BARD

FINAL REPORT

PROJECT NO. US-2508-94

Integrated Management of Tracheal mite, *Acarapis woodi*, and of Varroa mite, *Varroa jacobsoni*, Major Pests of Honey Bees

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BARD Final Scientific Report

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Evaluating Panel:

Project Title: Integrated Management of Tracheal mite, *Acarapis woodi*, and of Varroa mite, *Varroa jacobsoni*, Major Pests of Honey Bees

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Continuation of (Related to) Previous BARD Project:

☐ Yes

X ☐ No

Number:

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Publication Summary (numbers)

	Joint IS/ US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted)	0	3	3	6
Submitted, in review, in preparation	0	0	0	0
Invited review papers	1	0	0	1
Book chapters	1 (in press)	0	0	1
Books	0	0	0	0
Master theses	-	0	0	0
Ph.D. theses	-	1	0	1
Abstracts	0	0	0	0
Not refereed (proceedings, reports, etc.)	0	3 (1 video)	0	4

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/ US inventors	Total
Submitted	0	0	0	0
Issued (allowed)	0	0	0	0
Licensed	0	0	0	0

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Visits/ Meetings	1	1	0	2
Sabbaticals	0	0	0	0
Postdoctorates	0	0	0	0

- **Cooperation**, briefly explain whether synergistic, complementary or supportive.

Cooperation was synergistic, complementary and supportive. Mite problems in Israel and the U.S. have similarities and differences. Different laws dictating acaricide use impact what controls are legal in each country. Publication of the Annual Review of Entomology article and the book chapter best symbolize our very productive partnership.

Joint Authorship Papers (Invited Review):

Sammataro, D., U. Gerson & G.R. Needham. 2000. Parasitic mites of honey bees: life history, implications and impact. *Annu. Rev. Entomol.* 45: 519-548.

Join Authorship (Invited Book Chapter):

Needham, G.R., U. Gerson & D. Sammataro. Mite Biology. In *Mites of Honey Bees*, T. Webster & K. Delaplane, Eds., Dadant & Sons (in press).

Peer-Reviewed Journal Articles (US Authors):

Sammataro D. & G.R. Needham. 1996. Developing an integrated pest management (IPM) scheme for managing parasitic bee mites. *Amer. Bee J.* 136: 440-3.

Sammataro, D. & G.R. Needham. 1996. Host-seeking behaviour of tracheal mites (Acari: Tarsonemidae) on honey bees (Hymenoptera: Apidae). *Exp. Appl. Acarology*, 20: 121-136.

Sammataro D., G. Degrandi-Hoffman, G. Needham & G. Wardell. 1998. Some volatile plant oils as potential control agents for varroa mites (Acari:Varroidae) in honey bee colonies (Hymenoptera: Apidae). *Amer. Bee J.* 138: 681-5.

Proceedings Articles (US Authors):

Sammataro, D., P. Parker, & G.R. Needham. 1996. Using PCR-based RAPDs (Random Amplified Polymorphic DNA) to determine differences in tracheal mite *Acarapis woodi* (Rennie) (Acari: Tarsonemidae) populations. *Acarology IX: Proceedings*, 1994; Columbus, OH, pp. 143-48.

Sammataro, D., S. Cobey, B.H. Smith & G.R. Needham. 1996. Vegetable-Shortening Patties Control Tracheal Mites (Acari: Tarsonemidae) in Honey Bees (Hymenoptera: Apidae). *Acarology IX: Proceedings*, 1994; Columbus, OH, pp. 153-56

Bruce, W.A. and G.R. Needham. 1996. Effects of temperature and relative humidity on the water balance of *Varroa jacobsoni* Oudemans (Varroidae). *Acarology IX: Proceedings*, 1994; Columbus, OH, pp. 157-59.

Media Productions:

Sammataro, D. & R. Smith. 1994. VIDEOS: *Dissecting mites using the tracheal pull method* and 1995 *Host-seeking behavior of tracheal mites on honey bees*. Vesta Video Productions.

Ph.D. Dissertations (US Author):

Sammataro, D. 1995. *Studies on the Control, Behavior, and Molecular Markers of the Tracheal Mite (Acarapis woodi (Rennie)) of Honey Bees (Hymenoptera: Apidae)*. Ph.D. Dissertation. Columbus, OH: Ohio State University. 125 pp.

Peer-Reviewed Papers (Israeli Authors):

Dag, A., Slabezki, Y., Efrat, H., Kamer, Y., Yakobson, B.A., Mozes-Koch, R. and Gerson, U. 1997. Control of honey bee tracheal mite infestations with amitraz fumigation in Israel. *Amer. Bee. J.* 137: 599-602.

Mozes-Koch, R. and U. Gerson. 1997. Guanine visualization: a new method for diagnosing tracheal mite infestation of honey bees. *Apidologie*: 3-9.

Slabezki, Y., H. Efrat, A. Dag, Y. Kamer, B.A. Yakobson, R. Mozes-Koch and U. Gerson. 2000. The effect of honey bee tracheal mite infestation on colony development and honey yield of Buckfast and Italian honey bee strains in Israel. *Amer. Bee. J.* 140: 231-234..

ABSTRACT:

Objectives: The Israeli work plan regarding HBTM included: (a) producing a better diagnostic method; (b) following infestations during the season and evaluating damage to resistant bees and, (c) controlling HBTM by conventional means under local conditions. For varroa our plans to try novel control (e.g. oil patties & essential oils) were initially delayed by very low pest populations, then disrupted by the emergence of fluvalinate resistance. We monitored the spread of resistance to understand it better, and analyzed an underlying biochemical resistance mechanism in varroa.

The US work plan focused on novel management methods for both mites with an emphasis on reducing use of traditional insecticides due to resistance and contamination issues. Objectives were: (a) evaluating plant essential oils for varroa control; (b) exploring the vulnerability of varroa to desiccation for their management; and (c) looking for biological variation in HBTM that could explain virulence variability between colonies. Although the initial PI at the USDA Beltsville Bee Lab, W.A. Bruce, retired during the project we made significant strides especially on varroa water balance. Subcontracts were performed by Yoder (Illinois College) on varroa water balance and DeGrandi-Hoffman (USDA) who evaluated plant essential oils for their potential to control varroa. We devised an IPM strategy for mite control in the U.S.

Background: Mites that parasitize honey bees are a global problem. They are threatening the survival of managed and feral bees, the well-being of commercial/hobby beekeeping, and due to pollination, the future of some agricultural commodities is threatened. Specific economic consequences of these mites are that: (a) apiculture/ breeder businesses are failing; (b) fewer colonies exist; (c) demand and cost for hive leasing are growing; (d) incidences of bee pathogens are increasing; and, (e) there are more problems with commercially-reared bees. As a reflection of the continued significance of bee mites, a mite book is now in press (Webster & Delaplane, 2000); and the 2nd International Conference on Africanized Honey Bees and Bee Mites is scheduled (April 2000, Arizona). The first such conference was at OSU (1987, GRN was co-organizer). ***The major challenge is controlling two very different mites within a colony while not adversely impacting the hive.*** Colony management practices vary, as do the laws dictating acaricide use. Our basic postulates were that: (a) both mites are of economic importance with moderate to high infestations but not at low rates and, (b) once established they will not be eradicated. A novel strategy was devised that deals with the pests concomitantly by maintaining populations at low levels, without unnecessary recourse to synthetic acaricides.

Major Conclusions, Solutions, Achievements: A major recent revelation is that there are several species of "*Varroa jacobsoni*" (Anderson & Trueman 1999). Work on control, resistance, population dynamics, and virulence awaits knowing whether this is a problem. In the U.S. there was no difference between varroa from three locales in terms of water balance parameters (AZ, MN & PA), which bodes well for our work to date. Winter varroa (U.S.) were more prone to desiccation than during other seasons. Varroa sensitivity to desiccation has important implications for improving IPM. Several botanicals showed some promise for varroa control (thymol & origanum). Unfortunately there is varroa resistance to Apistan in Israel but a resistance mechanism was detected for the first time. The Israel team also has a new method for HBTM diagnosis. Annual tracheal mite population trends in Israel were characterized, which will help in targeting treatment. Effects of HBTM on honey yields were shown. HBTM control by Amitraz[®] was demonstrated for at least 6 months. Showing partial resistance by Buckfast bees to HBTM will be an important IPM tactic in Israel and U.S.

Implications: IPM programs are proposed (appended Sammataro & Needham 1996, Sammataro et al. 2000), but much remains to be learned about the mites and its host.

ACHIEVEMENTS:

Significance of Main Scientific Achievements: One of the main challenges for the beekeeper is detecting mite infestations, especially HBTM, so treatment can be initiated and good decisions made about moving bees. We predict that our mite guanine detection method will be quicker and less labor-intensive than other indirect HBTM diagnostic methods. For HBTM control, a single, three-part fumigation with amitraz effectively controls it for an entire season in Israeli (~ 6 mo) without negative effects on host bees. Use of resistant "Buckfast" bees under Israeli conditions should be an important, viable alternative to chemical treatments for HBTM. For varroa control, increased detoxification of tau-fluvalinate by monooxygenases has been proposed as a resistance mechanism, whereas esterases play a negligible role. Resistance varied within Israel from negligible to very resistant (Yas'resh mites). An immediate program of resistance management (RM) is needed in Israel, which emphasizes minimizing pesticide use while diversifying pest mortality factors, reducing selection pressure, managing susceptibility and predicting future resistance. Said another way, beekeepers need to use non-pesticide tactics where possible, apply additional pesticides with different modes of action than pyrethroids and select for bees tolerant of or resistant to mites. This is where the U.S. studies become important by proposing modifying the hive environment to impact mite water balance and applying novel active ingredients from plants. Plant ethereal or essential oils are candidate chemicals for the control of varroa because they are perceived as 'natural' compounds that will not contaminate hive products. Two oils (thymol and origanum) were promising but still inferior to Apistan, which is the best active ingredient in the U.S. for varroa control. Oils at the high dose are irritating to the bees so further tests are needed on concentrations and delivery systems before they can assume an important role for mite control. The net result is that beekeepers have some additional options to explore for mite management that include resistant Buckfast bees and a refined fumigation strategy (Israel).

Potential for Application:

Immediate Impact:

HBTM: The HBTM guanine detection technique can be implemented immediately so good management decisions can be made. Beekeepers can begin requeening colonies with the "Buckfast" strain of bees for HBTM management. Carnolian and select Italian bees should be considered in the US where continued use of vegetable oil/sugar patties is encouraged during peak mite infestations. This is the type of environmentally friendly approach we advocated at the outset. A single, three-part amitraz fumigation was very effective in Israel and should be coupled with using Buckfast bees. Random Amplified Polymorphic DNA could be used to discriminate between virulent HBTM based on banding patterns seen from different sources.

Varroa: Fluvalinate resistance is a developing problem in Israel and the US. Efforts to find alternative active ingredients are critical.

Medium-Term Application (5-10 yrs): Varroa control with essential oils is still several years from implementation. Further testing is needed to refine application techniques and evaluate effects on bees. Government approval is also needed. Modification of the hive environment to disrupt varroa water balance also needs testing on a colony level. A solar-powered ventilation device (Bee-Cool) was to be tested by Dr. Bruce but his retirement precluded this real-world experiment. Mite resistance to HBTM by Italian bees may be achieved by closed population breeding.

Estimated Economic Benefits: The main contribution of honey bees to agriculture is pollination. The value of bee-pollinated crops in IS and the US is in the billions of US dollars annually. It is very difficult to estimate the impact of improved mite management but it is significant.

BODY OF THE REPORT:

BACKGROUND

The mites that parasitize honey bees are a global problem as they are threatening the survival of managed and feral honey bees, the beekeeping industry, and due to the role of bees in pollination, the future of many agricultural crops. An appended book chapter (Needham et al., 2000) provides a layman's description of the mite problem and discussion of the mite's biology. Two other appended papers give overviews on parasitic mite biology and their control, one for beekeepers (Sammataro & Needham, 1996), and the other for scientists and professionals (Sammataro, Gerson & Needham, 2000). The honey bee tracheal mite (HBTM), *Acarapis woodi*, and *Varroa jacobsoni*, are the major honey bee pests in the USA and Israel. The former is a minute (0.2 mm) endoparasite of the bee tracheae (predominantly in the prothorax), and thus is very difficult to detect. Varroa is relatively large (ca 1 mm) ectoparasite primarily of immature bees within the sealed brood cells. Both cause considerable damage seen as decreased honey yields, declining brood production and overall hive decline.

The pesticide amitraz is used (as a fumigant) in Israel for HBTM control, whereas in the USA only menthol was registered for that purpose. Oil patties are also included as a control measure by many beekeepers. The pyrethroid fluvalinate (Apistan), applied in a slow-release formulation, was used in both countries against varroa as the project began.

HONEY BEE TRACHEAL MITE

Guanine Visualization is a Better Method to Detect HBTM

Introduction and Methods: Tracheal mite is an endoparasite whose infestations are not manifested by external symptoms. Consequently it is laborious to monitor pest populations, follow their year-around numerical trends and evaluate control efforts. Several direct or indirect methods have been proposed and implemented for positive HBTM recognition (Shimanuki and Knox, 1991). The method we devised is also indirect, based on the presence of guanine that is produce only by mites but not bees (Erickson et al., 1994). Guanine presence in honey bees that are free of varroa would therefore indicate an HBTM infestation. Details of the methodology are in the attached reprint (Mozes-Koch and Gerson 1997 *Apidologie* 28: 3-9).

Results: A preliminary estimate of HBTM presence by the dissection and guanine methods in bees of 3 of 4 infestation categories was promising with uninfested, medium-infested and heavily-infested bees. Data for bees with incipient infestations

were dissimilar, showing little fit between methods. Slight blemishes on the bees' tracheae could be due to causes other than mite attack, and a very early HBTM infestation may not result in marks. A comparison between various HBTM detection methods, including ELISA, indicated that all results were qualitatively consistent. HBTM presence was confirmed by all methods whenever bees were infested at or above 6%, the lowest level detectable by ELISA. Similarly, very low to no infestation was uniformly detected.

Discussion: Due to the minute size of HBTM and the equivocal symptoms of its early infestation, it is difficult to diagnose its presence. All diagnostic methods have advantages and disadvantages. Ours, although simple, is like other approaches and cannot not differentiate between *A. woodi* and other bee-colonizing *Acarapis* spp. Our method's inability to detect incipient HBTM is shared by all methods. Another disadvantage of the guanine method is that it is very time consuming for individual bees, because each thorax must be homogenized individually.

Scientific Implications: This method may be used as a quantitative tool in diagnosing infestations in individual bees (and colonies), as well as a qualitative tool in bulk apiary samples, following whole bee homogenization. We conclude it would be quicker and less labor-intensive than other indirect HBTM diagnostic methods.

Effectiveness of Amitraz on HBTM Infestations in Israel

Introduction: Amitraz fumigation has been the recommended means of controlling HBTM since it was first found in Israel (Gerson et al., 1994). Treatment consists of three fumigations, applied at weekly intervals, given either in the early morning or the late afternoon. Amitraz success abroad has been mixed, sometimes providing good control, at other times uneven control, while engendering complaints about deleterious side effects elsewhere. This pesticide was registered and used in the USA as Miticur® until 1993, when the manufacturer withdrew it due to post-treatment death of some colonies (Waller, 1993). No complaints were voiced about amitraz in Israel and this treatment, used by the majority of Israeli beekeepers, is quite satisfactory. No amitraz residues have been detected in honey from treated apiaries. However, we lacked data on its efficacy in colonies of different strengths, its effect on colony population and honey yields; plus we needed to optimize application dates.

Materials and Methods: Detailed methods and results are attached (Dag et al., 1997, *Amer. Bee J.* 137: 599-602). Briefly, experiments were conducted at three apiaries of Italian bees, located along the coastal plain of Israel. At two of these apiaries randomly

placed colonies were fumigated with amitraz. Rates were calculated as infested bees per colony on the sampling dates. To assess possible injuries to colonies, adverse effects were evaluated at one apiary by counting the number of populated combs and measuring the sealed brood area. At the third apiary the effect of pesticide on treated HBTM was determine directly by tallying living and non-living mites in bees' tracheae.

Results: The single three-part application of amitraz brought about significant declines in bee infestation rates (Fig. 1, appended, Dag et al. 1997). In addition, the percentage of infested bees declined significantly (Table 1). Mite mortality rates were 2.5 times higher than controls, remaining above that level throughout (Fig. 2). The rate of infested bees (Fig. 3) dropped below one-third a week after the first fumigation, but then fluctuated, never decreasing below 20% (70-80% in the control).

Discussion: These results clearly show that a single, three-part fumigation controls HBTM for an entire season, under Israeli conditions. The similar post-treatment situation of the combs and brood in treated and control colonies shows that amitraz, applied by fumigation, caused no immediate, discernible damage. Unfortunately this active ingredient is not available for use in the USA.

Impact of HBTM on Colony Development & Honey Yield of Buckfast & Italian Bee Strains in Israel.

Introduction: Honey bees show a wide range of susceptibilities to HBTM infestations. Resistant strains can be selected (Page and Gary, 1990). The 'Buckfast' strain is credited with possessing resistance to HBTM without affecting productivity (Brother Adam, 1987; Lin et al, 1996). We quantified HBTM infestation rates over a year and monitored damage to a local Italian bees (treated & untreated) and Buckfast bees. The detailed paper is appended (Slabezki et al. 2000, *Amer. Bee J.*).

Materials and Methods: An apiary with 30 heavily infested colonies was subdivided into three treatment groups of equal adult populations. They were provided either with Italian queens (n=20) or with Buckfast queens (n=10). Ten Italian colonies were fumigated with amitraz, before introducing mated and marked queens, whereas all other colonies were left untreated. Samples of ca 200 bees were collected from the outer populated combs of these colonies at monthly intervals and the thoraces of 35 bees in each subset were dissected and examined. Results were calculated as rates of infested bees per colony on each sampling date. Adult bees were collected monthly and examined for brood disease. Honey yield was recorded during the spring and

summer harvests. The colonies were examined for the presence of the original queens in December 1997, a year after the trial was started.

Results: HBTM levels in Buckfast colonies decreased rapidly from December 1996 to April 1997 and remained low (Fig. 1, appended ABJ, p. 232). In the untreated Italian colonies infestation remained high and steady until March and then declined, due to death of the most infested colonies. In the treated Italian colonies prevalence rates stayed low until July, and then began to increase. Bee populations of the three groups were similar. The treated Italian colonies built-up rapidly in the spring and the Buckfast colonies had a maximum of 15 combs in the spring (Fig. 2, ABJ, p. 233). Queen survival was affected more by the provenance of the queens than by the treatments. In the untreated Italian colonies only 1 out of the 5 colonies that had survived at the end of the year still carried the original queen, as did 2 out of the 9 surviving treated Italian colonies. But 8 out of 9 surviving original Buckfast queens remained at the end of the year. No honey could be harvested in spring or summer from 9 out of the 11 untreated Italian colonies; their mean spring yield was 1.0 kg (for all colonies) and 2.0 kg. in summer (Fig. 3, ABJ, p. 233). In the spring the treated Italian colonies produced an average of 21.9 kg, significantly more ($p < 0.05$) than the untreated Buckfast colonies (10.4 kg). Buckfast bees produced significantly more honey in the summer than the treated Italian colonies (9.6 vs 5.4 kg., $p < 0.05$). The total annual yields were statistically different. The levels of nosema infection and of varroa infestation were almost nonexistent in all bee groups through the experimental period.

Discussion: The recommended amitraz applications controlled HBTM for at least 6 mo (Fig. 1). The untreated Italian colonies suffered heavily: high mite infestations reduced colony populations (Fig. 2), honey yield (Fig. 3), and increased colony mortality. This is in contrast to other reports from our region (Orantes et al. 1997; Mossadegh and Bahreini, 1994). High mortality of colonies heavily infested by HBTM is well-documented in temperate regions (Baily and Ball, 1991; Otis and Scott Dupree, 1992), especially in susceptible strains (Danka et al., 1995).

Scientific Implications: This is the first demonstration of Buckfast bee resistance to HBTM under Mediterranean conditions. Such resistance may be due to a lower infestation rate of young compared with susceptible bees (Danka and Villa, 1996). Our uneven results may stem from initial high infestation levels (~50%), and/or that from variations in the Buckfast strains. Use of resistant bees under Israeli conditions should be a viable alternative to chemical treatments against HBTM. Resistance of the local

Italian colonies to HBTM may be achieved by closed-population breeding, as is done in the U.S. with Carniolian bees (Cobey 1997).

MOLECULAR TECHNIQUE FOR EVALUATING MITE VIRULENCE

Introduction: The endoparasitic tracheal mite has left a path of destruction worldwide since the beginning of this century. Infestation rates fluctuate widely between colonies, rising to over 90% in some colonies while never reaching 10% in others. Since HBTM were first described, their lethal effects on colonies seem to have different phases. First, many colonies are lost for a few years, then the mites seem to disappear altogether. Virulence of these acarines in honey bees is often reported only to dissipate after 10 to 20 years (Delaplane, pers. comm.). After many colonies have perished the remaining bees appear to tolerate infestation. Unfortunately many colonies are lost in the process, especially in northern climates. HBTM are not a problem where winters are mild.

The question is whether this fluctuation in mite tolerance is a sign of bee resistance, or if it is a reflection of decreasing mite virulence? If mites are too virulent, the colony may perish and the parasitism is not successful. Modulation of this parasitism is a natural process. If the mites modify somehow so they can live and keep their hosts alive, they will survive. We suspect that the mites are becoming less virulent and new molecular techniques may help facilitate discrimination between mite populations. This needs confirmation by a combination of DNA tests using systematic studies. To track this shift, which may not be visible morphologically but may be genetically based, a relatively new molecular method was chosen. Random Amplified Polymorphic DNA or *RAPDs* was developed by Williams et al. (1990). Using *RAPDs* we wanted to ascertain if there is a correlation between banding patterns for virulence with colony survival. (See appended manuscript Sammataro et al. 1996, *Acarology IX: Proceedings*).

Results and Discussion: We used the *RAPDs* technique to characterize several arthropods. We analyzed banding patterns visualized on agarose gels of honey-bee races, tracheal and other mites collected from infested *Apis* and *Bombus* bees, and cockroaches utilizing two band-scoring formulae. Distinct banding patterns among *A. woodi* mites from several apiaries suggest different biotypes in the mite populations. Comparing banding patterns of mites that kill the host hive with those that do not may identify less virulent *Acarapis* mites.

Scientific Implications: In the future this technique should be refined to elute a band of tracheal mite DNA from a *RAPDs* gel, which will be sequenced to manufacture a

primer and type a new infestation of mites in a colony. Eventually the mite primer could be used on honey bees from old, dried or archeological material, to determine mite subspecies and ancestry.

VARROA

Varroa Resistance to Apistan is Analyzed in Israel:

Introduction: The main pesticide used against varroa is the pyrethroid tau-fluvalinate, the active ingredient of Apistan®. Its efficacy against varroa is due to being tolerated by honey bees. Applied as a contact treatment, by pesticide-impregnated strips, fluvalinate was used in Israel since the mid-1980's (Yakobson and Efrat, 1988). Strips are placed within the hive for about six weeks. This efficacious mode of application continuously exposes the mites to the toxicant; Gerson et al. (1991) predicted that it would promote selection in favor of resistant individuals. Such resistance has since been found in Italy (Lodesani et al., 1995; Loglio and Plebani, 1992), France (Vandame et al., 1995), Poland (Londzin and Sledzinsky, 1996) and the USA (Elzen et al., 1999). The LC₅₀ (lethal concentration causing 50% death) for susceptible mites (from Udine in Italy and from two Austrian localities) lay between 15.9-18.5 ppm and for resistant individuals ~385-857 ppm (Milani, 1995). Mites resistant to tau-fluvalinate were cross-resistant to other pyrethroids. We suspected resistance in varroa when Apistan® ceased to kill the mites; relevant evidence is first presented, followed by efforts to elucidate the detoxification mechanism(s) involved. The following study is outlined in more detail because a manuscript has not been published.

Materials and Methods; Field studies: Surveys were taken in 1993 and 1997. Susceptibility to the toxicant was assayed using one strip of Apistan®. Mite levels were determined by placing white boards, thinly covered with vegetable oil, onto the hive floor (Herbert et al., 1989; Levin, 1985). Two drops of Taktic® (12.5% amitraz) were placed onto a strip of paper impregnated with KNO₃ and burned within the hive. Dropped mites were counted one hour later. Fluvalinate efficacy was determined by repeating the Taktic® fumigation 6 weeks later.

LC₅₀ tests: Mites were collected from two apiaries. At the first (Nehalim) fluvalinate had no effect on varroa and at the other (Yesodot), control was uneven. Tests were conducted in Petri dishes coated with paraffin into which tau-fluvalinate was added. Mites were assayed at four concentrations: 15, 25, 50, and 150 ppm (Milani, 1995). Groups of 45 mites (in 3 replicates of 15) were introduced onto the coated plates, which were placed in an incubator (30°C, 75% RH) for 6 hrs and allowed to move

freely. Survivors were transferred for 24 hrs to paraffin-coated Petri dishes, without tau-fluvalinate. These mites were offered 5 living bee larvae as food to ensure that mortality was not due to starvation. Total dead and live mites (mobile or paralyzed) were tallied under a dissecting microscope, and mortality scores were corrected (Abbott, 1925). LC_{50} values were obtained by calculating the logarithmic concentration of the toxicant. Mites that survived the toxicant were used for enzymatic activity tests (see below). Our hypothesis was that fluvalinate resistance was due to elevated rates of monooxygenase and/or esterase activity.

Microassay for Enzymes: Mites were from sealed brood (from Rehovot, untreated colonies; from Yas'resh, where fluvalinate had little effect and from Kfar Truman, where the chemical was effective), and kept at -80°C . For each test, 5 mites were homogenized in 200 μl buffer for 2 min, followed by Teflon-glass homogenization. The homogenate was centrifuged at 2.000g for 5 min and the supernatant filtered through glasswool, and microassayed for monooxygenases and esterases. Intensity of enzymatic activity was measured on a colorimeter at the reactions' endpoint (Vandame et al., 1995). In the monooxygenases microassay only *p*-nitroanisole (PNA) was used, after obtaining negatives results with other substrates. The reaction mixture included 75 μl varroa mite homogenate, 10 μl of an NADPH regeneration system (0.25 mM NADP^{+}), 2.5 mM glucose 6-phosphate, 1 unit of glucose 6-phosphate dehydrogenase and 1.5 mM PNA, brought up with the homogenization buffer to a final volume of 200 μl . Another part of the homogenate was diluted 1:100 in sodium phosphate buffer. The incubation mixture contained 100 μl of this homogenate, 80 μl homogenization buffer and 20 μl 0.5m M *p*-nitrophenyl acetate (PNPA), in a 200 μl volume. Because monooxygenases as well as esterases measure *p*-nitrophenol formation, they were appraised on the same microplate. Rate of *p*-nitrophenol formation was monitored at 405 nm, with a Thermomax microtiter plate reader at 30°C , using its kinetic mode, at 15-sec intervals during the 15 min incubation period. The extinction coefficient of $3.32 \text{ mM}^{-1}.\text{cm}^{-1}$ was used for specific activity calculations (Rose et al., 1995).

Results: Mean pre-treatment infestation by varroa in 1993 (taken as a pre-resistance census) was 100.7 mites/colony, dropping to 0.65 mites/colony after a treatment (Appendix, Table 1). The 1997 data were uneven, ranging from a huge decline in later mite numbers (Giv'at Chen), to a moderate drop (Kfar Malal), to doubling of varroa numbers (Nezer). Such uneven changes suggest an increasing decline in pesticide potency. To confirm, we used the LC_{50} tests, and then tried to determine the

detoxifying mechanism(s). The LC_{50} (probit mortality=5) of the Yesodot mites was 41.5 ppm (Appendix, Fig. 1), that for Nehalim mites was 92.1 ppm, verifying their resistance. Mites that survived this exposure were tested for enzymatic activity (Appendix, Table 2). High enzyme activity rates were detected in the Yas'resh mites as compared to the rates from Rehovot and Kfar Truman [a 20 fold increase in monooxygenases, a 1.5-2.5 fold increase in esterases (Appendix, Table 3)].

Discussion: The results are consistent with our hypothesis that the cytochrome P450 monooxygenase system detoxifies fluvalinate in the varroa mite in Israel. This system confers broad substrate specificity; arthropods that develop resistance to one toxicant in a group may subsequently become cross-resistant to chemically-related substances (Rose et al., 1995). An economically-important characteristic of P450 monooxygenases is that their levels increase following an organism's exposure to different toxicants (Lee and Scott 1989).

Our results indicate that the populations of varroa in certain Israeli apiaries are still acquiring resistance. This is consistent with findings from abroad concerning the LC_{50} base line for tau-fluvalinate in varroa (Colin et al. 1997; Faucon et al. 1996), and that some mite populations are almost wholly susceptible while others are either intermediate or completely resistant (e.g. Lodesani et al. 1995). Variability may have been due to different selection pressures operating in the various regions, to the various methods used, to assays being conducted in regions with different patterns of resistance onset, or from combinations thereof.

Hilleshaim et al. (1996) used inhibitors of microsomal monooxygenases and esterases, and believed that increased detoxification of tau-fluvalinate by monooxygenases was the resistance mechanism of varroa, whereas general esterases play a negligible role. Our results, obtained by a different approach, also suggest that the mites had a more efficient system of detoxifying fluvalinate than the susceptible ones (Table 2, 3).

SCIENTIFIC IMPLICATIONS We began this project by devising a unique indirect diagnostic method, based on the mite's guanine excretions. Then we compared the conventional chemical HBTM control method with the use of resistant Buckfast queens, and demonstrated, for the first time, the resistance of Buckfast to HBTM under Mediterranean conditions.

The situation in regard to varroa has become very serious due to two new, unrelated factors. The first was the emergence of resistance to fluvalinate, a factor that necessitated modifying our research efforts. The other was the recognition that the

biological entity known as *Varroa jacobsoni* may in fact be several species (Anderson and Trueman, 1999) Although this aspect lies outside the scope of our project, it will become of major concern in the future.

POTENTIAL IMPACT ON AGRICULTURE

The long-predicted emergence of varroa resistance to fluvalinate requires the application of vigorous resistance management (RM) practices. RM is a central feature of integrated pest management and supports the ideal of minimizing pesticide use. RM consists of diversifying pest mortality factors, reducing selection pressure, managing susceptibility and predicting the development of resistance. The first three components include the use of various non-chemical control methods, the application of additional pesticides (whether natural or synthesized), and selecting bees tolerant or resistance to the mite (Buchler, 1994). The present project has contributed to the first two tactics. Resistance onset in varroa now urgently requires developing a generally accepted bioassay method, which necessitates a better understanding of the mite resistance mechanism. We demonstrated that monooxygenases have a major role in detoxification of fluvalinate, whereas esterases play a minor role. This finding can be used to develop rapid field assay methods and is of importance in evaluating additional chemicals to control the pest.

Varroa Water Balance Disruption: Potential for Varroa Control?

Introduction: Varroa have a large surface area to volume ratio so they lose water very rapidly if exposed to low relative humidity. The beehive is a relatively stable environment so these tropical mites take advantage of this for their survival regardless of the surrounding habitat. The focus of this study was to characterize varroa water balance to determine if some weakness in their biology could be exploited for control. More specifically we wanted to understand (a) habitat preference and suitability for varroa; (b) the potential for spread into new regions; (c) the features crucial for survival, (d) is disrupting water balance a viable control method, and (e) to provide new information about the biology of varroa for IPM. The following water balance parameters were determined for varroa females: water pool size; water loss rate; and, capability to gain atmospheric water below saturation. Mites from three US states were studied: Arizona (AZ), Minnesota (MN) and Pennsylvania (PA) to see if there are regional differences in water balance characteristics. A population of fluvalinate resistant mites from PA were evaluated for any differences that could help explain their resistance to the acaricide.

Methods: Detailed methodology is given in the appended manuscript (Yoder et al. 1999, *Intl. J. Acarol.*). Permeability (leakiness) of female mites was determined by exposing them to 0%RH and room temperature, then weighing at 2 h intervals for five periods. Data is expressed as loss of water in percent per hour. Critical transition temperature was quantified by incubating killed mites at a range of temperatures then plotting data as an Arrhenius plot (rate vs reciprocal absolute temp.). The temperature where water loss accelerated abruptly was the CTT. This was determined for bee pupae as well. Water pool sized was measured by keeping mites at 0%RH, 90°C until a constant weight was reached. The difference between initial and final mass was the water pool size. Vapor uptake ability was determined by exposing mites to various relative humidities and monitoring mass changes at 12 h intervals. Relative humidity where the mass remained constant is interpreted as evidence for active water vapor absorption from unsaturated air. To pinpoint the site of uptake, mites were pre-desiccated then exposed to 93% RH for 6 d with mouth parts wax blocked or unblocked.

Results: Table 1 in the appended Yoder et al. (1999) summarizes our findings. We detected no significant differences with regard to size, water content, net water loss rate, weight loss tolerance during dehydration, critical transition temperature, and ability to absorb water vapor from air (critical equilibrium humidity; CEH). The CEH was between 85 and 93% RH (Fig. 1A). The mouth is the site of uptake (Fig. 1B). They did not drink water when offered as droplets. Mites survived net water losses of about 37% and their rate loss was 2% per h. Rate losses for females were 1/3 less when kept in sealed brood cells versus being outside. Mites live less than 18 h at 0%RH. CTT was 30°C for both mite and bee pupa (Fig. 2).

Discussion: Understanding water balance physiology of varroa may add a new dimension to future mite management strategies. The free-roaming female mite should be targeted for control by elevating hive relative humidity to overhydrate and thereby reduce feeding and possibly kill the mite. Varroa reproduction rate is also lower at high relative humidity. Alternatively, the fast water loss rate could be exploited by reducing hive humidity and increasing temperature to desiccate mites. These scenarios would have to be tested on a hive level for impact on host bees. Winter months would be the best for such a test as bees are hardier and mites more leaky. Finally, there was not correlation between water permeability and resistance to fluvalinate. It was thought that resistant mites may be less permeable in general to molecules.

Evaluation of Essential Oils for Varroa Management:

Introduction: Some volatile plant ethereal oils may control bee mites and other bee diseases (see citations in Sammataro et al. 1998). Many beekeepers are already trying their own experiments to disrupt mite life cycles. "Natural" plant oils are complex compounds that may have unwanted side-effects on bees and beekeepers and could contaminate hive products.

Methods: A detailed methodology is found in the appended paper by Sammataro et al. (*Amer. Bee J.* September 1998). We evaluated some oils to determine the short- and long-term effects on bees, and to begin development of application techniques that would reduce hive products from contamination. We selected oils reported to be beneficial to bees and repel or kill mites. We tested them at a 50% concentration.

Lab Trials: Briefly, adult varroa (females) were exposed to the volatile fumes of the oils first. Five mites per dish with 4 treatment and 4 control dishes per type of oil or compound. Numbers of live mites were tallied at 2 h intervals. This procedure was replicated three times and a t-test was performed to determine if mortality was different between treatment and control dishes.

Field Trials: Promising oils identified in lab assays were tested in two locations, the OARDC Extension Honey Bee Lab in Wooster, Ohio and an apiary in Medina, Ohio. Origanum , cineole, a thymol mixture and a commercial mixture of Bee Calm® were used in the field tests with olive oil serving as the control. Cardboard strips soaked with the test or control chemical were inserted between frames in the hives, just as Apistan® strips would normally be (1 strip/ 5 frames). Tests were conducted in 4-8 hives per candidate treatment. Test colonies were examined and frames of brood and bees were recorded and overall fitness observed before and after the experiment. Mite counts on sticky boards after the oil treatment were divided by the number of mites counted prior to the treatment (=ratio of mites after/mites before treatment). An analysis of variance (ANOVA) was performed on the average mite ratios from each colony to determine if the treatments differed from each other or the controls.

Results: For lab assays mortality in controls ranged between 6.7 and 28.6%. Of the oils tested, origanum, thymol , clove, bay and tea tree were the most effective miticides and, After six hrs mite mortality was clearly greater than the controls; the results are summarized in Table 1 of the appended paper. Bee Calm, cineole and cinnamon were moderately effective, and patchouli was the least effective. Of the four compounds tested in the field only Bee Calm® differed from the olive oil control in its effect as a miticide (Table 3). Thymol and origanum were as effective as Bee Calm®, but not

significantly different from the control. In all cases, counts on the sticky boards were the greatest after Apistan® was inserted into the colonies.

Discussion: Bee Calm® was one of the least effective miticides in the petri dish assay but was the most effective in the field tests. All of the cardboard strips were chewed up by the bees, suggesting that the delivery system be improved before additional essential oil tests are performed. Despite the apparent progress with these compounds Apistan® strips were still much superior to any of the test treatments. Impact of these materials on colonies needs further investigation. In general these introduced compounds were not well received by the bees as evidenced by their fanning at the hive entrance. More work needs to be done on different concentrations of these and other materials.

Scientific Implications: Apistan® strips remain the best option for varroa control in the US and Israel, although serious problems with resistance are developing. It was hoped that one or more of the essential oils would provide the next generation of active ingredient for varroa management. Following this study it is clear we are far from finding the next 'silver bullet' for mite control. Assuming that because something is of plant origin means it will be user friendly and effective is not correct. Bees reacted very poorly to these compounds and most likely they will alter the honey flavor and contaminate the other hive products as well.

SUMMARY OF IPM STRATEGY FOR THE US (SAMMATARO ET AL. 1996, 2000)

How are beekeepers going to keep colonies that survived the winter from succumbing to parasitic mites? Assuming that both mites (varroa and tracheal) are prevalent almost everywhere, it is essential for beekeepers to have a plan to reduce mite levels and to keep bees healthy.

Our four-year project objective was to develop an integrated pest management (IPM) program for managing mites and bees while reducing use of synthetic acaricides. We proposed using both existing and experimental treatments, with novel tactics, as part of regular hive maintenance to keep mite populations below the economic injury levels. In our approach, we are treating the colony unit as a crop of bees and brood that needs to be sampled and treated at intervals, before the population of pests (in this case the parasitic mites) become too high and kills the colony.

IPM is a strategy used by many farmers and growers to control pests in their crops without relying exclusively on one chemical or treatment, but rather on managing the pest or pests with a variety of tactics, including chemical, behavioral, mechanical and others. The key components of such a scheme include: 1) knowing the life cycles and

seasonal rhythms of the pest(s), 2) measuring the economic injury level (the lowest number of mites that will cause damage) and economic damage (how many mites must be present before controls are justified), and 3) timing the different control measures to coincide with the most susceptible time of the pest's life cycle. Controls include pesticides, natural enemies, host resistance or tolerance, modifying the environment and impairing the pest's reproductive capacity.

There are several reliable chemicals that control mites; but they must not be abused or their effectiveness will diminish. While resistant lines and strains of bees will help in the long-term, it should be remembered that the mites may be changing along with the bees. We hope to "keep up" with the arms race between bees and mites.. We cannot afford to let nature take its course (i.e. not treating bees), or there will not be enough bees to make honey and to pollinate commercial crops.

First, we realize that there are two mites to manage and their biology, requirements and the nature of their damage is quite different. Colonies should be tested twice a year. Another problem associated with varroa mites (and maybe when both mites are present) is the *Bee Parasitic Mite Syndrome* studied by many researchers. In general, the common factor is varroa and the colony declines; there is no one separate cause for hive death, though microbial and viral infections are present.

CONTROL of TRACHEAL MITES IN THE US

A chemical that helps control these mites is menthol crystals, available in 1.8 oz packets (50g), which is enough to treat one colony. The main problem with menthol is its temperature-dependence for the vapors to work effectively in the colony. If the outside temperature is too hot, the menthol will sometimes drive the bees out of the hive; if too cold, the crystals will not vaporize. For menthol to work properly, the air temperature should be around 70°F (21.1°C) for at least two weeks; under the right conditions, menthol vapors will kill tracheal mites in the bee's tubes. The continuous presence of vegetable shortening (like Crisco) or oil and white sugar (or powdered sugar) patties, placed on the top bars of the broodnest frames, was investigated by us and reduces tracheal mite populations. Extender patties made with Terramycin control bacterial infections bees may get while under stress. These are the only two treatments for tracheal mites in the U.S. However, the Buckfast strain of bees shows some tolerance to tracheal mites, which means that the mite levels are lower than in other strains and under most circumstances, will not kill bees.

CONTROL of VARROA

Currently, there is one chemical registered in the U.S. to control varroa and that is fluvalinate sold in plastic-impregnated *Apistan Strips*®. It is an effective contact poison that kills only varroa. Label instructions require the strips must be removed before supering for honey.

Biological and Cultural controls for varroa mites now being used include:

- Restricting brood rearing by caging the queen and removing capped brood, then treating adult bees with Apistan® to kill mites.
- Trapping mites in drone-comb brood (freeze); this reduces mite levels in a colony.
- Blowing hot air into hives at 108-118°F (42-48°C), used with a sticky board; heating frames of bees to kill mites is effective, but not practical for beekeepers with more than a few colonies. BeeCool hive ventilators may discourage mites by lowering relative humidity but this needs to be tested.
- Some botanical oils show promise but their use, dosage and delivery still need refinement.

Tolerance Factors, or breeding bees tolerant of varroa, is currently underway. Many researchers have noted certain characteristics about mite and bee behavior that are helpful in suppressing varroa levels. This inventory of behavioral traits is by no means complete, but indicates where future research will be concentrated. Look for any one or combination of these behaviors in colonies:

- **Hygienic behavior:** bees open cells with mites, remove infested brood.
- **Grooming behavior:** bees remove mites from each other, killing the mites with their mandibles (seen in some strains of Carniolan bees).
- **Length of post-capping stage:** bees pupate in fewer days (less than 12); the longer pupae are in the cells, the more time mites have to develop. African worker bees will emerge in 11 days, while Europeans do so in 12 days. This may be heritable, but don't forget that mites could also adapt to develop faster too. Smaller sized worker comb cells (5.0 mm in diameter) may also help in accelerating pupal development.
- **Brood attractiveness:** so far, European bees are highly attractive to varroa, with the exception of the German Black Bee (*A. m. mellifera*) and maybe the Africanized bee. Also, mites reproduce better on drones (90-97% of the time) than on workers (47-84% of the time).

• **Low mite fecundity:** mites are not reproducing or are only replacing themselves. The mechanisms of this are currently unknown. Some scientists are beginning to suspect that the plastic in frames or foundations may be one of the causes.

• **Length of phoretic period:** mites are attached to adult bees for a longer period of time; since this is a non-reproducing stage, the mites are more at risk from being groomed or picked off.

Chart 2. Sequence of Suggested Treatment Times for Bee Mites. This chart is a hypothetical representation of bee brood and mite populations over a bee season. It is offered as a guide to beekeepers, so they can visualize the relationship between mite population levels as they fit in with bee colony management practices.

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Appendix

Unpublished Data

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APPENDIX

Legend for Figure 1. Log dose vs. probit mortality of varroa mites treated with tau fluvalinate from two sites [squares: Yesodot (Y); diamonds: Nehalim (N)] with reduced response to Apistan®.

Table 1. Infestation levels of varroa mite before (b) and after (a) being treated with one strip of Apistan® for six to eight weeks, 1993 and 1997.

Site	Date	# of colonies sampled	Mite drop after fumigation Average	Standard deviation
Kfar Malal	26.07.1993 (b)	21	100.7	191.7
	08.09.1993 (a)	29	0.65	1.1
Giv'at Chen	27.07.1997 (b)	12	33.3	16.9
	13.09.1997 (a)	12	2.5	4.3
Kfar Malal	27.07.1997 (b)	17	36.1	24.9
	13.09.1997 (a)	17	10.7	22.1
Ga'ash	10.08.1997 (b)	4	50.5	25.1
	10.10.1997 (a)	4	57.5	26.7
Yas'resh	13.07.1997 (b)	29	58.2	63.9
	15.09.1997 (a)	29	82.9	99.8
Nezer	17.07.1997 (b)	17	32.5	26.9
	17.09.1997 (a)	17	76.0	55.0

Table 2. Enzyme (monooxygenase and general esterase) activity of homogenates of varroa mites from Nehalim and from Yesodot that had survived exposure to various pesticide rates (average and SD of 4 samples for 25 and 50 ppm treatments and of 2 samples for 150 ppm).

Source and treatment	monooxygenase nmol/min/ μ g	Esterase nmol/min/ μ g
Yesodot 25 ppm	0.65 $\pm$ 0.11	329.0 $\pm$ 11.5
50 ppm	3.13 $\pm$ 1.05	356.8 $\pm$ 39.0
150 ppm	6.34 $\pm$ 0.50	389.3 $\pm$ 25.0
Nechalim 25 ppm	1.98 $\pm$ 1.10	252.9 $\pm$ 11.4
50 ppm	4.37 $\pm$ 0.73	301.6 $\pm$ 9.0
150 ppm	4.90 $\pm$ 1.10	368.8 $\pm$ 13.8

Table 3. Enzyme (monooxygenase and general esterase) activity in homogenates of varroa mites collected from Yas'resh, Rehovot and Kfar Truman - 1998 (three different colonies from each site); (average and SD of 4 samples).

Sites	Esterase nmol/min/ μ g	Monooxygenase nmol/min/ μ g
Yas'resh	451 $\pm$ 40	4.38 $\pm$ 0.7
Rehovot	206 $\pm$ 8.4	0.23 $\pm$ 0.022
Kfar Truman	168 $\pm$ 12.7	0.336 $\pm$ 0.17