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שם המחקר : זיהוי ובחינת אקריות טורפות לפיתוח ממשק הדברה

כנגד נמטודות צמחוניות בשדות פתוחים ובבתי צמיחה אורגניים

Identification and evaluation of soil borne predatory mites for nematode control in open and protected organic cropping systems

מוגש לקרן המדען הראשי במשרד החקלאות

ע"י: אריק פלבסקי - palevsky@volcani. agri. gov.il, שירה גל, מרכז מחקר נווה יער, מכון וולקני; סיגל בראון מיארה פטיסיה בוקי– המחלקה לאנטומולוגיה, מכון וולקני; שירלי גזית - האירגון לחקלאות ביולוגית ואורגנית.

תקציר

<u>הצגת הבעיה</u>: בחקלאות האורגנית נמטודות טפילות הן קבוצה חשובה הגורמת לנזקים כלכליים. הנחת היסוד שלנו היא שקיימות בארץ אקריות טורפות שוכנות קרקע המסוגלות להדביר דרגות נעות של נמטודות צמחוניות, ועל ידי כך להפחית את אוכלוסיתן ואת הנזק לצמחים.
<u>מטרות</u>: 1. איסוף, גידול, וזיהוי של מיני אקריות העשויות לטרוף דרגות נעות של נמטודות צמחוניות. 2. קביעת כושר הטריפה של מינים של אקריות טורפות במעבדה. 3. איתור מזונות אלטרנטיבים לאקריות הטורפות בקרקע. 4. הערכת יכולת הטריפה של נמטודות עפצים על ידי האקריות, בנוכחות מזונות אלטרנטיביים שונים.
<u>שיטות ותוצאות עיקריות</u>: נאספו דגימות קרקע מתוכן הופקו והוגדרו אקריות טורפות באמצעים מקרוסקופיים ועל ידי ריצוף גנטי. שישה מתוך 20 מיני אקריות שהוגדרו מהתת סידרה Gamasina, נלקחו לניסויי טריפה של נמטודות חופשיות בכלים סגורים, ושלושה לניסויים בעציצים עם צמחי עגבניה. בניסוי מעבדה במדיום של וורמקוליט מורטב בכלים סגורים הראנו הפחתה מובהקת בהשרדות הנמטודות ע"י יישום של מספר מיני טורפות. בניסויי העציצים נמצא שהיתה הפחתה מובהקת בנזק הנמטודות לצמח בנוכחות האקריות הטורפות, עם מזון אלטרנטיבי של אקריות טרף או נמטודות חופשיות בקרקע. בסרטוני וידאו שצילמנו תועדו אקריות מהמינים *Lasioseius floridensis*, *Stratiolaelaps scimitus*, ו- *Gaeolaelaps aculeifer* בטריפה של נמטודות מהסוג *Panagrellus*.
<u>מסקנות</u>: מספר מיני אקריות טורפות נמצאו יעילות בהפחתת הנזק שנגרם מנמטודות העפצים. בהמשך אנו מציעים לערוך ניסויים נוספים לשיפור תנאי הקרקע בעציצים, כדי ליצור תנאים אופטימליים לנמטודות החופשיות המהוות מזון אלטרנטיבי, ולאקריות הטורפות במערכת. על ידי כך תוגבר היעילות של ההדברה הביולוגית של הנמטודות המזיקות.

מעריכים מומלצים לבדיקת הדו"ח המדעי:
פרופ' יגאל אלעד, ד"ר סטנלי פרימן, פרופ' אורי גרזון, פרופ יצחק שפיגל
הממצאים בדו"ח זה הינם תוצאות ניסויים. הניסויים <u>לא</u> מהווים המלצות לחקלאים

חתימת החוקר

תאריך: 28/02/2016

Introduction and general hypothesis

Phytoparasitic nematodes are important soil pests of agricultural crops in Israel and abroad, inflicting substantial economic losses (Agrios 1997). Root knot nematodes (RKN), *Meloidogyne* spp. are bio-trophic endo-parasitic nematodes that attack roots of a wide range of plants, including important agricultural crops (Perry et al. 2009), spending most of their life cycle as sessile life stages, inside the root. In contrast, nematodes of the genus *Pratylenchus* spp. are endo-parasites that, following egg hatch, do not lose their ability to move in and out of roots (Pinkerton et al. 1988). What is common to the species of these two genera is their high reproduction rate, ability to reach high densities, subsequently causing significant root damage, yield loss and even plant death.

Predatory mites, especially of the family Phytoseiidae are widely used for the control of above-ground pests (Gerson et al. 2003). In contrast, there is very limited information on the ecology of acarine predators found in soils and their respective potential for the biological control of soil pests. While a range of soil-inhabiting predatory mites belonging to the sub orders Prostigmata and Mesostigmata are known from abroad (Epsky et al. 1988; Gerson 2015; Walter 1987; Walter et al. 1986; Walter et al. 1987; Walter and Ikonen 1989; Walter and Kaplan 1991; Walter et al. 1993), only few studies were conducted in Israel, mostly by Costa, primary focused on the acarine fauna of the litter and rodent parasites (Costa 1966a; Costa 1966b).

Although synthetic nematicides are still used by conventional growers, usually applied prophylactically, they are by definition forbidden in organic farming. In contrast to conventional farming, where a pesticide is used to control a pest, organic farming aims at enhancing host plant resistance and reaching a balance between beneficial and pathogenic organisms. Clearly an important component in this approach is the conservation of natural enemies and the first steps are to identify and evaluate native species.

The general hypothesis of this project is that soil predatory mites will prey on mobile stages of phyto-parasitic nematodes in the soil, thereby reducing nematode density.

Note to reviewers: Following the provisos of the Organic Agriculture Scientific Panel of the Chief Scientist of the Ministry of Agriculture, and the consequent reduction to 2 years we removed the field trials, originally planned for year 3.

Objectives

1) Collect and identify indigenous acarine species as potential candidates for phyto-parasitic nematodes using morphological and molecular tools. 2) Evaluate predation of mobiles stages of the RKN *Meloidogyne javanica* in a simple no choice system. 3) Identify and assess the potential of alternative prey for the conservation of soil predatory mites. 4) Evaluate predator control of RKN in potted plants and assess the effect of soil structure on predatory mite efficacy.

Methods

1) Collect and identify indigenous acarine species using morphological and molecular tools

Because morphological identification is time consuming, requires expertise and keys are based on adult females we decided to add a component of molecular taxonomy. This became feasible through the collaboration on another project focused on the evaluation of soil mites as predators of the red poultry mite, a pest of egg laying hens. The connection between these two projects made even more sense when we found that mites extracted from the soil of organic poultry houses were worthy candidates for the evaluation as predators of nematodes, at least based on the literature.

For barcoding we collected soil samples from selected sites based on our soil mite survey conducted in 2014 (supported by the Israel Taxonomic Initiative). Samples were placed in Berlese funnels (approximately 500 gm per funnel), with 40-watt tungsten lamps as heat sources, for five days and mites were extracted to 95% ethanol. In the lab, ethanol samples were poured into a large Petri dish (25 cm in diameter) and sorted to isomorph types under a dissecting microscope, keeping each sample separate, thereby retaining their collection identity of date and location. Five of each isomorph (per date and location, when available) were imaged one by one with a high resolution digital camera mounted on a stereo microscope equipped with a motorized z

stack (Leica M205) for extended focal imaging (EFI). Then mites were placed individually into wells with 30 µl 95% ethanol in 96-Well PCR Plates (each mite, identifiable by its row, column and microplate number) were sent by courier to the Canadian Center for DNA Barcoding (CCDB) in Guelph Ontario, Canada (http://ccdb.ca/). Collection data (collector, location, date, host), taxonomic information and images of each mite were uploaded to the Barcode of Life Data system (http://v4.boldsystems.org/index.php/default), a process that must be completed before the CCDB will accept microplates for sequencing.

At the CCDB, specimens were sequenced for the barcode region of COI using standard invertebrate extraction, amplification and sequencing protocols outlined in Ivanova et al (Ivanova et al. 2007). Glass fibre extraction was followed by recovery of voucher specimen. A cocktail (1:1 ratio) of LepF1/LepRI (Hebert et al. 2003) and LCO1490/HCO2198 primers were used for PCR, and DNA extracts were subsequently archived at −80°C at the Centre for Biodiversity Genomics (CBG). The vouchered specimens were stored in 95% EtOH and returned to the NYRC.

Contigs were assembled and edited using Codon Code Aligner v. 4.2.7, and aligned by eye in MEGA 6.03 . Each sequence meeting minimum quality criteria (>500 base pairs, <1% ambiguous sites, free of contamination and stop codons) was assigned a Barcode Index Number (BIN) by BOLD (Ratnasingham and Hebert 2013). Sequences were further validated by building a Neighbor-Joining tree in BOLD using the Kimura-2-Parameter distance model. The tree was inspected for unexpected placement of taxa which might indicate contamination or analytical error. These sequences were flagged and filtered from the DNA barcode library on BOLD.

The vouchered specimens, upon their return to Newe Yaar, were sorted by BIN for morphological identification**.** At least 5 female specimens (when available) were mounted in Hoyers for identification using a light microscope, fitted with DIC optics. The remaining adult female specimens, when deemed suitable, were prepared for scanning electron microscopy, following standard procedures of drying and coating. Immatures with the same BINS were mounted as well.

2) Evaluate predation of mobiles stages of RKN in a simple no choice system

For the establishment of live cultures, mites were extracted from the soil with Berlese funnels to closed containers (60 ml) fitted (2.5 cm hole cut through screw top lid with heated hole punch) to the bottom of the funnels. Containers were 2/3 filled with plaster of Paris (made of dental plaster and active carbon, mixed at a volume ratio of 9:1) and wetted down to provide moisture to the extracted mites. Containers were replaced daily and inspected for the presence of live mites. Cultures were then established with approximately 5 individuals appearing to be of the same species based on their morphology (iso-morphic). Mites were reared in a soil mix in plastic 60 ml closed containers. The screw cover lid was ventilated with a 10 mm diameter hole covered with 40 micron mesh. For additional protection from contamination, each 60 ml container was placed in a standard plant tissue culture container, ventilated with the same mesh, placed on a plastic upside down cover with its rim filled with lactic acid serving as a moat. Rearing units were fed twice a week with the sugar mite *Carpoglyphus lactis,* kept at room temperature, and humidity was added by spraying the soil with a hand held water mister. Cultures of six species were established (Table 1).

Table 1: Family, genus and species of predatory mites identified and reared for nematode predation evaluation.

Family	Genus species
Blattisociidae	*Lasioseius floridensis*
Macrochelidae	*Macrocheles* sp.
Laelapidae	*Gaeolaelaps aculeifer*
Parasitidae	*Parasitus hyalinus*
Parasitidae	*Parasitus fimetorum*
Rhodacaridae	*Protogamasellopsis corticalis*

The experimental unit for the evaluation of nematode predation consisted of the ventilated 60 ml container, containing 20 mg of vermiculite (vermiculite 20, Agrical, Habonim, Israel), humidified with water at a ratio of 1:1, 500 nematode motile juveniles (stage J2) and 20 individuals of the predatory mite species. The humidified vermiculite medium was chosen because it allowed both the nematodes and the predatory mites to move freely and facilitated extraction. For the control the same experimental unit

was used except no predatory mites were added. We arrived at a rate of 20 predators per container following the first experiment where we compared 5 individuals to 20. Treatments (candidate species and control) were replicated 4-5 times per experiment. For the preparation of second stage juvenile (J2) of the root knot nematode *M. javanica* RKN, tomato infected roots were harvested and egg extraction was performed with 0.05% (v/v) sodium hypochlorite (NaOCl), followed by sucrose flotation, as described by Hussey and Baker (1973). Eggs were then collected and placed on 25-µm pore size sieves in 0.01 M 2-(*N*morpholino) ethanesulphonic acid (MES) buffer (Sigma-Aldrich) darkness for 3 days, and hatching J2s were collected. To determine the volume needed to apply 500 J2s, density was assessed in four samples of 5 micro-liters. Accordingly, the designated volume was added to the humidified vermiculite. Predatory mites were aspirated into 20 microliter tips using a vacuum pump, twenty individuals per tip and placed open in the 60 ml container, allowing the mites to leave the tip and enter the vermiculite. Containers were placed in a climate chamber at 25ºC in darkness for 48 hours. Nematodes were extracted to water with Baermann funnels for 24 hours at room temperature. Extracted nematodes were kept in a refrigerator till they were counted under a microscope. A total of six experiments were conducted (Table 2). ANOVA was used analyze the predation levels of species for each experiment.

Table 2: Nematode predator species compared to a non-treated control in the six experiments conducted. In experiments 2-6 the predator release rate per replicate was 20 individuals.

Exp.	Nematode predator species
1	*Lasioseius floridensis - rate 5 vs. 20 individuals*
2	*Macrocheles* sp.
3	*Gaeolaelaps aculeifer*
4	*L. floridensis, G. aculeifer, Parasitus hyalinus*
5	*L. floridensis, G. aculeifer, Protogamasellopsis corticalis*
6	*P. hyalinus, Parasitus* sp. (deutonymphs), *P. corticalis*

3) Identify potential alternative prey for soil predatory mites

In our original proposal, we proposed using the sugar mite *Carpoglyphus lactis* as alternative prey and suggested using starch wastes to promote this

predator in greenhouses in the third year of our project. As the project was only funded for two years, this component was removed. Instead, in the first potted plant experiments we use *C. lactis* along with the medium developed for its mass rearing (bran and added ingredients). The problem with *C. lactis* is that it colonizes the soil litter and does not enter the soil, as it is not a soil mite but a pest of storage products. Species of free-living non-parasitic nematodes (FLNPN) could certainly be better suited as potential alternative prey since they live in the soil adjacent to the roots of plants. To test the prey suitability of FLNPN we used the free living nematode *Panagrellus redivivus* as prey, and observed and recorded predation behavior of *Gaeolaelaps aculeifer, Stratiolaelaps scimitus and Lasioseius floridensis*. Clips were recorded in collaboration with Eitan Recht from the Plant Protection and Inspection Services (Israeli Ministry of Agriculture, Bet Dagan) and Orly Oren (Prof. Itamar Glazer's lab, Plant Protection, ARO) using a new high resolution digital microscope (Hirox KH-8700).

4) Evaluate predator control of RKN in potted plants and assess effect of soil structure on predatory mite efficacy

Two experiments were conducted in plastic pots (800 cc).

Exp. 1 was setup on 20/12/16. Each pot comprised one replicate of each treatment. The bottom of the pot was covered with tuff and filled with potting soil composed of red loam: vermiculite (5:1 by volume). One young tomato plant with 4 leaves, CV Avigail (www.hazera.com) known to be susceptible to RKN, was planted in each pot. For the two predator species *Lasioseius floridensis* (Blattisociidae) and *Protogamasellopsis corticalis* (Rhodacaridae), three treatments, replicated 5 times were carried out: 1) non-treated control, 'clean plant', with no RKN or predators. 2) Inoculated with J2 of RKN. 3) Treatment '2' plus the release of predators. The experiment was conducted in 2 climate rooms at the Newe Ya'ar Research Center, one room for each predator species at 16L:8D, *L. floridensis* 20.3°C ±3.3, 60.6% RH ±6.5; *P. corticalis* 23.6°C ±3.4, 47.6% RH ±7.0. Plants were grown under high intensity discharge fluorescent grow lamps. Pots were placed at random, mounted on an upside down saucer (the center drilled for drainage) its rim serving as a trough, filled with oil to prevent contamination between treatments. Plants were watered individually from the top of the pot, every 2-3 days with 100-200

ml of distilled water and fertilized once a week with a prepared liquid mix of NPK 20-20-20. To establish predator populations before nematode inoculation (treatment 3 only), 0.2 gm of a commercial bran mix containing approximately 500 individuals of *C. lactis* (used for rearing *A. swirskii* by BioBee-http://www.biobee.com/biological-ipm/solutions/bio-swirskii/) were placed on the soil 2 cm from the plant, along with 20 female predators (day 0). On day 7, treatments 2 and 3 were inoculated with approximately 3000 nematodes/plant. On day 21 an additional predator release and application of *C. lactis* was performed, following which applications of *C. lactis* were conducted fortnightly (days 35 and 49, only for treatment 3). Exp. 1 ended on 21/02/2017, day 63 (56 days from nematode infestation), upon which the upper plant material of each plant was cut and discarded and the below soil stem and roots were washed in a pail. Nematode infestation was ranked using a Gall Index (Oka et al. 1999) and eggs were extracted from roots. Gall index rank values (GI) were analyzed with the non-parametric Wilcoxon signed-rank test, comparisons performed between pairs of treatments. Egg values were analyzed with ANOVA. To confirm mite presence and absence in respective treatments, mites were sieved from the liquid wash and soil, with a 99µm sieve.

Exp. 2 was setup on 26/4/2017. In this experiment we added two elements to the experimental design of Exp. 1 and evaluated a third species *Stratiolaelaps scimitus*. First, we hypothesized that the relatively fine particle size characteristic of loam impeded predator movement and consequently reduced control efficacy. Thus, all treatments, except for treatment 5 were planted in pure vermiculite, successfully used in the arena experiments, which we know poses no problem as we rear *S. scimitus* in this medium. Secondly, we hypothesized that the free-living-non parasitic-nematode (FLNPN) *Panagrellus redivivus* (reared on oats and extracted to water) will be a better alternative prey than *C. lactis* as it lives inside the soil. Additionally, to avoid washing the predators from the pot when irrigating from above, we irrigated from the saucer below the plant (which was mounted on the same upside down saucer with the moat filled with oil) and fertilized once a week. Accordingly, the following five treatments, replicated 5 times, were conducted:

1) non-treated control, ‘clean plant’. 2) Inoculated with J2 of RKN. 3) Treatment ‘2’ plus the release of *S. scimitus* and *C. lactis*. 4) Treatment ‘2’ plus the release of *S. scimitus* and FLNPN. 5) Same as treatment 4 but the potting soil was composed of red loam: vermiculite (5:1 by volume), as performed in Exp. 1. The experiment was carried out in a climate controlled room at 16L:8D, 24.4°C ±1.7, 62.5% RH ±7.4. Plants were grown under high intensity discharge fluorescent grow lamps and pots were placed at random. On day 1 we added to the soil: To treatment 3, *C. lactis* in surplus and 30 individuals of *S. scimitus*; To treatments 4 and 5 approximately 10,000 FLN and 30 individuals of *S. scimitus*. On day 11 all treatments, except treatment 1, were inoculated with 3500-4000 J2 RKN. On day 13 alternate prey and predators were applied a second time at the same rates on treatments 3-5, respectively. On 18/6/2017, day 53 (42 days from nematode inoculation) the experiment ended and processed as described above for Exp 1. With the exception that mites from the potting soil were extracted with Berlese funnels.

Results

1) Collect and identify indigenous acarine species using morphological and molecular tools

Seventeen species of mites (three to genera) belonging to the suborder Gamasina, were identified molecularly and morphologically, uploaded to the BOLD, and listed in Table 3. Of which we have assessed 6 species in small arenas in no choice tests and 3 in potted plant trials.

Table 3: Species of mites (three to genera) belonging to the suborder Gamasina, identified molecularly and morphologically, uploaded to BOLD http://v4.boldsystems.org/index.php/default.

Family	Species
Blattisociidae	*Blattisocius mali* (Oudermans, 1929)
Blattisociidae	*Lasioseius floridensis* Berlese, 1916
Digamasellidae	*Dendrolaelaps lobatus*
Laelapidae	*Androlaelaps casalis* (Berlese, 1887)
Laelapidae	*Gaeolaelaps aculeifer* (Canestrini 1883)
Laelapidae	*Stratiolaelaps scimitus* Womersley, 1956
Melicharidae	*Proctolaelaps parascolyti or near*
Melicharidae	*Proctolaelaps sp.*

Parasitidae	*Coleogamasus sp.*
Parasitidae	*Gamasodes spiniger* (Oudemans, 1936)
Parasitidae	*Parasitus hyalinus* (Willman, 1949)
Parasitidae	*Parasitus consanguineus* Oudemans & Voigts, 1904
Parasitidae	*Parasitus fimetorum* (Berlese, 1903)
Parasitidae	*Parasitus kempersi* Oudemans, 1902
Parasitidae	*Poecilochirus sp.*
Macrochelidae	*Macrocheles merdarius* (Berlese, 1889)
Macrochelidae	*Macrocheles peniciliger* (Berlese, 1904)
Macrochelidae	*Macrocheles muscaedomesticae* (Scopoli, 1972)
Macrochelidae	*Macrocheles scutatus* (Berlese, 1904)
Rhodacaridae	*Protogamasellopsis corticalis* Evans & Purvis, 1987
Rhodacaridae	*Rhodacarellus silesiacus* Willmann, 1935

2) Evaluate predation of mobiles stages of RKN in a simple no choice system

In the results presented below we presumed that nematode predation occurred when the number of nematodes extracted in the predator release treatments were significantly lower than the number of nematodes extracted in the no release control treatment.

In the first experiment we compared nematode predation following the release of 5 versus 20 individuals of *L. floridensis.* Nematode predation attained by 20 predators/replicate was significantly higher than the control, whereas the predation level afforded by 5 individuals/ replicate did not differ from the control or the higher rate (P= 0.0218; Table 4). Thus the subsequent experiments were conducted at the rate of 20 predators/replicate.

Table 4: Experiment 1, mean number of root knot nematodes RKN *Meloidogyne javanica* extracted following 48 hours of predation by 5 and 20 individuals/replicate of *Lasioseius floridensis.* Means followed by a different letter indicate a significant difference with $\alpha \leq 0.05$.

Treatment	Mean RKN *M. javanica* extracted
20 individuals of *L. floridensis*	73.4 b
5 individuals of *L. floridensis*	119.4 ab
No release of predators	166.4 a

Similarly significantly more nematodes survived in the no release treatments compared to the release of 20 individuals of *Macrocheles* sp. ($P < 0.0001$) and Gaeolaelaps queenslandicus ($P < 0.0001$), respectively (Table 5).

Table 5: Experiments 2 & 3, mean number of root knot nematodes RKN *Meloidogyne javanica* extracted following 48 hours of predation of *Macrocheles* sp. and *Gaeolaelaps aculeifer,* respectively. Means followed by a different letter indicate a significant difference within each experiment with $\alpha \leq 0.05$.

Exp.	Treatment	Mean RKN *M. javanica* extracted
2	*Macrocheles* sp.	21.8 b
	No release of predators	344.4 a
3	*Gaeolaelaps aculeifer*	40.7 b
	No release of predators	86.7 a

In experiments 4 through 6 we compared between 3 predator species and the no release control, adding each time one species that was not previously evaluated (Table 6). In experiments 4 ($P = 0.0026$) and 5 ($P = 0.0011$) *L. floridensis* and *G. aculeifer* continued to show significantly higher predation levels compared to the control, as did the *P. corticalis*. In contrast *P. hyalinus* did not differ from the control. Finally in experiment 6 we evaluated the deutonymphs of another *Parasitus* species (evidently *P. fimetorum*). All species differed significantly from the no release control ($P = 0.0005$), including *P. hyalinus* that did not differ significantly from the control in experiment 5.

Table 6: Experiments 4-6, mean number of root knot nematodes RKN *Meloidogyne javanica* extracted following 48 hours of predation of the predator species *Lasioseius floridensis, Gaeolaelaps aculeifer, Parasitus hyalinus, Protogamasellopsis corticalis and Parasitus* sp. (deutonymphs). Means followed by a different letter indicate a significant difference within each experiment with $\alpha \leq 0.05$.

Exp.	Treatment	Mean RKN *M. javanica* extracted
4	*Lasioseius floridensis*	76.4 b
	Gaeolaelaps aculeifer	57.6 b
	Parasitus hyalinus	85.6 ab
	No release of predators	117.3 a
5	*Lasioseius floridensis*	78.2 b

	Gaeolaelaps aculeifer	69.6 b
	Protogamasellopsis corticalis	64.4 b
	No release of predators	108.6 a
6	*Parasitus hyalinus*	55.4 b
	Parasitus sp. (deutonymphs)	53.8 b
	Protogamasellopsis corticalis	51.8 b
	No release of predators	82.2 a

3) Identify potential alternative prey for soil predatory mites

As far as we know, these are the first high quality videos of nematode predation ever recorded (the video files are included on the disc submitted to the chief scientist, along with the hardcopy of this proposal). Predation behavior of the three species (Video 1. *Stratiolaelaps scimitus*; Video 2. *Gaeolaelaps aculeifer*; Video 3. *Lasioseius floridensis*) was remarkably similar, and can be described in four sequential steps: 1) the predator catches the nematode with one chelicera (evidently, between the fixed and movable digits); 2) tugs it from the surface; 3) using both chelicerae it spins the nematode into a ball (which probably includes cutting into the nematode's integument); and 4) sucks the nematode's body fluids into the opening of the pharynx (located at the tip of ventral surface of the deutosternum). Free living nematodes, such as the *P. redivivus* can be found in abundance in soils free of pesticides and can serve as an excellent source of alternative food to maintain predatory mites (Carrascosa et al. 2015; Heidemann et al. 2014). Soil predators can then serve as a standing army for protection against plant parasitic nematodes and arthropod (mite and insect) pests. However, clearly more research is needed to determine predator species compatibility to soil types, suitability of free living nematodes and pest species.

4) Evaluate predator control of RKN and assess the effect of soil structure on RKN infestation and predatory mite efficacy

Exp. 1: Mites were recovered from the predator treatments: *Lasioseius floridensis* 16.4±4.6 (mean±SE per pot) and *Protogamasellopsis corticalis* 51.2±17.9 but not from the two non-release treatments (1 & 2). Thereby confirming the establishment of predators in their respective treatments and no contaminations in the non-release treatments. Nematode infestation on the

RKN treatment only (Trt. 2) reached a high gall index (GI) of 4, whereas the control treatment (Trt. 1) remained clean with a GI of 0 (Table 7). The release of *Lasioseius floridensis* (Trt. 3) significantly reduced the GI to 3, but did not significantly affect the number of RKN eggs/gr roots. In contrast, the predator release of *P. corticalis* did not affect the GI, nor did it significantly affect the RKN eggs/gr roots.

Exp. 2: This experiment was terminated after 7 weeks, a week less than Exp. 1. Mites were recovered from the release treatments (Trt. 3, 4 and 5) and not from the non-release treatments (Trt. 1 and 2) but levels were very low (means followed by SE: Trt 3. 3.2±1.3; Trt 4. 0.4±0.4; Trt. 5 1.8±0.9) compared to Exp. 1. Probably because the soil was too wet due to the mode of irrigation. However the results pertaining to nematode control assessed by the gallining index (GI) of this experiment are particularly interesting and novel. The release of *S. miles* with the FLNPN potted in vermiculite yielded the same result as the control (which received no RKN) and was significantly different from release of *S. miles* with the FLNPN potted in Loam-Vermiculite (5:1) (Trt. 4), the latter being similar to RKN only (Trt. 2). The release of *S. miles* with *C. lactis* in vermiculite however, did not differ from any of the treatments that received RKN (Trt. 2,4 and 5).

Table 7a: Exp. 1 – Seven weeks post inoculation of the root knot nematode RKN *Meloidogyne javanica.* Mean number of predators, median gall index recorded from roots of tomato plants CV Avigail, and RKN eggs/gr roots for treatments: 1) No RKN. 2) RKN. 3) RKN with predator and *Carpoglyphus lactis* releases. All treatments were planted in a soil mix of loam-vermiculite (5:1). Two trials were conducted in parallel, in two identical rooms, one with *Lasioseius floridensis* and the second with *Protogamasellopsis corticalis.* Means followed by a different letter indicate a significant difference within each column with $\alpha \leq 0.05$.

Trt. No.	Treatment	Mites	GI	Eggs/gr roots
1	Control	0	0 c	0 b
3	RKN+ *L. floridensis* + *C. lactis*	16.4	3 b	106407 a
2	RKN	0	4 a	154190 a
1	Control	0	0 c	0 b
3	RKN+ *P. corticalis* + *C. lactis*	52	3 ab	56744 ab
2	RKN	0	3.5 a	103749 a

Table 7b: Exp. 2- P-values for non-parametric Wilcoxon signed-rank test, comparisons performed between pairs of treatments.

	Level		**- Level**	
Trt. No.	Treatment	Trt. No.	Treatment	**p-Value**
1	Control	2	RKN	0.0071
3	RKN+L. floridensis	2	RKN	0.0434
1	Control	3	RKN+L. floridensis	0.0065
1	Control	2	RKN	0.0071
3	RKN+P. corticalis	2	RKN	0.6873
1	Control	3	RKN+P. corticalis	0.0100

Table 8a: Exp. 2 - Six weeks post inoculation of the root knot nematode RKN *Meloidogyne javanica.* Median gall index for tomato plants CV Avigail for five treatments: 1) Control. 2) RKN. 3) RKN plus *Stratiolaelaps scimitus* and *Carpoglyphus lactis*. 4) RKN plus *S. scimitus* and the free living non parasitic nematode (FLNPN) *Panagrellus redivivus. 5)* RKN plus *S. scimitus* and FLNPN. Treatments 1-4 were planted in vermiculite whereas treatment 5 was planted in a soil mix of loam-vermiculite (5:1). Means followed by a different letter indicate a significant difference within each column with α ≤ 0.05.

Trt. No.	Treatement	Soil mix	Median
1	Control	Vermiculite	0 d
4	RKN+*S. miles*+FLNPN	Vermiculite	0 cd
3	RKN+*S. miles*+*C.lactis*	Vermiculite	0.5 abc
5	RKN+*S. miles*+ FLNPN	Loam-Vermiculite (5:1)	1 ab
2	RKN	Vermiculite	1.5 a

Table 8b: Exp. 2- P-values for non-parametric Wilcoxon signed-rank test, comparisons performed between pairs of treatments.

	Level			- Level		p-Value
	Treament			Treament		
2	RKN	Vermiculite	1	Control	Vermiculite	0.0071
3	RKN+*S. miles*+*C.lactis*	Vermiculite	2	RKN	Vermiculite	0.1175
4	RKN+*S. miles*+FLNPN	Vermiculite	2	RKN	Vermiculite	0.0285
5	RKN+*S. miles*+FLNPN	Loam-Verm.	2	RKN	Vermiculite	0.9142
5	RKN+*S. miles*+FLNPN	Loam-Verm.	4	RKN+*S. miles*+FLNPN	Vermiculite	0.0181
4	RKN+*S. miles*+FLNPN	Vermiculite	1	Control	Vermiculite	0.1770
5	RKN+*S. miles*+FLNPN	Loam-Verm.	1	Control	Vermiculite	0.0073
3	RKN+*S. miles*+*C.lactis*	Vermiculite	1	Control	Vermiculite	0.0232
5	RKN+*S. miles*+FLNPN	Loam-Verm.	3	RKN+*S. miles*+*C.lactis*	Vermiculite	0.0634
4	RKN+*S. miles*+FLNPN	Vermiculite	3	RKN+*S. miles*+*C.lactis*	Vermiculite	0.2040

Discussion and Summary

The molecular tools that we have produced in this study for the identification of species will give the independence biocontrol researchers so dearly need to continue working with these species, without having to depend on the support of trained mite taxonomists.

A simple bioassay using humidified vermiculite as a medium allowed to screen for nematode predation potential. Nematode survival was significantly lower than the no release control in all species of predators evaluated (except for *P. hyalinus* in experiment 5, but was significant in experiment 6). As P values were considerably lower than 0.01 in almost all cases, we believe this bioassay to be reliable. Having said that, it appears that the bioassay can be improved as J2 survival in the no release treatment varied considerably between experiments.

Lasioseius subterraneus (another species of *Lasioseius*), has been reported to feed on more than 100 J2 of *M. javanica* per day (Walter et al. 1993). As there were 20 predators per replicate, and the initial number of J2 500, it appears that our experimental setup yielded substantially lower predation levels for *L. floridensis*. The commercially available species *Gaeolaelaps aculeifer*, supposedly similar or identical to the local strain studied in the present study, was observed feeding on egg masses of *Meloidogyne chitwoodi* (Inserra and Davis 1983). For the genus *Parasitus* we did not find any indication of its potential for feeding on plant feeding nematodes. However in a recent study focused on predatory mites in mushroom houses, designed to evaluate the range of prey suitability for *Parasitus bituberosus*, the shortest developmental time was obtained on the rhabditid nematode diet (Szafranek et al. 2013). Similarly numerous *Macrocheles* species have been reported to prey on free living nematodes (Carrillo et al. 2015). Species of Rhodacaridae also feed on many free living nematodes (Carrillo et al. 2015) and may be more promising candidates than species of Parasitidae and Macrochelidae as they are substantially smaller and may therefore be more able to enter deeper into the rhizosphere populated by plant parasitic nematodes.

Our direct recording of *S. scimitus*, *G. aculeifer*, and *L. floridensis* demonstrated their ability to feed on the FLNPN *P. redivivus.* This

accomplishment opens the doors to addressing further questions such as: What parameters may affect the suitability of FLNPN as alternative prey for soil mite predators? FLNPN species, available nutrients, production costs, etc. Clearly More behavioral studies are needed to record the predation of gall forming and free living plant parasitic nematodes.

In our first potted plant trials we reached relatively high gall index (GI) values of 4 and 3.5 in the RKN treatments and 3 in both predator treatments. While *L. floridensis* did significantly reduce the GI by one level, the roots were still substantially damaged. We hypothesized that the small spaces between the silica particles of dense loam could be impeding the movement of the predators. Consequently, in the second experiment we decided to assess the effect of the potting mix density on nematode control, using vermiculite as the ultimate aerated potting mix vs. the dense loam mix. Additionally to enhance the predator 'size' effect, we switched to the relatively large predator *S. scimitus*. Indeed our results demonstrated that the predator had no effect in the dense loam but did significantly reduced the GI in vermiculite. This result is very important because it indicates that the manipulation of soil structure towards a more aerated/fluffy texture, for example by annual applications of compost commonly practiced in organic agriculture, can enhance nematode biocontrol. Additionally it appears that the FLNPNs are better alternative prey than the storage mite *C. lactis*. Utilizing and preferring FLNPN over *C. lactis* as alternative prey also fits with organic agricultural practices because populations of FLNPN are enhanced by compost treatments (Thoden et al. 2011).

One important issue that remains to be addressed is the mode of irrigation in the potted plant experiments. Manually irrigating from the top of the soil (as done in Exp. 1) can flush the predators from the pot. Whereas irrigating from the bottom without drainage (Exp. 2) keeps the soil constantly wet and enhances salinity. Maintaining a moist soil is crucial for studying the populations dynamics of predators, FLNPN and plant parasitic nematodes, over extended periods. In the present study, we were able to overcome this problem because biological control took place immediately after the RKN J2s were added. Clearly, the motile J2 that were fed upon did not make it into the plant roots whereas those who escaped predation did. However, in a real

situation, where plants are constantly exposed to RKN J2, a stable population of predators is needed to protect the plant roots.

In summary, in this study we have uncovered numerous indigenous potential candidates for nematode control. Additionally, our research indicates that the efficacy of commercially available soil predators (in Europe), such as *G. aculeifer* and *S. scimitus* (found naturally in Israel, but not yet produced) can be enhanced by the augmentation of FLNPN and soil structure manipulations. To improve and develop the biological control of plant parasitic nematodes we suggest that further research focus on the manipulation of soil structure and nutrients to conserve FLNPN and soil predatory mite populations.

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סיכום עם שאלות מנחות

אנא פרט מהם הניסויים שנעשו תוך השוואה לתכנית העבודה המתוכננת והתאמתם למטרות המחקר כפי שהופיעו בהצעה המקיפה
הושגו מטרות 3-1: 1) נאספו וזוהו 20 מינים של אקריות קרקע פוטנציאליות לשימוש כאויבים טבעיים לנמטודות צמחוניות. 2) נערכו ניסויים במעבדה לבחינת יעילות הטריפה של דרגת J2 של נמטודות העפצים (RKN) על ידי ששה מינים שונים של אקריות טורפות. 3) בניסויים בעציצים נבדקה יעילות שלושה מיני אקריות טורפות בנוכחות מזון אלטרנטיבי, בקרקעות עם מבנה שונה, ונאמד הנזק לצמח.
מהם עיקרי הניסויים והתוצאות שהושגו בתקופה אליה מתייחס הדו"ח?
20 מיני אקריות פוטנציאלים זוהו באמצעים מיקרוסקופים ובאמצעות ריצוף גנטי. בניסויי טריפה של נמטודות היתה הפחתה מובהקת בכמות הנמטודות. בניסויים בעציצים היתה הפחתה מובהקת בנזק לצמח בנוכחות אקריות וטרף אלטרנטיבי לאקריות. הייתה השפעה מובהקת של מבנה הקרקע על יעילות ההדברה הביולוגית.
בעקבות הניסויים שנעשו, אנא פרט והסבר כיצד הושגו מטרות המחקר בתקופת הדו"ח או חלק מהן
זיהוי מיני האקריות בוצע קודם על בסיס מולקולרי ולאחר מכן בעזרת מומחים לטקסונומיה מחו"ל. היתכנות הטריפה בוצעה בניסויים בארנות ללא בחירה. הוכחת הפחתת הנזק נערכה בניסוי עציצים. שיתוף הפעולה האמיתי בין המעבדה של פלבסקי ובראון מיארה היה קריטי לביצוע כל הניסויים והוביל לתוצאה מאוד מוצלחת.
בהתאם להצעה המקיפה, ציין מה התבצע מתוך טבלת המשימות ואבני דרך, כולל אבני דרך כמותיות (סעיפים-IV VI) ומהם הקריטריונים שפורטו בהצעה המקיפה כבוחן להצלחת המחקר אכן הושגו
הועדה של חקלאות אורגנית קיצצה את התוכנית משלוש שנים לשנתיים, ביקשה להוריד את ניסוי השדה ולהוכיח שאקריות טורפות יכולות להפחית את האוכלוסיות של נמטודה צמחונית והנזקים שהיא גורמת. בהתאם לכך התרכזנו בניסויים בארנות ובעציצים. הראנו שמגוון של מיני אקריות טורפות יכולים להפחית אוכלוסיות של RKN, ושניים מתוך שלושה מינים של אקריות טורפות הפחיתו את רמת הנזק.
מהן המסקנות המדעיות ומהן ההשלכות לגבי יישום המחקר והמשכו בעתיד?
ההדברה הביולוגית של נמטודות צמחוניות ע"י אקריות הטורפות מושפעת ע"י מבנה הקרקע וטיב המזון האלטרנטיבי החליפי. נמטודות חופשיות בקרקע עשויות להיות יותר מתאימות מהאקרית המחסן *Carpoglyphus lactis* כמזון חליפי. יש להתאים את מין הטורפת, מזון חליפי ומבנה הקרקע על מנת למקסם את ההדברה הביולוגית.
מהן הבעיות שנותרו לפתרון ו/או שינויים (טכנולוגיים, שיווקיים ואחרים) שחלו במהלך העבודה ומה אמורה להיות ההתייחסות בהמשך?
יש לבחון: א) תוספות קרקע ליצירת סביבה אופטימלית לאקריות הטורפות ולמזון האלטרנטיבי שלהן. ב) מיני טורפות נוספים להדברת הנמטודה יוצרת העפצים והנמטודה האנדופרזיטית הנודדת. ג) כמזון חליפי התאמה בין מיני נמטודות חופשיות לא טפיליות למיני אקריות טורפות.
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פרסום הדו"ח: אני ממליץ לפרסם את הדו"ח: (סמן אחת מהאופציות)
➢ ללא הגבלה (בספריות ובאינטרנט)
➢ האם בכוונתך להגיש תוכנית המשך בתום תקופת המחקר הנוכחי? לא