Virulence and Efficacy of Different Entomopathogenic Nematode Species against Western Flower Thrips (*Frankliniella occidentalis*)

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Virulence and efficacy of five species and strains of the entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae: *Steinernema riobravis, Steinernema feltiae* strains Ger. and UK, and *Heterorhabditis bacteriophora* strains HP88 and IS5, against the prepupal and pupal stages of the western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), were investigated in the laboratory. Although all these nematodes controlled WFT to some extent, they differed in efficiency. The heterorhabditid nematode *H. bacteriophora* strain HP88 was more specific to the soil-inhabiting WFT stages (36–49% thrips mortality). The steinernematid nematodes *S. riobravis* and *S. feltiae* strains Ger. and UK had only a slight effect (10% mortality) on prepupal and pupal populations of WFT, and *H. bacteriophora* strain IS5 had the least effect of all. A possible reason for such species variation is suggested and discussed.
KEY WORDS: Western flower thrips; *Frankliniella occidentalis*; virulence; efficacy; entomopathogenic nematodes; *Steinernema riobravis*; *Steinernema feltiae* strains Ger. and UK; *Heterorhabditis bacteriophora* strains HP88 and IS5; biological control.

INTRODUCTION

The western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is one of the most common thrips species in the world, infesting many field crops, and ornamental and horticultural plants (16). WFT was probably introduced into Israel in 1988 and spread within a very short time throughout the country (4,14).

The life cycle of *F. occidentalis* includes eggs, first and second stages of larvae, prepupae, pupae and adults. The larvae and adults feed mainly on flowers and leaves, and rarely also on fruits. The pupal stages stay in hidden parts of the plants or descend to the ground. They may move only when they are disturbed, but do not feed (5).

Chemical control of WFT is very difficult due to its cryptic habitat and because the insect has developed tolerance to many pesticides (13). To date, a reliable method of biological control of WFT has been developed for only very few agricultural crops. Several efficient predators of WFT have already been described, among them mites (*Amblyseius* spp.) and bugs (*Orius* spp.). The mites consume only small larvae, whereas the bugs feed on either larvae or adults. These predators are used in commercial programs to control the flower and foliage-feeding stages of the WFT (6,8,12).

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The prepupae and pupae of WFT stay mainly in the ground for about one-third of the thrips' developmental time. Thus it is important to search for biological control agents which act in this habitat. Entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae are currently used as biological control agents for soil-inhabiting insect pests (10); these nematodes are lethal insect parasites and thus promising bioinsecticides. They are mutually associated with bacteria of the family Enterobacteriaceae. The third-stage infective juveniles (IJs) of these nematodes locate and infect suitable insect hosts. Within a few hours after penetration, the nematodes release the bacteria into the hemocele of the host. The bacteria multiply there, killing the insect within 24–48 h. Although association between the WFT and entomophilic nematodes has been reported (11,20), only Tomalak (18,19) found that the WFT is susceptible to the entomopathogenic nematode *Steinernema feltiae.* In his experiments the parasite's effectiveness varied greatly among nematode strains and ranged from 3.7% to 72.6%.

The objective of this study was to investigate the virulence and efficacy of five species and strains of steinernematids and heterorhabditids on the prepupal and pupal stages of the WFT.

MATERIALS AND METHODS

Stock culture of nematodes

The nematodes were reared *in vivo* on larvae of the wax moth, *Galleria melonella* Walker, according to Woodring and Kaya (21). Details on the nematodes used in this research are given in Table 1.

TABLE 1. List of the tested nematode species and strains

Genus	Species	Strain	Obtained from
Steinernema	*riobravis* (Cabanillas and Poinar)		Ecogen Inc., Langhorne, PA, USA
Steinernema	*feltiae* (Filipjev)	UK	"
		Ger.	Dr. Ehlers, Christian Albrecht Univ., Kiel, Germany
Heterorhabditis	*bacteriophora* (Poinar)	HP88	Biosys, Columbia, MD, USA
		IS5	Israel, new isolate

Stock culture of WFT

The stock culture of WFT was maintained on leaves of kidney bean (*Phaseolus vulgaris* L.). Stems with the first true leaves were immersed in 25-ml vials closed with parafilm membranes to prevent insects from falling into and drowning in the water. Each vial was introduced into a 720-ml glass jar with a round piece of foam-rubber sponge on the bottom, serving as the site for the thrips' pupation. Twenty young females were caged for 2–3 days in each of these jars, which were kept at 25±2°C, 70±5% r.h. and a 16:8 L:D photoperiod. The females were then removed and the jars were held further under the same conditions for the thrips to hatch and develop. Leaves were added as necessary.

To obtain prepupae and pupae of WFT for bioassays, the sponge on the bottom was

replaced by small strips of twisted paper-toweling that were occasionally moistened. This procedure was found suitable for collecting these stages of the pest.

Bioassays

The first assay was conducted in plastic containers (volume, 100 ml; approximately 25 cm^2 bottom surface). Each container contained 15 ml of sterilized sandy soil (layer *ca* 0.7 cm thick) mixed with one of the five nematode species or strains. Each treatment consisted of 0 (control), 500, 1000, 5000 or 10,000 IJs in 1 ml of water per container (or 0, 20, 40, 200 and 400 IJs/cm^2 surface, respectively). Twenty mixed prepupae and pupae of WFT were placed in each container and a piece of bean leaf was added to the container as food for emerging adults; there were ten replications. The containers, closed by plastic lids with a few small holes to allow for ventilation, were kept for 9 days under the WFT rearing conditions described above. Virulence of the nematodes on the WFT was estimated according to the number of emerged adults which were caught by an aspirator on the 5th, 7th and 9th day from the start of the experiment.

In the second bioassay the same containers were used, but the lids were removed and the containers were filled with 75–80 ml of sterilized sandy soil (layer *ca* 4.0 cm thick), in which cotton seeds were sown. When the cotton was at the cotyledon stage, the containers were transferred into 720-ml glass jars, which were closed with a fine cotton cloth held in place with the rims of jar lids. Fifteen mated females of WFT were caged in each of these jars for 2 days at 25±2°C. Under these conditions prepupation occurs usually after 10 days, and it was then that nematodes were placed on the soil surface. In this experiment three nematode species were used: *S. riobravis*, *S. feltiae* strain UK, and *H. bacteriophora* strain HP88, which had been the most effective ones in the first assay. The nematodes were applied in 1 ml of water per container at the following concentrations: 0 (control), 40, 200 and 400 IJs/cm^2 soil surface. The nematode's effect on thrips mortality was determined as in the previous tests (on the 5th, 7th and 9th day), by counting emerging adults. Each treatment was carried out in ten replicates.

Effect of nematodes on WFT reproduction

The effect of nematodes on WFT reproduction was determined only with the thrips emerging from the treatments infested with 40 and 200 IJs/cm^2 in the second assay. Twenty 5–6-day-old mated WFT females that had survived the nematode treatment were caged in a fresh glass jar with cotton seedlings (as above) free of nematodes. The females were removed after 3 days, during which time they had laid eggs. The number of emerged adults of the second generation was recorded. This experiment was carried out in ten replicates.

Statistical analysis

Dose response data were analyzed by the Probit Procedure of the SAS System (17). LC_{50} and LC_{90} values (numbers of IJs/cm^2 surface causing 50% and 90% mortality, respectively, of the thrips) were determined and estimated. Statistical significance was determined by analysis of variance and Dunnet's Multiple Range Test, at $P<0.05$ (17). The results of each experiment were corrected for the control mortality or the number of emerging thrips in the control, according to Abbott's (1) formula.

RESULTS

Bioassays

All nematodes tested (except *H. bacteriophora* IS5) in the first bioassay showed reasonable virulence against prepupae and pupae of thrips. The susceptibility of WFT to *S. feltiae* UK and *H. bacteriophora* HP88 was rather high at all nematode concentrations: from 500 to 10,000 IJs/container (35.5–50.0% and 41.8–73.4% mortality, respectively) (Fig. 1). Pathogenicity of *S. feltiae* Ger. and *S. riobravis* was high only at concentrations of 5000 and 10,000 IJs/container (56.1–75.0% thrips mortality for *S. feltiae* and 36.5–71.7% for *S. riobravis*). Virulence of *H. bacteriophora* IS5 at different concentrations against thrips was very low (maximum mortality, 15.4%) (Fig. 1). The number of thrips adults which emerged (from 200 tested prepupae and pupae) in the controls of the first bioassay ranged from 134 to 152.

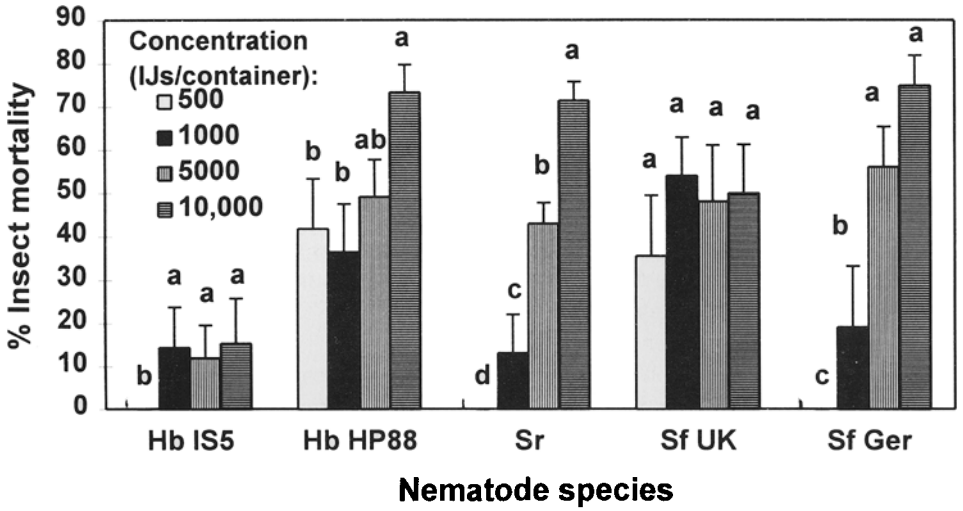

Fig. 1. Mean percent mortality of *Frankliniella occidentalis* prepupae and pupae after inoculation by five nematode species/strains (first assay; 100-ml plastic container). Hb IS5 = *Heterorhabditis bacteriophora* strain IS5; Hb HP88 = *H. bacteriophora* strain HP88; Sr = *Steinernema riobravis*; Sf UK = *Steinernema feltiae* strain UK; Sf Ger = *S. feltiae* strain Ger.

The LC_{50} and LC_{90} values calculated from the first bioassay are summarized in Table 2. The lowest LC_{50} value (number of IJs causing 50% mortality) was represented by *H. bacteriophora* HP88 and the highest by *S. riobravis* (143.3 and 262.6 IJs/cm^2, respectively). The virulence rates of *S. feltiae* Ger. and UK were quite similar (LC_{50}= 182.0 and 205.1 IJs/cm^2, respectively). The LC_{50} for *H. bacteriophora* IS5 could not be determined because of the strain's low virulence.

TABLE 2. Dose effect of five different entomopathogenic nematode species/strains on prepupae and pupae of the western flower thrips, *Frankliniella occidentalis*

Nematode species/strain	LC_{50}[z] (95% FL[y])	LC_{90}[z] (95% FL)[y]
H. bacteriophora HP88	143.3 (73.2–598.4)	742.5 (434.9–3003.5)
H. bacteriophora IS5	-[x]	-[x]
S. feltiae Ger.	182.0 (NE)[w]	564.8 (NE)[w]
S. feltiae UK	205.1 (NE)[w]	5170.2 (NE)[w]
S. riobravis	262.6 (200.2–645.9)	578.1 (457.9–2046.7)

[z] LC_{50} and LC_{90} expressed as number of infective juveniles per cm^2 surface area.
[y] Fiducial limits, FL, are given in parentheses.
[x] Not effective.
[w] NE, could not be estimated.

In the second bioassay the WFT pupated at a soil depth of 0–4.0 cm. In this trial only *H. bacteriophora* strain HP88 caused a relatively high rate of thrips mortality at all concentrations tested (36.1%, 42.1% and 48.8% at concentrations of 40, 200 and 400 IJs/cm^2, respectively) (Fig. 2). Two other nematode strains, *S. riobravis* and *S. feltiae* UK, were less effective (maximum level of thrips mortality was only 19.9%) (Fig. 2). In the controls of this bioassay (each consisting of ten replicates), from 578 to 667 thrips adults were collected.

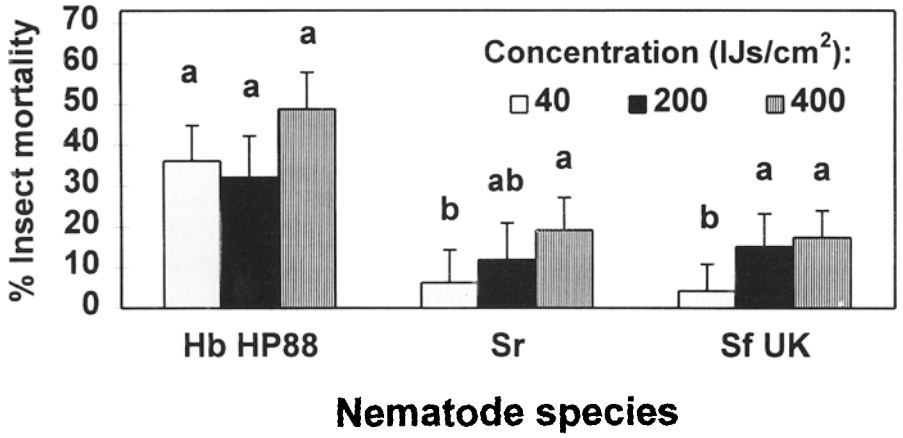

Fig. 2. Mean percent mortality of *Frankliniella occidentalis* prepupae and pupae after inoculation by three nematode species/strains (second assay; 720-ml glass jar). Hb HP88 = *Heterorhabditis bacteriophora* strain HP88; Sr = *Steinernema riobravis*; Sf UK = *Steinernema feltiae* strain UK.

Effect of nematodes on WFT reproduction

The number of progeny of the thrips surviving the *H. bacteriophora* HP88 test at 40 and 100 IJs/cm^2 was significantly less than that of the progeny from the control thrips

(67.9–68.2 and 111.1 thrips emerged per jar, respectively). The reduction of the new thrips generation by *H. bacteriophora* strain HP88 was approximately 40% compared with the control (Table 3). On the other hand, the females that emerged from prepupae and pupae exposed to *S. riobravis* and to *S. feltiae* UK produced similar and rather high numbers of thrips progeny that did not differ significantly from the number in the control (89.9–95.3 and 97.4–99.6 thrips/jar, respectively, *vs* 111.1 thrips/jar in the control). The population reduction of a new thrips generation was only 14.2–19.1% for *S. riobravis* and 10.4–12.3% for *S. feltiae* UK compared with the control.

TABLE 3. Effect of the three entomopathogenic nematodes on the second generation of the western flower thrips, *Frankliniella occidentalis*

Nematode species/strain	Concentration (IJs/cm^2)	Number of thrips progeny ±S.E. (per jar)	Reduction of thrips progeny (%)
S. riobravis	40	95.3±6.7	14.2
	200	89.9±3.9	19.1
S. feltiae UK	40	99.6±3.9	10.4
	200	97.4±3.2	12.3
H. bacteriophora HP88	40	68.2±7.8*	38.6*
	200	67.9±7.4*	38.9*
Control	–	111.1±10.1	–

*Significantly different (at $P<0.05$) from the control and other nematode species/strains (by Dunnet's test).

DISCUSSION

Entomopathogenic nematodes demonstrate great variation in their ability to reduce insect populations and some of the species/strains may be highly specific (3,10). Our results in the first assay proved that all nematodes tested have at least some controlling effect on prepupae and pupae of WFT. *S. riobravis, S. feltiae* Ger. and *H. bacteriophora* HP88 were very effective at 10,000 IJs per container (equals 4x10^6 IJs/m^2) (Fig.1), but this concentration is considered very high and not economically feasible. On the other hand, effective control was obtained also at lower and more economic concentrations of all nematodes except for *H. bacteriophora* IS5, which was the least effective strain.

In the second bioassay, when the nematodes were applied to the soil of plants kept in cages, effective control was obtained only with *H. bacteriophora* HP88. Two other nematode species, *S. riobravis* and *S. feltiae* UK, gave only negligible control of the thrips, even at a concentration of 10,000 IJs per container (equals 400 IJs/cm^2). The host's location in the soil probably determines the activity and effectiveness of these nematodes. According to Alatorre-Rosas and Kaya (2) and Choo *et al.* (7), *H. bacteriophora* searches for hosts and generally infects deeper in the soil profile, whereas *S. carpocapsae* waits and infects hosts near the soil surface. High efficiency of heterorhabditid nematodes was reported against the sweetpotato weevil, *Cylas formicarius* (F.) (15), and against larvae of the Japanese beetle, *Popillia japonica* Newman (9).

In our investigations, too, the heterorhabditid nematode *H. bacteriophora* HP88 is so far the most promising agent for further studies of controlling the underground stages of

WFT. It reduced the emergence of adult thrips by up to ~40% compared with the control and reduced the number of progeny produced by the surviving adults. It may be that following additional experiments, entomopathogenic nematodes will be considered for an integrated WFT control program in greenhouses, in which predatory *Orius* spp. bugs and predatory *Amblyseius* spp. mites are released to control the foliage-feeding pest stages.

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