LABORATORY EVALUATION OF STEINERNEMATID AND HETERORHABDITID NEMATODES FOR CONTROL OF THE BEETLE *MALADERA MATRIDA*

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The potential use of steinernematid and heterorhabditid nematodes against the beetle *Maladera matrida* Argaman (Coleoptera: Scarabaeidae) was determined under laboratory and greenhouse conditions. Infective juveniles (IJ's) of the nematode *Heterorhabditis* sp., Hp88 strain, mixed with soil at a concentration as low as 50 IJ's/cm^3 soil, resulted in 86% control. No increase in control was obtained with higher nematode concentrations. Soil surface application of the nematodes at concentrations of 160 and 640 IJ's/cm^2 was sufficient to obtain 87% and 86% mortality, respectively, even at a depth of 40 cm below the soil surface. The *Heterorhabditis* sp. Hp88 strain was found to be the most pathogenic to the beetle grubs at 25°C. Lower control levels of 30–47% were achieved by *Heterorhabditis* sp. HL81 strain, *S. feltiae* 'All' strain and *S. bibionis* CR strain. The nematode *Heterorhabditis* sp. BS strain did not have any effect on insect mortality. However, at a temperature of 16°C, the HL81 strain of *Heterorhabditis* sp. was the most effective. Grubs 3–5 wk old were found to be the most susceptible developmental stage of infection of *Heterorhabditis* sp. Hp88 strain. Pupae, which were exposed to the nematodes in the same experimental regime, were not affected. The results obtained in the present study suggest that *M. matrida* is an attractive candidate for biological control by entomoparasitic nematodes.

KEY WORDS: *Maladera matrida*; beetles; Steinernematidae; Heterorhabditidae; entomoparasitic nematodes; pathogenicity bioassay.

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INTRODUCTION

The beetle *Maladera matrida* Argaman (Coleoptera: Scarabaeidae) was first recorded In Israel in 1983, damaging ornamental plants (1,11). The beetle population increased rapidly and spread very quickly. Huge populations of adult beetles were found on chrysanthemum, geranium, gerbera, castor bean, rose and marigold plants as well as on apple, apricot, peach, pear, plum, avocado, citrus and macadamia trees. These polyphagous pests feed on young leaves and flowers, causing severe damage. White grubs of the beetles were responsible for severe damage to sweet potato tubers in the field and to ornamental plants in pots. Chemical control did not prevent damage or spread of the pest (8).

Entomoparasitic nematodes of the families Steinernematidae and Heterorhabditidae offer a potential alternative to insecticides for control of white grubs. The successful control of soil insects attacking ornamentals and numerous other crops was reviewed by Kaya (9) and Poinar (13). Previous research had demonstrated the susceptibility of the white grub of Japanese beetle (*Popillia japonica* Newman) to the nematode *Steinernema glaseri* (Steiner) (3). More recently, it was shown that *Maladera* species are also susceptible to nematode infection (10,12). Moreover, the decline in production costs due to improved mass-rearing techniques (2) and environmental safety of the entomoparasitic nematodes make them attractive candidates for use against insect pests.

The present study was conducted to determine the potential use of steinernematids and heterorhabditids against the beetle *M. matrida* under laboratory and greenhouse conditions.

MATERIALS AND METHODS

Nematodes of *Heterorhabditis* spp., Hp88 and HL81 strains, as well as *Steinernema feltiae* (Filipjiv) (=Neoaplectana carpocapsae), 'All' strain, were obtained from "Biosys", a biological control company located in Palo Alto, California, U.S.A. *S. bibionis*, CR strain, and *Heterorhabditis* spp., BS strain, are nematodes recently found in Israel (Glazer and Poinar, in preparation for publication). These Israeli strains were reared on an artificial medium according to the methods described by Bedding (2). *M. matrida* beetles were obtained from laboratory rearings at the Gilat Experiment Station. Pathogenicity bioassay: three grubs (5 wk old) of *M. matrida* were placed on the bottom of a glass tube (10 cm long, 2.5 cm diam). The insects were covered with sterilized sandy soil up to the 7 cm level. Nematodes were added to the tube in water suspension, either by mixing with the soil or by topical application to the soil surface. The treated tubes were covered with plastic caps and placed at 25°C. Insect mortality was recorded after 120 h. Dead insects were dissected and nematode presence in their body cavity was determined. The treatments were done in 21 replicates, each of which consisted of one tube with three insects.

This bioassay was used to elucidate the following aspects of nematode-insect interaction: (*a*) The range of effective nematode concentrations was determined by mixing Hp88 with soil at concentrations ranging from 25 to 800 infective juveniles (IJ's) per cm^3; control consisted of nematode-free soil. (*b*) The effectiveness of topical application of nematodes on the soil surface was evaluated using Hp88, which was applied at concentrations of 0, 40, 160 and 640 IJ's per cm^2. Soil mixed with 200 IJ's/cm^3 was used as the control. (*c*) The pathogenic effect of different nematode species (listed above) was determined with 50 IJ's/cm^3 at 25°C. The pathogenicity of Hp88 and HL81 was also compared at 16°C. (*d*) Susceptibility of different *M. matrida* developmental stages was evaluated by placing white grubs as well as adult beetles in soil mixed with IJ's of Hp88 at a concentration of 50 nematodes per cm^3.

Nematode effectiveness on grubs at various soil depths was determined in the following test: 4- to 5-wk-old grubs were placed in plastic net bags filled with 100 cm^3 soil (four grubs per bag). Wheat seedlings were added to the bags as food for the grubs. The bags were transferred to three plastic containers (40 cm deep, 35 cm in diam) filled with soil. Groups of four bags were placed at different depths in the containers (5,15,25,40 cm). In the first container, IJ's of Hp88 were mixed with the soil at a concentration of 200 nematodes per cm^3. In the second container the nematodes were applied to the surface of the soil at a concentration of 200 IJ's per cm^2. The third container was not inoculated with nematodes. The containers were placed at 28°C, in the dark, and the soil in them was maintained moist (70% of field capacity). After 10 days the mortality of the grubs from the various treatments was recorded. Dead insects were dissected and nematode presence in their body cavity was determined. Each one of the various treatments included seven replicates.

RESULTS

Figure 1 demonstrates the effect of increased concentration of *Heterorhabditis* sp. Hp88 strain IJ's, mixed with soil, on the mortality of 5-wk-old *M. matrida* grubs. At a concentration of 25 IJ's/cm^3 soil, 55% mortality was recorded. A concentration as low as 50 IJ's/cm^3 soil resulted in 86% control. Similar control levels were obtained with higher nematode concentrations.

The effect of soil-surface application of nematodes on grub mortality is shown in Figure 2. With 40 IJ's/cm^2 only 37% mortality was recorded. However, concentrations of 160 and 640 IJ's/cm^2 were sufficient to obtain 87% and 86% mortality, respectively. Mixing nematodes with soil at a concentration of 400 IJ's/cm^3 resulted in similar mortality (88%).

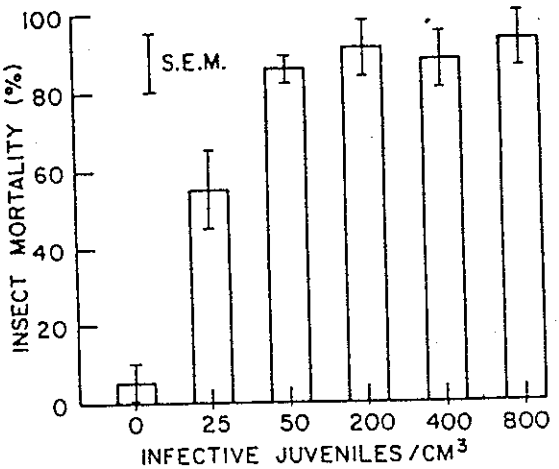

Fig. 1. Mortality of 5-wk-old grubs of *Maladera matrida* in a soil bioassay with different concentrations of the entomoparasitic nematode *Heterorhabditis* sp. Hp88 strain, mixed with the soil. Insect viability was determined 120 h after inoculation.

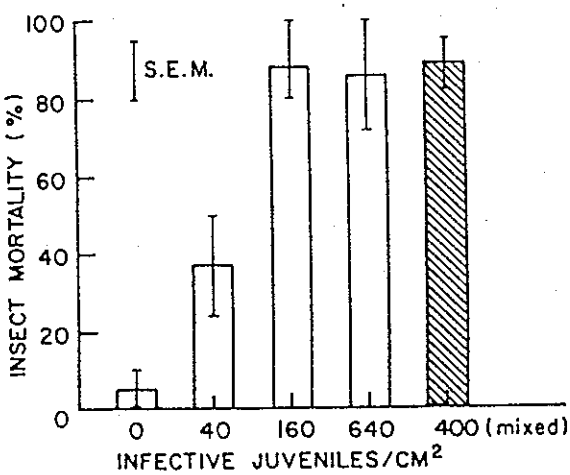

Fig. 2. Mortality of 5-wk-old grubs of *Maladera matrida* in a soil bioassay with different concentrations of the entomoparasitic nematode *Heterorhabditis* sp. Hp88 strain, applied on the soil surface. Insect viability was determined 120 h after inoculation.

A comparison between the pathogenic effect of various nematode strains on *M. matrida* grubs is presented in Figure 3. The highest mortality (86%) was obtained with *Heterorhabditis* sp. Hp88 strain. Lower control (30–47%) was achieved with

Heterorhabditis sp. HL81 strain, *S. feltiae* 'All' strain and *S. bibionis* CR strain. The *Heterorhabditis* sp. BS strain did not have any effect on insect mortality.

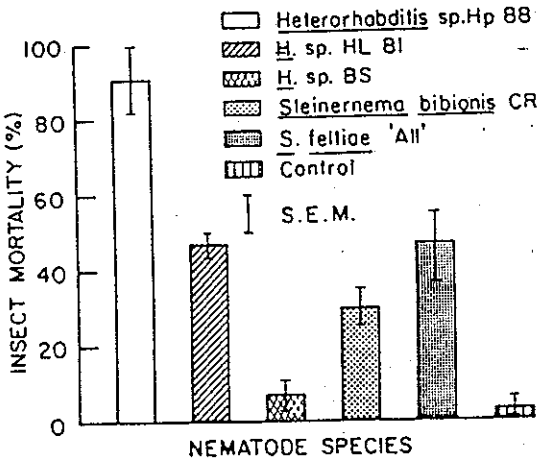

Fig. 3. Mortality of 5-wk-old grubs of *Maladera matrida* in a soil bioassay with different entomoparasitic nematode strains mixed with the soil at a concentration of 50 infective juveniles/cm^3. Insect viability was determined 120 h after inoculation.

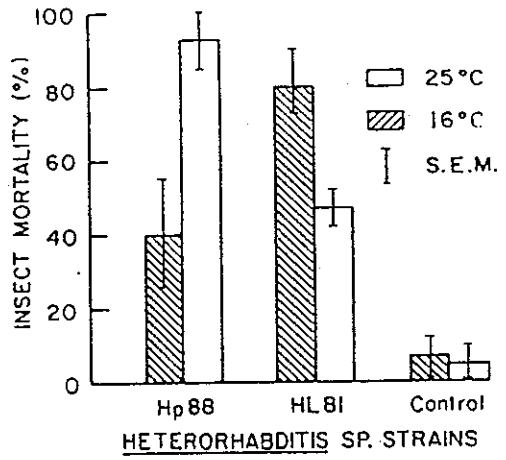

Fig. 4. Mortality of 5-wk-old grubs of *Maladera matrida* at different temperatures, in a soil bioassay with various *Heterorhabditis* strains mixed with the soil at a concentration of 50 infective juveniles/cm^3. Insect viability was determined 120 h after inoculation.

The effect of temperature on the pathogenicity of two heterorhabditid strains is shown in Figure 4. At 25°C, *Heterorhabditis* sp. Hp88 strain induced 93% mortality and only 47% mortality was obtained with the HL81 strain. However, at 16°C, the effectiveness of the Hp88 strain was reduced 2.2-fold to 40% mortality, while the HL81 strain effected 80% control, which is almost double that achieved at 25°C.

The susceptibility of different *M. matrida* developmental stages to infection of *Heterorhabditis* sp. Hp88 strain is illustrated in Figure 5. The highest mortality, of 90%, was obtained with 5-wk-old grubs; the effect of nematodes on 3-wk-old grubs was slightly less (77%). Young grubs (1 wk old) had the lowest mortality effect, of 37%. In addition, 60% of the adults were also killed by the nematodes. Pupae, which were exposed to the nematodes in the same experimental regime, were not affected.

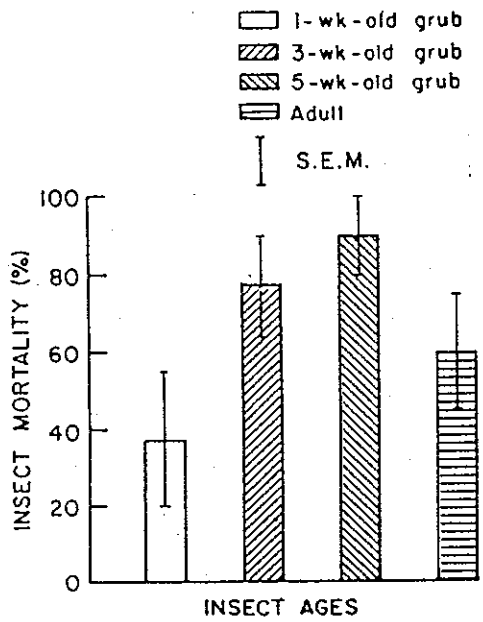

Fig. 5. Mortality of *Maladera matrida* at different developmental stages in a soil bioassay with the entomoparasitic nematodes *Heterorhabditis* sp. *Hp88* strain, mixed with the soil at a concentration of 50 infective juveniles/cm^3. Insect viability was determined 120 h after inoculation.

The effect of the nematode *Heterorhabditis* sp. Hp88 strain on the mortality of 5-wk-old grubs at various soil depths is presented in Table 1. Mortality levels ranging between 75% and 83% were obtained at all depths when the nematodes were mixed with the soil. When the nematodes were applied on the soil surface, similar results were

obtained even at the 40 cm depth. Nematodes at various developmental stages were found in the body cavities of 85–90% of the dead insects.

TABLE 1

EFFECT OF THE NEMATODE *HETERORHABDITIS* SP. HP88 STRAIN ON THE MORTALITY OF 5-WK-OLD *MALADERA MATRIDA* GRUBS AT VARIOUS SOIL DEPTHS (% OF TOTAL, ± S.E.)

Depth (cm)	*Topical application (200 IJ's/cm²)*	*Control*	
		Nematode mix in soil (200 IJ's/cm³)	*Nematode-free soil*
5	75 ± 6	83 ± 6	0
15	83 ± 5	92 ± 4	0
25	83 ± 7	75 ± 7	8 ± 6
40	78 ± 5	83 ± 7	8 ± 6

DISCUSSION

The initial pathogenicity studies in the soil bioassay conducted here indicate that grubs of *M. matrida* are highly susceptible to infection of the *Heterorhabditis* sp. Hp88 strain. A high mortality level was achieved with relatively low nematode concentrations, both when the nematodes were mixed with the soil and when they were placed on the soil surface.

The results obtained regarding nematode efficacy against the beetle *M. matrida* are in line with previous studies of different *Maladera* species and other scarabs (10,12). The most detailed research on the potential efficacy of entomoparasitic nematodes against white grubs was conducted with the Japanese beetle *P. japonica*. The results had indicated that nematodes of the genus *Heterorhabditis* Khan, Brooks and Hirschmann were the most effective against the grubs of this pest as compared with other nematode species (4,14,16). Our data indicated, similarly, that the heterorhabditid nematodes are the most pathogenic against *M. matrida* grubs (Fig. 3). Furthermore, a comparison between the pathogenic effect of various heterorhabditid strains against *M. matrida* grubs indicated that *Heterorhabditis* sp. Hp88 strain is the most virulent at 25°C, but at a lower temperature the HL81 strain had shown a significantly higher pathogenic effect than Hp88. The efficacy of HL81 at lower temperatures is known from Simons and van der Schaf (15). Therefore, this strain would be useful for grub control during the colder seasons. Although in most of our experiments *Heterorhabditis* sp. Hp88 was used, strain alternation should be considered for practical purposes.

Previous studies with the Japanese beetle, *P. japonica*, had also shown that nematode concentrations of 4–5 billion IJ's per acre in field tests (4,14) and of 150–200 IJ's per cm^2 in pots (16) provided sufficient control. The effective nematode concentration found in the present work falls within this range (160 IJ's/cm^2 is equivalent to 4.6 billion/acre).

Kushid *et al.* (12) found that older grubs of *M. japonica* are more susceptible to nematode infection than younger stages. Our data with *M. matrida* also show that the last instar is the most susceptible developmental stage of the insect. These findings should be taken into consideration when planning the timing of nematode application.

It has been shown here that nematodes of the *Heterorhabditis* Hp88 strain which were applied on the soil surface sought out and infected the grubs as deep as 40 cm below the surface. Since in nature the late instar of *M. matrida* penetrates to this depth in order to pupate, there is a great advantage to the nematodes over chemicals. Some work has been done on the movement of nematodes in the soil. Studies with *S. glaseri* indicated that after application to the soil surface, this nematode tends to disperse downward; the presence of wax moth pupae 10 cm below the soil surface tended to increase the downward movement of the nematodes (6). In sandy loam soil, *Heterorhabditis heliothidis* applied to the soil surface tended to stay at or near the surface, again with greater downward movement in the presence of wax moth pupae below the soil surface, over a 5-day period of observations (7). Similar results were obtained with *S. feltiae* (5). The ability of *Heterorhabditis* sp. strain Hp88 to infect the insect so deep below the soil surface should be attributed to the ability of the infectives to move in the soil and to locate the target insect. The downward movement of *Heterorhabditis* sp. strain Hp88 shown here was probably stimulated by the presence of grubs at intervals of 10–15 cm in depth.

The results obtained in the present study suggest that *M. matrida* is an attractive candidate for biological control by entomoparasitic nematodes. However, field trials and more efficacy tests are needed prior to practical use of the nematodes against this pest. Further research is currently being conducted along these lines.

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