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# *Cosmolaelaps simplex* (Berlese), a Polyphagous Predatory Mite Feeding on Root-knot Nematode *Meloidogyne javanica* and Citrus Nematode *Tylenchulus semipenetrans*

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**Abstract:** Biological aspects of the laelapid mite *Cosmolaelaps simplex* (Fox) was studied for the first time after feeding on one acarid mite species *Caloglyphus rodriguezi* Samsinak and egg masses of two plant parasitic nematodes *Meloidogyne javanica* Treub and *Tylenchulus semipenetrans* (Cobb) at 26°C. Also, the effect of adding of mite individuals or aldicarb to citrus seedlings infected with citrus nematodes *T. semipenetrans* was investigated under greenhouse conditions. The laelapid mite successfully completed its life span feeding on the previous preys. The developmental time of *C. simplex* averaged 8.4, 8.8 and 10.6 days for male immatures and averaged 10.3, 12.6 and 13.9 days for females when they were fed on acarid mites and egg masses of both *M. javanica* and *T. semipenetrans* at 26°C, respectively. Larvae didn't feed and the mite passed through two nymphal stages before adulthood. Males had a shorter life span than females where they lasted for an average of 37.6, 42.0 and 44.9 days for males and 45.7, 45.9 and 48.6 days for females after feeding on the same previous food sources, respectively. Arrhenotoky parthenogenesis was observed where un-mated females put eggs that gave males. Egg production was higher when *C. simplex* fed on the acarid mites where the net reproductive rate (Ro) was 20.46, while it decreased to 13.62 and 13.10 after feeding on egg masses of root-knot and citrus nematodes, respectively. The predatory mite had a mean generation time (T) of 22.24, 21.07 and 20.22, while the net rate of natural increase ($r_m$) was 0.135, 0.123 and 0.127 after feeding on the aforementioned preys, respectively. Enhanced citrus seedlings growth were achieved when citrus seedlings were treated with the nematicide aldicarb and followed by those treated with mite individuals added at the same time of nematode inoculation. Compared to the nematode-alone control, all mite treatments and aldicarb significantly restricted reproduction of citrus nematode. Nematode populations ranged from 1386 to 1665 J2/100 $cm^3$ soil for the mite-treated plants compared to 4011 J2/100 $cm^3$ soil for the nematode untreated control. Moreover, the predatory mite *C. simplex* showed a preferable response to the acarid mites but it also significantly reduced citrus nematode *T. semipenetrans* populations under greenhouse conditions.

**Key words:** *Cosmolaelaps simplex*, *Caloglyphus rodriguezi*, *Meloidogyne javanica*, *Tylenchulus semipenetrans*, life table parameters, biology

## INTRODUCTION

Plant parasitic nematodes are widespread and cause serious losses to most agricultural crops. Nematodes are often managed with chemical nematicides which can contaminate agro-ecosystems. Natural antagonistics of nematodes and biocontrol agents may provide an alternative to the use of pesticides for nematode management. Considerable information available in the literature has documented the effectiveness of several biological agents to manage plant parasitic nematodes[1-5]. The acari exhibit various associations with soil fauna especially nematodes. Since beneficial soil mites especially predatory species must be conserved in the field to promote a more stabilized pest and natural balance[6]. The potential of using the predaceous mites as biological control agents in controlling plant parasitic nematodes have rarely been studied so far. Little is also known about the habits of many organisms that form together the belowground food web[7]. Accordingly, several trials had demonstrated the ability of some soil mites to reduce nematode populations[8-13]. Mesostigmatid mites represent an important component of the belowground food web, where they are generally considered to be predators feeding on small arthropods, worms and nematodes[11,14]. Previously, it was found that nymphs and adults of *Lasioseius scapulatus* (Kennet) had the ability to capture, consume and complete its entire life cycle on the root knot nematode *Meloidogyne incognita*[9]. Also, the suitability of egg

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masses of *Meloidogyne* spp. as food source of the ascid mite species *L. dentatus* (Fox) was studied where it was found that the predatory mite successfully completed its whole life span on egg masses[8].

On the other hand, the mesostigmatid family Laelapidae Berlese is considered one of the most important groups of soil predators, where it usually feeds on nematodes[15].

Genus *Cosmolaelaps* Berles consists of a large group of free-living predators in soil[16-18]. The adult female of *C. simplex* was firstly described without giving any illustration of morphological characters[9] but it was recently re-described the female and described the male for the first time for samples collected from soil including leaf litter under citrus trees at Lack Alfred, Florida, USA[20].

The present investigation was conducted to determine the biological aspects of *C. simplex* for the first time and also to evaluate its effectiveness in reducing some of the co-existence organisms such as root knot nematode *M. javanica* Treub and citrus nematode *T. semipenterans* in comparison with the acarid mite *C. rodriguezi* Samsinak under laboratory conditions. Also, a greenhouse study was conducted to study the effect of adding mite individuals or aldicarb for control of citrus nematode, *T. semipenetrans* on citrus seedlings.

## MATERIALS AND METHODS

**Rearing of predatory mite in laboratory:** Laboratory culture of the predatory laelapid mite *C. simplex* was originated from soil samples including leaf litter under citrus trees at Lake Alfred County, Florida. Mite samples have been maintained on the acarid mite species *Caloglyphus rodriguezi* as a food source in rearing units which was previously described[21]. The acarid mite was maintained in laboratory on moulded cheese and bread.

Newly deposited eggs of *C. simplex* had been singly placed in smaller rearing units (5 cm in diameter), where immature stages were provided with one of the following food types during their entire life span:

-Developing stages of the acarid mite *C. rodriguezi*
-Egg masses of root - knot nematode *M. javanica*
-Egg masses of citrus nematode *T. semipenetrans*

All rearing units were kept in an incubator at 26±1°C and 65%±5 RH. In all cases, incubation period, duration of developmental stages (in days), number of surviving mite individuals and egg production were recorded daily. Life table parameters of the predatory mite *C. simplex*, which was fed on three different foods, were calculated[22,23] by using the Basic Computer Program[24] where:

L = Number of female alive
x = Actual female age (in days)
Mx = Female progeny/female (mothers) /day x (specific fecundity rate)
Lx = Rate of survival at day x
Ro = The net reproductive rate ($\sum Lx\ Mx$)
T = The mean generation time ($\frac{\sum Lx\ Mx\ .\ x}{\sum Lx\ Mx}$)
$r_m$ = The intrinsic rate of natural increase
$e^{rm}$ = The finite rate of increase

**Greenhouse experiments:** A group of 24 plastic pots (25 cm in diameter) containing sand loam sterilized soil (1:1) and previously transplanted with citrus seedlings of sefi orange similar in length (approximately 30 cm each). The transplanted pots were divided into six groups, 4 pots each, where the first group was infected with approximately 1000 of juvenile stages of citrus nematode *T. semipenetrans*/pot. The second group was infected with nematodes 15 days after adding 200 individuals of *C. simplex*/pot. The third group received the mites at the same time of nematode inoculation, while the fourth one received the same number of mites 15 days after nematode inoculation. The nematicide, aldicarb (10 g/pot) was used in the fifth group which was previously infected with nematodes. The sixth group was left without any application and received the same agricultural tactics and used as control.

Ninety days after nematode inoculation, citrus seedlings were carefully taken off where data dealing with shoot length and fresh weight for both shoot and root systems were recorded. After that, roots were carefully washed and left free of soil, where total number of egg masses per root was recorded. Moreover, the second juvenile stages $J_2$ found in 100 mL of soil samples was estimated for each nematode treatment after extraction by sieving using modified Baermann's technique[25]. On the other hand, soil samples of about 250 g each were also subjected for mite extraction by the aid of the modified Tullgern's funnels[2] where, the average number of mite/250 g of soil was calculated.

In all cases, data were subsequently analyzed by Duncan's multiple rang and ANOVA tests, where the reproduction index of predatory mite = final mite population (PF)/ initial mite population (PI).

## RESULTS AND DISCUSSION

**Biology and life table parameters of *Cosmolaelaps simplex*:** Under laboratory conditions of 26°C and 65% RH, it was noticed that the incubation period of eggs of *C. simplex* ranged from 2.20 to 2.70 days for both sexes

Table 1: Developmental time (days) of different stages and adult longevity of *Cosmolaelaps simplex* fed on three different foods and kept at 26°C and 65% RH

| | | | | Developmental stages | | | | Adults | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Food source | Sex ratio (%) | Sex | Eggs | L | N1 | N2 | T | Preovi-posion | Ovi-posion | Post-oviposion | Longevity | Life span |
| *Caloglyphus* | 61 | O | 2.2±0.06 | 2.9±0.00 | 2.8±0.2 | 2.7±0.1 | 8.4±0.8 | - | - | - | 29.2±1.1 | 37.6±1.6 |
| *rodriguezi* | | O | 2.7±.03 | 2.8±0.00 | 3.1±0.1 | 4.4±0.3 | 10.3±1.2 | 4.3±0.2 | 26.3±1.2 | 4.8±0.3 | 35.4±1.6 | 45.7±1.9 |
| *Meloidogyne* | 54 | O | 2.3±.04 | 2.2±0.20 | 2.9±0.1 | 3.7±0.2 | 8.8±1.3 | - | - | - | 33.2±1.3 | 42.7±1.9 |
| *javanica* | | O | 2.6±0.03 | 3.4±0.10 | 3.8±0.2 | 5.4±0.1 | 12.6±1.2 | 5.6±0.6 | 21.8±0.9 | 5.9±0.4 | 33.3±1.3 | 45.9±1.6 |
| *Tylenculus* | 56 | O | 2.4±0.03 | 2.8±0.08 | 3.8±0.1 | 4.0±0.3 | 10.6±1.1 | - | - | - | 34.3±1.4 | 44.9±1.4 |
| *semipenetrans* | | O | 2.6±0.06 | 3.8±0.10 | 4.3±0.3 | 5.8±0.2 | 13.9±1.4 | 6.2±1.1 | 22.3±1.2 | 6.2±0.8 | 34.7±1.1 | 48.6±1.6 |

L=Larva, N1=Protonymph, N2=Deutonymph, T=Total immature stages

(Table 1). Because of no previous information about different biological aspects isn't available about this mite species, it was felt necessary to note most of its biology where *C. simplex* is considered to be oviparous mite. Adult female lays its eggs singly on the upper thin layer of soil, which covers the substrate. No changes in size or gross chorion structure during the incubation period were observed. Moreover, both sexes of *C. simplex* passed through larval, protonympal and deutonymphal stages before reaching adulthood. Larvae didn't feed on any of the tested food sources. Similar results were previously obtained with the gamasid mite species *C. claviger* that belonging to the same genus[27]. While it was mentioned before that most larvae of mesostigmatid mites must feed to develop into protonymphs[7,8]. The newly hatched larvae were observed relatively sluggish, pale yellow in color and move very slowly.

Male larva lived for 2.2 to 2.9 days while female ones durated from 2.8 to 3.8 days, respectively (Table 1). Concerning feeding, it was observed that *Cosmolaelaps* mite individuals showed common feeding behavior where they approached acarid mites and egg masses of nematodes with their first pair of legs. After that, they elevated slightly, pinned down the prey with their pedipalps and penetrated by using rostrum and chelicerae then devoured the preys. After feeding, the predator were always noticed rubbing their mouth parts several times with its first pair of legs for cleaning. Generally, a mite individual was observed feeding on a single prey individual of *C. rodriguezi* in approximately 40-50 sec. On the other hand, mite individuals were observed feeding on the gelatinous matrix and body fluids of eggs of both nematodes *M. javanica* and *T. semipenetrans*. Female protonymphs lasted 3.1, 3.8 and 4.3 days while male protonymphs lasted 2.8, 2.9 and 3.8 days when they fed on the acarid mite *C. rodreguezi*, egg masses of *M. javanica* and *T. semipenetrans*, respectively. Concerning deutonymphal stage, data clearly showed that female deutonymphs took 4.4, 5.4 and 5.8 days, while males lasted 2.7, 3.7 and 4.0 days before reaching the adulthood when they were provided with the same aforementioned food sources, respectively. Accordingly, it is clear that male life cycle was shorter than females where it ranged from 8.4 to 10.6 days for males but it prolonged from 10.3 to 13.9 days for females, respectively (Table 1). The previous data indicate that a diet of egg masses of both plant parasitic nematode species obviously prolonged the developmental period of *C. simplex*. Data also showed that there was significant difference between the effects of both nematode species as food sources on the developmental period of the tested predaceous mite. From the previous results, it can be concluded that *C. simplex* can be considered as a polyphagous predatory mite, which can survive and reproduce on a wide range of food sources. Similarly, it was previously found that *C. claviger* fed and reproduced when it was provided with the bulb mite *Rhizoglyphus* echinopus, collembolan, house fly eggs and larvae as well as free living nematodes[27]. Also it was found that its reproductive potentiality was higher by feeding on mites or insects than on nematodes[27]. Moreover, it was found that about 18 mesostigmatid mite species fed vigorously on the nematode species *Cephalopus* sp. (Rhabiditida) under laboratory conditions. Most of these species preferred the vermiform nematodes and showed also a positive response to the obese-forms like females of *Meloidogyne* spp. and cyst nematodes *Heterodera* spp. that were sedentary[28]. After that, it was experimentally proved that *Lasioseius dentatus* (Fox) survived and successfully reproduced by feeding on fungi, acarid mites and root-knot nematodes[8]. Contradictory, *Macrocheles muscadomestica* (Scopoli) didn't reproduce when it was supplied with acarid mites or collembolans and it had a successful biology on nematodes[29]. In the adulthood, mating was observed between males and females where its posses lasted an average of 45 min. Some of the unmated females were contradictory observed laying eggs that gave only males. Therefore, it can be concluded that arrhenotoky parthenogenesis commonly occurs in *C. simplex*. These findings agree with previous ones[30-32] that arrhenotoky are apparently common in mesostigmatid mite species especially the

Table 2: Life table parameters of *Cosmolaelaps simplex* fed on three different food sources and kept at 27°C and 65% RH

| Food source | T | Ro | $r_m$ | $e^{rm}$ |
|---|---|---|---|---|
| *Caloglyphus rodriguezi* | 22.24 | 20.46 | 0.135 | 1.145 |
| *Meloidogyne javanica* | 21.07 | 13.62 | 0.123 | 1.132 |
| *Tylenchulus semipenetran* | 20.22 | 13.10 | 0.127 | 1.135 |

family Laelapidae to which *C. simplex* belongs. On the other hand, it was reported that both mesostigmatid mite species *Lasioseius yousefi* and *L. berlesei* reproduce by theylotokous parthenogenesis where un-mated females give only females[7]. As in most mesostigmatid mites, males of *C. simplex* generally lived for shorter time than females where males lived for an average of 29.2, 33.2 and 34.3 days while females lived for 35.4, 33.3 and 34.7 days and took an average of 4.3, 5.6 and 6.2 days before starting to lay eggs when they fed on acarid mites and both nematode species, respectively. Accordingly, male life span averaged 37.6, 42.0 and 44.9 days while female life span were 45.7, 45.9 and 48.6 days by feeding on the same aforementioned foods, respectively (Table 1). It was also noticed that the total percentage of daughter females represented 61, 54 and 56% from the whole mite populations. However these percentages were represented later in calculating the life table parameters.

From the previous results, it was clearly noticed that the laelapid mite *C. simplex* fed and successfully reproduced during its life span when it was provided with diets of acarid mite and egg masses of both plant parasitic nematodes. Also, it was previously found that *L. scapulatus* (Gamasida: Ascidae) fed and reproduced on juvenile stages and adults of the nematode *Aphelenchus avenae* where each adult female was able to lay at least 23 eggs during only 6 days[9]. On the other hand, it was also stated that the laelapid mite species *Hypoaspis aculeifer* (Canestrini) fed and developed on egg masses of the cyst nematode *Heterodera* sp. and root-knot nematodes *Meloidogyne* sp.[10]. Moreover, a successful biology of *L. dentatus* was obtained where it was fed on two species of acarid mites and egg masses of root-knot nematodes under laboratory and greenhouse conditions[8, 11].

Concerning the life table parameters that obtained from the biological studies of *C. simplex*, data clearly clarified that the mean generation time (T) at 26°C and 65% RH was affected by food source. It was also noticed that a diet of egg masses of *T. semipenetrans* gave the shortest T time (20.22 days), while it was prolonged to 21.07 and 22.24 days by feeding on *M. javanica* and acarid mite *C. rodriguezi*, respectively (Table 2).

With regard to the survivorship of the laelapid mite *C. simplex*, Lx curves followed the I pattern in which survival of immature stages was nearly 100% (Fig. 1). The

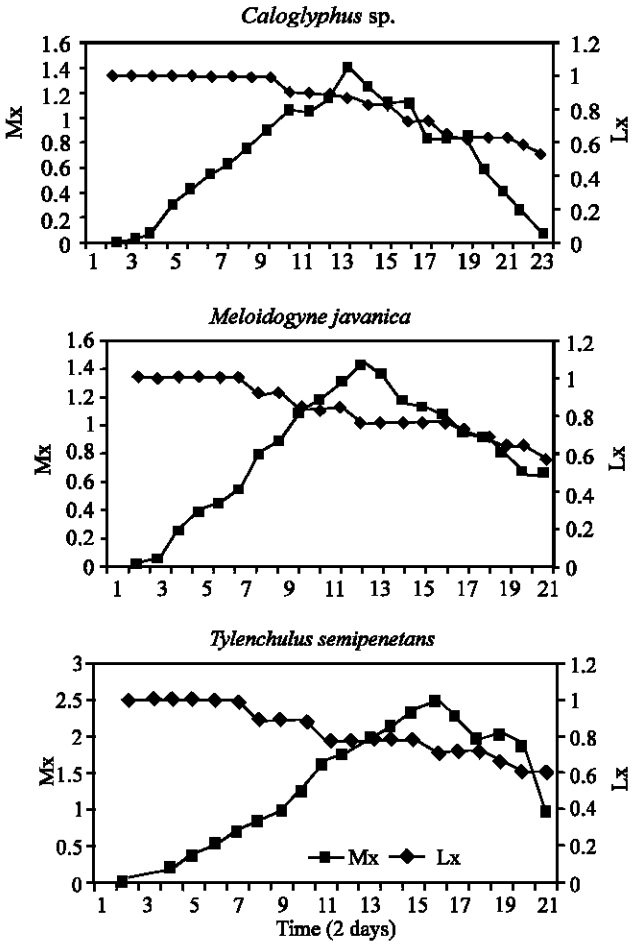


Fig. 1: Age-specific fecundity (Mx) and survivorship (Lx) of *C. simplex* fed on *C. rodreguezi* and egg masses of both nematodes *M. javanica* and *T. semipenetrans* at 26°C

fecundity of *C. simplex* was clearly affected by food source where the net reproductive rates Ro values averaged 13.62 and 13.10 when the predatory mite fed on egg masses of root-knot nematode and citrus nematode,

Table 3: Plant response after the infection of citrus nematode *Tylenchulus semipenetrans* in the presence of the predatory mite *Cosmolaelaps simplex* or aldicarb under greenhouse conditions

| Treatments | Shoot length (cm) | Shoot weight (g) | Root weight (g) |
|---|---|---|---|
| Nematode alone | 38.70c | 18.07d | 21.25ab |
| Nematodes + mites at the same time | 45.70b | 38.92ab | 23.22a |
| Mites 15 before nematode inoculation | 40.47b | 31.75c | 21.75ab |
| Mite 15 days after nematode inoculation | 42.18b | 36.40b | 22.30ab |
| Nematode + aldicarb | 60.00a | 40.25a | 23.30a |
| Control | 61.10a | 38.66a | 24.04a |

*Means in a column followed by the same letter(s) are not significantly different (p= 0.05)

Table 4: Citrus nematode *Tylenchulus semipenetrans* development in citrus seedlings in the presence of the predatory mite *Cosmolaelaps simplex* or aldicarb under greenhouse conditions

| Treatment | No. nematodes/100 $cm^3$ soil (J) = X | No. mites/250 $cm^3$ soil = X | Reproduction index of mites (PF/PI) |
|---|---|---|---|
| Nematode alone | 4011.00d | - | - |
| Nematode + mites at the same time | 1386.00b | 133.20a | 1.63b |
| Mites 15 before nematode inoculation | 1665.00c | 121.60a | 1.21a |
| Mite 15 days after nematode inoculation | 1417.00bc | 116.40a | 1.46ab |
| Nematode + aldicarb | 614.00a | - | - |

X = mean of 4 replicates, PF = extracted mite population, PI = initial mite population,
*Means in a column followed by the same letter(s) are not significantly different (p= 0.05) according to LSD test.

respectively (Table 2). These values obviously increased to reach 20.46 by feeding on the acarid mite *C. rodriguezi.* That means, although, acarid mites caused the longest generation time but it gave the highest rate of egg deposition. It was previously found that Ro values of *L. dentatus* were higher by feeding on acarid mites than feeding on the egg masses of root-knot nematodes[8]

Concerning the net rate of natural increase ($r_m$), data showed that feeding on the acarid mite gave also the highest rate ($r_m$=0.135) and followed by feeding on citrus nematodes (0.127) and then root-knot nematodes (0.123). The same trend was obtained with regard to the finite rate of increase ($e^m$) where its values averaged 1.145, 1.135 and 1.132 when *C. simplex* fed on acarid mites, egg masses of citrus nematodes and root-knot nematodes, respectively (Table 2).

**Greenhouse experiments:** The results showed the potential of the predatory mite *C. simplex* in repressing *T. semipenetrans* reproduction. Significantly enhanced shoot growth was observed in aldicarb (60.0 cm) and in all the mite-treated citrus plants as compared to the *T. semipenetrans* untreated control (38.7 cm) (Table 3). Root weight was slightly enhanced in aldicarab and all mite-treated plants but this response was not significant compared to the plants with nematodes only. The citrus plants with no nematodes had the greatest shoot length (61.1 cm) and shoot weight (38.7 cm). Compared to the nematode-alone (control), all mite treatments and aldicarb significantly restricted reproduction of *T. semipenetrans* (Table 4). Nematode populations ranged from 1386 to 1665 J2s/100 $cm^3$ soil for the mite-treated plants compared to 4011 J2s/100 $cm^3$ for the nematode untreated control. The aldicarb treated plants significantly had the lowest nematode populations (614 J2s/100 $cm^3$ soil) (Table 4).

That means aldicarb application as a chemical compound or mite addition as a biological agent to citrus plants, that had been previously infested with citrus nematodes, significantly suppressed nematode populations (Table 4).

Similarly, it was found before that aldicarb and addition of the predatory mite species *L. dentatus* either at the same time or 40 days after root-knot nematode inoculation had significant improvement in shoot length, weight and root weight of tomatoes seedlings under green house conditions[11]. However, it was noticed that there was a slight increase in shoot length when the mites were added to the soil 11 days before nematode inoculation. Also, there was no significant difference between the effect of all mite treatments and control on the root weight but they obtained significant differences between the effect of chemical and biological control methods on nematode population[11].

Concerning the reproduction index of *C. simplex,* it was noticed that it was at its highest level when the predatory mites were added to the treatments at the same time of nematode inoculation (PF/PI=1.63) and followed by 1.46 and 1.21 for mites that were added 15 days after and before nematode inoculation, respectively (Table 4). Also, there were no significant differences between the effect of application time of mites added to citrus seedlings 15 days after nematode inoculation and either mites added at the same time or mites added 15 before inoculation on the reproduction index of *C. simplex.* Whereas, there was significant difference between the effect of adding mites at the same time of inoculation and those that were added before inoculation on the reproduction index of *C. simplex* (LSD 0.33). Similarly, it was previously found that the reproduction index of the ascid predatory mite *L. dentatus* was higher when mite individuals were added to tomato seedlings 40 days after root-knot nematode inoculation[24]. That may be due to the mite species and its feeding behavior as well as to the biological aspects of nematodes. Therefore, it can be concluded that *C. simplex* had a better response which directly represented by its reproductive potentiality and capability to reducing citrus nematode populations when it was added at the same time of nematode inoculation. In other word, the predatory mite *C. simplex* had the chance to search the developmental individuals of citrus nematodes and feed on them before they can reach the root system and become more difficult for the predator. These results are in harmony with the previous findings where it was found that the developmental stages of root-knot nematodes were eaten by *L. scapulatus* (*L. dentatus*) under laboratory and greenhouse conditions[8,9,32].

Finally, it can be concluded that the laelapid mite *C. simplex* could be considered as a biological control

agent, which may limit populations of both citrus nematode and root-knot nematodes. Moreover, mite capability to feed, survive and reproduce on nematodes can be integrated with other control tactics and further field work in this area is highly warranted.

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