

Evaluation of Entomopathogenic Nematodes for Biocontrol of the European Corn Borer, *Ostrinia nubilalis*, on Sweet Corn in Israel

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The potential of entomopathogenic nematodes for biological control of the European corn borer (ECB), *Ostrinia nubilalis* (Hübner), was evaluated under laboratory, greenhouse and field conditions. The 'All' and 'Mexican' strains of *Steinernema carpocapsae* (Weiser) and the 'HP88' strain of *Heterorhabditis bacteriophora* Poinar were compared in both dose response assays (5, 50 and 500 infective juveniles [IJ] per petri dish containing five 5th-instar ECB eggs; 72 h of incubation) and exposure time assays (3, 6 and 9 h of incubation). In the dose response assays the highest rates of ECB killing resulted from infestation with the Mexican strain of *S. carpocapsae*. In the exposure time assays there were no significant differences between the killing rates of the three nematode strains. Sweet corn plants (*Zea mays* var. *saccharata*) grown in a greenhouse, were infested with ECB neonates and 4 days later sprayed with a suspension of the Mexican strain of *S. carpocapsae* (50,000 IJ per plant). The number of ECB larvae found on treated corn plants after one week was significantly ($P=0.05$) lower (3- to 5-fold) than the number found on untreated plants. Similar treatment in the field significantly reduced the rate of economic ear damage from 20% to 5%.

KEY WORDS: Entomopathogenic nematodes; *Heterorhabditis bacteriophora*; *Steinernema carpocapsae*; *Ostrinia nubilalis*; European corn borer; biological control; sweet corn.

INTRODUCTION

The European corn borer (ECB), *Ostrinia nubilalis* (Hübner), is a major pest of corn, *Zea mays* L., in Europe and North America (17) and also in Israel (6,23,25). ECB is difficult to control by contact insecticides because the larvae bore into plant tissues shortly after hatching (17). In addition, pesticide residues in food are becoming increasingly unacceptable to consumers. These constraints have encouraged the search for biological control methods for this pest.

Entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* are parasites that carry mutualistically associated entomopathogenic bacteria (*Xenorhabdus* spp. and *Photorhabdus* spp., respectively) in their intestines (2,7). The infective juveniles (IJ) actively seek and invade insects (21). Infected insects die of septicemia following the release of the symbiotic bacteria by the invading nematodes (1). These nematodes are used

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as biological control agents for soil-inhabiting insect pests (11,19); attempts to use them to control foliage-feeding insects have usually produced disappointing results (18). This is probably because the foliar environment has low humidity, high temperatures and intense sunlight, which are unfavorable conditions for nematode survival (5). However, insects were successfully controlled by nematodes above ground when they resided in protected sites on plants (5). Neonate larvae of ECB often migrate to leaf axils, and remain in these protected and moist sites for a few days (8). Therefore, we also tested the ability of the nematodes to control them in those niches on corn plants.

Previous studies have indicated the possibility of using entomopathogenic nematodes to control corn pests. In laboratory tests, ECB was killed effectively by the DD-136 strain of *Steinernema carpocapsae* (Weiser). However, these nematodes have failed to control overwintering larvae in cornstalk debris under field conditions (22). When nematodes of the 'All' and 'Mexican' strains of *S. carpocapsae* were sprayed on ears of sweet corn infested with the fall armyworm, *Spodoptera frugiperda* (J.E. Smith), and the corn earworm, *Helicoverpa zea* (Boddie), the pest populations were reduced by 53% compared with untreated plants (27). The 'Agriotos' strain of *S. feltiae* (= *carpocapsae*) gave 92% control of the Asian corn borer, *Ostrinia furnacalis* (Guenée) (16), which is a close relative of ECB.

In this study we first selected the most effective nematode strain for ECB control in the laboratory testing. For this purpose the All and Mexican strains of *S. carpocapsae* and the 'HP88' strain of *Heterorhabditis bacteriophora* Poinar were used. Then we evaluated the performance of the most effective nematode on infested plants under various environmental conditions.

MATERIALS AND METHODS

Insects and nematodes

ECB larvae were reared on a meridic diet as described by Melamed-Madjar and Raccach (24). We used 5th instar larvae (17 days old; head width 1.6 ± 0.2 mm) for the laboratory studies and eggs at the black-head stage (shortly before hatching) for the on-plant studies. The Mexican and All strains of *Steinernema carpocapsae* (Weiser) (Rhabditida: Steinernematidae), and the HP88 strain of *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae), were originally obtained from "biosys" (Columbia, MD, USA) The nematodes were reared on an artificial medium according to a method described by Bedding (3). In all tests, infective juveniles (IJ) of nematodes were suspended in deionized water to obtain the desired concentration. Deionized water alone was applied to the control groups. During laboratory studies, insects and nematodes were kept at $25 \pm 2^\circ\text{C}$ and 16:8 L:D in 5-cm-diam petri dishes. After infestation, petri dishes were sealed with parafilm to maintain a humid environment.

Dose-response assay

Nematode suspensions with concentrations of 10, 100 and 1000 IJ/ml were prepared. Ten treatments (including control) were carried out, in three replicates (= petri dishes) per treatment with five ECB larvae per replicate. One-half ml of suspension was applied to a 5-cm-diam filter-paper disc (Whatman no. 1) placed in a petri dish (*i.e.*, 5, 50 and 500 IJ/dish). ECB larvae were added shortly after that. The insects were inspected and dead

larvae were recorded and removed every 24 h for 3 days. The entire assay was repeated three times.

Exposure-time assay

ECB larvae were exposed to nematodes (500 IJ/dish) for periods of 3, 6 and 9 h (ten treatments, six replicates, five ECB larvae per replicate). At the end of each exposure period, ECB larvae from two petri dishes of each treatment were rinsed and transferred to clean petri dishes. Insect mortality was recorded 48 h after initial exposure. The entire assay was repeated three times. Dead ECB larvae from the third run were kept under moist conditions for 3 days, then dissected individually in deionized water. The numbers of nematodes in these larvae were determined 20 h after dissection with the aid of a stereoscopic microscope.

Screenhouse and greenhouse assays

Sweet corn (*Zea mays* var. *saccharata* (Sturter) Bailey, cv. 'Jubilee') plants were grown in large planters (150×40×40 cm, ten plants/planter) in a screenhouse (white screen, 30 mesh) during June through September 1993 (trials 1 and 2), or in 10-l planting pots in a greenhouse during October 1994 to January 1995 (trial 3). Plants at the late whorl stage were infested with 100 ECB eggs on a piece of waxed paper which was attached to the underside of the second-from-top leaf. Four days after infestation, plants were sprayed with a suspension of the Mexican strain of *S. carpocapsae* (5000 IJ/ml) in water from the top downwards, using a hand sprayer. Each plant was sprayed with 10 ml at dusk, to provide better survival conditions for the nematodes (two treatments including control, 10–13 plants per treatment). All plants grown together in a planter received the same treatment. Six days after spraying, plants were dissected and the numbers of ECB larvae found on individual plants were recorded. Only a very small portion of the larvae developed in the tassel. Those larvae were omitted because they were unlikely to cause any damage to the plant.

Field trial

Small experimental plots (8×15 m) of sweet corn were planted at Bet Dagan on May 1 (trial 1) and June 5 (trial 2), 1995. Tasseling dates were June 19 and July 16, respectively. Plants were grown under conditions similar to those in commercial fields (irrigated and enriched with 300 nitrogen units/ha; 60,000 plants/ha). There were four plots for each treatment, arranged in a random block design. One week after tasseling, ten plants were randomly selected in each plot and each plant was infested with 40 ECB eggs at the black-head stage (*i.e.*, within 24 h of hatching). Eggs (on a piece of waxed paper) were attached to either the underside of the second leaf above the ear (trial 1) or to the ear itself (trial 2). Four days after infestation, the ears on each plant were sprayed with a 10 ml suspension of the Mexican strain of *S. carpocapsae* (5,000 IJ/ml) in aqueous solution of 6% Folicote (Asia-Riesel, Ramat Gan, Israel) and 0.05% Tween 80 (Sigma Chemicals, St. Louis, MO, USA), using a hand sprayer. Nematodes were sprayed again, at dusk, to provide better conditions for their survival. Control plants were sprayed with 10 ml of the aqueous solution of 6% Folicote and 0.05% Tween 80. At harvest time, the percent economic damage (for processing corn, *i.e.*, damage to the body of the ear excluding the top 2 cm) was determined for each group.

Statistical analysis

Data obtained during laboratory studies were normalized by an arcsine of square root transformation. The quantity (dose) of IJ per dish was subjected to logarithmic transformation. The significance of differences in strain effect were determined by analysis of variance (ANOVA); in on-plant larval mortality, by Student's t-test; and in economic damage, using a single factor ANOVA. The significance level for all analyses was $P < 0.05$. Treatments were ranked using Duncan's multiple range test (9).

RESULTS

Dose-response assay

The three different experiments were considered as randomized blocks. Statistical analysis of the overall study showed that the Mexican strain of *S. carpocapsae* caused a significantly higher death rate than the two other strains (df 2, 80; $F=9.1$; $P < 2 \times 10^{-4}$). In the control treatment, none of the larvae died. The differences among the ECB mortality rates caused by the three strains were most noticeable after 48 h of exposure (Fig. 1).

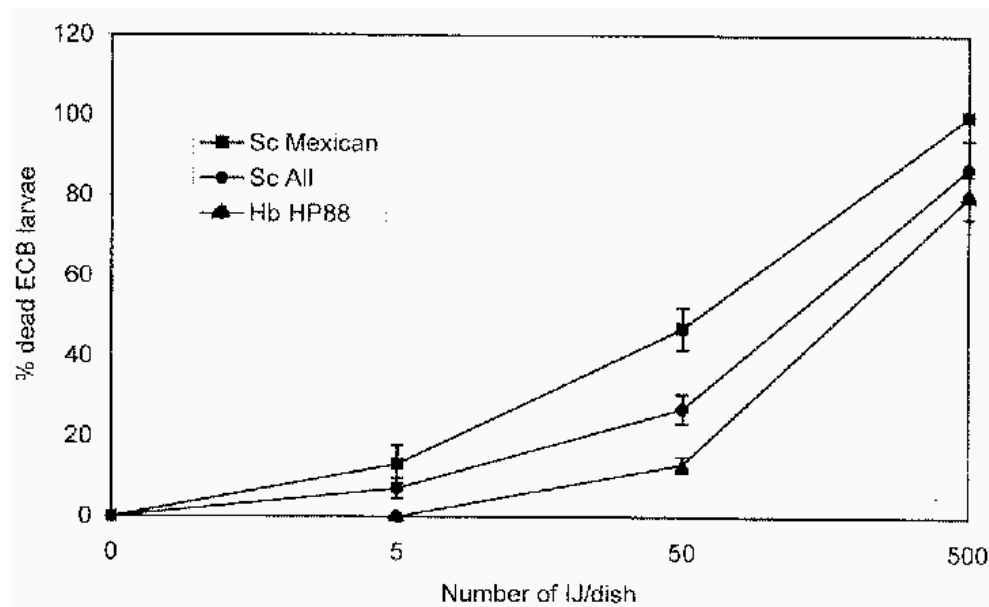


Fig. 1. Percent of dead European corn borer (ECB) larvae (mean of three petri dishes, five larvae per dish \pm SEM as bars) 48 h after exposure to infective juveniles (IJ) of entomopathogenic nematodes (*Steinernema carpocapsae*, Sc; *Heterorhabditis bacteriophora*, Hb) at various doses. Data were normalized by an arcsine of square root transformation.

Exposure-time assay

Data obtained from the three experiments were combined, since no significant differences were found among them (Fig. 2). No significant differences were found in ECB killing rates among the three nematode strains. Overall, ECB larval death rate was

significantly higher after 9 h exposure to the nematodes than after 3 or 6 h exposure. The number of nematodes found in dead ECB larvae ranged between 0 and 44, and no relationship could be established between these numbers and the time of exposure.

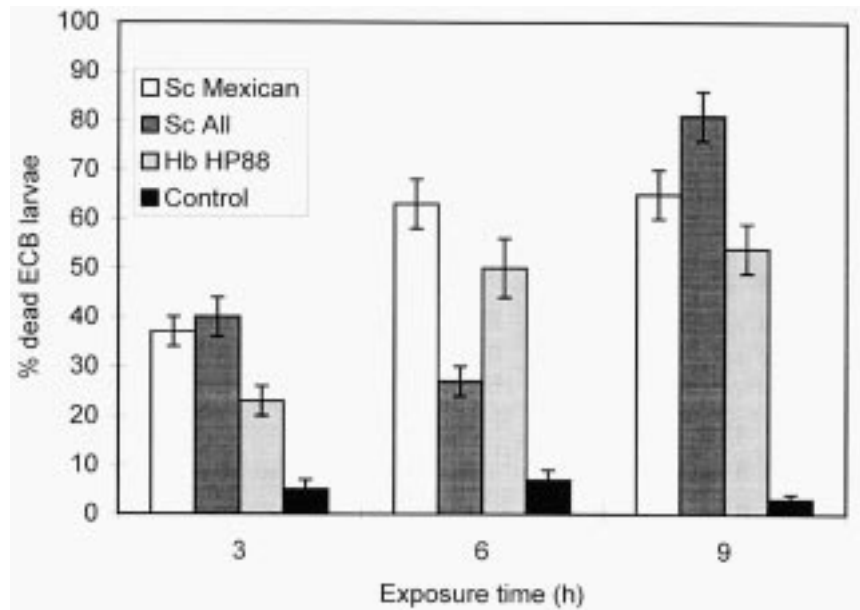


Fig. 2. Percent of dead European corn borer (ECB) larvae (mean of two petri dishes, five larvae per dish; SEM as bars) after various exposure times to entomopathogenic nematodes (*Steinernema carpocapsae*, Sc; *Heterorhabditis bacteriophora*, Hb) at an infestation rate of 500 infective juveniles per petri dish. Data were normalized by an arcsine of square root transformation.

Screenhouse and greenhouse assays

The number of ECB larvae found on corn plants that had been sprayed with nematode suspension was significantly lower in all three trials than that found on the control plants (Table 1). In trial 3 approximately half of the ECB larvae developed on the tassel, which was not sprayed with nematodes. Therefore, these larvae were not included in the analysis of the results.

TABLE 1. Mean number (\pm SD) of European corn borer larvae per plant^z 6 days after the plants were sprayed with a suspension (5000 IJ/ml) of the Mexican strain of *Steinernema carpocapsae* nematodes in water

Treatment	Trial 1	Trial 2	Trial 3 ^y
Nematodes	2.8 (\pm 1.8) a ^x	3.4 (\pm 3.3) a	1.9 (\pm 1.6) a
Water	7.9 (\pm 4.3) b	18.7 (\pm 6.1) b	3.3 (\pm 2.1) b

^z n=10–13 plants; each plant was infested with 100 ECB eggs 4 days before spraying.

^y Larvae that developed in the tassel were not included.

^x Within columns, means followed by different letters are significantly different (Student's t-test, $P=0.05$).

Field trial

Infestation directly on the ear resulted in greater economic damage than infestation on the second leaf above the ear. In both trials, treatment of the ear with nematodes reduced the economic damage significantly (Table 2).

TABLE 2. Mean percent (\pm SD) of economic damage caused by European corn borer larvae per plot^z at harvest time, after the ears were sprayed with a suspension (5000 IJ/ml) of the Mexican strain of *Steinernema carpocapsae* nematodes in aqueous solution of 6% Folicote and 0.05% Tween 80

Treatment	Trial 1 ^y	Trial 2 ^x
Nematodes	0.0 (\pm 0.0) a	5.3 (\pm 6.1) a ^w
Water	6.8 (\pm 5.0) a	19.7 (\pm 5.7) b

^zn=four plots, ten plants per plot; each plant was infested with 40 ECB eggs 4 days before spraying.

^yEggs were attached to the second leaf above the ear.

^xEggs were attached to the ear itself.

^wWithin columns, means followed by different letters are significantly different (Student's t-test, $P=0.05$).

DISCUSSION

The efficacy of various nematode species or strains for controlling a particular insect pest may differ significantly (4,10,20). Efficacy is influenced by the rate of IJ penetration into the insect, the time it takes to release the symbiotic bacteria, and the virulence of the latter (12). In the present study, when ECB was exposed to various dosages of nematodes in the laboratory for 48 h, the Mexican strain of *S. carpocapsae* killed the greatest number of larvae. Although direct relationships were found between results obtained in dose-response and exposure-time assays in studies of the effect of nematodes on other lepidopteran pests (13,26), in the present study no such relationships were found. This may be due to the fact that exposing ECB for up to 9 h (as compared with the 48 h of exposure during the dose-response assay) was insufficient to distinguish among the killing effects of the three nematode strains tested. Indeed, within the exposure-time assay, ECB larval death rate gradually increased and was significantly higher after 9 h of exposure than after 3 or 6 h.

Since the Mexican strain of *S. carpocapsae* had given the best control of ECB in the dose-response assay and had been shown to be more tolerant to low relative humidity conditions than the other strains tested here (14,15), it was chosen for the on-plant assay, aimed at controlling ECB neonates during the tasseling to green silk stages of the corn plant. These neonates usually feed in the leaf axil (8). The corn leaves act as funnels, directing applied fluids such as nematode suspensions to the axil. Therefore, the likelihood of contact between the nematodes and young ECB larvae is high. Because ECB larvae remain in this vulnerable site for only a few days after hatching (17), accurate timing of application is essential for effective control. The effectiveness of control also depends on the length of time the nematodes can remain infective on the plant (residual effect). He *et al.* (16) reported that under field conditions in China, entomopathogenic nematodes remained infective on corn plants for 5 to 7 days. Richter and Fuxa (27) obtained similar results in Louisiana (USA) when they applied nematodes to ears of sweet corn in the field.

In this study, application of the Mexican strain of *S. carpocapsae* to the corn ears under field conditions reduced the economic damage significantly. In both trials treatment

reduced the ear damage to less than 5.5% which is the maximum level of damage accepted by the processing plants. This reduction was achieved despite adverse weather conditions for the nematodes (mid summer) and a relatively high infestation rate.

This has been a pilot study to decide whether additional research on this control method is warranted. The results of our study, together with previous reports, indicate that entomopathogenic nematodes may be used successfully for the control of ECB. Additional studies are needed for developing commercial methods of application and evaluation of economic feasibility.

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