EVALUATION OF THREE ENTOMOPATHOGENIC NEMATODES (STEINERNEMATIDAE) FOR THE CONTROL OF THE MEDITERRANEAN CLIMBING CUTWORM, Spodoptera littoralis UNDER LABORATORY AND GREENHOUSE CONDITIONS

Hussein, H. M.; J. Jersáková; J.M. Webster and Z. Mráček

1 Pests & Plant Protection Department, National Research Centre, Al Tahrir St., Dokki, 0202 Cairo, Egypt
2 Department of Theoretical Ecology, Institute of Systems Biology and Ecology, Czech Academy of Sciences and University of South Bohemia, Branišovská 31, 370 05 České Budějovice, Czech Republic
3 Laboratory of Insect Pathology, Institute of Entomology, Czech Academy of Sciences, Branišovská 31, 370 05 České Budějovice, Czech Republic
4 Department of Biological Sciences, Simon Fraser University, Burnaby, B.C. V5A 1S6, Canada

ABSTRACT

The virulence and invading efficiency of three Steinernema nematode species on the last larval instar of Spodoptera littoralis (Boisd.) as well as, average number of damaged leaves and consumed leaf area were investigated in laboratory and greenhouse experiments. The results proved that the indigenous, Mediterranean species, Steinernema apalae, at 500 and 1500 IJs/pot was the most virulent species in laboratory and greenhouse experiments respectively. It also showed significantly higher invading efficiency than did other nematode species, except that when used at a high concentration of 2700 IJs/pot in greenhouse. The numbers of adult nematodes found in the cadavers of S. littoralis larvae were significantly different among nematode species at 500 IJs/dish in the laboratory and 1500 IJs/pot concentration in the greenhouse. The number of adult females found in the cadavers of this insect larvae was always higher than the number of males. Foliage application of S. apalae resulted in a significant reduction in the number of damaged leaves and revealed a lower index of damage as compared with that in the control. As conclusion, S. apalae has significant the capability of controlling potentiality in the management of the Mediterranean climbing cutworm S. littoralis in Egyptian and European greenhouses.

Keywords Steinernema spp., Spodoptera littoralis (Boisd.), Biological control

INTRODUCTION

ENTOMOPATHOGENIC NEMATODES (EPNs) in the families Steinernematidae and Heterorhabditidae are of considerable interest because of their use as biological control agents of insect pests (Poinar 1979, Woodring and Kaya 1988), that are an environmentally acceptable alternative to chemical control methods. The infective 3rd - stage juvenile (IJ) of these nematodes, the only soil free-living stage, carries mutualistic bacteria in its intestine (Boemare et al. 1993). Upon encountering a susceptible insect host,
the IJ enters it through a natural body opening (mouth, anus, or spiracles) or, rarely, through the thin intersegmental membrane and releases its mutualistic bacteria into the hemocoel, which then causeS a lethal septicaemia (Kaya et al. 1993).

Several species of the genus Spodoptera are recognised as quarantinable plant pests by UK and EU legislation. These include Mediterranean climbing cutworm Spodoptera littoralis (Boisl.), which occurs in Mediterranean Europe and Africa. Mediterranean Europe and in glasshouses in northwest EU, on, for example, imported

S. littoralis is a destructive insect pest of wide spectrum of forage crops and vegetables in subtropical and tropical zones and has the potential to cause a serious damage to field cultivation as well as in greenhouses (Salama et al. 1970). The larval stages feed on a wide range of plants, including edible ones produce and ornamentals. Many Spodoptera populations developed extremely resistant strains to many pesticides and control measures become exceptionally difficult to control. It is important that a comprehensive management program including the utilization of entomopathogens has to is implemented (Glazer et al. 1991).

Despite the ability of EPNs to parasitize many different insect species, including many economic pests (Poinar, 1979), they have been only sporadically used to control Spodoptera spp. Larvae of the fall armyworm, S. frugiperda, and of the black cutworm, Agrotis ipsilon Hufnagel, were found susceptible to infection by Steinernema carpocapsae (Weiser) in both laboratory and field trials, as shown by Capinera et al. (1988) and Richter and Fuxa (1990). However, in tests by Episky and Capinera (1993) using the Mexican strain and the All strain of S. carpocapsae only 10-50% of the nematodes infected S. frugiperda. The percentage of infective juveniles invading the host (invasion efficiency) was positively related to the increase in the period of host exposure and to the number of hosts per arena. However, it was negatively related to increase in a substrate surface area per host, and or been not affected by the applied number of nematodes

S. littoralis is a major pest in Egyptian cultivation and chemical control commonly fail to decrease this pest. Therefore, we focused our research on testing some entomopathogenic nematodes biological control measure, such as entomopathogenic nematodes. A laboratory and greenhouse experiments were conducted to determine the efficacy of three steinernematid nematode species (including one indigenous species from the Mediterranean region) against S. littoralis larvae. in the event that the indigenous species might be more effective than the imported (foreign ones ) in the control of Spodoptera species than the other two (Duncan et al. 2003)

MATERIALS AND METHODS

Nematode cultures. The entomopathogenic nematodes Steinernema oregonense Liu & Berry (from Oregon, USA), S. arenarium Artyukhovsky (from Russia) and S. apulii Trigiani, Mráček & Reid (indigenous species, from Italy) were propagated by passing through the late instar of the (Greater wax moth) Galleria mellonella L. adopting of Dutky et al. (1964). Infective
juveniles were harvested from nematode water traps as described by White (1929). The IJs were stored for three weeks in darkness at 15°C in deionized water and then examined microscopically for their viability before being used in the experiments.

**Insect culture.** The Mediterranean climbing cutworm, *S. littoralis*, was obtained from a laboratory colony reared on an artificial diet (Premix diet [Manduca premix-Heliothis premix] produced by Stonofly Industries, Inc. USA.). This diet consisted of Premix powder (25% w/w), tap water (75% w/w or v/v), 2ml acetic acid and 1 ml formalin (37% formaldehyde) mixed together in blender. The insects were reared in an insect rearing room in 15-cm-diameter petri dishes at 25±2°C, 65±5% RH, and photoperiod of 16:8h(L: D ).

**Laboratory studies – Bioassay procedure.** Groups of newly ec lysed last instars larvae of *S. littoralis* (ten larvae/ groups) were starved for 2-4h . Then exposed each, in each a Petri dish, to one of the three different species of EPNs for 48h. An aqueous suspension of EPNs at a dose of 500 IJs per dish was applied to a 9-cm-diameter water moistened filter paper (Whatman no.1) and placed in each petri dish. Five replicates/dish. Were used (10 larvae each ) per each inoculum concentration.

After exposure to the nematodes, both dead and living insect larvae were rinsed in tap water and transferred to other petri dishes. Final insect mortality was recorded 72h after the initial exposure. Dead larvae were kept in moist conditions for an additional 3-4 days, to obtain the 1st adult nematode generation, then dissected individually in deionized water. The number of male and female nematodes in the larval cadavers was counted using a stereoscopic microscope, Arsenal model SZP 1102 ZOOM.

Invasion efficiency, estimated according to Epsky and Capinera (1993), as the percentage of IJs that successfully invaded the host was calculated from the number of adult nematodes recovered from the dissected cadavers (summed from all hosts in trials with multiple hosts per petri dish arena) divided by the number of IJs applied per dish (nematode dose), multiplied by 100.

**Greenhouse tests.** The experiments were conducted in the greenhouse at the Institute of Entomology (České Budějovice, CZ) on one-month-old potato, Superior cultivar, plants. Each 6-litre pot containing three potato plants, each with 6 to 8 leaves, were maintained under conditions resembling the field situation in Egypt during early spring (20-25°C, 70-80% RH, and 14:10 L:D).

The nematode dose, of either 1500 IJs or 2700 IJs per pot in aqueous solution, was sprayed over the top of each plant and onto the soil surface using a hand sprayer. Nematodes were applied after sunset (just before feeding activity of the *Spodoptera* larvae) in an aqueous suspension of 25 ml/plant, and then each pot was infested with 10 newly last instar larvae of *S. littoralis*. Each pot was covered with a white muslin cage to prevent larval escape. Control treatment pots were sprayed with the same volume of uncontaminated water.

Larval mortality was recorded after 3 days and nematode invading efficacies as well as the number of male and female nematodes per larval cadaver determined five days post treatment. Leaf damage caused by insect larvae was evaluated in those plants sprayed with 2700 IJs. Estimation leaf
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damage was based on the number of leaves damaged or consumed by feeding insect larvae in each replicate, according to Glazer et al. (1992), where four categories represented (1) 0-25%, (2) 26-50%, (3) 51-75%, and (4) 76-100% consumption of the leaf area. Consumed area of all leaves per pot was expressed as an index of damage and calculated as the average of percentages of consumed leaf areas from all damaged leaves per pot.

Statistical analyses. The effect of different concentrations of IJs on the larval mortality, IJ efficiency, and the number of male and female nematodes in larval cadavers were determined using One-way ANOVA in both laboratory and greenhouse experiments. The differences between nematode species were tested using Post-hoc comparisons with Tukey HSD test. The differences in larval mortality, IJ invading efficiency and the number of male and female nematodes in larval cadavers at 1500 IJs/pot and 2700 IJs/pot concentrations were tested using Two-way ANOVA with interactions (concentration* and nematode species). arcsine transformations for proportions was not used as data normality and variance homogeneity did not violate ANOVA assumptions.

The effect of foliage application of three nematode species on the mean number of damaged leaves and total consumed leaf area per replicate was compared with that in the control, using t-tests for independent samples with the associated Bonferroni correction.

RESULTS

Laboratory experiments. At a concentration of 500 IJs/dish, S. apuliae caused significantly higher mortality of the insect larvae than did the other nematode species (Table 1). S. apuliae had also the highest invading efficiency, which corresponds to the highest mean number of established nematodes in larval cadavers (Table 1, 2).

Table 1. The impact of three Steinernema nematode species on Spodoptera littoralis larval mortality and nematode invading efficiency in laboratory and greenhouse experiments using different concentrations of infective juveniles (IJ) (mean ±SE).

<table>
<thead>
<tr>
<th>Effects</th>
<th>S. apuliae</th>
<th>S. oregonense</th>
<th>S. arenarium</th>
<th>N</th>
<th>F-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 IJs/dish in laboratory:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of mortality</td>
<td>100.0±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66.0±2.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74.0±5.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5</td>
<td>29.6</td>
<td>0.0001</td>
</tr>
<tr>
<td>% invading efficiency</td>
<td>60.8±1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.5±1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.6±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5</td>
<td>292.2</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

1500 IJs/pot in greenhouse:

| % of mortality | 70.0±3.2<sup>a</sup> | 44.0±2.4<sup>b</sup> | 36.0±2.4<sup>b</sup> | 5 | 43.1 | 0.0001 |
| % invading efficiency | 15.9±0.3<sup>a</sup> | 1.6±0.6<sup>b</sup> | 10.1±0.6<sup>c</sup> | 5 | 166.6 | 0.0001 |

2700 IJs/pot in greenhouse:

| % of mortality | 65.0±5.4<sup>a</sup> | 40.0±0.0<sup>b</sup> | 40.0±3.2<sup>b</sup> | 10 | 9.32 | 0.0018 |
| % invading efficiency | 5.2±0.9<sup>a</sup> | 2.3±0.4<sup>a</sup> | 3.2±0.3<sup>a</sup> | 10 | 3.45 | 0.0551 |

<sup>a</sup> indicates the number of petri dish arena replicates. Different letters indicate the significant differences between nematode species at P < 0.05 level in Tukey HSD test.

Greenhouse experiments. At both concentrations of 1500 IJs/pot and 2700 IJs/pot in greenhouse experiments, S. apuliae caused significantly higher mortality of S. littoralis larvae than did the other nematode species (Table 1).
While the mortality caused by *S. oregonense* was almost similar to that obtained by *S. arenarium* at 1500 and 2700 JJs/pot concentrations (Two-way ANOVA, factor concentration: $F_{1,29} = 1.21; P = 0.28$; interaction concentration*nematode species: $F_{2,29} = 1.59; P = 0.22$).

**Table 2. The average number of male and female *Steinernema* nematode species found per *Spodoptera littoralis* larval cadaver in laboratory and greenhouse experiments using three different concentrations of infective juveniles (IJ) (mean ±SE).**

<table>
<thead>
<tr>
<th>Effects</th>
<th>S. apuliea</th>
<th>S. oregonense</th>
<th>S. arenarium</th>
<th>N</th>
<th>F-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 JJs/dish in laboratory:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of nematodes</td>
<td>$31.8±1.6$ $^a$</td>
<td>$22.7±1.1$ $^b$</td>
<td>$18.4±1.7$ $^b$</td>
<td>5</td>
<td>21.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Sex ratio (F/M)</td>
<td>$1.4±0.1$ $^a$</td>
<td>$1.5±0.1$ $^a$</td>
<td>$1.6±0.1$ $^a$</td>
<td>5</td>
<td>1.8</td>
<td>0.2097</td>
</tr>
<tr>
<td>1500 JJs/pot in greenhouse:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of nematodes</td>
<td>$34.6±1.3$ $^a$</td>
<td>$6.3±2.3$ $^b$</td>
<td>$44.9±1.6$ $^c$</td>
<td>5</td>
<td>126.3</td>
<td>0.0001</td>
</tr>
<tr>
<td>Sex ratio (F/M)</td>
<td>$1.4±0.1$ $^a$</td>
<td>$6.0±1.9$ $^b$</td>
<td>$1.4±0.1$ $^a$</td>
<td>5</td>
<td>5.8</td>
<td>0.0174</td>
</tr>
<tr>
<td>2700 JJs/pot in greenhouse:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of nematodes</td>
<td>$22.2±1.9$ $^a$</td>
<td>$22.1±4.8$ $^a$</td>
<td>$26.1±2.8$ $^a$</td>
<td>10</td>
<td>0.5</td>
<td>0.6050</td>
</tr>
<tr>
<td>Sex ratio (F/M)</td>
<td>$1.1±0.1$ $^a$</td>
<td>$1.2±0.2$ $^{ab}$</td>
<td>$1.8±0.2$ $^b$</td>
<td>10</td>
<td>5.8</td>
<td>0.0120</td>
</tr>
</tbody>
</table>

*N* indicates the number of petri dish arena replicates. Different letters indicate significant differences between nematode species at $P < 0.05$ level in Tukey HSD test. F – female, M – male

Similar to the laboratory experiment, *S. apuliea* had significantly higher invading efficiency at 1500 JJs/pot concentration than did the other nematode species. However, no significant difference in invading efficiency was observed at 2700 JJs/pot concentration, even though *S. apuliea* again reached the highest value (Table 1). The invading efficiency was significantly higher at the 1500 JJs/pot concentration than at the 2700 JJs/pot concentration for both *S. apuliea* and *S. arenarium* (Two-way ANOVA, factor concentration: $F_{1,29} = 81.17; P= 0.0001$; interaction concentration*nematode species: $F_{2,29} = 29.29; P = 0.0001$).

Statistical analysis showed significant differences between the number of established adult nematodes found in the larval cadavers of *Spodoptera* treated with different nematode species at the 500 JJs/dish in the laboratory and at the 1500 JJs/pot treatment in the greenhouse. No significant differences were observed at 2700 JJs/pot concentration (Table 2). Comparing the number of successfully established nematodes in the larval cadavers in both concentrations proved that it was significantly higher at the 1500 JJs/pot than at 2700 JJs/pot concentration in the *S. apuliea* and *S. arenarium* spp. (Two-way ANOVA, factor concentration: $F_{1,29} = 5.40; P = 0.027$; interaction concentration*nematode species: $F_{2,29} = 21.62; P= 0.0001$) treatments. The total number of nematodes in the cadavers was significantly correlated with the larval mortality in all three dosage treatments (Pearson correlation coefficients: 500 JJs/pot = 0.86, 1500 JJs/pot = 0.61 and 2700 JJs/pot = 0.84). The number of adult females found in the cadavers of *Spodoptera* larvae was always higher than the number of males, but the differences in sex ratio between nematode species were inconsistent across concentrations (Table 2; Two-way ANOVA, factor concentration: $F_{1,29} = 7.27$.  

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\[ P = 0.011; \text{ interaction concentration*nematode species: } F_{2,20} = 7.62, P = 0.002. \]

**Foliage application.** Foliage application of *S. apuliae* resulted in a significant decrease in the number of damaged leaves and a lower index of damage compared with the control (t-test: 2.98, df = 18, \( P = 0.008 \); t-test: 2.32, df = 18, \( p = 0.032 \); respectively). The effect of other nematode showed insignificant differences from the control (t-tests: df = 13, \( P > 0.05 \)). The overall feeding activity of *Spodoptera* larvae was reduced by 43% in *S. apuliae*, 15% in *S. arenarium* and 7.5% in *S. oregonense* as compared with the control (Table 3).

Table 3. The average number of damaged leaves and the percentage of consumed leaf area caused by *Spodoptera littoralis* larvae (mean ±SE) in control and after foliage application of three *Steinernema* species

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>No. of damaged leaves</th>
<th>Consumed leaf area category</th>
<th>Index of damage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0-25%</td>
<td>26-50%</td>
</tr>
<tr>
<td><em>S. apuliae</em></td>
<td>10</td>
<td>10.8±1.4</td>
<td>3.2±0.4</td>
<td>2.3±0.5</td>
</tr>
<tr>
<td><em>S. arenarium</em></td>
<td>5</td>
<td>15.6±1.2</td>
<td>3.6±0.3</td>
<td>3.0±0.4</td>
</tr>
<tr>
<td><em>S. oregonense</em></td>
<td>7</td>
<td>17.0±1.4</td>
<td>4.0±0.4</td>
<td>3.2±0.4</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>18.0±2.6</td>
<td>4.0±0.4</td>
<td>2.7±0.5</td>
</tr>
</tbody>
</table>

*Index of damage is equivalent to the sum of consumed leaf areas from all damaged leaves per replicate. N indicates the number of pot replicates; * shows significant impact of nematode application versus control at \( P < 0.05 \).*

**DISCUSSION**

The experiments proved that entomopathogenic nematodes infect *S. littoralis*. *Steinernema apuliae* caused 100% mortality of the target *Spodoptera* larvae followed by *S. arenarium* and *S. oregonense*. The mean number of established adult nematodes in each arena was also higher in *S. apuliae* than in the other two species. Moreover, the invading efficiency of *S. apuliae* reached 61%, among the tested nematode species, most probably due to the Mediterranean origin of *S. apuliae*. This may be due to thermal adaptation of *S. apuliae* to the warm, and dry Mediterranean climate, whereas *S. oregonense* and *S. arenarium* originate from colder regions in the temperate zones of the north-west of USA and Russia. The pre-assumption that these two non-indigenous nematode species would not be so effective in the control of *Spodoptera* larvae was confirmed by our experimental results. Such natural cold or heat adaptability is a well-known feature among nematodes (Shapiro et al. 1996 and Mrácek et al. 1997). Similar results have been reported by Glazer et al. (1991). In their laboratory experiments, the most virulent EPN to *S. littoralis* was an indigenous *Heterorhabditis* sp. originating from Israel. Thus, it is clear that *S. apuliae* has the capacity to effectively invade the target host, proliferate within the host and causing up to 100% of the larval mortality.

A similar pattern of results was obtained from the greenhouse experiment, where *S. apuliae* showed higher invading efficiency and
achieved a higher number of established nematodes in the larval host than did the other two species tested. Thus indicating successful development of the nematodes in the cadaver, as well as a higher mortality of the target pest. However, all these parameters were significantly lower in the greenhouse than those obtained in the laboratory. This is not unusual, as nematodes sprayed on plants in the greenhouse scatter the nematodes in large surface areas (plants and soil) which decrease the chance of such a fortuitous contamination and consequently lowers the mortality of the hosts. However, *S. apuliae* exhibited significantly successful invading efficiency at 1500 IJs/pot, but not at 2700 IJs/pot, since the mean number of nematodes in each cadaver in laboratory and greenhouse study was 60.8±1.7 and 15.9 ±0.3, respectively. This is a good indicator that *S. apuliae* has a reasonably high invading efficiency and has the ability to search for the host and to attack it in a situation resembling field conditions.

Upon the application of a higher concentration of nematodes (2700 IJs/pot) in the greenhouse a wide range of mortality between replicates was observed using *S. apuliae* (40 – 100%). The invading efficiency varied between 1.6 – 10.8% (5.2±0.9%) in average.

The present results at 1500 IJs/pot coincide with Epsky and Capinera (1993) findings, who observed that 10-50% of applied nematodes successfully infected the host. The lower number of *S. apuliae* in a cadaver does not mean that the efficiency of *S. apuliae* is less, because one successfully developed adult or male/female nematode pair makes this cadaver a source for a new generation capable of infecting another pest larva in a soil.

A high positive correlation was observed in the mortality of target insects and the number of nematodes recovered from their cadavers at all tested concentrations of IJs. Hence, the higher the invasive efficiency, the greater the number of nematodes developing in the host ensuring a successful elimination of the target organism. Interestingly, a greater proportion of female than male nematodes were developed in the cadavers. This may have been due, in part, to the overlooking of some much smaller males. *S. littoralis* larvae feed on the foliage of plants, similar to that of many economically important insect pests which destroy the plant canopy. In the past, applying nematodes to foliage faced many desiccation limitations adding anti-desiccants (Webster and Bronskill 1988, MacVean et al. 1982). However did not entirely showed satisfactory results when applied in the field. Much higher EPN efficiency has been achieved in more limited habitats, such as in greenhouses where the high humidity plus using anti-desiccants can keep infective juveniles viable for several hours or even days (Williams and Walters 2000, Glazer et al. 1992, Glazer and Navon 1990).

Foliar applications in greenhouse experiments on indicate that spraying potato plants with an aqueous inoculum of *S. apuliae* reduced pest feeding activity and leaf damage by 43% in due to high mortality of the pest larvae. This is similar to the leaf damage 46% reported by Glazer et al. (1992). The high effectiveness of the indigenous species, *S. apuliae*. Against *S. littoralis* has been reported also by Glazer et al. (1991).
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*S. littoralis* larvae spend most of their life-cycle at the soil surface around host plants and climb these plants during the night to feed. The application of nematode's at evening, either on the foliage or soil surface extends their survival period and increases the opportunity for these nematodes to find and infect their target hosts. In the past, field applications usually failed to suppress the target hosts even though when applied in more humid temperate zones. Based on the results of our studies we conclude that Mediterranean *S. apulias* could provide more effective management than other EPN species of the Mediterranean climbing cutworm in Egyptian and European greenhouses and be better also in open field areas.

Acknowledgement:

We would like to thank the Czech Ministry of Education for the supporting the COST 850.001 project to Zdeněk Mráček, and Simon Fraser University, Burnaby, Canada for support to John M. Webster. Also I am grateful to Dr. Maller M.M. for his efforts and kindly help.

REFERENCES


تقسيم ثلاث منها النيماتودا المعرضة الحشرية من عائلة 
في مكافحة يرقات حوض البحر الأبيض المتوسط المتفصّلة 
مكاشفة الورقة (دوّدة ورق القطن) تحت Spodoptera littoralis 
الورقة كلها ومختلفة.

هشام محمد حسين، يانا بارسكوفا، جيمس، م. وبيستر، زدينك مرانشيك
قسم الأفلام ووفاية النباتات - المركز القومي للبحث

تم دراسة القوة التأثيرية وكفاءة النمو لثلاث منها عائلة نيماتودا
ضمن نمط الورق الباهي ليرقات حوض البحر الأبيض المتوسط المشابهة
الورقة (دوّدة ورق القطن) وكذلك كمية الضرر الناتج عن تغذية اليرقات على أوراق نباتات
البطاطس، والتي تتناثى على مدى الورقات سواء بالبيتامونا أو غيره من الورقات (كنتشول).
قد أجريت التجارب والمراقبات بكل من الورق والصوب ذاتية التحكم في الحرارة والاضاءة.
ورصدت النتائج أن الأنواع من الورقات التي تتبع نمط الورقة المتوسط من جنس
الورقة عند تركيز 500،000 فرد شتات أعلى كفاءة في النمو، وكذلك في الصوب apuliae
و لكن عند تركيز 2700 فرد شتات / أصبح. وقد وجد أن عدد الأفراد الكاملة للتخطيط
من الورقات داخل جثة اليرقات من دوّدة ورق القطن كانت مختلفة المعمولية تجاوزاً لنوع الورقات المستخدمة، و أن عدد الأفراد الكاملة لتخطيط الإنتاج
في جثة الورقة أعلى من عدد النازير. كذلك
أوجدت نتائج متنوعة Nematema apuliae
في حجم الضرر الناتج للمجموع الورق الج창.
وعند تغذية اليرقات عند مراقبة بكمياتها العالية (كنتشول). من تلك نتائج أن النيماتودا من نوع S. apuliae
قد تشكل من عوامل المكافحة المتكاملة لدودة ورق القطن في مصر والصوب في أوروبا.