We examined the effect of Mavrik™ Aquaflow, incorporated into

plywood inserts, on the mortality of the Varroa mite. 14 to 16 days after placing Mavrik inserts inside the brood-nest, the population level of Varroa was reduced to less than 10 mites/colony. As a result of this study the control of Varroa in Israel is based now on 2 annual applications of Mavrik inserts. This treatment is efficient and less labor intensive (5-6 days vrs 50 days/500 colonies/year for amitraz fumigation). Contrary to previous years, in 1987 no colony losses due to Varroa were reported (see Appendix 1).

f. Specific Objective: The effect of short- and long-term exposure of bee colonies to fluvalinate inserts on bee population, honey yields and residues of fluvalinate in honey and wax.

The placement of fluvalinate inserts in bee colonies for 6 weeks and 6 months resulted in similar levels of Varroa and honey bee populations. However, in colonies exposed to inserts for 6 months honey yields were lower and fluvalinate residues in wax and honey were higher than in those exposed for 6 weeks (see Appendix 2).

g. Specific Objective: The effect of placement of fluvalinate inserts at hive entrances versus brood-nest on Varroa populations and on accumulation of fluvalinate in honey and wax.

The placement of fluvalinate inserts at hive entrances resulted in effective control of Varroa and in lower levels of fluvalinate residues in wax and honey than in colonies with inserts placed in the brood center. Placement and the removal of inserts at the entrances saved both time and labor (see Appendix 2).

h. Specific Objective: Monitoring for pesticide resistance in Varroa.

The possibility of the development of pesticide resistance in Varroa prompted this preliminary study, in which current levels of two detoxification systems, namely esterases and glutathione-S-transferase, were assayed. The results establish basic levels of these enzymatic systems in the pest. Changes in these "baseline"

values will be used to monitor for the incipency of pesticide resistance in Varroa (see Appendix 3).

i. Specific Objective: Non-chemical methods of Varroa control.

(1) Dusts. Dr. William Ramirez, University of San José, Costa Rica, informed us about his promising results of controlling the Varroa mite using various dusts inside bee colonies. We carried out a field experiment at the beeyard of Ein Hamifraz from August 14 to September 2 1987, to test the effect of dust on the population level of Varroa. Sixteen colonies occupying about 10 frames and containing 6-8 brood frames were randomly divided into four groups, four colonies in each. Combs in each colony were dusted with 100 gr of dust on August 14, 20 and 28. The effect of sugar powder, talcum and flour on the population of Varroa on adult and developing bees was examined by: (a) removing 100 prepupae and pupae from brood combs of each colony; (b) counts of dead mites inside bottom traps; (c) fumigation of 500 adult bees with amitraz; (d) fumigation of the entire colony after 3 weeks of treatment on Sept. 2. Of the three different dusts, sugar powder gave the best results. There was a slight decrease in the population level of the mites during the 3 weeks of treatment. No statistically significant differences between the 3 treatments and the controls were found after 3 weeks, as based on final fumigation with amitraz. Counts of Varroa on developing bees showed even less consistent results. No further trials with dusts were carried out as the results were not promising and the method is labor intensive.

(2) Alystin. (Bay Sir 8514, Mobay Chemical Corporation Kansas City, MD) is a chitin inhibitor which has shown to terminate brood production in colonies of honeybees. Dr. Herbert Elton, kindly provided us the sample of Alystin. We used the concentrations of 250, 500 and 250 mg of 25% WP in 2 liters sugar solution which were fed during January to 6 bee colonies in Rehovot. No inhibition of brood rearing or mortality of developing bees was observed. The concentrations used in Rehovot were not efficient

enough, presumably because of high activity of field bees and availability of pollen during the winter season.

(3) Removal or destruction of brood, restriction of queens' egg laying were evaluated.

(a) Removal of brood combs from colonies into "bank" colonies which were fumigated with amitraz or fluvalinate. The broodless colonies were treated with amitraz or fluvalinate.

(b) Mechanical destruction of capped brood

(c) Restricting queens inside a broodless super or caging inside a mailing cage for about 4 weeks. These treatments were examined at Ayelet Hashakhar commercial beeyard, and in general a substantial reduction of the *Varroa* population was observed. However, since the above mentioned methods are time and labor intensive, they are suitable for a hobbyist but not for a commercial beekeeping operation.

(4) Plant extracts of Labiatae. The acaricidal effect of several compounds characteristic to Labiatae is well known: Menthol is used in the US to control Acarapis woodi and Thymol to control Varroa in several European countries.

We have made an attempt to find a possible candidate between several Labiatae compounds for integrated control of Varroa under a subtropical climate.

Thymol, Menthol, Carvacrol, Origanum and p-Cymene were used to examine the effect on Varroa (mortality) and on adult honeybees (repellence, excitation, absconding) during summer months.

(a) Laboratory screening of the above compounds was carried out in Petri dishes. 0.2 ml of compounds or dilutions of 1:1, 1:5 and 1:10 in Ethanol were applied to filter paper strips and the mortality of mites was established.

(b) Observation hives. 0.5 ml of the compound or one of the dilutions (as above) were placed in a small glass vessel (2 cm diam) on the top bar, the fallen Varroa were counted on the filter paper spread on the bottom.

Of the five Labiatae compounds, Origanum oil (Sigma, St Louis, MO) showed both acaricidal activity and the slightest excitation and

repellency effects on honey bees. This compound was further tested in bee colonies.

(c) Bee colonies. Several methods of application of Origanum oil were examined: fumigation (the oil was deposited on filter paper strips impregnated with KNO_3) and filter paper discs soaked with oil, did not give satisfactory results. Trials with plywood inserts (4 X 20 X 180 mm) which were soaked for 24 hrs in Origanum oil diluted with EtOH (1:1) and suspended in the center of the brood nest, were conducted but the mortality of Varroa was not high.

One highly infested colony with Varroa (no.1) and one slightly infested (no.2) were used to study the effect of direct evaporation of Origanum oil diluted in Ethanol (1:1). The solution was placed in a Petri dish on the bottom board (10 ml/dish) of colony 1. A count of fallen mites during 24 hrs before the beginning of Origanum evaporation, was made on July 28. Consequently, daily counts of dead Varroa on the bottom inserts were made for one week. The results show that in colony No 1, there was about a 3 fold increase in the number of dead mites one day after the application of the treatment.

To elucidate the long-term effect of evaporation of Origanum oil we used another two colonies. In the treated colony, a Petri dish was placed on the bottom board, filled with Origanum diluted with EtOH (1:1) and refilled during the trial whenever necessary.

The results in Fig.1 show that the number of fallen Varroa on the first day, June 23, in colony 1 (treated) was about 1000, and in colony 2 (control) it was 205 mites. Daily counts of fallen mites were made for 5 weeks and their weekly averages are shown in Fig.1. On July 27, both colonies were fumigated with amitraz. The results show that in the control, untreated colony there was an increase from 205 to 780 of fallen Varroa during the trial. The effect of Origanum oil is evident in the treated colony: there was a steady decrease in the number of mites from about 1000 to 58 upon fumigation with amitraz on July 27.

Origanum oil as a natural product seems to be a promising

Fig. 1: The effect of *Origanum* oil on *Varroa* in bee colonies during 5 weeks. No. of fallen *Varroa* on the 1st day (June 23) and the last day (July 27) is indicated for the treated and control colonies. The *Varroa* count on July 27 was made following colony fumigation with Anitraz.

candidate for integrated control of Varroa, especially in hot climates, since it did not have any particular adverse effect on the behavior of bee colonies during summer time.

The application of Origanum oil in Petri dishes during the treatment for 4-5 weeks was labor intensive, therefore the formulation (pure or diluted) and the application methods will have to be explored. It should be emphasized that Origanum oil is relatively expensive.

j. Specific Objective: Attractiveness of developing bees to Varroa.

Attractiveness of various immature stages of workers and drones to adult Varroa females were examined. Drone and worker larvae of 3rd, 4th and 5th stage prepupae, unpigmented and brown pupae were removed from combs, cleaned with a filter paper strip soaked with distilled water and then transferred into Petri dishes (14 cm diam) and deposited in 6 age-groups, each consisting of three individuals. Adult female mites were collected from worker prepupae and pupae which were removed from untreated colonies. Fifteen mites were immediately placed at the center of a Petri dish. The dishes were placed inside an incubator at 35°C. Observation of the number of mites attracted to each age-group of bees were made every 20 min for 2 hrs. The observations were repeated 4 times.

The results in Fig.2 and 3 show that the most attractive stage of both castes is the 5th larva.

The attractiveness of worker and drone 5th stage larvae was determined by removing them from worker and drone cells. In each Petri dish 4 worker and 4 drone 5th stage larvae were placed at 0.5 cm from the edges and 15 mites at the center of the dish. For other details, see age-groups. The observations were made in 4 repetitions. The results in Fig.4 show that 5th stage drone larvae are more attractive than worker larvae.

Fig. 2: Attraction of 15 Varroa adult females to drone 3rd, 4th and 5th stage larval, prepupal, unpigmented pupae and brown pupae.

Fig. 3: Attraction of 15 Varroa adult females to worker 3rd, 4th and 5th larval stage, prepupae, unpigmented and brown pupae.

Fig. 4: Attraction of 15 Varroa adult female mites to 4 worker and 4 drone 5th stage larvae. Counts of mites attracted to each group of larvae was made every 20 mins. for 120 mins.

k. Specific Objective: The effect of weight, volume and surface of worker and drone larvae on the attraction of adult female, Varroa mites.

Worker and drone 3rd, 4th and 5th stage larvae were removed from their cells in combs, and then were placed in groups of 6 larvae for each age group in a Petri dish (13.5 cm diam.). Adult female Varroa mites were removed from combs containing infected honeybee prepupae and pupae and then 15 adult female mites were placed at the center of each Petri dish. The Petri dishes containing the larvae and the mites were maintained at 33-34°C, and the number of mites present on the larvae was counted every 10 mins for 2 hrs. At the end of each observation the body weight, volume and surface of each larvae was established as follows:

Weight: with Sartorius Analytical Balances.

Volume: by immersing 6 larvae into a 10 ml graduated cylinder which was filled with 7 ml distilled water.

Surface: by blotting the larvae with ink, which was then absorbed by a soft tissue, and the surface of the instained tissue was established using a millimeter paper.

(a) The effect of body volume. Body volume of the bioassayed drone and worker larvae was 1.65 and 1.50 ml, respectively. The results in Fig. 5 show that the number of Varroa mites attracted after 40 min to 5 drone and 10 worker 5th stage larvae was similar.

(b) The effect of body weight. Body weight of the bioassayed drone and worker larvae was 1.140 and 1.445 gr, respectively. The results in Fig. 6 show that the number of Varroa mites attracted after 40 min to 4 drone larvae was 5.5 ± 2 and to 13 worker larvae was 6.2 ± 1.6 . The slightly higher attraction of the mites to worker larvae may be to the fact that their body surface was larger than that of drones (1.890 mm^2 vrs. 1.120 mm^2).

(c) The effect of body surface. Two groups of drone and worker 5th stage larvae with similar body surface of 1140 mm^2 and 1010 mm^2 , respectively, were exposed to the mites for 40 min. The results in Fig. 7 show that the number of Varroa mites attracted

Fig. 5: Effect of body volume of drone (1.65 ml) and worker (1.50 ml) 5th stage larvae on the attraction of 15 adult female Varroa. Mites which were present on the larvae were counted at the end of 40 mins. exposure in Petri dishes.

Fig. 6: Attraction of 15 Varroa adult females to drone and worker 5th stage larvae, the total body weight of each group was 1.440 gr and 1.445 gr respectively. Count of mites were made at the end of a 40 mins. exposure in Petri dishes.

Fig. 7: The effect of similar body surface of drone and worker larvae on the attraction of Varroa. Two groups of drone and worker 5th stage larva, with a similar body surface of 1140 mm² and 1010 mm², respectively, were bioassayed for 40 mins. With 15 adult Varroa females and the counts of mites present on larvae were made at the end of the exposure.

by each group of larvae was similar.

Weight, volume and surface of 3rd, 4th and 5th stage of worker and drone larvae are summarized in Table 1. It is evident from the results that the body weight, volume and surface of 5th stage drone larvae are higher than that of worker larvae of the same stage, these can explain the higher attractiveness of drone larvae.

1. Specific Objective: Collect cuticular fractions from 5th stage worker larvae and bioassay for kairomonal activity.

We examined the possibility of extracting larval kairomones which are responsible for the attraction of the Varroa mite.

1. Solvents; 2. Bioassay of cuticular extracts.

(1) Solvents. We used water, ethanol, petrol-ether (b.p.40-60°), hexane, hexane-ethanol (1:1) to select the most suitable solvent for the extraction of cuticular components with kairomonal activity to the Varroa mite. To collect the cuticular components, each larva was gently wiped with a filter paper strip (9 cm²) which was soaked with 1 ml solvent. Two series of extractions were carried out and the extracts were bioassayed for Varroa attraction.

Series 1. Each larva was successively wiped with three different solvents and in the following order: Trial 1- Water, Ethanol, Hexane; Trial 2- Hexane: Ethanol (1:1), Hexane, Water; Trial 3- Petrol-ether, Hexane, Ethanol. Extracts were collected from 24, 5th stage, worker larvae.

Three filter paper strips soaked with different solvents for successive extractions of larvae were placed inside Petri dishes (13.5 cm int. diam.) and 23 Varroa mites were placed in the center of each of the dishes. The number of mites present on each strip was counted every 15 min for 140 min. Typical results of kairomonal activity of larval extracts are shown in Fig. 8. It emerges that ethanolic and hexanolic extracts attracted the mites already after 15 mins. Except for water, all solvents used for the preparation of extracts in all three trials were attractive to the

mites, ethanol being the most attractive. In general, any solvent which was used for the third successive extraction from the same larva, showed a reduced kairomonal activity.

Series 2. Each larva was extracted with one solvent only. Five batches of ten larvae in each, were extracted with each of the following solvents: Water, Ethanol, Hexane, Hexane:Ethanol (1:1), Petrol-Ether.

The bioassays were carried out as described in the above section (Series 1). Fig. 9 shows a typical result: the kairomonal components of larval cuticle can be collected with all the solvents examined, but not with water.

In another series of bioassays we have tested the attraction of Varroa to control paper strips soaked with the different solvents used: ethanol showed the lowest attraction.

(2) Attraction of Varroa mites to worker and drone 5th stage larvae and to their cuticular extracts

(a) Intact worker and drone larvae. Four worker and 4 drone 5th stage larvae were placed in two groups, 7 cm apart from each, in Petri dish (7.5 int. diam) and 15 adult female Varroa mites were deposited at the center of the dish. Number of mites present on larvae of each group was counted every 20 mins. for 120 mins. The results in Fig. 10 show that drone larvae were more attractive to Varroa than worker larvae of the same instar.

(b) EtOH extracts. Twenty drone and 20 worker, 5th stage larvae, were wiped with filter paper soaked with EtOH. The extracts were concentrated under nitrogen, and applied to filter paper strips. These were placed inside Petri dish (7.5 cm diam) near the walls. 20 Varroa mites were placed in the center of the dishes and their presence on the filter paper strips was established every 20 mins. for 60 mins. The mean values of 2 repetitions in Fig. 11, show that drone larvae extracts were more attractive to Varroa than that of worker larvae. No mites were found near the dry filter paper or EtOH soaked filter papers.

Table 1: Weight, volume and surface of 3rd, 4th and 5th stage of worker and drone larvae

(a) worker larvae

Larval Stage	Weight (mg)	Volume (ml)	Surface (mm ²)
3rd	20	0.02	—
4th	56	0.06	35
4/5th	104	0.10	85
5th	140	0.18	143

(b) Drone larvae

Larval Stage	Weight (mg)	Volume (ml)	Surface (mm ²)
3rd/4th	200	0.320	180
4th	266	0.300	262
4/5th	305	0.260	220
5th	333	0.200	235

fig. 8 Attraction of 23 Varroa females to cuticular extracts of 24, 5th stage, worker larvae. Water, ethanol and hexane were used as solvents. Each larva was successively wiped with a filter paper soaked with one of the solvents and in the same order.

fig. 9 Attraction of 23 Varroa females, to cuticular extracts of 5th stage worker larvae. The solvents were water, ethanol and hexane. Each larva was extracted with one solvent only. Ten larvae per each solvent were used to prepare the extracts.

Fig. 10 Attraction of 15 Varroa adult female mites to worker and drone 5th stage larvae. Counts of mites attracted to each group of larvae was made every 20 mins. for 120 mins.

Fig. 11 Attraction of 20 adult female Varroa mites to ethanolic extracts of 20 worker and 20 drone 5th stage larvae. Counts of mites attracted to filter paper strips were made every 20 mins. for 60 mins.
1 - Control, dry filter paper strips; 2 - Control, EtOH soaked strips. 3 - EtOH extract of worker larvae; 4 - EtOH extract of drone larvae.

m. Specific objective: GC-MS analysis of the cuticular components of 5th stage worker and drone larvae.

Cuticle of 5th stage drone and worker larvae was extracted with EtOH using filter paper as described above. The extracts were concentrated under nitrogen and 1 μ l samples injected for analysis (DB-1, 30 m long, capillary column was programmed from 50°C to 220°C at 5°C/min.) using Finnigan Mat 4600 GC-MS equipped with Incos Data Septem with NBS Gas Spectra Library. Separation by GC of worker and drone cuticular components is shown in Figs. 12 and 13. Table 2 lists the cuticular components as characterized by mass spectra library. Most of the worker and drone larvae cuticular components are branched alkanes. This portion of the project was terminated because in 1987 the French team from Bures-sur-Yvette reported in Science 245: 638-639, by Yves Le Conte et al., that they have isolated and identified the larval substances which are attractive to Varroa.

n. Specific Objective: Develop laboratory rearing method for workers.

We have modified our method for rearing queens in the laboratory to the purposes of this research. 5th stage larvae were removed from their cells and transferred into the artificial cells as follows: Depressions (5.2 mm diam., 17.0 mm long) were made with a hot metal tool in polystyrene blocks. The larvae were deposited on the bottom of the depression, the blocks containing seven depressions were covered with a microscope slide, and placed inside an incubator at 35°C and 36% RH. The blocks were treated with 0.08% Mycostatin and 0.08% Thiomersal. The dimensions of the artificial cells allowed successful molts and the emergence of adult bees. This method is useful for artificial infestation of developing bees with mites under controlled conditions, and the microscope slide facilitates direct observations of the mite. It will be used in the study of Varroa metabolic inhibitors.

RTIC
 13/12/99 11:10:00
 DATA: 17 #1
 SAMPLE: 29-2-C
 COND: 29-2-C
 FLOW: 1.2504
 LABEL: N 0.4.0 QUANT: A 9.1.0 J 0 BASE: U 2A. 3
 DATE: 17 #1
 CALL: RONY #2
 FROM: 29-2-C TO 1000

A. mellifera L. ligustica Workers

Fig. 17. GC-MS scans of 210H cuticular extract of worker larvae.

RIC
 03/13/89 12:22:00
 SAMPLE: -DB-5.EI
 CONDS.: DB-5.EI
 RANGE: 0 1.3503
 DATA: LS #1
 CALL: RONY #2
 SCANS 500 TO 3503
 LABEL: N 8.4.0 QURN: 4.9.1.0 J 0 BASE: U 20. 3

2720.

A. mellifera L. ligustica Drones

Fig. 13 GC-MS scans of EtOH cuticular extract of drone larvae.

Table 2: GC-MS characterisation of compounds present in cuticular EtOH extracts of 5th stage worker and drone larvae

Workers

<u>Scan</u>	<u>Compound</u>	<u>Formula</u>	<u>M.W.</u>	<u>n/z</u>
2439	O-Decyl-Hydroxylamine	C ₁₀ .H ₂₃ .O.N	173	57
2619	2, 4, 6-Trimethyl-Octane	C ₁₁ .H ₂₄	156	57
2650	2, 9-Dimethyl-decane	C ₁₂ .H ₂₆	170	57
2728	4, 8, 12-Trimethyl - 3, 7, 11-Tridecatrienitrile	C ₁₆ .H ₂₅ .N	231	69
2812	3-Ethyl - 2, 7-Dimethyl-Octane	C ₁₂ .H ₂₆	170	57
2847	3-Ethyl - 2, 7-Dimethyl-Octane	C ₁₂ .H ₂₆	170	57

Drones

<u>Scan</u>	<u>Compound</u>	<u>Formula</u>	<u>M.W.</u>	<u>n/z</u>
2025	2- Propyl - 1-Heptanol	C ₁₀ .H ₂₂ .O	158	43
2243	2, 4, 6-Trimethyl-Octane	C ₁₁ .H ₂₄	156	57
2437	3, 5-Dimethyl-Octane	C ₁₀ .H ₂₂	142	43
2472	2, 4, 6-Trimethyl-Octane	C ₁₁ .H ₂₄	156	57
2624	Octacosane	C ₂₈ .H ₅₈	394	57
2654	Pentatriacontane	C ₃₅ .H ₇₂	492	57
2737	4, 8, 12-Trimethyl 3, 7, 11-Tridecatrienitrile	C ₁₆ .H ₂₅ .N	231	69
2817	2, 4, 6-Trimethyl-Octane	C ₁₁ .H ₂₄	156	57
2858	2, 6, 10-Trimethyl dodecane	C ₁₅ .H ₃₂	212	57
3073	17-Pentatriacontene	C ₃₅ .H ₇₀	490	43
3180	3-Ethyl - 2, 7-Dimethyl-Octane	C ₁₂ .H ₂₆	170	57
3541	17-Pentatriacontane	C ₃₅ .H ₇₀	490	43

Description of Cooperation

The Varroa mite was first found in Israel in October 1984 and experience was gained here both in the use of detection and chemical control methods, which were elaborated during the first year of this project. When the mite was detected in the US in Florida at the end of 1987, the results of our field and laboratory work were transmitted to Dr. M. Shimanuki at Beltsville, and published in January 1988 in the American Bee Journal.

A comparison of Varroa detection methods used in the US (ether roll) and in Israel (bottom board insert) has been carried out at Beltsville and Rehovot, and the fluvalinate smoke strip method and bottom board inserts have been adapted by the U.S. and Israel bee industries.

There was a fruitful exchange of ideas, information and technologies between Rehovot and Beltsville, especially during Dr Shimanuki's visit in Israel in May 1988. Dr Shimanuki lectured to researchers, extensionists and beekeepers in Rehovot, on the control of Varroa and on the present status of this mite, as well as other honeybee pests in the U.S. During his field trips to commercial beeyards in the Coastal Area, Western and Upper Galilee and the Jordan Valley, Dr Shimanuki evaluated the detection, treatment and control methods used and was exposed to the effect of climatic zones on the population dynamics of bee colonies and the Varroa mites. Dr Shimanuki also visited research laboratories at the University of Tel-Aviv and the Veterinary Institute at Bet-Dagan.

Dr Lensky visited Beltsville in February 1989 and had a fruitful exchange of information with Dr Shimanuki and his team on metabolic inhibitors and other non-chemical control methods. He also visited Varroa infested bee yards in Florida with Dr Sanford and Dr Cromroy.

Evaluation and Benefits to Agriculture

In accordance with the objectives of the research (detailed in our original proposal), better understanding of the detection and control of Varroa in the US and in Israel was achieved and the techniques are applied in beekeeping industry: fluvalinate smoke strips and bottom board inserts, fluvalinate inserts or strips and Queen Tabs. Monitoring for Varroa may reduce the incidence of treatment with fluvalinate. Effective control of Varroa has been achieved by placement of inserts at hive entrances instead at the center of a brood-nest. This technique was less labor intensive and the chemical analyses for fluvalinate showed that it is more safe than the suspension of inserts or strips in the brood-nest. The application of fluvalinate in bee colonies for periods longer than 6 weeks resulted in fluvalinate residues both in wax and honey. Monitoring for Varroa resistance to pesticides can be based on two detoxification systems. The use of Origanum oil may be applicable as a part of integrated control of Varroa. Partial characterization of honey bee 5th stage larvae kairomones and of Varroa ecdysteroids may contribute to better understanding of the attraction of mites to honey bees as well the possible use of specific antimetabolites to control this pest. Collection of mites from all over the world (17 countries) and a morphometric study of the variability among the females of Varroa have indicated that the origin of the mite detected in North America is from Brazil. A new species of Varroa and three biotypes have been identified.