דו"ח **מסכם**

לתכנית מחקר מספר 131-1815-15

דרכים חדשות להקטנת נזקי אקרית הוורואה בדבורת הדבש במכוורות ישראל

מוגש לקרן המדען הראשי במשרד החקלאות

ע"י

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שמות השותפים למחקר, שטח הפעולה של כל משתתף, תיאור קצר של חלקו במחקר

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3. פיתוח תוכנית להדברה משולבת של אקרית הוורואה על בסיס חומצה פורמית ושמנים אתרים: אריק פלבסקי, אקרולוג, palevsky@volcani.agri.gov.il, שירה גל, אקרולוגיה, מרכז מחקר נווה יער, מנהל המחקר החקלאי; יוסי סלבצקי ואוהד אפיק מדריכי דבורים משה"מ.
4. זיהוי, אפיון וטיפול כנגד ווירוסים מועברים על ידי אקרית הוורואה: נור צ'חנובסקי, וירוסים פתוגנים לחרקים, ninar@volcani.agri.gov.il, המחלקה לאנטומולוגיה, מנהל המחקר החקלאי, סופיה לוין (דוקטוראנטית) טל ארז.
5. שיבוש התנהגות בחירת הפונדקאי על ידי אקרית הוורואה: סורוקר ויקטוריה, התנהגות ותקשורת כימית, sorokerv@agri.gov.il, נורית אליאש (תלמידה לתואר שלישי - ניסויי התנהגות הוורואה ואלקטרופיסיולוגיה) ניטין סינג (בתר דוקטור ניסויי התנהגות הוורואה ואלקטרופיסיולוגיה של דבורים), לאוניד אנשלביץ (אלקטרופיסיולוגיה ותכנון מערכות ניסוי), יוסי קמר ואיליה זידמן (טיפול בכוורות ובחינת חומרים משבשים בכוורות) מנהל המחקר החקלאי, בית דגן; אריקה פלטנר (Erika Plettner, Simon Fraser University, Canada).

תקציר

הצגת הבעיה: ללא הדברה יעילה של אקרית הוורואה לא ניתן לקיים מכוורת מסחרית. נתיב ההדברה הכימית נראה מתקרב למבוי סתום, מצב המחייב פתרונות מהירים לטווח קצר ופתרונות לטווח ארוך.
מטרות: 1) בדיקת ממשקי הדברה קיימים, בדיקת עמידות ובדיקת קוטלי אקריות שעדיין לא נבחנו במכוורת. 2) בחינת מעורבותם של חיידקים בעמידות הוורואה לתכשירי הדברה. 3) פיתוח תוכנית להדברה משולבת של אקרית הוורואה על בסיס חומרים ידידותיים. 4) זיהוי, אפיון וטיפול כנגד ווירוסים שמועברים, מופעלים ומוגברים על ידי האקרית. 5) שיבוש ההכרה הכימית בין אקרית הוורואה לדבורה.
שיטות ותוצאות עיקריות של קבוצות המחקר: 1) פותח מבחן מעבדה לבחינת רגישות הוורואה למגוון אקריצידים ומספר תכשירים ובראשם פנזקווין נמצאו יעילים בהדברת וורואה ובטוחים לשימוש עבור הדבורים, אולם יעילותו בתנאי שדה לא הוכחה. בנוסף, נבחנה לראשונה בארץ עמידות גנטית של אקריות לפלוולינאט ונמצא שיותר מ- 50% מהאקריות נושאת את הגן לעמידות ונערך ריצוף של גן הקולטן לאוקטופאמין, במטרה לבחון התפתחות אקריות עמידות לאמיטרז, התכשיר העיקרי כיום כנגד הוורואה.

2) טיפול בקומאפוס גרם לנפילה של כ-70% מכלל האקריות הפורטיות שבכוורת ולא נמצא הבדל באחוז האקריות שנפלו בין כוורות ביקורת לכוורות שטופלו באנטיביוטיקה. לא נמצאה תמיכה להשערה שמקור העמידות של הוורואה לקומאפוס הוא באוכלוסיית החיידקים הסימביונטים של האקריות. 3) מספר האקריות הפורטיות הופחת באופן מובהק בטיפולים בחומצה פורמית ובתימול יחסית לרמות האוכלוסייה לפני הטיפול, אך נגרמה פגיעה מסויימת בוולד. השיטה של שימוש במיכלים לשיחרור מבוקר של חומצה פורמית נמצאה כיעילה ביותר מבין השיטות שנבחנו. המטען הוירלי הופחת בטיפול בחומצה פורמית במשך שנתיים בכפר רות, לא הושפע בטיפול באמיטרז ועלה בביקורת. 4) האקרית מכילה כמות עצומה של וירוס עיוות הכנפיים DWV המשתכפל בה, בהשוואה לדבורה שאליה נטפלה. בנוסף זהינו הופעה של וירוס חדש DWV-VDV1 ריקומבינננטי בין ווירוס האקרית VDV1 לוירוס DWV, שהוא ככל הנראה אלים יותר עבור הדבורים וגורם להחמרת הנזק הנובע מנגיעות באקריות. נבחנה מערכת מודל לטיפול אנטיוויראלי בדבורים להקטנת נזקי הוירוסים אולם בשלב זה לא הוכחה יעילות הטיפול בתנאי שדה. 5) נערכה סריקה רחבה של חומרים בעלי פוטנציאל לשיבוש יכולת החישה של הוורואה. בניסויי מעבדה נמצאו שישה חומרים בעלי השפעה שפעלו במנגנונים שונים ובהם פגיעה ביכולת האקרית למצוא את הדבורה, שיבוש יכולתה להבחין בין דבורה מטפלת לדבורה משחרת ופגיעה ישירה באקריות. נבנתה מערכת ניסוי של מיני-כוורות לבדיקת יעילות החומרים בתנאי שדה, אך עד כה לא הוכחה יעילותם בשטח.

<u>מסקנות:</u> 1) קיימים תכשירים ישנים בעלי התכנות להדברת הוורואה, אולם דרושה השקעה רבה בפיתוח שיטות ליישומם בשטח. פותחו כלים מולקולאריים שיאפשרו מעקב אחר התפתחות העמידות לתכשירים הקיימים 2) תוצאות המחקר בשילוב המסקנות ממחקר 1 מחלישים את ההשערה שמקור העמידות של הוורואה נובע מהתפתחות אוכלוסיית חיידקים סימביונטים המקנים עמידות 3) יישום חומצת נמלים בכוורות במיכלים לשיחרור מבוקר נמצא כבעל פוטנציאל להתמודדות עם הוורואה ויתכן ואף ישירות עם המטען הויראלי, אולם עדיין נחוצה עבודה למציאת ממשק הטיפולים הנכון לאורך השנה בתנאי שדה . 4) קיימת חשיבות רבה להבנת מערכת יחסי הגומלין שבין האקרית, הדבורה והעברת הווירוסים שביניהן. יש מקום להמשך פיתוח של מערכת לבדיקת חומרים אנטיוויראליים לדבורים. 5) נמצאו חומרים המשבשים את התנהגות הוורואה ובטוחים לדבורים אך עדיין יש להמשיך ולבחון את יעילותם, בעיקר בשדה. נבנתה מערכת יישומית של מיני-כוורות ככלי לבחינת יעילות תכשירים בשדה, אך גם כאן יש עוד מקום לשיפורים.

<u>מעריכים מומלצים לבדיקת הדוח המדעי</u>

1. פרופ' אורי גרזון. 2. ד"ר ארנון דג. 3. מר יצחק מלכה

הצהרת החוקר הראשי:

הממצאים בדו"ח זה הינם תוצאות ניסויים. הניסויים לא מהווים המלצות לחקלאים:

חתימת החוקר

תאריך: 30/6/2017

Final Research report for project number 131-1815-15
Project titled:
Novel methods for controlling the *Varroa* mite in honey bee hives in Israel
Submitted to the Chief Scientist of Agriculture of Israel

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WP-I Monitoring resistance and identifying new chemicals for *V. destructor* control
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WP-II Determining the role of symbionts in *V. destructor* resistance –
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WP-III Identifying optimal timing for formic acid and thymol treatments - Eric Palevsky and Shira Gal, Newe-Ya'ar, ARO; Ohad Afik, Shlomi Zarhin and Yossi Slabezki, Extension service, Shaham, MOAG.
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WP-IV Determining the effects of *V. destructor* on virus virulence and evaluate potential antiviral treatments – Chejanovsky and Sofia Entomology, Bet Dagan, ARO;
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WP-V identifying and evaluating compounds to disrupt the orientation of *V. destructor* with respect to finding its bee host - Soroker et al. Entomology ARO.
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General Introduction

The heavy losses of honeybee colonies reported worldwide represent a serious threat to agriculture since one third of the world's food crops depend on honey bee-mediated pollination. It is clearly accepted that the ectoparasite *Varroa destructor* along with transmitted honeybee viruses are the leading causes of elevated rates of mortality and colony collapse worldwide. The recent development of *V. destructor* resistance to almost all known synthetic acaricides in Israel and abroad has demonstrated the importance of developing a sustainable approach to control this pest. To accomplish this general objective this multidisplinary project includes the following workpackages (WP) each with its specific objectives: WP-I monitoring resistance and identifying new chemicals for *V. destructor* control; WP-II determining the role of symbionts in *V. destructor* resistance; WP-III identifying optimal timing for formic acid and thymol treatments. WP-IV determining the effects of *V. destructor* on virus virulence and evaluate potential antiviral treatments; WP-V identifying and evaluating compounds to disrupt the orientation of *V. destructor* with respect to finding its bee host.

WP-I Monitoring resistance and identifying new chemicals for *V. destructor* control

Introduction

Current Varroa control in Israel is based almost entirely on the acaricide Amitraz (formamidine). Synthetic pyrethroids have been used such as tau-fluvalinate and organophosphates such as coumaphos, but these were lost to resistance some years ago. Official and unofficial reports are available regarding Varroa resistance to amitraz (Maggi et al. 2010), with no available knowledge regarding the biochemical or molecular basis for resistance. Amitraz is applied at least twice per year, with a treatment duration of six weeks before strips are removed and disposed of. Consequently, there is a constant fear of resistance development, especially as it seems that some Varroa populations already show initial levels of resistance. It was thus urgent to map the spatiotemporal response of Varroa populations in Israel to amitraz, and other previously used compounds (Mozes-Koch et al. 2000), and to identify other acaricides that could be alternated with amitraz to prevent or delay resistance. It was also important to understand the nature of resistance to tau-fluvalinate and whether the same resistance mechanisms that exist in Israel are similar to those reported in Europe. Another important objective was to monitor whether resistance to Amitraz exists in Israel and if so to test whether this

resistance has a molecular basis that could be monitored, as the molecular basis for resistance to this compound is not yet reported.

Research objectives 1)Develop and establish a bioassay for testing the response of Varroa to acaricides for resistance monitoring in Israel, and for testing new acaricides. 2)Screen new acaricides for efficacy against Varroa and toxicity for bees, and evaluate their potential to be used in alternation with existing acaricides mainly amitraz. 3)Study the biochemical and molecular basis for Varroa resistance to acaricides mainly amitraz.

Materials, Methods and Results

Bioassays were developed and used for Varroa and honeybee response to acaricides. Two bioassays were developed: the first consisted of a Petri dish, with the cover ventilated via a metal mesh, and the bottom of the dish filled with 2% agar mixed with the acaricide at the desired concentration to be tested (Fig. I.1A). For testing honeybee response to acaricides, the system consisted a plastic vial ventilated via holes in the cover. The cover included a glass vial for providing sugar water for honeybee feeding (Fig. I.1B). In the bottom of the vial, filter paper mixed with the tested acaricide at the desired concentration was provided. Both methods were standardized and worked well for proper evaluation of mortality among Varroa and honeybees, and were used during the course of this project for resistance monitoring, evaluating new acaricides and various experiments.

Collections were made in several locations in Israel, for testing Varroa response to amitraz. Locations were mainly chosen based on information provided about decrease in Varroa susceptibility to amitraz treatments or where treatments were not conducted. Each season, the efficacy of amitraz was variable. Table I.1 shows all the collections made in the different locations, the different concentrations tested for Varroa response to amitraz and the mortality rates recorded after different time points. Following these trials, it was decided that the assay duration be extended to 18 hours.

During the first and second years, the list of acaricides that appears in Table I.2 was tested for efficacy against varroa and toxicity to adult honeybees, using the bioassay described in Fig. I.1B. These acaricides alongside with fenazaquin, were chosen based on their low toxicity to honeybees as indicated by the producer. As seen in Fig. I.2, while amitraz and vertigo (abamectin) showed high toxicity that reached up to 90% mortality after 6 days, the rest of the new tested acaricides caused very low levels of mortality even after 9 days, suggesting that those acaricides are good candidates to be tested for

their efficacy against Varroa. In two separate experiments, fenazaquin at a concentration of 0.05% showed efficacy equivalent to amitraz, and in a second experiment it showed higher efficacy compared to defender and acrimite (Table I.3), suggesting that this compound was the leading one for further tests on honeybee hives. Subsequently we evaluated in commercial hives the efficacy of fenazaquin against Varroa and its safety to honeybees by applying two strips per hive, fumigation and by spraying the frames from above. While no toxicity to honeybees was recorded, no efficacy against Varroa was observed. Thus, we recommend that further research be conducted to determine suitable rates and forumulations.

Molecular identification of the resistance to fluvalinate and Amitraz. The collected individuals from the field were subjected to RNA extractions and cDNA synthesis from each individual. The DNA was then used to amplify ~2,100 base pairs from the voltage-gated sodium channel (VGSC) gene in a hot spot region known to harbor mutations for resistance against pyrethroids. The mutation L924V that was reported to confer resistance to fluvalinate was detected in more than 50% of the tested individuals (Fig. I.3). This molecular analysis identified further mutations (Fig. I.4) that could be related to the resistance. Furthermore, a highly sensitive qPCR assay is still under development to screen for resistance individuals of Varroa in a rapid and more accurate test.

For amitraz, the full length coding sequence of the octopamine receptor gene, the traget for amitraz was recently cloned from populations collected in Israel. The gene is 1,101 base pairs long and encodes for a protein of 366 amino acids (Fig. I.5). Several primers (Table 1.4) were used for the cloning and some were designed for qRTPCT assay from suspected resistant and susceptible populations that were previously collected from Israel and that will be collected in 2017. Currently, sequences from both suspected resistant and susceptible populations are being obtained and analyzed to identify possible differences in the sequences that could be correlated with resistance.

<u>Summary</u>

While bioassays on field collected Varroa populations showed varying levels of susceptibility to amitraz, it generally still seems to be effective, because even if the resistance is carried by field populations, some other conditions such as climatic, nutritional and others may still be in favor of susceptible populations. Our molecular study suggests widespread existence of the mutation L925V, shown to confer resistance to fluvalinate in the VGSC gene, despite cessation of application for several years by most bee keepers. Hopefully the continued banning of this product and education

disseminated by extension agents to halt usage will eventually lead to an increase in the proportion of susceptible mites. The reuse of amitraz on a large scale will probably lead to selecting for more resistance populations that carry the resistance and this will increase the possibility for widespread resistance nationwide. In collaboration with Dr. Joel Gonzales from Spain we cloned the full length sequence of the Octopamine receptor gene, the target for amitraz, from Israeli Varroa populations. We also designed primers for performing a quantitative assay to measure the levels of gene expression of this gene in possible resistant and susceptible populations.

WP-II Determining the role of symbionts in *V. destructor* resistance

Introduction

Bacteria that can break down chemicals, and specifically organophosphates, are found in different environments, including in soils and water (Paris and Lewis 1973; Shelton and Somich 1988). Lately, it has been found that symbiotic bacteria in insects can break down organophosphates and thus render resistance to their insect hosts (Kikuchi et al. 2012; Tago et al. 2015). Of specific interest to our study, bacteria have been found in Varroa that can break down oxalic acid and are suspected to be involved in the need for a greater number of treatments of oxalic acid to control Varroa in colonies (Maddaloni and Pascual 2015). We therefore hypothesized that symbiotic bacteria of Varroa mites may also contribute to the evolving resistance of the mite to acaricides**.** We focused on coumaphos, which is the latest acaricide that *Varroa* mites evolved resistance to in Israel. Research objective Our research aim was to compare the survival of Varroa mites when treated with coumaphos, between untreated mites and those pre-treated with wide-range antibiotics, in both laboratory conditions and at the colony level.

Materials, Methods and Results

Cultivating symbiotic bacteria: We managed to cultivate bacteria that is found in Varroa mites that are resistant to coumaphos and not found in coumaphos-susceptible colonies and identified them as α-proteobaterium, previously isolated from honeybee gut (AC: AJ971850). However, when we tested by HPLC whether these bacteria can dissociate coumaphos to its derivative compounds found previously in other bacteria, we could not distinguish between coumaphos and its derivative compounds. We also searched by PCR for known genes in the bacteria that are involved in dissociation of organophosphorus compounds, but we did not find such genes.

Rearing Varroa mites on agar plates: Given the above technical difficulties, we changed our approach to rearing Varroa mites in the lab and testing whether resistant mites are more susceptible to coumaphos in the presence of broad-spectrum antibiotics. We managed to optimize (controlling temperature and humidity) a protocol developed in the Ghanim lab (as part of WP1), showing that mites survive best (even 44 hours) when grown on agar plates rather than in artificial queen-raising cells, and when 10 rather than 4 mites were raised together (Table II.1). Using this technique, we found that Varroa mites from our untreated colonies were in fact susceptible to coumaphos, with about 80% of the mites dying when raised on agar with 10 ppm coumaphos. Interestingly, survival of Varroa from sick hives was a mean (± SE) of 17.8% (± 0.07), whereas from healthy hives survival was 24.4% (± 0.03). Without coumaphos, survival was 65.7 (± 0.10), and 87.7 (± 0.03), for sick and healthy hives, respectively.

Antibiotics delivery to *Varroa* mites on agar plates: To test whether we can deliver antibiotics to Varroa mites through pre-pupae fed with antibiotics, we fed honey bee brood with royal jelly containing different concentrations of tetracycline. Tetracycline is a broad-spectrum antibiotic already used in hives for the prevention of bacteria diseases. After feeding full-grown larvae with royal jelly containing tetracycline, we waited 2 days for the cells to be sealed, pooled them out from their cells as pre-pupae, and squeezed them on two known bacteria growth culture media. We did not see consistent results in cultivated bacteria from pre-pupae previously fed or unfed with tetracycline. Hence, we have not yet found a reliable marker for knowing if we did succeed in antibiotics delivery.

Effect of broad-spectrum antibiotics on Varroa resistance to coumaphos at the colony level: We conducted two replicates of the experiment. In each replicate, we created two similar colonies, containing only phoretic Varroa, on bee bodies. To the treatment colony (A in replicate 1 and D in replicate 2; Fig. II.1) we added antibiotics (1 ppm Tetracycline, 10 ppm Piperacycline, and 100 ppm Ciprofloxacin), whereas to the controls (B and C) we did not add antibiotics. We treated the colonies with coumaphos by inserting a Check-mite strip into each hive. We then monitored hourly the number of mites that fell, for the following 12 h. In order to assess the total number of mites that were in the colonies at the beginning of the experiment (and how many did not respond to the coumaphos treatment), the following day we treated the colonies with oxalic acid, and after two days, another treatment with oxalic acid, and in addition we treated with amitraz (Glabitraz stick), tau-fluvalinate (Apistan strip), and coumaphos (Check-mite strip).

The number of Varroa mites that fell during the various stages of the two replicates is shown in Figure II-1. Overall, the percent of phoretic mites that fell after coumaphos treatment was between 64% and 73%. In replicate 1, the percent mites that fell after the first coumaphos treatment relative to all the mites that were in the colony (as counted after delivery of all the treatments) was similar in the antibiotics-treated (A) and control (B) colonies (Likelihood ratio chi-square test, $X^2 = 2.8$, $N = 1141$, $P = 0.09$). In replicate 2, surprisingly, a relatively higher percent of mites fell in the control (D) than in the antibiotics-treated (C) colony colonies (Likelihood ratio chi-square test, $X^2 = 10.4$, $N = 1848$, $P = 0.001$). Interestingly, mites began falling soon after first treatment with coumaphos, to a peak at around 4 h after treatment; most mites had fallen by about 8 h (Figure II-2).

Summary

We developed some techniques to test our hypotheses, but we did not find that broad-range antibiotic treatment reduced the ability of Varroa mites to resist coumaphos treatment. It may be that greater concentrations of antibiotics are needed to perceive an effect. Future directions could also be to test bacteria of the genus *Burkholderia,* recently isolated from Varroa, characterized, and cultured in the lab (Tago et al. 2015). This genus contains species able to break down organophosphates in diverse insects (Kikuchi et al. 2012; Tago et al. 2015), and could potentially break down coumaphos.

WP-III Evaluation of alternative measures for Varroa control

Introduction

Formic acid (FA) is the only compound that provides effective control of Varroa in the sealed brood cells. However it also can be lethal to bee larvae in the unsealed brood cells, to the freshly emerging adult bees following pupation and to the queen. Additionally high ambient temperatures can increase mortality incidence and low ambient temperatures could cause the treatment to be less effective. Amrine and colleagues developed a short term method for FA applicaton where FA 50% was applied on a thick filter card placed on spacers, above the brood frames. The essence of this methodology is that within 24 hours FA vapors return to pre treatment levels (Amrine and Noel 2006; Amrine et al. 2007).

While essential oils have been shown to significantly impact Varroa populations in the hive, they can only affect the Varroa that are exposed, as the essential oil volatiles do

not penetrate the sealed brood cells (Umpierrez et al. 2011). Thus their efficacy is dependant on a steady state of volatiles within the hive.

Objectives-Develop an integrated management program for Varroa control based on formic acid and essential oils.

Methods and Materials

Field trials were conducted with commercial growers, comparing alternative products to the standard commercial treatment of amitraz and a non treated control (Table III.1). Two methods using diluted technical grade FA were evaluated. In years 1 and 2 we used the short term FA method developed by Amrine and colleagues (Amrine and Noel 2006; Amrine et al. 2007) and in years 3 and 4 (January through February) we assessed the evaporator developed by Nassenheider (http://bee.nassenheider.com/artikels/view/1/0/0/0/0/149) (Picture III.1). This system is composed of a 300 ml container, a wick and an evaporating tray. The wick comes in 3 sizes to allow for regulation of the evaporation rate, a function of the ambient temperature. Together with the management of Nassenheider we designed an extra small wick (now the 4th size), which was tested and proved suitable for our summer conditions.

In year 3 we also evaluated the FA slow release formulation 'Mite-Away Quick Strips™ MAQS' (https://www.brushymountainbeefarm.com/downloads/MAQSapplication.pdf). The thymol product (made available by the bee keeper Anna Barenboim) consisted of thin wood strips impregnated with thymol (made in the Ukraine, Picture III.2). In two story hives, 3 strips were placed between the frames of the nest box, following the same methodolgy used for the standard amitraz sticks.

In year 3, via the chemical company http://www.luxembourg.co.il/ we attempted to import the French thymol product Apigaurd. Unfortunately, the Israeli customs stopped the shipment because its papers were not in order and it was returned to France.

Additionally, After the first year of our project, we were asked by the steering committee to evaluate the product Hopgaurd (http://www.imkerverband.info/kalender/HopGuard%20-%20Usage.pdf), based on hops beta acids.

Prior to treatment hives were assessed, which included hive weight, number of combs populated and number of combs with open brood, the latter two parameters serving as indicators of hive health. Phoretic Varroa levels were estimated by amitraz fumigation with paper slips. Two post treatment evaluations were conducted. The first, to estimate negative impact on hive health, including queen loss, oviposition and number of combs

populated, performed one week post treatment. The second, aimed at assessing hive health six weeks post treatment, we measured hive weight, frame counts and phoretic Varroa populations as done prior to the treatment. In year 1 only, we estimated mortality of Varroa within brood 1-4 days after treatment, by opening 100 drone cells per hive and counting the number of live and dead Varroa. In years 2 and 3, for the evaluation of treatment effect on viral load, we sampled a 50 ml vial of bees from each replicate of FA, Amitraz and control, before and six weeks post treatment. Viral loads were assessed as described in package WP-IV.

Results

Mean % mortality of Varroa in brood cells was very high in the FA short term treatments on one and two stories, ranging from 88-98% in comparison to 3-18% in the Amitraz and non treated controls, respectively (Figure IIIa.1, 2014) . While the mean number of phoretic mites was significantly lower in the FA treatment compared to the non treated control, efficacy was inferior to the Amitraz treatment (Figure IIIa.2, 2104). Clearly the most problematic result following the application of the FA short term treatment was queen loss (Figure IIIa.3, 2104). Mean number of phoretic mites were significantly reduced in the short term FA and thymol treatments compared to Varroa levels prior to treatment (Figures III-4, 2015). While this result was encouraging, the efficacy was in consistent and inferior to the standard Amitraz treatment (Figures III-5, 2015). Unfortunately, this was not the case in the Hopgaurd treatment, as mite populations were invariably higher in the post treatment evaluation, and basically similar to the non treated control (Figure III-6, 2015). In 2016 in Kefar Ruth all treatments were significantly better than the non-treated control. In Yad Mordechai the Galbitraz (amitraz) sticks were significantly more effective than the MAQS slow release pads, while the Nassenheider evaporator did not differ signficantly from the Galbitraz. Similarly in Dan both treatments (the Nassenheider evaporator and Galbitraz) differed significantly from the control. In the trial conducted from January to February 2017, control attained with the Nassenheider evaporator was similar to Galbitraz. Brood in the hives exposed to FA at 6 weeks post treatment was substationally less homogenous than the Galbitraz and control, but after 8 weeks, these differences were no longer distinguishable.

Summary

Both thymol and the short term FA treatments significantly reduced mite levels compared to the non-treated controls. However their level of efficacy was never

comparable to the Galbitraz treatments. In contrast Hopguard had no effect on Varroa control. This result is similar to those reported from the USA and Canada (Oliver 2014; Vandervalk et al. 2014). While the short term FA treatments was relatively safe on two stories it was very problematic on one story as queen loss was very substantial (Fig.III.3). In contrast the Nassenheider evaporator evaluated in 2016 and 2017 proved to be substantially more promising with respect to efficacy, nearing that of Galbitraz. Regarding the evaporation rate of FA, the large wick is evidently suitable for winter applications, based on the last trial conducted in Kefar Sirkin, Jan.-Feb. 2017. However from April through October we suggest using the extra small wick specifically designed for our hot temperatures in Israel. This will allow for a continuos release of FA over a two week period, approximately 20 cc/day of 65% FA. Interestingly it was recently shown that FA released from the Nassenheider evaporator has no negative impact on cognitive bias in honey bees (Schlüns et al. 2017). Essentially meaning that bees are not liable to flee from the hive after their first exposure to FA, providing the release rate is not excessive. Another very important advantage of using the Nassenheider evaporator kit is that all of its components are re-usable and that the user has complete control of the product (the user sees that the 300 ml bottle is full before placing it on the tray and can confirm the wick size). In contrast, MAQS and thymol formulations are black boxes. It is impossible for the grower to know, monitor or adjust the release rate of these products. Having said that it is very important to stress that FA 65% is dangerous and appropriate measures must be taken when transporting and handling the Nassenheider evaporator kits. Additionally, because it is a liquid product, care must be taken to prevent spillage, meaning the hives must be relatively level.

WP-IV Identification characterization treatment viruses transmitted by *V. destructor*

Introduction

Varroa destructor bear viruses and appears to facilitate the appearance of more virulent strains of the Deformed Wing Virus and *Varroa* destructor virus-1 (DWV and VDV-1, respectively) (Neumann et al. 2012). In this work package we characterized the above viral load of Varroa and its interaction with the honey bee. Following this characterization

we evaluated the impact of anti-Varroa and antiviral treatments on the virulence of DWV and VDV-1.

Research objectives

I-Characterize the viral load of DWV and VDV-1 of Varroa and its interaction with the honey bee in Varroa infested colonies.

II- Evaluate the impact on the health of honey bee colonies of a combination of anti-Varroa and anti-viral treatments.

Materials and Methods

To characterize the loads of DWV, VDV-1 and recombinant f DWV-VDV-1 we utilized quantitative RT-PCR with primers that we developed from sequences we obtained throughout our study (Table IV.1). This methodology was implemented as well to evaluate viral loads in Varroa-treated colonies. Also, we used laboratory bioassays to evaluate the impact of the antiviral treatments on the health and viral loads of Varroa-infested honey bee adults. For that purpose we used a fine brush to place Varroa from highly infested colonies on the back of healthy adult bees (30 bees with 2-3 mites in acrylic cages) fed on a sucrose solution plus or minus AV (antiviral) treatment. We monitored daily bee survival and DWV levels in antiviral treated and untreated individuals.

Results and Conclusions

Since the most common viruses transmitted by Varroa mites are DWV and VDV-1 we analyzed the load of these viruses in individual symptomatic bees and their parasitizing Varroa. High copy numbers of DWV and VDV-1 were detected in honey bees and Varroa from our study-colonies. The mites showed higher number of viral copies per µg RNA compared to the parasitized bee. Our results demonstrate that the DWV virus and VDV-1 replicate in Varroa mites. These results correlated well with recent reports that Varroa may not only enhance replication of DWV by immune-suppressing the honey bee but also it may stimulate appearance of more virulent strains of DWV. These strains may emerge from recombination between DWV and VDV-1 that bear high homology. We tested for the presence of DWV-VDV-1 recombinants in honey bees and Varroa using an RT-PCR approach based on a combination of primers from DWV and VDV-1 and found a recombinant DWV-VDV-1 virus that replicated in Varroa and its parasitized bee. We determined the copy number of DWV, VDV-1 and the DWV-VDV-1 virus in colonies treated and untreated against *V. destructor* with specific primers for each virus that we developed (Table IV.1). The number of copies of DWV-VDV-1 and VDV-1 were

significantly higher than DWV in colonies untreated for Varroa than in treated colonies, 10^8-10^{13} copies vs 10^6-10^7 copies, respectively (Fig.IV.1)

Our results offer an explanation for the observation made by bee growers that following initial infestations where Varroa parasitize colonies in an apiary, less mites are required to cause subsequent parasitic events and collapse of other colonies. In the primary infestation Varroa encounters endogenous low virulent asymptomatic DWV in the body of the honey bee. Then DWV is stimulated for replication to high amounts, "changes" by recombination through a process of virulence-selection and it is transmitted to the same and other colonies of the apiary by phoretic Varroa. This synergism of a more virulent virus with the Varroa mite turns the colony much more sensitive to collapse while infested with lower numbers of mites. Also, our findings support previous inferences that hive-infestation by Varroa facilitated appearance of a recombinant virus and that treatment against Varroa may keep virus copies low. We noticed that the number of copies of recombinant DWV-VDV-1 virus and VDV-1 were similar. This fact did not allow us to isolate each virus in order to determine its virulence in separate bioassays. However, they sustain our conclusion that this/these strains are more virulent. This is supported by a recent paper that identified a DWV-B (VDV-1) as a dominant virulent strain (McMahon et al. 2016).

Antiviral and anti-Varroa treatments. To evaluate the impact on the health of honey bee colonies of antiviral and anti-Varroa treatments we: 1. Measured the activity of the antiviral AV against DWV in bioassays with Varroa-infested adults. In this case, we used generalist primers that did not distinguish between DWV, VDV-1 and recombinant and all fell in our determinations in the category DWV. AV was not able to protect honey bee adults carrying high levels of DWV from death following Varroa infestation and showed a negative impact on the survival of adult bees bearing DWV (Fig.IV.2). Moreover, individual determination of the number of DWV copies in AV-treated or untreated bees did not change, independently of the presence or absence of Varroa (Table IV.2).

2. We tested the effect of Formic Acid on viral loads of adult bees. This demanded extensive qualitative and quantitative RT-PCR determinations in Kfar Ruth, Yad Mordechai and Dan colonies exposed to various treatments (Tables IV. 3-6). Tests for the main honey bee viruses Acute Bee Paralysis virus (ABPV), Black Queen Cell virus (BQCV), Chronic Bee Paralysis virus (CBPV), Israeli Acute Bee Paralysis (IAPV), Kashmir Bee virus (KBV), Sacbrood virus (SBV), VDV-1 and DWV in Kfar Ruth samples

showed that DWV was the most prevalent virus involved in colony losses during Varroa infestation (since BQCV does not affect adult bees, Table IV.3). Then, in Yad Mordechai and Dan, and in all quantitative measurements we tested only for DWV (using DWV generalist primers). In Kfar Ruth, Yad Mordechai and Dan the qualitative assessment did not enable estimation of treatments on DWV (Tables IV. 4-6), so we utilized quantitative RT-PCR to measure DWV loads following the anti-Varroa treatments. In Kfar Ruth Formic Acid had a positive impact diminishing DWV copies by several logs in two consecutive years, distinctly from Amitraz-Galbitraz or MAQS-treated colonies and untreated control (Figs. IV.3 and IV.4). But neither in Yad Mordechai nor in Dan did we see the same trend (Figs.IV. 5 and 6). In conclusion, our antiviral treatment (AV) was not able to protect honey bees against DWV in bioassays with Varroa-infested adults. From the above anti Varroa treatments only formic acid resulted in consistent significant reduction of DWV copies in Kfar Ruth, but not in Yad Mordechai or Dan. Further experimentation with FA will be necessary to understand this difference.

WP-V identifying and evaluating compounds to disrupt the orientation of Varroa with respect to finding its bee host

Introduction

This research focused on search and evaluation of Varroa disrupting chemicals. Aiming to detect chemicals that will decrease honeybee infestation by the mite specifically by disrupting its host sensing. All the compounds were provided (under agreement) by Prof. Erika Plettner, Simon Frazer University Vancouver Canada. Most of the chemicals are under patenting process.

Materials & Methods & Results

The present study used electrophysiological, behavioral, bee and mite mortality assays as well as in hive experiments.

Electrophysiological assays: Recordings of eletrophysiological activity (EAG) were carried out on the Varroa foreleg (the site of its olfactory sensory organ) as commonly done by us to screen the Varroa sensing disruptive compounds (Elaish et al., 2014; Singh et al., 2014). Briefly, a foreleg was stimulated by sequential puffs of air, a positive stimulus (nurse bee head space or (E)-β-ocymene odor), a mixed stimulus (positive stimulus + synthetic test compound) and the pure reference stimulus. Pure compounds were delivered on filter paper (Whatman No 1) in a Pasteur pipette. Filter papers were treated with 1μl of a solution of the compound in hexane.

Our results clearly show that compound 3c[*3,6*] causes significant inhibition of the Varroa foreleg's response to honey bee headspace, no difference in host preference but low selection of any of the bees. No effect of this compound was found on the response of bee antennae to nurse volatiles. This was similar to the effect of DEET. The other disruptive chemicals studied in details were cy[*4,1*] and cy[*2,2*]**,** in order to isolate the active isomer of these compounds. Significant difference in the EAG activity of the isomers was found: *Varroa* foreleg's response to bee headspace significantly decreased by enantiomer (+)-cy[*4,1*], ($F_{3,20}$=5.58, P= 0.006, ANOVA) whereas the antipode (-) had no effect. Interestingly, in case of cy[*2,2*]**,** the effects were opposite: enantiomer (-)-cy[*2,2*] significantly affected foreleg responses ($F_{3,20}$=5.5, P=0.006, ANOVA) while the opposite enantiomer was found inactive (Pinnelli et al. 2016).

Effect of compounds on bee mortality: The effect of compounds: **3c**[*3,6*], **3b**[*2,2*], racemic cy[*4,1*] and DEET at 300 µg, on survival of bees was tested in hoarding cages as described by Medrzycki et al. (Medrzycki et al. 2013). The test was conducted in eight to ten replicates. Survival of bees in control and treated groups at the end was similar (79-90%). The safety of other compounds to bees was confirmed from experiments in which bees and Varroa were exposed together to the chemicals (described below).

Acaricidal effect of 3c[*3,6*] on free moving mites was tested on six concentrations (compound prepared in hexane): 1 µg/µl, 10 µg/µl, 25µg/µl, 50µg/µl, 75µg/µl and 100µg/µl). The assay was conducted in glass petri dish (90 mm diameter and 17 mm deep glass) containing moistened filter paper. 10 µl volume of the compound was placed on Parafilm (5x5cm) on the plates cover; 10 µl of pure hexane were used as a control. Experiment started once 10 active mites (freshly collected from bees) were placed in each dish. Experimental and control plates were kept in incubators at (28-30^{0}C) and RH (50-70%) and the mites survival was monitored at 30 minutes interval for 4 hours.

Acaricidal effect of the compounds on phoretic mites. In this experimental setup (Picture V.1) five emerging bees loaded with two Varroa mites per bee were introduced into each plate. The bees were provided with candy (60% of Pollen and 40% sugar). The experiment with **3c**[*3,6*] was conducted using the same set of dilutions of 3c[*3,6*] and under the same conditions, described above. Subsequently the series of 15 compounds were screened blindly using 10 µl or 30 µl volume of each compound (100 or 300 nmol respectively prepared in hexane: ethyl acetate (1:1) in 10 mM concentration according to instructions) in a mix of with 10 µl or 30 µl volume control mix were placed on Parafilm (5x5cm) on the plates cover. Experimental and control plates were kept in incubators at

(28-30^0 C) and RH (50 -70%). The falling mites and their activity were noted at every 30-60 min intervals for 4 hours and after additional 20 hours.

Acaricidal effect of **3c[***3,6***]** on Varroa mites was proven in two laboratory bioassay. The results clearly showed (Fig.V.1) that control groups where mites either free moving or phoretic mites maintained active throughout the experimental period, whereas mites in the treated groups showed mortality that increased with incubation time intervals depending on the compound amount. The highest mortality was achieved at 100 µg/µl. In addition, we have also observed changes in the behavior of the free moving mites. It changed from active movement, to inactive, to erratic "dancing" movement, to paralysis and finally death, during the course of incubation in the presence of a chemical. The effect was apparently achieved without direct contact. The chemical is volatile and as such was perceived by mites. In the phoretic mites the effect was manifested in a drop off of the effected mites (Fig.V.2). In the control group the mites occasionally fell off bees but reattached rather fast and most were on bees by the end of the experiment. On the other hand, in the group treated with 100 µg/µl most of the mites dropped of bees, were incapable of climbing on the bees once they descended from the host bee and were found dead at the end of the incubation period.

Screening blindly the effect of 15 compounds including 3c[3,6] on mites survival confirmed the acaricidal effect of 3c[3,6] and revealed three additional acaricidal compounds (Table V.1). In particular, these are 3c[6,6], 3c[3,3]and 3c[4,6]. Additional somewhat effective chemicals were (3c[4,3] and Thy-For. It is important to note that none of the tested compounds had negative effects on the honeybees.

Effect of compounds on free foraging honeybee colonies

The experiments were conducted on healthy colonies (mini hives that contain a quarter of normal hive populations, with a net bottom for Varroa monitoring). Bee condition was assessed by visual hive inspection for queen presence and function, bee populated frames and brood area, food stores and disease symptoms. Prior to the beginning of experiments Varroa populations were monitored for natural fall to bottom board for 24 days. Based on the colony strength and Varroa infestation, the groups were divided into three groups for the experiment.

Four types of formulation of chemicals were tested in the hives: in parafilm dispenser (DEET), on wooden sticks (3b[2,2]) and 3c[3,6] and 3b[2,2] either in wax mold or as a sprayable solution.

Exp 1: Eight hives were used for the experiment, 4 hives were treated with 1.1mg of DEET dissolved in 6µl of hexane, and the control 4 hives were treated with the same amount of hexane. Both DEET and Hexane were introduced to the hives in an handmade dispenser. The dispenser was made from a folded piece of parafilm (10*5cm), and was hanged using a paper clip between two brood frames in the middle of the hive (Picture V.2). The honeybee population was monitored twice: in the beginning of the experiment and in the end, after 1 week. Throughout the experiment, the hives were monitored daily for Varroa population by counting the number of 24-hours-free-falling-mites on an oiled bottom board. The results indicated no negative effect of DEET on the honeybee population, or on the number of free falling mites, suggesting that the dose is probably too low to be effective, especially as bees were chewing the parafilm. Due to concerns in the literature with using DEET we focused on evaluations of other active chemicals and assessments of other application means.

Exp 2: **Testing 3b**[2,2] **on wooden sticks**

In this application we tested 3b[2,2](1,3-diethoxybenzene) applied on wooden sticks covered with parafilm for slow release relative to control hexane. The 10 µl of hexane solution or of 3b[2,2](10 µg/ µl) was applied on each stick. Two sticks were placed in between the brood frame (Picture V.3). Free falling mites were recorded daily. The experiment was conducted on 12 colonies: 6 control and 6 experimental. Over the 24 days of the experiment the daily Varroa fall dropped in both control and experimental colonies. In experimental colonies the final Varroa numbers were a bit lower but not statistically different from the control (t test).

Exp 3: Evaluation of **3c**[3,6] and **3b**[2,2] presented in wax molds

The pure chemicals were confirmed in NMR and GC/MS analysis. Both compounds were added to the appropriate amount of warm (molten) Bee wax, stirred vigorously, and cast in small molds wetted with a layer of water. Bees wax was used as a control for the experiments. Each bees wax device contained 17 mg of **3c**[3,6] or 25-30mg of **3b**[2,2]. Experiment was conducted on 11 colonies, three for each treatment and 5 colonies in the control group. Two devices were introduced into each hive, fixed in the brood frame (Picture V.4): Group A, **3c**[3,6] (1-allyloxy-4-propoxybenzene); Group B, **3b**[2,2](1,3-diethoxybenzene), Group C: Control (Bee Wax). Free falling mites were recorded daily for 24 days (Fig.V.3). Varroa infestation at the end of the experiment was assessed by Varroa fall following Amitaz smoking and was found not different between the groups, but more variable in the control. No significant differences between the groups were

observed, not in Varroa counts and not in the colony strength. However, bees were found to manipulate the wax and glue it to the comb or chew and remove it from the colony, suggesting that wax mold is not a good substrate.

Exp 4: **Testing sprayable formulation**

The soluble 2% solutions were diluted in distilled water to 0.2% each contained 2mg/ml of a compound. Nine healthy colonies were divided into three groups, three colonies each. Group C: **3c**[3,6] (1-allyloxy-4-propoxybenzene); Group B: **3b**[2,2](1,3-diethoxybenzene); Group A: Control (Bee Wax). All the frames were sprayed two times with 13 days interval about 60 ml first time and 77 ml the second time (Picture.V.5). Free falling mites were recorded daily for 3 weeks. The experiment is in progress. All the colonies appear to function normally. Changes in Varroa counts are shown on Fig.V.4. The variability between colonies is high and it seems that following spraying Varroa fall increases. Varroa infestation at the end of the experiment is planned to be assessed by Amitraz smoking.

Summary

During the three years of this project via electrophysiological (EAG) and behavioral techniques we screened over 20 chemicals and identified 6 compounds that specifically disrupt Varroa host sensing and host selection in laboratory assays, but their mode of action is not the same. While in the presence of dialkoxynenzene **3b**[*2,2*] and ether **cy**[4*,1*] most of the mites' preference changed from nurses to forgers, but the total number of mites reaching any host remained similar. In the presence of DEET the preference did not change but only a minimal amount of mites reached any of the hosts. Two effects at the level of the sensory organ were detected by the EAG: 1) short-term inhibition-decreased responses to bee volatiles when the compound was given simultaneously and 2) long-term inhibition- decreased responses to the following stimulation by bee volatiles. The long term effect was found predictive of behavioral changes (ELiash et al. 2014; Singh et al. 2014). However, due to effects of DEET, on inhibition of acetylcholinesterase in both insects and mammals (Corbel et al. 2009), we looked for safer alternatives, and selected 1-allyloxy-4-propoxybenzene, 3c[*3,6*] DEET analog (Plettner, unpublished). Indeed the effects of 3c[*3,6*] on Varroa were similar to DEET. In its' presence hardly any mites reached any of the hosts. Moreover, 3c[*3,6*] proved acaricidal to both free moving and phoretic mites. This finding led to further identifications of related compounds that proved acaricidal in laboratory assays. None of

the tested compounds had negative effect on the honeybees. The effect of different compounds on mites survival after 24 hours is presented in Table V.2.

Experiments in free foraging colonies are challenging, as not only that the colonies are variable in the infestation levels and strength, but pure compounds were also a limiting factor. So far, we tried four different methods of applications in small colonies, but were unable to demonstrate an effect on Varroa infestation, probably due to low amounts of the compounds or their formulation. However, it seems that we have built the experimental protocol that will be used in future studies.

General Summary

Work packages I and III have worked together to provide immediate solutions to the pending threat of resistance to amitraz. WP I monitored resistance to amitraz in populations from various locations and started to develop molecular tools for resistance screening. In search for further mutations WP1 completed the cloning of the amitraz target gene. One mutation that confers resistance to fluvalinate was confirmed in Israeli populations. New acaricides were tested in the lab and one of them (Fenazaquin) was further tested under semi-field conditions.

WP3 extensively evaluated formic acid (FA) formulations/applications methods and found the new Nassenheider evaporator to perform better than the products/methods that were previously available. Additionally, in collaboration with the Nassenheider staff, an extra small wick was designed for the hot Israeli summer, which is now commercially available for the Israeli growers. Integrating FA applications to Varroa control programs could be an important step in amitraz resistance management.

WP II developed a colony-level bioassay for monitoring effects of antibiotics on susceptibility of Varroa mites to Coumaphos, but did not find an effect of wide-range antibiotics at the concentrations tested.

WP III and WP IV demonstrated in one location for two consecutive years that viral loads were significantly reduced in the FA treatment, whereas in the amitraz treatment they remained stable and in the control treatments, viral loads increased. More research is needed to determine the long-term effect of FA treatments on hive health, in the anticipation that effective Varroa control in the sealed cells in mid-season can reduce the viral load and enhance hive health. In contrast, antiviral treatments, developed in WP4 against DWV in Varroa-parasitized bees did not prevent replication of the virus and death of infested bees. In WP V experiments conducted in specially adapted mini-hives,

indicated safety of DEET, 3b [2,2] and 3c [3,6] to honeybees at the doses tested. However, no clear negative effects on Varroa were recorded. In future research, active compounds will need to be further tested in combinations and at higher concentrations.

WP-I Monitoring resistance and identifying new chemicals for Varroa control

2.1 research objectives	1. Developing bioassays for evaluating Varroa response to existing and new acaricides, and assessing the response of honeybees to acaricides. 2. Resistance monitoring of Varroa to acaricides especially amitraz 3. Study the biochemical and molecular basis of resistance to amitraz and fluvalinate.
2.2 results	Resistance bioassays for Varroa were developed. Resistance monitoring was performed for populations from various locations. Varroa samples were collected for biochemical and molecular assays. One mutations that confers resistance to fluvalinate was confirmed in Israeli populations. Cloning the target gene for amitraz was completed to search for mutations that might confer resistance. New acaricides were tested and one of them was tested under semi-field conditions.
2.3 conclusions	There is a decrease in the susceptibility to amitraz among local populations as was previousley reported, emphasizing the importance in maintaining a resistance monitoring program. In the lab we have identified acaricides that are safe for honeybees and toxic to Varroa. Thus we hypothesize that effective formulations can be developed for Varroa control under field conditions.
2.4 deviations	No deviations

WP-II Determining the role of symbionts in *V. destructor* resistance

2.1 research objectives	To compare the survival of *Varroa* mites treated with Coumaphos, between untreated mites and those pre-treated with wide-range antibiotics.
2.2 results	Coumaphos treatment caused about 70% of phoretic *Varroa* mites to fall; most mites fell within 8 h, with a peak at 4 h, and almost all mites fell by 12 h. Wide-range antibiotics did not affect the percent of mites that fell due to Coumaphos treatment.
2.3 conclusions	We did not find an effect of wide-range antibiotics at the concentrations that we used.
2.4 deviations	No deviations.

WP-III Identifying optimal timing for formic acid and thymol treatments

2.1 research objectives	Develop an integrated management program for Varroa control based on formic acid (FA) and essential oils.
2.2 results	Mean number of phoretic mites were significantly reduced in the FA and thymol treatments compared to *Varroa* levels prior to treatment.

2.3 conclusions	While the short term FA treatments was relatively safe on two stories it was very problematic on one story as queen loss was substantial. In contrast the Nassenheider evaporator evaluated in 2016 and 2017 proved to be substantially more promising with respect to efficacy, nearing that of Galbitraz. Regarding the evaporation rate of FA, the large wick is evidently suitable for winter applications. However from April through October we suggest using the extra small wick specifically designed for our hot temperatures in Israel.
2.4 deviations	No deviations.

<u>WP-IV</u> <u>Identification characterization treatment viruses transmitted by *V. destructor*</u>

2.1 research objectives	Identification and characterization of the viral load of Varroa destructor isolated from treated- and untreated-colonies. Evaluation of the impact on the health of honey bee colonies of a combination of anti-Varroa and anti-viral treatments.
2.2 results	We characterized virulent DWV, VDV-1 and recombinant virus strains that emerged from *Varroa*-infested hives. We tried antiviral treatments and evaluated the impact of anti-varroa treatments on viral loads (DWV, VDV-1 and recombinant strains).
2.3 conclusions	Two viral strains a VDV-1-DWV recombinant and VDV-1 become dominant in hives highly infested with Varroa. Our antiviral treatments against DWV in Varroa-parasitized bees could not prevent replication of the virus and death of infested bees. Formic acid (FA) applied as an acaricide significantly reduced virus titers in one of the three apiaries examined (Kfar Ruth) over two consecutive years. Further experimentation with FA is necessary to determine the mechanism responisble for this effect.
2.4 deviations	None

<u>WP-V identifying and evaluating compounds to disrupt the orientation of *Varroa* with respect to finding its bee host</u>

2.1 research objectives	Identify new *Varroa* control agents, particularly those disrupting Varroa selection of bee host but not affecting bee chemosensing and behavior.
2.2 results	1. Tested cpmpounds differed in their effect on Varroa. In host choice experiments three compounds disrupted host selection and two (DEET and 3c [3,6])decreased ability to reach the host. The latter was found to have acaricidal activity in phoretic and free-moving mites. 2. Enantiopure disruptive compounds (cy[4,1] and cy[2,2]), were identified**.** 3. None of the Varroa disrupting or acaricidal compounds were toxic to bees. 4.Experiments conducted in mini-hives adapted for Varroa monitoring indicated safety of DEET, 3b [2,2] and 3c [3,6] to honeybees at the doses tested. No clear negative effects on Varroa were recorded.

2.3 conclusions	a. Several active compounds were identified: three disrupted host selection, and five componds had acaricidal activity in laboratory assays. b. The fact that pure enantiomers give the same long-term electrophysiological responses than the racemate, eliminates the need to synthesize the more expensive pure enantiomers c. None of the Varroa active compounds are harmfull to bees. d. To achieve activity in hive conditions active compounds need to be further tested in combinations and higher concentrations.
2.4 deviations	Test for acaricidal effect were not originaly planned

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סיכום עם שאלות מנחות

נא להתייחס ל<u>כל</u> השאלות <u>בקצרה ולעניין</u>, ב-3 עד 4 שורות לכל שאלה (לא תובא בחשבון חריגה מגבולות המסגרת המודפסת).

שיתוף הפעולה שלך יסייע לתהליך ההערכה של תוצאות המחקר.

הערות: דוחות שיוגשו בסטטוס פרסום "**מוגבל רק לספריות**" יחשבו ע"י הקרן כדוחות **שאינם מוגבלים** לפרסום ויפורסמו באתר המדען הראשי של משרד החקלאות ופיתוח הכפר.

אנא פרט מהם הניסויים שנעשו תוך השוואה לתכנית העבודה המתוכננת והתאמתם למטרות המחקר כפי שהופיעו בהצעה המקיפה
I. פיתוח מבחני מעבדה ביולוגיים לבחינת תגובת הוורואה והדבורים לאקריצידים קיימים וחדשים, ניטור עמידות הורואה לאקריצידים קיימים ולימוד הבסיס הביוכימי והמולקולארי לעמידות לאמיטראז ופלואלינאט.
II. פיתוח מערכת למעקב אחר נפילת ורואה בכוורת שלמה במשך מספר שעות ובדיקת שרידות הוורואה בעקבות טיפול בקומפוס בכוורת בשילוב עם טיפול באנטיביוטיקה רחבת טווח.
III. בחינת יעילות ההדברה של תכשירים אלטרנטיביים כנגד אקריות והשפעתם האפשרית על רמת הנגיעות בווירוסים בכוורת.
IV. אפיון אוכלוסיית וירוסי עיוות הכנפיים הפוקדים את אקרית הוורואה, תוך שימוש בטכניקות המולקולריות שבתוכנית, ובדיקת השפעת טיפול אנטיוויראלי ואנטי-וורואה על כמות וירוס עיוות הכנפיים בדבורים ובכוורות.
V. בחינת השפעת חומרי שיבוש על הוורואה ובירור השפעת חומרים אלה בכוורות
מהם עיקרי הניסויים והתוצאות שהושגו בתקופה אליה מתייחס הדו"ח?
I. פותחו מבחנים ביולוגיים ונבחנו אקריצידים חדשים. תכשיר אחד נמצא יעיל בתנאי מעבדה ונבחן בתנאי שדה. נערך ניטור לעמידות במספר מקומות בארץ ונמצאה עמידות לפלוואלינט. נמצאה המוטציה האחראית לעמידות לפלוואלינט בארץ ונעשה שיבוט לגן המטרה של האמיטראז לבחינת מוטציות שיכולות להביא לעמידות בעתיד.
II. מעקב מתמשך אחר נפילת ורואה פורטית מכוורת שלמה, הראה שרוב האקריות נופלות בשמונה השעות הראשונות שלאחר תחילת הטיפול בקומפוס, עם שיא כעבור ארבע שעות, וסיום הנפילה לאחר 12 שעות. לא נמצאה השפעה של אנטיביוטיקה רחבת טווח על שרידות האקריות בכוורות מטופלות בקומפוס.
III. מספר האקריות הפורטיות הופחת באופן מובהק בטיפולים בחומצה פורמית ובתימול בהשוואה לרמות האוכלוסייה לפני הטיפול, אך טיפולים אלה היו יעילים פחות מטיפול באמיטראז. המטען הוויראלי של הדבורים הופחת בעקבות טיפול בחומצה פורמית, נותר ללא שינוי בטיפול באמיטרז ועלה בביקורת ללא טיפול.
IV. פותחה שיטה כמותית לבדיקת וירוסים אלימים בכוורות ונבדקו כוורות מטופלות ולא מטופלות כנגד וורואה בעזרת RT-PCR. זוהו שני וירוסים אלימים בכוורות הלא מטופלות: VDV-1 והווירוס הריקומבیננט DWV-VDV-1. הטיפול האנטיוויראלי לא עזר כנגד ווירוסים ומהטיפולים כנגד וורואה רק חומצה פורמית הפחיתה את כמות וירוס עיוות הכנפיים בכוורות.
V. נבחנה השפעת אנאלוג של DEET על שרידות הוורואה במעבדה ונמצא שעליה במינון החומר פוגעת בשרידות אקריות פורטיות. נסרקו 14 חומרים לפעילות כנגד הוורואה וחלקם נמצאו קוטלי וורואה, מבלי שפגעו בשרידות הדבורים. שני חומרים נבדקו בכווריות בשדה במספר שיטות אפליקציה, אך לא הביאו לירידה ברמת הוורואה.
בעקבות הניסויים שנעשו, אנא פרט והסבר כיצד הושגו מטרות המחקר בתקופת הדו"ח או חלק מהן
I. עבור בדיקת תכשירים חדשים, המטרות הושגו באמצעות מבחנים ביולוגיים במעבדה ועבור הערכת העמידות לתכשירים ישנים הושגו המטרות על-ידי דגימת אוכלוסיות דבורים בשדה וביצוע מבחנים מולקולריים.
II. יצירת כוורות עם מבנה יחודי שאפשר קבלת מידע מדוייק על מספר האקריות בכוורת לפני הטיפול ובסיומו, בשילוב עם טיפול אנטיביוטי לדבורים.
III. סדרה של ניסויי שדה באזורים שונים ברחבי הארץ, עבודה עם כוורות בעונות שונות וברמות חוזק שונות ובשימוש במגוון שיטות יישום לחומרים השונים.

IV. מטרת מחקר 1: זיהוי ואפיון ווירוסים שמועברים, מופעלים ומוגברים על ידי האקרית - ראה שאלה קודמת. מטרת מחקר 2: בדקנו השפעת טיפול אנטיוויראלי ואנטי-וורואה על כמות וירוס עיוות הכנפיים בדבורים ובכוורות עם וורואה במבחן ביולוגי במעבדה ובכוורות בשטח שטופלו באקריצידים (נסויים בכפר רות, יד מרדכי ודן) בהתאם.

V. סדרה של ניסויי מעבדה עם אקריות ודבורים לבדיקת חומרים משבשי חישה וניסויי שדה במערכת של מיני-כוורות שפותחה לצורך הניסוי.

בהתאם להצעה המקיפה, ציין מה התבצע מתוך טבלת המשימות ואבני דרך, כולל אבני דרך כמותיות (סעיפיםIV-VI)
ומהם הקריטריונים שפורטו בהצעה המקיפה כבוחן להצלחת המחקר אכן הושגו

I. בשנה 1 פותח מבחן ביולוגי לבדיקת רגישות וורואה לאקריצידים. בשנים 2 ו-3 נבחנה רגישות האקריות לאקריצידים הנמצאים בשימוש ונמצאה רגישות לאמיטראז אך עמידות לפלואלינט.

II. טיפולים אנטיביוטיים רחבים לא השפיעו על רמת העמידות של האקריות לקומפוס ולא נמצאו הבדלים באוכלוסיית החיידקים הסימביונטיים בין אקריות עמידות ורגישות לקומפוס

III. בהתאם לאבני הדרך נבחנו מספר שיטות ליישום חומצה פורמית ונמצאה שיטה חדישה וזמינה שהיתה ויעילה יותר משיטות קודמות. כמו-כן נבחנו מספר פורמולציות של שמנים אתריים (צמחי תבלין) אך לא נמצא טיפול מומלץ שנותן תוצאות עקביות.

IV. אפיינו את אוכלוסיית וירוסי עיוות הכנפיים הפוקדת את אקרית הוורואה ואת השפעת הטיפולים האנטי ויראליים על המטען הויראלי שמועבר לדבורים.

V. בשנים 1 ו-2 נבחנה השפעת חומרים משבשי חישה של וורואה על וורואה ועל דבורים בתנאי מעבדה, בהתאם לאבני הדרך. בשנה 3 נבחנה השפעתם של חומרים נבחרים בשדה ברמת אוכלוסיית דבורים ואקריות.

מהן המסקנות המדעיות ומהן ההשלכות לגבי יישום המחקר והמשכו בעתיד?

I. נראה שקיימת ירידה ברגישות לאמיטראז כפי שדווח בעבר, אולם צריך להמשיך את הניטור בכל הארץ לקביעת מקומות מדוייקים לעמידות ורמתה. ישנם כנראה אקריצידם חדשים שניתן אולי להכניס לשימוש, אולם הדבר דורש בחינה מעמיקה בתנאי שדה, ובדיקת בטיחות התכשירים לדבש ולדבורים.

II. לא נמצאה השפעה של אנטיביוטיקה רחבת טווח על עמידות הוורואה לתכשיר קומפוס, בטווח הריכוזים שנבדקו, ואין תמיכה להשערה שחיידקים סימביונטיים עמידים מקנים לוורואה עמידות כנגד תכשירי הדברה.

III. יישום חומצת נמלים בכוורות במיכלים לשיחרור מבוקר נמצא כבעל פוטנציאל להתמודדות עם הוורואה ויתכן ואף ישירות עם המטען הויראלי, אולם עדיין נחוצה עבודה למציאת ממשק הטיפולים הנכון לאורך השנה בתנאי שדה

IV. הטיפול בחומצה פורמית הפחית את המטען של DWV במכוורת כפר רות אבל לא הראה את אותן התוצאות ביד מרדכי ודן

V. נמצאו חומרים המשבשים את התנהגות הוורואה ובטוחים לדבורים אך עדיין יש להמשיך ולבחון את יעילותם, בעיקר בתנאי שדה.

מהן הבעיות שנותרו לפתרון ו/או שינויים (טכנולוגיים, שיווקיים ואחרים) שחלו במהלך העבודה ומה אמורה להיות ההתייחסות בהמשך?

I. בחינת האקריצידים החדשים בתנאי שדה. דרוש שימוש בפורמולציות עמידות לתנאים בכוורות ובחוץ. נדרש כנראה עירוב של חברות כימיה המעוניינות לבחון את התכשירים שלהן לטיפול בבעיית הוורואה בענף הדבורים.

II. לא נמצאה השפעה של אנטיביוטיקה בריכוזים שנבחנו, אך יש מקום לבדוק ריכוזים גבוהים יותר. בעקבות תגלית חדשה של חיידקים שמפרקים חומרים מבוססי זרחן בחרקים, ונמצאים גם בוורואה, יש לבחון את יכולת החיידקים הללו לפרק קומפוס.

III. חומצה פורמית בשחרור מושהה נבדקה בניסויים נקודתיים. עדיין נותר להתאים ממשק שנתי לשימוש בחומצה פורמית כטיפול יחיד או בשילוב עם אמיטראז, במטרה להפחית את השימוש באמיטראז ולהקטין את הסיכון להתפתחות עמידות.

IV. הפחתת אוכלוסיות אקרית הוורואה ומניעת התרבות וירוסים, בעיקר וירוס עיוות הכנפיים, בעזרת שילוב של אקריציד יעיל יותר ובניית ממשק עבודה שיותאם לממצאי המחקר.

V. יצירת כמות מספקת של כוורות אחידות מבחינת אוכלוסיה ונגיעות בוורואה לצורך ביצוע הניסוי וכמות מוגבלת של החומר הסינטטי לביצוע הבדיקות בכוורות.

הפצת הידע שנוצר בתקופת הדו"ח: **פרסומים בכתב** - ציטט ביבליוגרפי כמקובל בפרסום מאמר מדעי; **פטנטים** - יש לציין שם ומס' פטנט; **הרצאות וימי עיון** - יש לפרט מקום, תאריך, ציטוט ביבליוגרפי של התקציר כמקובל בפרסום מאמר מדעי.

כלל הממצאים עד כה הוצגו בשני כנסים למגדלי דבורים, ובסיכומי ישיבות המיזם שהוצגו בפני ועדת ההיגוי ונציגי המדען הראשי.

IV. Chejanovsky N (2016). Monitoring virulent DWV strains in Varroa infested colonies. Institute of Bee Health, Bern, Switzerland.

Chejanovsky N, Slabezki Y. (2017). Honey bee viruses - Pathogenesis, mechanistic insights and possible management projections in "Beekeeping - from Science to Practice". Vreeland RH, Sammataro D. (Eds), Springer (978-3-319-60635-4).

V. Singh NK, Eliash N, Pinnelli GR, Plettner E, Soroker V. (2015). Specific disruption of Varroa chemosensing. In Congreso Internacional De Actualización Apícola, Poebla, Mexico. 73-77.

Plettner, E., Pinnelli G.R., Eliash, N., Singh K. N., and Soroker, V. (2016) The chemical ecology of host-parasite Interaction as a target of Varroa destructor control agents. Apidologie doi:10.1007/s13592-016-0452-8.

Pinnelli GR, Singh KN, Soroker V, Plettner E. (2016) Synthesis of enantiopure alicyclic ethers and their activity on the chemosensory organ of the ectoparasite of honey bees. J. Agr. Food Chem. 64:8653–8658

➢חסוי – לא לפרסום: **מצורף מכתב הסבר**

החומרים המדווחים ב- WP-5 הם בבעלות השותפה הקנדית למחקר וחסויים כל עוד לא דווח אחרת.

State of Israel \ Ministry of Agriculture and Rural Development

Eric Palevsky
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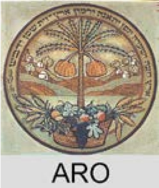

אריק פלבסקי
המעבדה לאקרולוגיה,
המחלקה לאנטומולוגיה,
מינהל המחקר החקלאי
מרכז מחקר נווה יער
ת.ד. 1021, רמת ישי, 30095

27/06/2017

To Chief Scientist Office,
Ministry of Agriculture

Re: **Request that Final report will not be published**
Justification for confidentiality

Greetings,

As scientific director of project number 131-1815-15, titled ‘Novel methods for controlling the *Varroa* mite in honey bee hives in Israel’ I am requesting that all parts pertaining to WP 5 - identifying and evaluating compounds to disrupt the orientation of *V. destructor* with respect to finding its bee host - Soroker et al. Entomology ARO, will not be published. The request for confidentiality is to protect the property rights concerning novel compounds with acaricidal activity, as it is Soroker’s intention to patent them.

Sincerely,

Eric Palevsky

Dr. Eric Palevsky, Acarologist,
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Ministry of Agriculture, P.O. Box 1021, Ramat Yishay 30095, Israel
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Web page - http://www.agri.gov.il/en/people/723.aspx

Table I.1 Amitraz efficacy against varroa in various collections made

Collection Location	Amitraz concentration tested (ppm)	Mortality (%) after (hours):			
		5	18	24	42
Dan	Control			50±8a	85±6a
	25			85±4b	97±2b
	50			88±5b	98±2b
Tsrifin1*	Control	0a	53±24a	60±20a	
	10	0a	80±12b	93±7b	
	50	13±13b	93±7b	100b	
	100	40±0b	67±7b	100b	
Tsrifin2	Control			10±6a	72±4a
	25			63±14b	100b
Tsrifin3	Control		10±3a		31±3a
	25		20±6b		92±2b
	50		24±11b		100c
Kvotsat Shiler	Control	15±9a	45±9a	55±5a	
	10	13±13a	100b	100b	
	50	13±13a	87±7b	100b	
	100	25±13a	100b	100b	
Zitim1*	Control		12±7a	12±7a	
	25		30±6b	93±3b	
Zitim2	control		37±20a		80±10a
	25		73±3b		100b
	50		60±10b		100b
Boaz	control		27±17a		53±9a
	25		93±3c		100b
	50		57±7b		100b
Dror Gal	control		70±6a		87±3a
	25		83±7a		97±3b
	50		87±3a		100b
Ein Harod	control		23±3a		80±11a
	25		67±13b		87±13a
	50		100bc		100a
Lavi	control		57±26a		87±13a
	25		93±3b		100a
	50		87±7b		100a

* Three collections from Tsrifin and two collections from Zitim, in different dates, were tested.

Table I.2 Acaricides tested for toxicity against honeybee and varroa

Toxicity to honeybees	Formula	Group	Active ingredient	Commercial name
Slightly toxic		Sulfite ester	Propargite	Omite
Not Acutely Toxic		Tetrazine	Clofentezine	Apollo
Not Acutely Toxic		---	Acequinocyl	Xmite
None toxic		Organotin	fenbutatin oxide	Acrimite
None toxic		organochlorine	Dicofol	Acarine
---		---	cyflumetofen	Defender
---		---	Fenazaquin	Majister

Table I.3 Efficacy of new tested acaricides against varroa collected from Tsrifin in a lab bioassay

Experiment 1		Experiment 2	
treatment	**Mortality (%)**	**treatment**	**Mortality (%)**
Control	15±3a	Control	10±2a
Amitraz 25 ppm	61±6b	Magister 0.05%	93±7c
Amitraz 50 ppm	73±8b	Defender 0.05%	31±7b
Magister 0.05%	64±7b	Acramite 0.05%	28±4b

Table I.4 List of primers used to clone the octopamine receptor gene (provided by Joel Gonzales)

Primer Name	Sequence 5'-3'	Length	Tm (ºC)
128 F	GATAACGCTTGCCCGAGGTA	20	59.9
1376 F	GCATGGCGTTCAAGAAGACC	20	59.8
1445 R	CGTCACACTTTACTTTCACAAATC	24	57
1596 R	TTTGCTGTTGCCGCTTCTTC	20	60
1643 R	GATCCATCCGACCGCTGAAT	20	60
1733 R	CGTATCCGTGGGCGTGATTA	20	60
261 F	GCGTCGGTTGAAATCGGAAG	20	59.9
588 F	CGCGATGACATTCAATGCGT	20	60
74 F	CACGAACGACAAAACCGGTC	20	60
819 F	GCTCATATCCTTCGTGCCCA	20	59.9
905 R	ACACACTCGTTCGGATGGTC	20	60

Table II.1 Varroa mites survival rate under laboratory conditions. Mites were held on agar plates or within artificial cells. 10 mites or 4 mites were fed on 2 pre-pupae. n= represents the number of replicates. Plates were held in an incubator, 32°C, 45-50% humidity. Reported are means with standard errors in parentheses.

Treatment	0 hours	20 hours	25 hours	44 hours
(A) 10 *Varroa*; 2 prepupa on agar (n=3)	100%	93.3 (SE 0.03)	93.3 (SE 0.03)	80.3 (SE 0.10)
(B) 10 *Varroa*; 2 prepupa in queen cells (n=3)	100%	53.3 (SE 0.20)	36.7 (SE 0.07)	20 (SE 0.10)
(C) 4 *Varroa*; 2 prepupa on agar (n=6)	100%	83.3 (SE 0.08)	66. 7 (SE 0.11)	66. 7 (SE 0.11)
(D) 4 *Varroa*; 2 prepupa in queen cells (n=8)	100%	46.9 (SE 0.10)	46.9 (SE 0.10)	28.1 (SE 0.11)

Table II.2 *Varroa* mites survival rate on different coumaphos concentrations from healthy and sick hives. Mites were held on agar plates. Plates were treated with different coumaphos concentrations (0, 10, 50, 100, 200 ppm). n= represents the number of replicates. 10 mites were fed on 2 pre-pupae. Plates were held in an incubator, 32°C, 45-50% humidity. Survival rate was examined after 20 hours. Reported are means with standard errors in parentheses.

Coumaphos concentration (ppm)	% Survival rate from healthy hives (n=5-6)	% Survival rate from sick hives (n=4)
0	87.73 (0.03)	65.68 (0.10)
10	24.38 (0.03)	17.78 (0.07)
50	4.00 (0.02)	
100	8.00 (0.04)	16.46 (0.07)
200	10.22 (0.03)	

Table IIIa.1. Trials conducted on one and two stories with alternative control measures compared to the commercial standard amitraz and a non-treated control (when available). FA-Amrine-24 hour formic acid treatment; FA-Nassenheider evaporators 1-2 weeks treatment.

Date	Story	Treatments	Location	Hive
26.06.2013	2	FA-Amrine, Amitraz, Control	Sdeh Eliezer	Raikain
11.08.2013	1	FA-Amrine, Amitraz, Control	Road 90-899 Junction	Dan
19.2.2014	1	FA-Amrine, Amitraz, Control	East of Kefar Szold on the pipe line road	Dan
16.3.2014	1	FA-Amrine, Amitraz, Control	Road 3411 adjacent to Zikim, Karmiya and Yad Mordechai	Yad Mordehai
7.5.2014	2	FA-Amrine, Amitraz, Control	Road 6953 north of Jo'ara	Ein Harod
10.9.2014	1	FA-Amrine, Amitraz, Control	Road 9977 north of Margaliot	Dan
28.6.2015	2	FA-Amrine, Hop Guard, Thymol sticks, Amitraz, Control	Kefar Ruth	Shai Spector
19.8.2015	1	Thymol	Alon Hagalil	Zvika Ofir
11.8.2015	1	Thymol sticks, Hop Guard	Dan	Dan
10.8.2015	1	Thymol sticks, Hop Guard	Kefar Ruth	Shai Spector
7.7.2016	1 1/2	FA-Nassenheider evaporators, Amitraz, Control	Kefar Ruth	Shai Spector
11.7.2016	2	FA-Nassenheider evaporators, Amitraz	Road 3411 adjacent to Zikim, Karmiya and Yad Mordechai	Yad Mordechai
1.9.2016	1	FA-Nassenheider evaporators, Amitraz, Control	Road 918 south of Gonen	Dan
5.1.2017	1	FA-Nassenheider evaporators, Amitraz, Control	Kefar Sirkin	Shabtai Simon

Table IIIa.1. Mean phoretic Varroa drop per hive immediately following amitraz fumigation 6 weeks post treatment with formic acid (FA) or Amitraz, Kefar Sirkin, February 2017.

Treatment	Hives	Mean phoretic Varroa drop	SE
Galbitraz (amitraz)	13	2.7	1.11
Formic acid	9	6.2	2.07
Control	5	199.6	53.39

Table IV.1 Primers for quantitative detection of DWV, VDV-1 and DWV-VDV-1- recombinants

DWV	**sequence**	**Tm**
DWVfor2553	ATTTGGCAGAGGGATTGTTG	56
DWVqRTRev2747-new	CAGGAGCACAACCTACAGGA	60
VDV-1		
VDVqRTFw6111	TGGCTAATCGACGTAAAGCA	56
VDVqRTRev6299B	ACTAATCTCTGAGCCAACACGT	60
Recombinant DWV-VDV-1		
Rec-5026-F	AGCCGCTCAAAAACCGAAAC	58
Rec-5329-R	ATGTGCCGCAAACACTCTCT	58

Table IV.2 DWV genomic copies in adult bees

Individual group	Time of death (days)	Viral copies
Varroa	6	9.47E+10
Varroa+AV	7	3.68E+10
AV	13	5.42E+11
Control	15	1.61E+11
Varroa	16	2.17E+09
Varroa+AV	13	2.56E+10
AV	15	1.25E+11
Control	17	1.44E+11
Varroa	20	9.58E+10
Varroa+AV	22	1.61E+09
AV	20	4.03E+10
Control	21	2.03E+11

Table IV.3 Determination of Honey bee viruses in Kfar Ruth by qualitative RT-PCR.

					Virus				
Treatment	**Hive**	**sample date**	**IAPV**	**SBV**	**ABPV**	**BQCV**	**CBPV**	**VDV**	**DWV**
Control	41	7.7.16				POS			POS
		17.8.16				POS			POS
	44	7.7.16	POS			POS			POS
		17.8.16	POS						POS
	45	7.7.16	POS			POS			POS
		17.8.16							POS
	49	7.7.16				POS			
		17.8.16				POS			POS
	51	7.7.16							
		17.8.16				POS			POS
	54	7.7.16				POS			
		17.8.16	POS		POS	POS			POS
	55	7.7.16				POS			POS
		17.8.16	POS						
	65	7.7.16	POS			POS			POS
		17.8.16				POS			POS
Max	4	7.7.16	POS			POS			
		17.8.16				POS			POS
	7	7.7.16				POS			POS
		17.8.16				POS			POS
	8	7.7.16				POS			
		17.8.16				POS			
	9	7.7.16				POS			POS
		17.8.16	POS			POS			POS
	10	7.7.16				POS			POS
		17.8.16				POS			POS
	12	7.7.16	POS			POS			
		17.8.16				POS			
	13	7.7.16	POS			POS			POS
		17.8.16				POS			POS
	14	7.7.16				POS			POS
		17.8.16				POS			
Amitraz- Galbitraz	15	7.7.16	POS			POS			
		17.8.16				POS			POS
	21	7.7.16				POS			
		17.8.16	POS			POS			
	26	7.7.16	POS						
		17.8.16				POS			
	31	7.7.16					POS		POS
		17.8.16				POS			
	40	7.7.16				POS			
		17.8.16	POS			POS			
	42	7.7.16	POS			POS			
		17.8.16				POS			
	43	7.7.16	POS		POS	POS			POS
		17.8.16				POS			POS
	58	7.7.16	POS			POS			
		17.8.16				POS			
Formic acid	17	7.7.16	POS		POS	POS			POS
		17.8.16				POS			
	19	7.7.16			POS	POS			
		17.8.16				POS			POS
	27	7.7.16							
		17.8.16				POS			POS
	28	7.7.16	POS			POS			POS
		17.8.16	POS			POS			POS
	29	7.7.16							
		17.8.16							POS
	32	7.7.16	POS		POS	POS			POS
		17.8.16	POS		POS	POS			POS
	34	7.7.16				POS			POS
		17.8.16				POS			

Positive sample, POS. Negative sample, empty space.

Table IV.4 Summary of DWV positives in Kfar Ruth determined by qualitative RT-PCR

		DWV Positive	
Treatment	**Number of hives**	**7.7.16**	**17.8.16**
Amitraz- Galbitraz	8	3	2
Formic acid	7	4	4
Max	8	5	5
Control	8	5	7

Table IV.5 Summary of DWV positives in Yad Mordechai determined by qualitative RT-PCR

		DWV Positive	
Treatment	**Number of hives**	**11.7.16**	**22.8.16**
Amitraz- Galbitraz	6	4	3
Formic acid	7	3	3
Max	7	6	6

Table IV.6 Summary of DWV positives in Dan determined by qualitative RT-PCR

		DWV Positive	
Treatment	**Number of hives**	**31.8.16**	**10.10.16**
Amitraz- Galbitraz	7	0	2
Formic acid	10	1	2
Control	6	0	4

Table V.1 The comparative effect of 15 chemicals on mite survival. The data are percentages of mites per treatment. Number of mites tested are indicated in brackets for each treatment. Acaricidal compounds appear in bold.

	% of surviving mites 24 hrs post treatment (n)	
Treatment	10µl	30µl
Hex: EtAc	80 (40)	100 (60)
3c{3,6}	**100 (20)**	**7.5 (40)**
3b{3,6}	95 (20)	90 (20)
3a{3,6}	85 (20)	100 (20)
3c{1,6}	95 (20)	95 (20)
3c{2,6}	90 (20)	80 (20)
3c{4,6}	**100 (20)**	**33 (30)**
3c{n5,6}	90 (20)	100 (20)
3c{3,3}	**80 (20)**	**22.5 (40)**
3c{4,4}	95 (20)	100 (20)
3c{3,n5}	95 (20)	95 (20)
3c{4,n5}	35* (20)	95 (40)
3c{4,3}	95 (20)	45 (20)
3c{6,6}	**55 (20)**	**0 (40)**
Thy-For	90 (20)	**55 (40)**
Thy_2Ox	80 (20)	90(20)

* mites drowned in bee food

Table V.2 List of tested chemicals that showed disruptive of acaricidal activity

Compound code	Full name	Varroa chemosensation (EAG)		Toxicity Varroa	Effect on host preference	Toxicity Bees
		Disruption (-/+)	Dose response (effective dose, µg)			
cy{4,1}	Cyclopentene Ether- butyl methyl	+	0.1	–	shift	–
3b{2,2}	1-ethoxy-3-ethoxybenzene	++	0.01	–	shift	–
cy{2,2}	1-ethoxy-5-(2'ethoxyethyl) cyclopent-2-ene	++	++	–	shift	–
DEET	*N*-Diethyl-*meta*-toluamide,	++	?	+?	no change	–
3c{*3,6*}	1-allyloxy-4-pentoxybenzene	++	NT	++ (1 µg)	no change	–
3c{*4,6*}	Not provided	NT	NT	+	NT	–
3c{*3,3*}	Not provided	NT	NT	+	NT	–
3c{*4,3*}	Not provided	NT	NT	+	NT	–
3c{*6,6*}	1,4-diallyloxybenzene	NT	NT	++	NT	–
Thy-For	Thymyl formate	NT	NT	+	NT	–

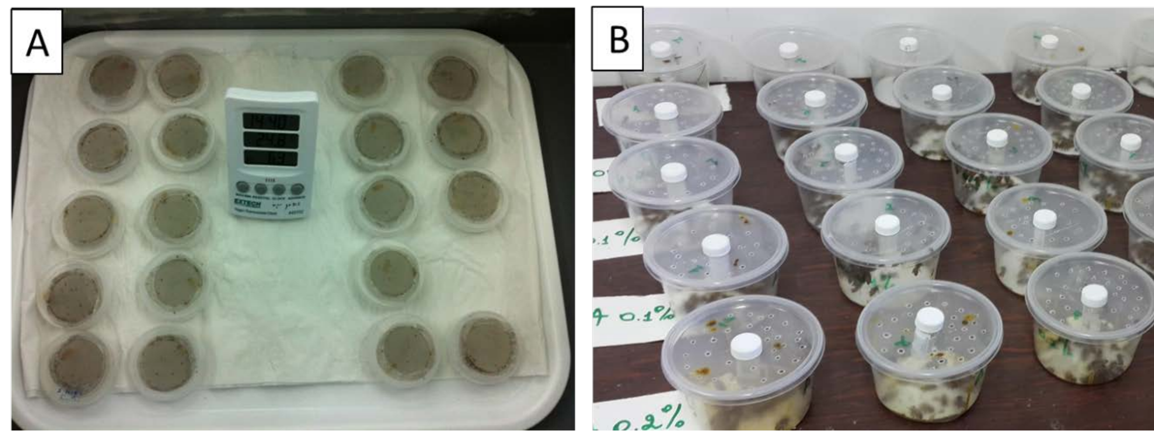

Fig. I.1 bioassays for testing the response of varroa (A) and honeybees (B) to acaricides (see text for more details)

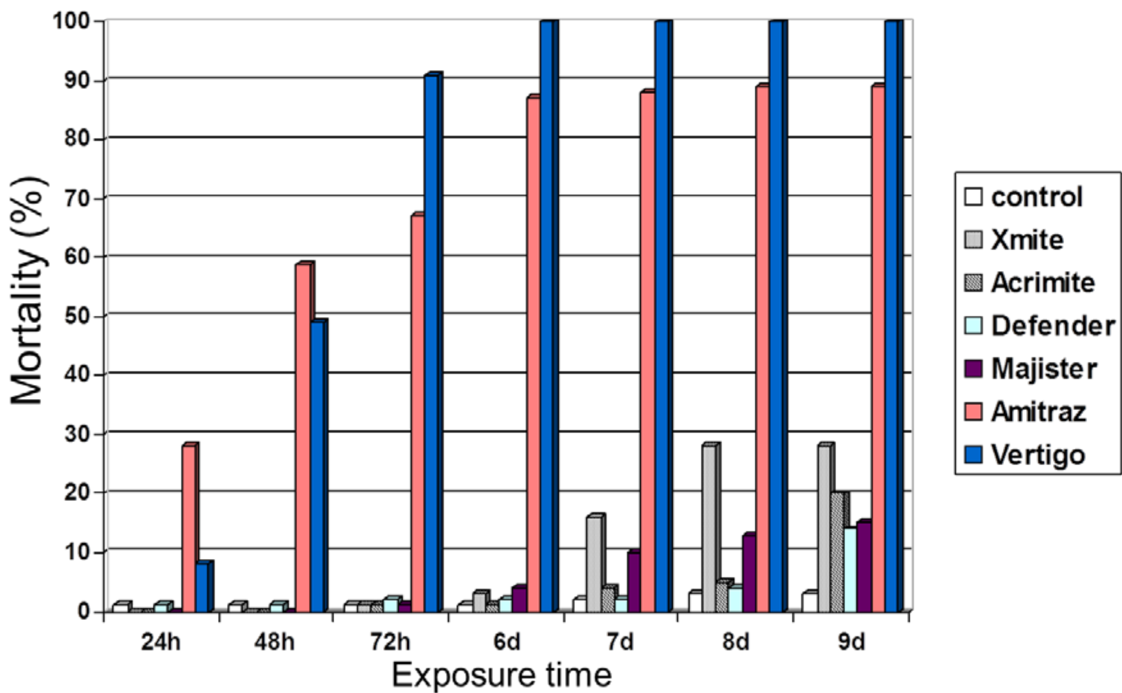

Fig. I.2. Toxicity of various acaricides against honeybee adults compared to amitraz and no treatment control.

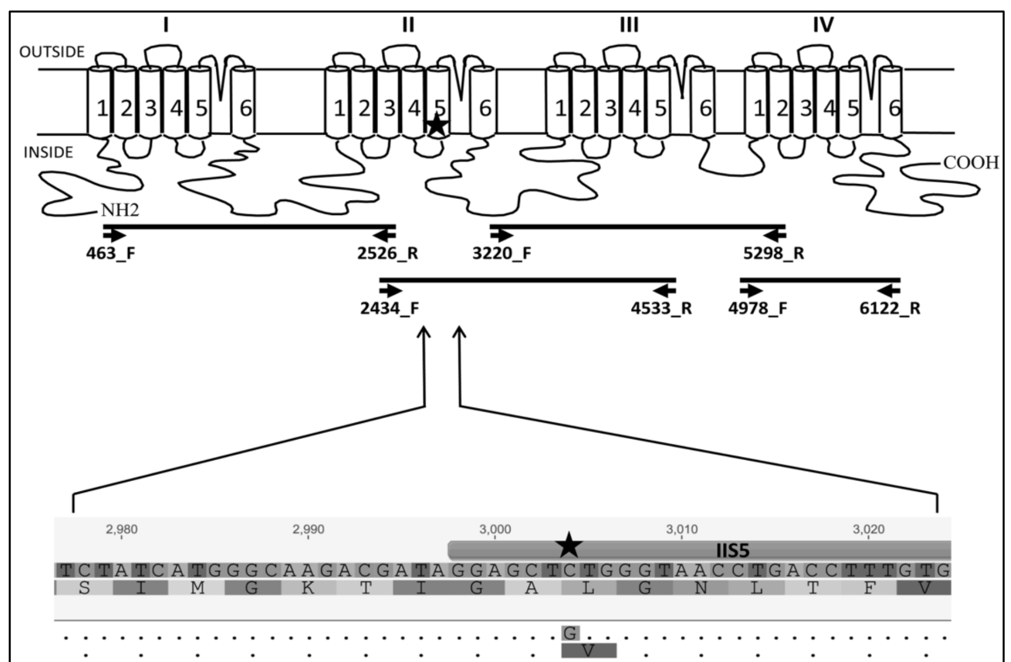

Fig.I.3. Schematic diagram of the Varroa destructor sodium channel gene and position of the mutation L925V. The diagram of the sodium channel protein showing the four main domains (I–IV) and proposed folding of the membrane segments (S1–S6) within each domain. The nucleotide and amino acid sequences of the IIS4–IIS5 linker & IIS5 helix flanking the L925V mutation that confers resistance to fluvainate. (Gonzales-Cabrera et al. 2013).

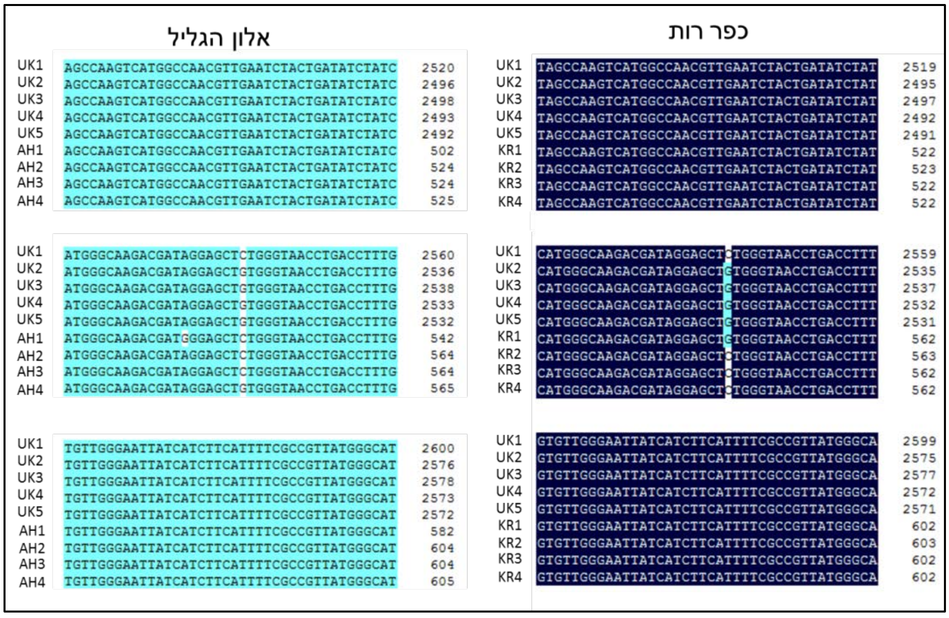

Fig.I.4. Sequences from varroa individuals from UK (UK 1-5) and from Kfar Ruth (KR1-4) and from Alon Hagalil (AH1-4) showing the position of the L925V mutation in some of the populations that confers resistance to fluvalinate.

Gene sequence (in red) 1,101 base pairs

CTCTCTCTCCCTCTTTCTTTCTCTATCTCCGTTTCTTAGCGTCCGAACCTATCTGAAGCTTAACGGTGTTTACCACGAAC
GACAAAACCGGTCCGCGCAGGAGCTCGTCGTTCAACCTGTCCCGTTCGATAACGCTTGCCCGAGGTACTCTTGCTTGGCGC
CCTCTGCAATAAAAACAGCAACAACAGGAACAACGAGGCAGGTTCGTTGCTTACGTTGTCGTCCCTGTCGCGGTGGTTC
TTCCGGTCGTGGTAGTGGTTGCGTCGGTTGAAATCGGAAGAAGGCGCCAGGCTGCCCCGCGACGTTGCCTCGGACGCTGG
CCAACGCTGGACGTGATACCGAATGTCGGTAGAAGCGGGGGCAACCAGCCCCGCCGATATTGCCACCGCCGCCGCTGCCG
ACAGCCCAGAATGGCAGACGTTGGCAAAGACGATCGCCAAGTCGGTGGTGCTGATCTCGATCATATTGACGGCCATCTTCGG
CAATCTGCTCGTCGTCACATCAGTGATAAGGCACCACAAGCTGCGAGTCACCACCAACTATTTTATCGTATCGCTGGC
GTTAGCGGACGCTCTCGTCGCCCTGTTCGCGATGACATTCAATGCGTCGTTCACCATTTCGGGCAAGTGGATGTTCAATC
AGGTGGTCTGCGATTTTTGGAATTCGTGCGATGTCCTTTTTTCGACAGCGTCGATTATGCACCTATGCTGCATATCTGTC
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Protein sequence, 366 amino acids

MSVEAGATSPADIATAAAADSPEWQTLAKTIAKSVVLISIILTAIFGNLLVVTSVIRHHKLRVTTNYFIVSLAL
ADALVALFAMTFNASFTISGKWMFNQVVCDFWNSCDVLFSTASIMHLCCISVDRYYAIIKPLEYPTKITTK
RVFIMLALAWTVSLLISFVPIFTGWYTTEEHRQWLADHPNECVFKVNKYYAIISSSISFWIPCSVMLFTYW
RIYLEATRQEKMLCKTQMGPGGGEQQVIHAPPHRNSHGAEDTESGQSTPTKRNITKMKREHKAAKTLG
IIMGAFILCWLPFFLWYVTTSLCAPTDCPCPEVVVDLLFWIGYINSSLNPIIYAYFNRDFRMAFKKTLQDLF
CSYK ICESKV

Fig.I.5. Octopamine receptor gene and protein sequences cloned form varroa mites in Israel. The gene is 1,101 base pairs in length and encodes a protein with 366 amino acids.

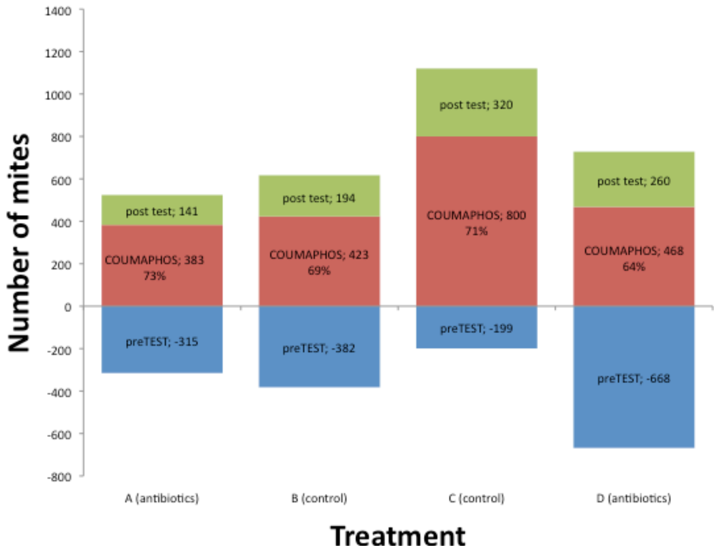


Fig.II.1. The number of Varroa mites that fell to the bottom of the hive during replicate 1 (A,B) and replicate 2 (C,D). Colonies A and D were treated with antibiotics, whereas colonies B and C were untreated controls. Shown are number of mites that fell before treatment (preTEST, blue), during 12 h after treatment with Coumaphos (COUMAPHOS, red), and at the end of the experiment after treatment with oxalic acid, Amitraz, Tau-fluvalinate, and again with Coumaphos (post test, green). Percentages shown are for number of mites falling after Coumaphos treatment relative to all mites in the colony at the beginning of treatment (red / red + green).

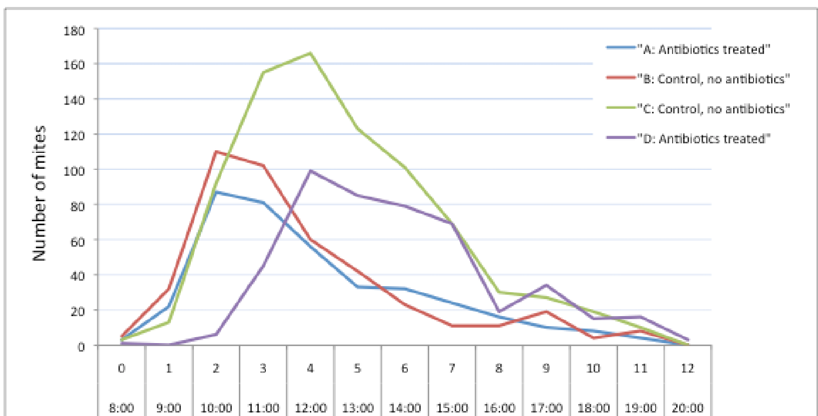


Fig.II.2. The number of Varroa mites that fell to the bottom of the hive during 12 h after treatment with Coumaphos. A Check-mite strip was inserted into each hive at 0800. In the experimental conditions in which all mites were phoretic, peak falling occurred at around 4 h post treatment, declined by 8 h, and ceased almost completely by 12 h.

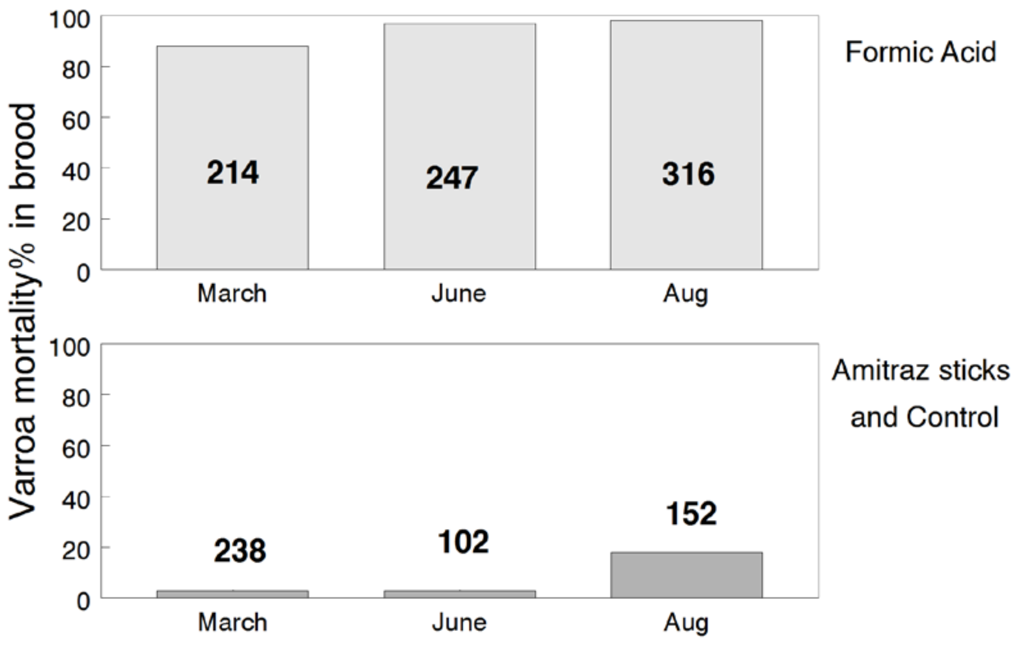


Figure III.1. Mean *Varroa* % mortality in brood 1-4 days post treatment. Top figure treated with formic acid, lower figure treated with Amitraz sticks or non treated controls. Numbers in or above columns indicate the total number of Varroa found and assessed in the respective treatments per season, 2014.

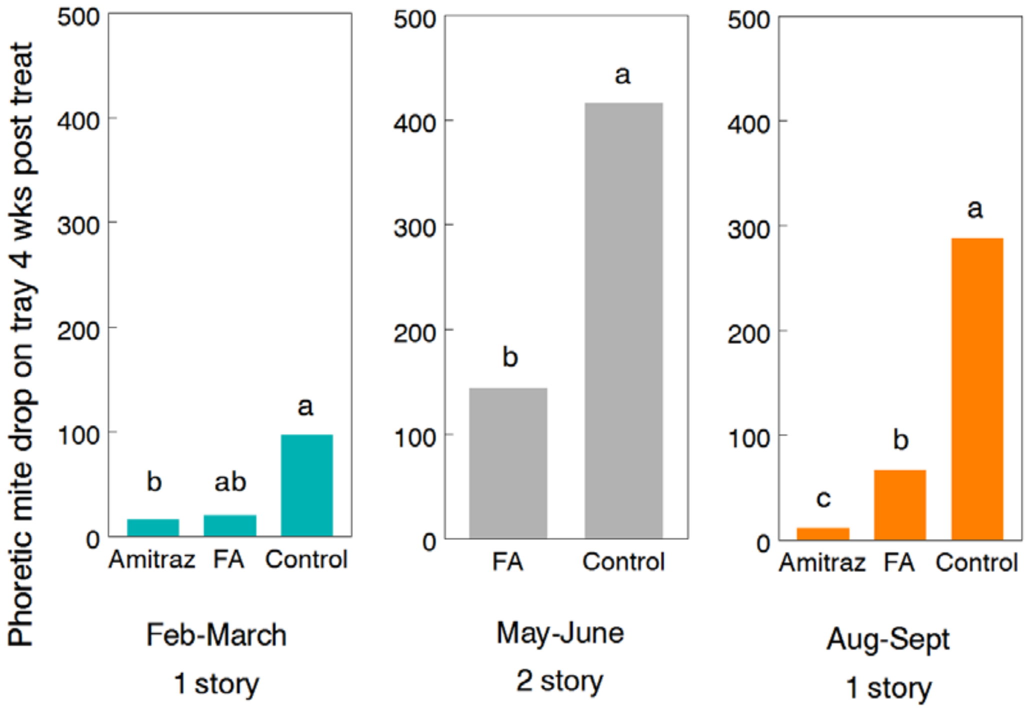


Figure III.2. Mean phoretic Varroa drop per hive immediately following amitraz fumigation 3-4 weeks post treatment with formic acid (FA) or Amitraz, 2014.

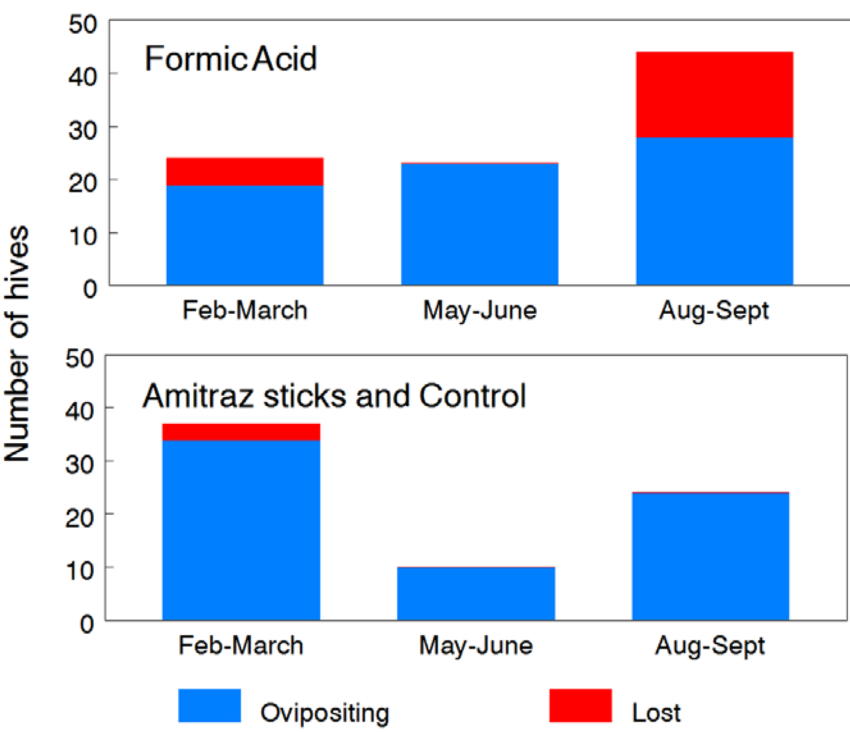


Figure III.3. Number of hives with ovipositing queens and hives that lost their queens, 3-4 weeks following treatment with formic acid (FA) or Amitraz and non-treated control, 2014.

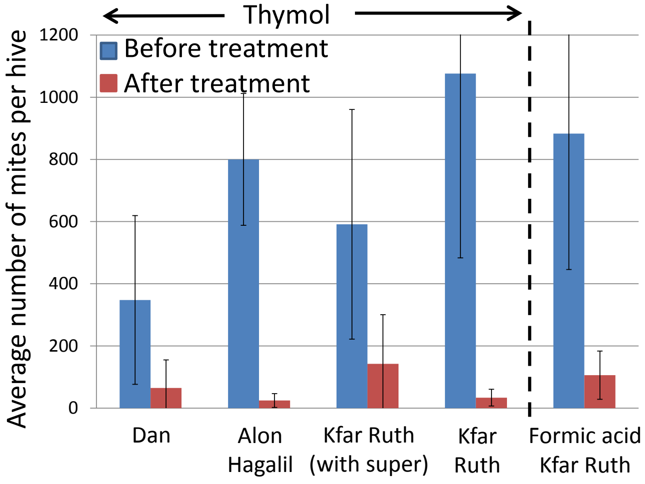


Fig III.4. Efficacy of varroa treatments using thymol and formic acid (with super) measured by mite fall one hour after fumigation with amitraz, summer 2015.

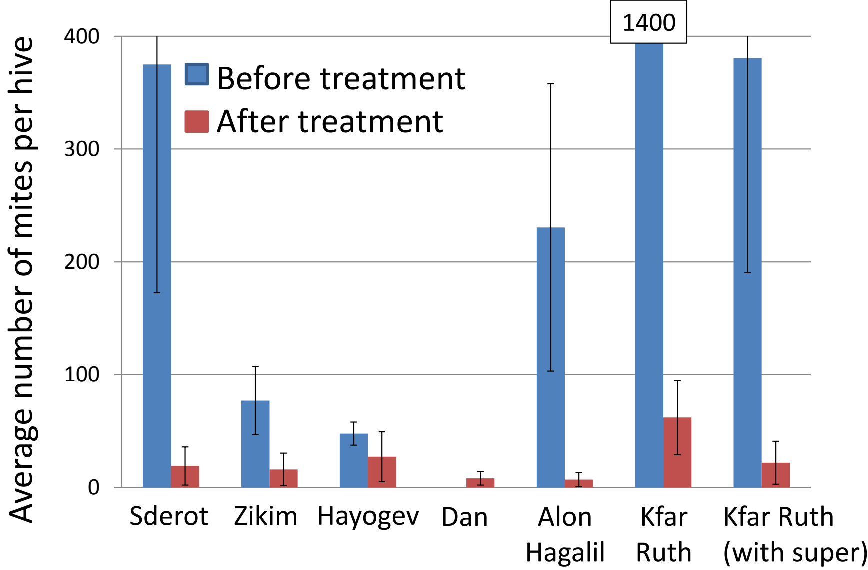


Fig III.5. Efficacy of varroa treatment using amitraz (Galbitraz sticks) measured by mite fall one hour after fumigation with amitraz, summer 2015.

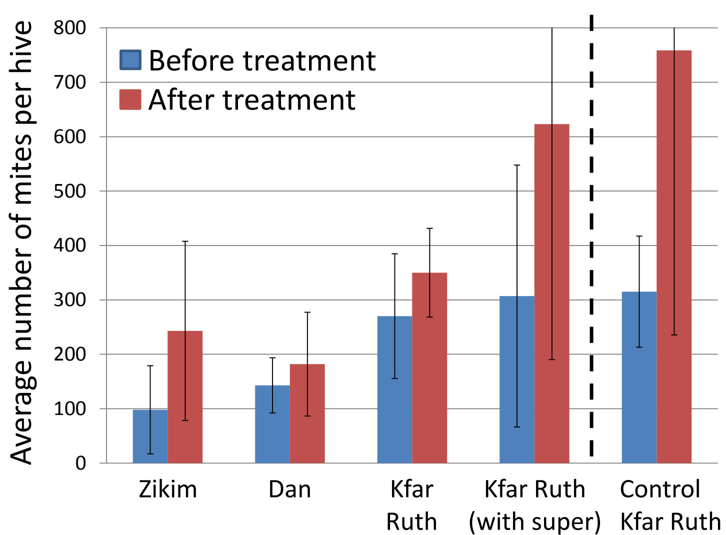


Fig III.6. Efficacy of varroa treatment using Hop-Guard (Hop beta acids) compared to non treated control hives measured by mite fall one hour after amitraz fumigation, summer 2015.

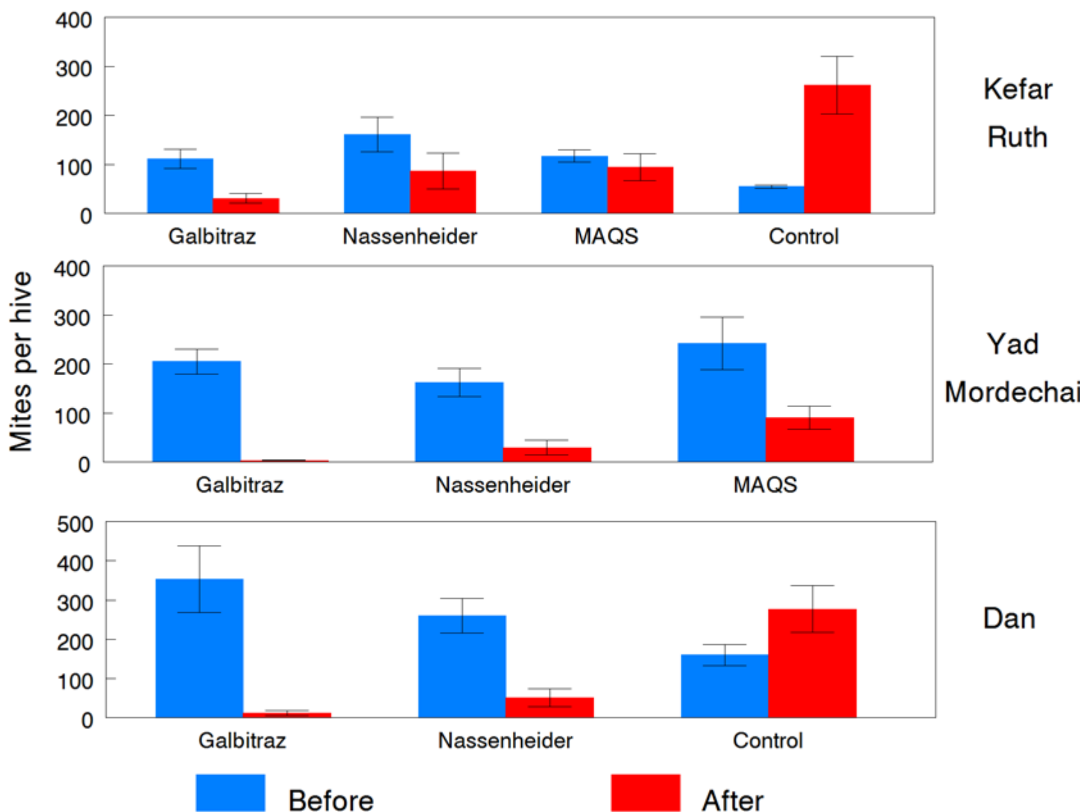


Fig.III.7. Efficacy of varroa treatment using the Nassenheider evaporator, MAQS slow release pads and Galbitraz (amitraz sticks) compared to the non treated control hives measured by mite fall one hour after amitraz fumigation, Kefar Ruth, Yad Mordechai and Dan 2016 (see Table III.1 for date, location, and format [with or without super] for each experiment).

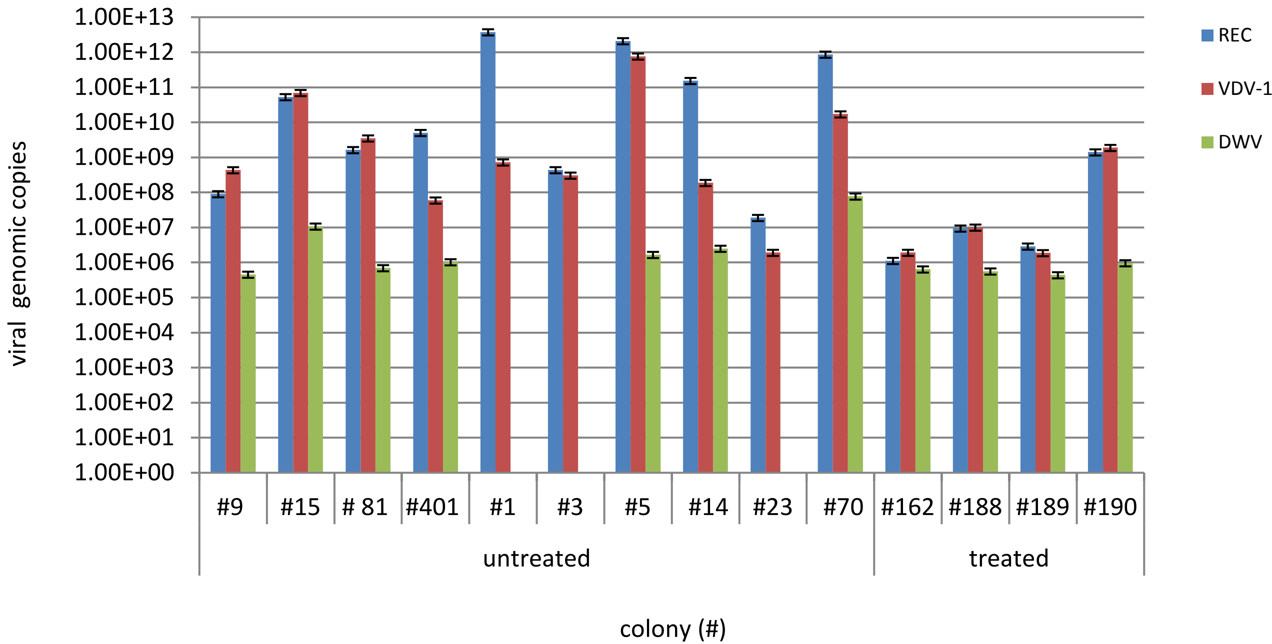
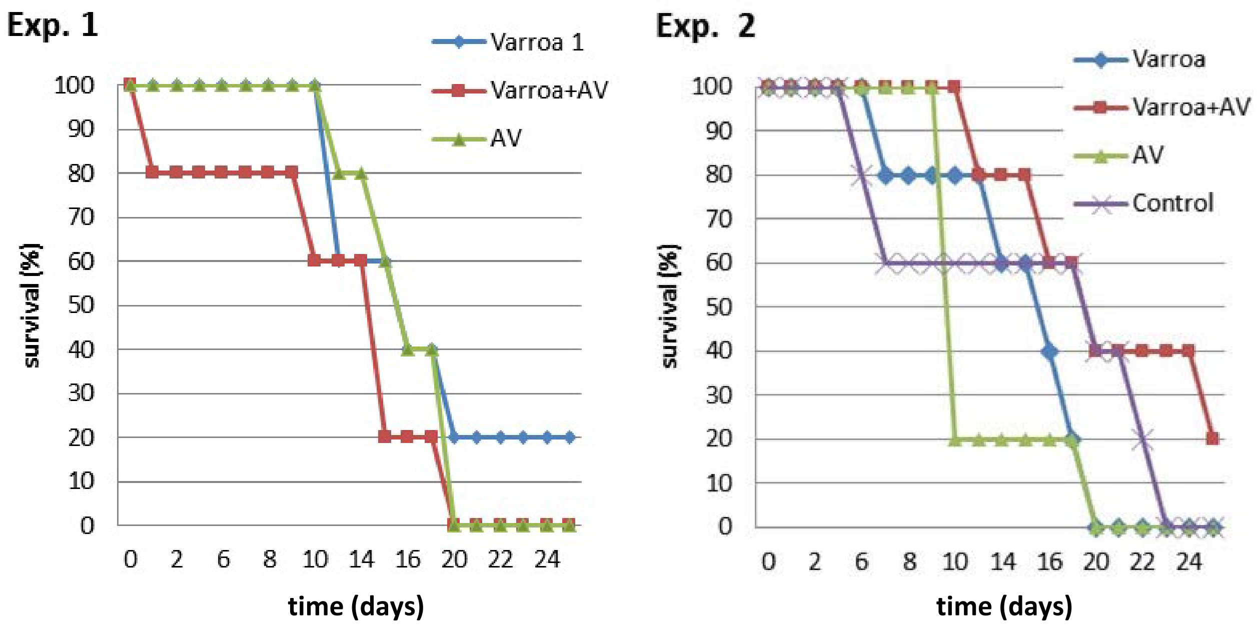


Fig. IV.1 . Genomic copies of DWV,VDV-1 and DWV-VDV-1 recombinant virus (Rec) from pools of adult bees in colonies untreated and treated against *Varroa destructor.*



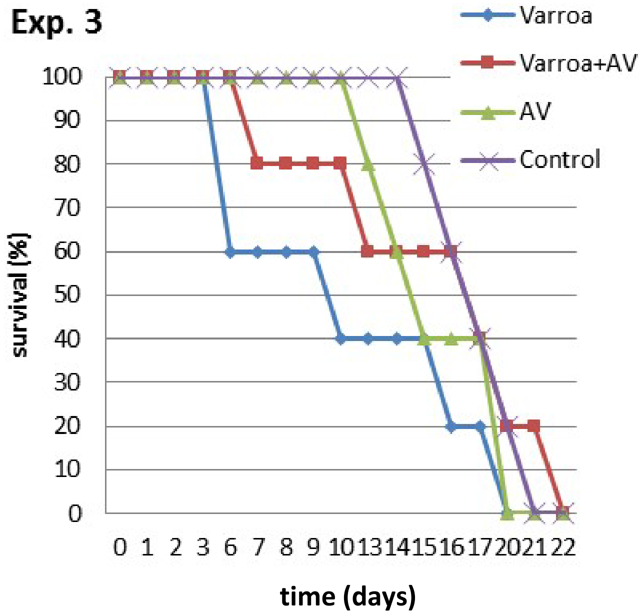


Fig.IV.2. Survival of DWV-infected honey bee adults with or without Varroa following treatment with AV.

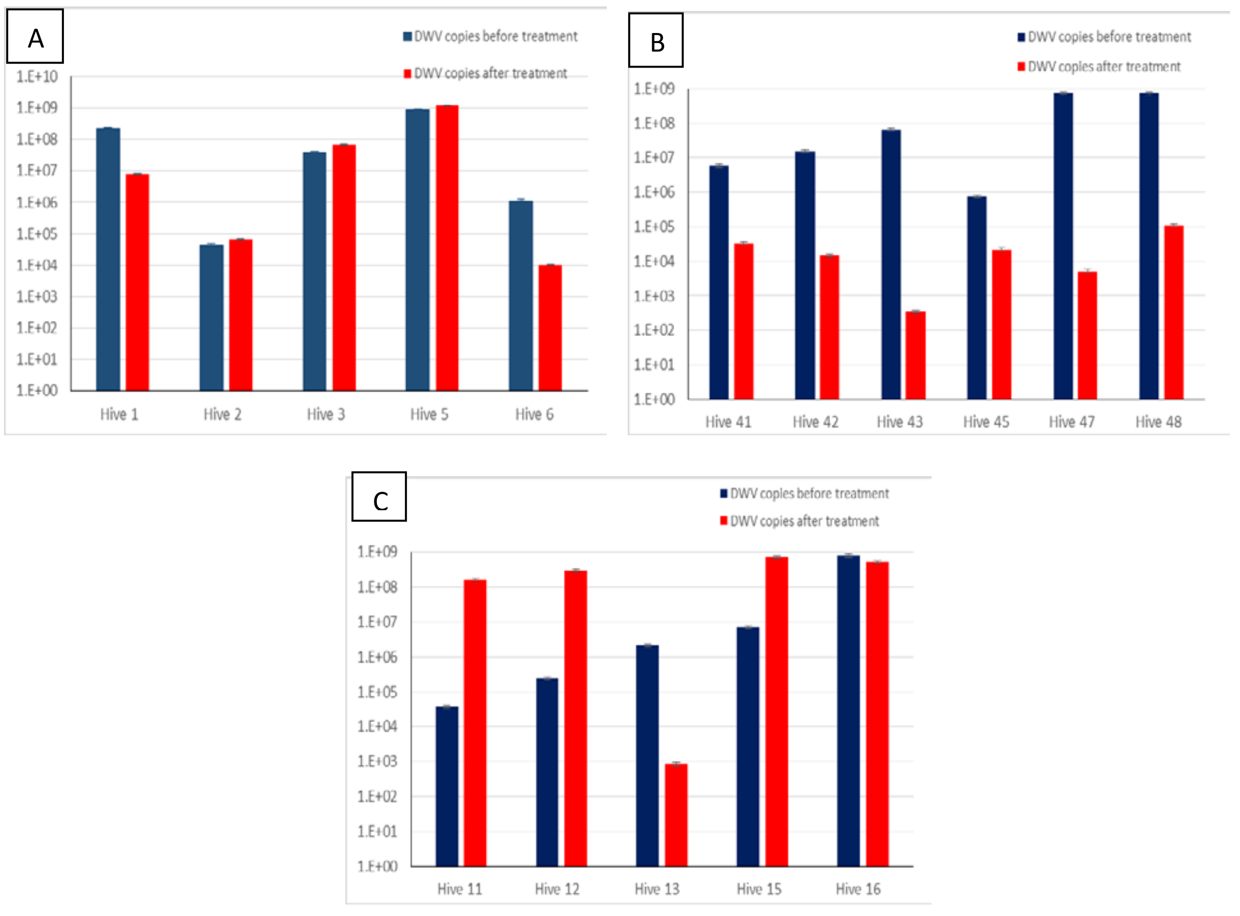


Fig.IV.3. Genomic copies of DWV (including VDV-1-DWV recombinants) in hives subjected to Varroa treatments: A. Amitraz. B. Formic Acid. C, untreated control, Kefar Ruth, summer 2015.

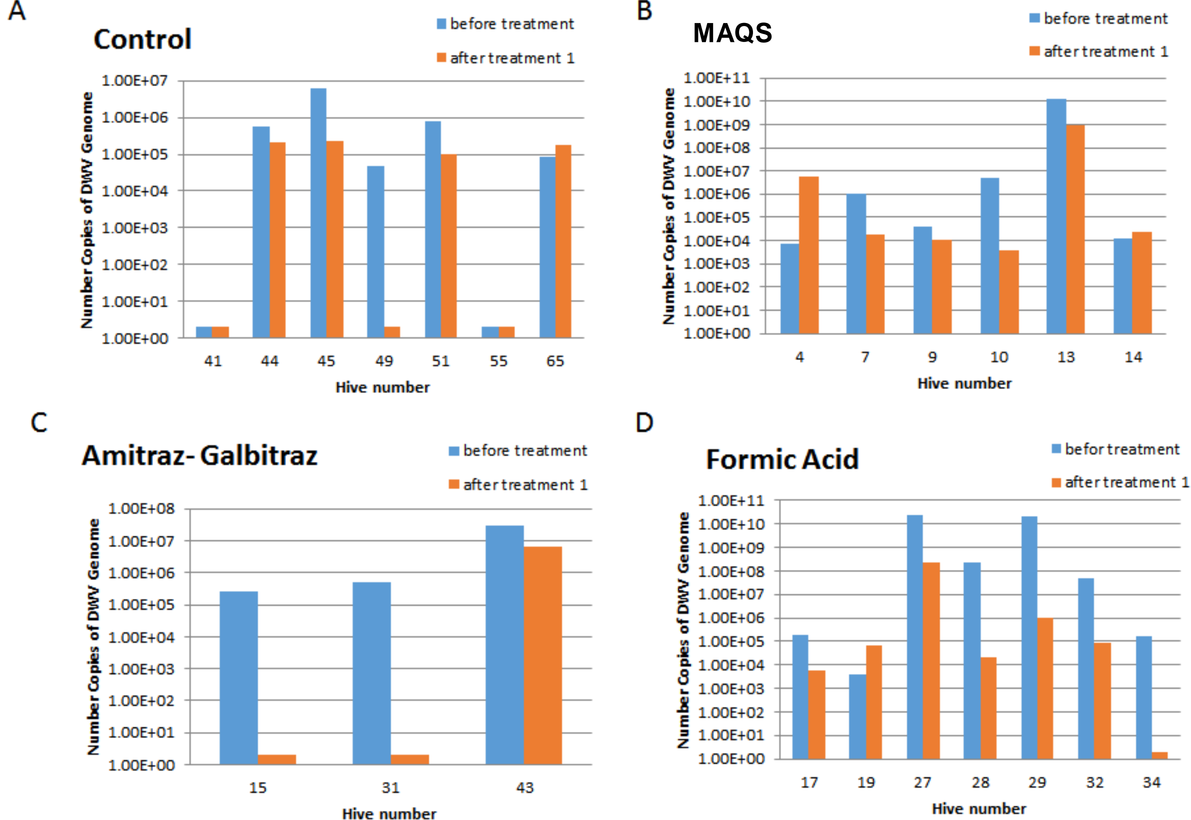


Fig.IV.4. DWV levels in Kfar Ruth, 2016, colonies treated and untreated against Varroa.

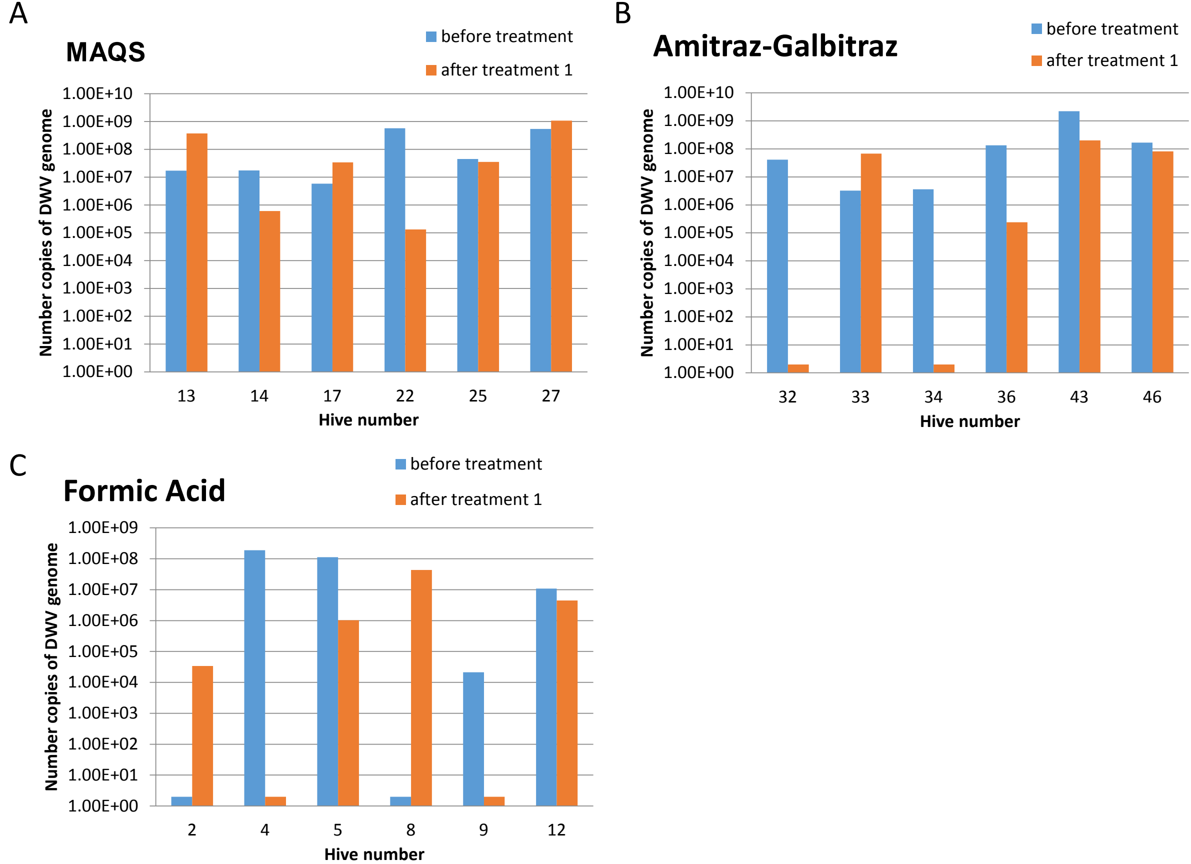


Fig. IV.5. DWV levels in Yad Mordechai, 2016, colonies treated and untreated against Varroa.

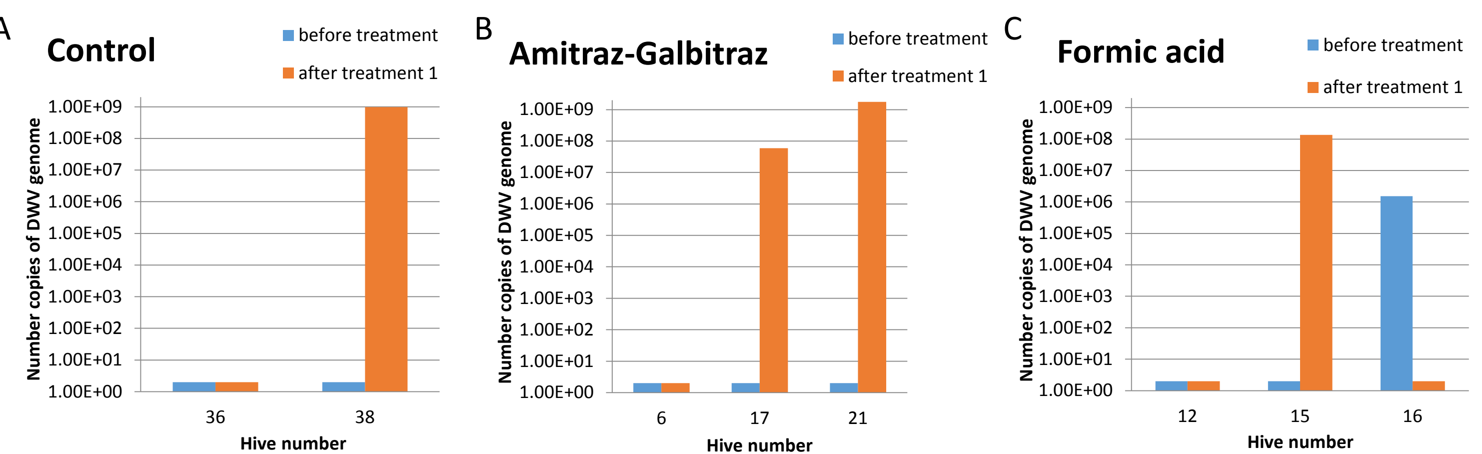


Fig.IV.6. DWV levels in Dan, 2016, colonies treated and untreated against Varroa.

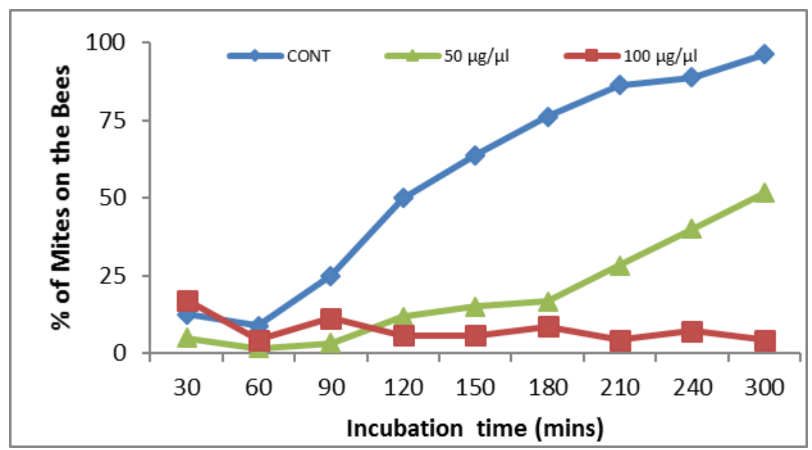


Figure V.1. The effect of 3c{3,6} µg/µl doses on survival of free moving Varroa mites.

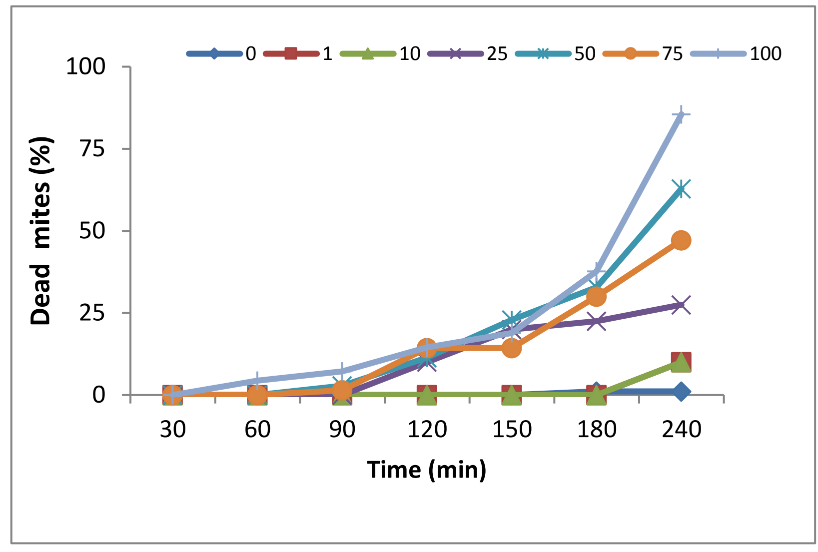


Figure V.2 The effect of 3c{3,6} µg/µl on the fall of phoretic Varroa mites from their host honeybee worker.

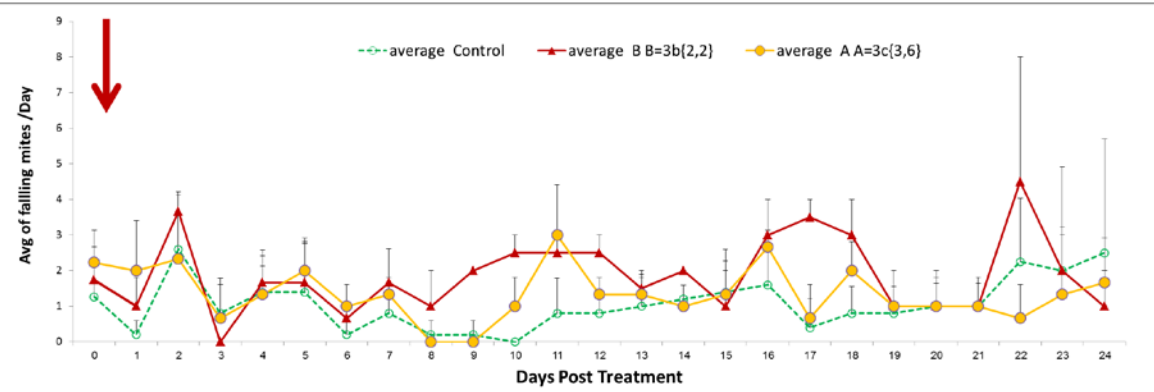


Fig V.3. Changes in Varroa fall/day following the treatment with the wax mold formulation. The arrow indicates treatment time: Each line represents average of Varroa fall +SD in 5 control colonies and 3 hives in each of treatment colonies: B - **3b**{2,2}(1,3-diethoxybenzene); c-**3c**{3,6} (1-allyloxy-4-propoxybenzene).

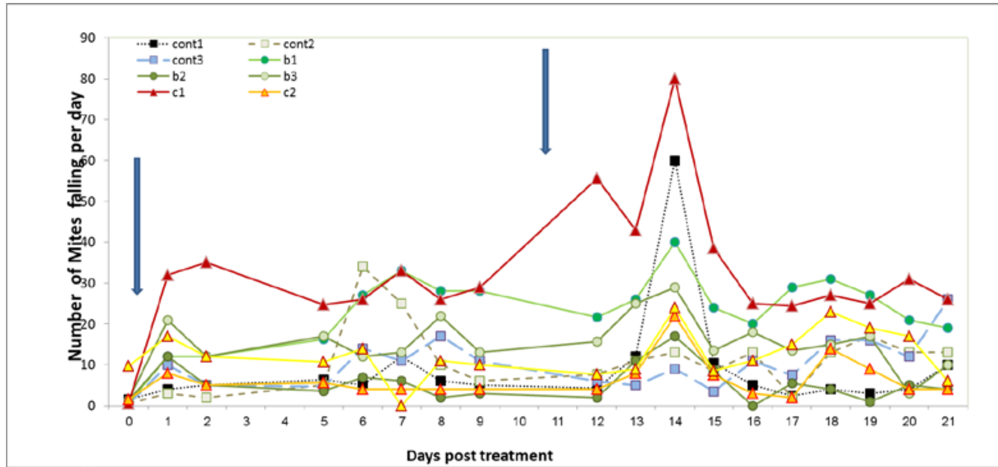
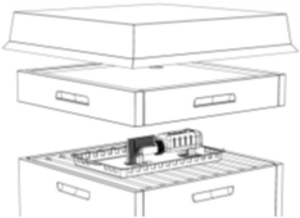


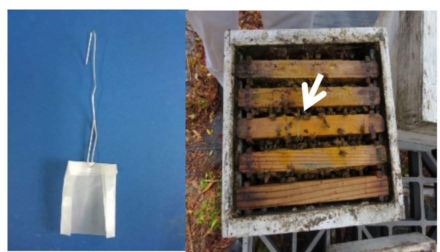
Fig V.4. Changes in Varroa fall/day following the treatment with the sprayable formulations. Arrows indicates treatment times: Each line represents a colony: a-control hives; b-**3b**{2,2}(1,3-diethoxybenzene); c-**3c**{3,6} (1-allyloxy-4-propoxybenzene).



Picture.III.1 Nassenheider evaporator placed below an upside feeder.

Picture.III.2. Slow release thymol formulations.

Picture.V.1. Acaricidal bioassay on the phoretic mites



Picture V.2. Evaluation of DEET effect in the hive, using parafilm (10*5cm) dispensers.

Picture V.3: Testing chemical application on wooden sticks covered with parafilm.

Picture V.4. Application of chemicals in wax mold

Picture V.5 Application of sprayable formulation on and between the frames