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Characterization of biological control traits in the entomopathogenic nematode *Heterorhabditis mexicana* (MX4 strain)

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Abstract

Our objective was to estimate the biocontrol potential of the recently discovered species, *Heterorhabditis mexicana* (MX4 strain). In laboratory experiments, we compared *H. mexicana* to several other entomopathogenic nematodes for virulence to several insect pests, environmental tolerance (to heat, desiccation, and low oxygen levels), and host-seeking ability. *H. mexicana* expressed low or intermediate capabilities in all traits except in host-seeking ability and virulence to the yellow mealworm, *Tenebrio molitor*, for which no differences among nematodes were detected. *Steinernema carpocapsae* caused greater mortality in *Agrotis ipsilon* than *H. mexicana* and *Heterorhabditis bacteriophora* 1 day after exposure. *Curculio caryae* mortality in *S. carpocapsae* treatments was higher than *H. mexicana* treatments but not different from *Heterorhabditis megidis*. *Steinernema riobrave* strains caused greater mortality in *Diaprepes abbreviatus* than *H. mexicana*, *Heterorhabditis indica*, *H. bacteriophora*, *Steinernema feltiae*, and *S. carpocapsae*. Heat tolerance was more than five-fold greater for *S. riobrave* than it was for *H. mexicana*, *H. indica*, and *H. bacteriophora*. Desiccation tolerance was greater in *H. bacteriophora*, *S. carpocapsae*, and *S. feltiae* than *H. indica* and least in *H. mexicana*. Mortality of nematodes following exposure to low oxygen levels was lowest in *S. riobrave* followed by *Steinernema glaseri* and higher in *H. mexicana*, *H. bacteriophora*, and *H. indica*. The apparent superiority of *S. riobrave* in heat and low oxygen tolerance is likely to be advantageous in biocontrol programs. We conclude that *H. mexicana* exhibits a cruiser type of search strategy and, relative to other entomopathogenic nematodes, generally possesses moderate abilities in biocontrol traits.
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Keywords: Beneficial traits; Biological control; Entomopathogenic nematode; *Heterorhabditis mexicana*; *Steinernema*

1. Introduction

Entomopathogenic nematodes are biological control agents in the families Steinernematidae and Heterorhabditidae (Adams and Nguyen, 2002). These nematodes kill insects with the aid of a mutualistic bacterium, which is carried in their intestine (*Xenorhabdus* spp. and *Photorhabdus* spp. are associated with *Steinernema* spp. and *Heterorhabditis* spp., respectively) (Boemare, 2002). The nematodes generally complete 2–3 generations within the host, after which free-living infective

juveniles (IJs) emerge to seek new hosts (Adams and Nguyen, 2002). Although entomopathogenic nematodes are (collectively) pathogenic to a wide variety of insect pests [e.g., some nematode species can infect more than 100 different hosts in the laboratory (Poinar, 1979)], successful commercialization has been limited to relatively few targets (Grewal and Georgis, 1999; Shapiro-Ilan et al., 2002).

Discovery of new entomopathogenic nematode species can substantially expand or improve the utility of these organisms in biological control. For example, discovery of *Steinernema riobrave* (Cabanillas et al., 1994) resulted in improved control of *Diaprepes abbreviatus* (L.) (Duncan and McCoy, 1996; Shapiro-Ilan et al.,

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2002), discovery of *Steinernema scapterisci* (Nguyen and Smart, 1990) led to enhanced suppression of mole crickets, *Scapteriscus* spp. (Parkman et al., 1994), and the recent discovery of *Steinernema scarabaei* (Stock and Koppenhöfer, 2003) promises to expand nematode control of white grubs (Koppenhöfer and Fuzy, 2003a). Once a new species is discovered, characterization of certain biological and ecological properties may facilitate an estimation of the newly described organism's potential as a biocontrol agent (Koppenhöfer and Fuzy, 2003b; Koppenhöfer and Kaya, 1999; Koppenhöfer et al., 2000).

During a recent soil survey conducted to isolate new strains of *S. riobrave* (Stuart et al., 2004), a new entomopathogenic nematode species, *Heterorhabditis mexicana* (Nguyen et al., 2004) was discovered in Tamaulipas, Mexico. The MX4 strain of the new species was isolated from one of 10 sites surveyed and was found in only a single sample taken near an irrigation canal. The objective of the present study was to estimate the relative biocontrol potential of the MX4 strain. We measured virulence to several insect pests that are targets or potential targets for entomopathogenic nematode control (Shapiro-Ilan et al., 2002): the black cutworm, *Agrotis ipsilon* (Hufnagel), pecan weevil, *Curculio caryae*, (Horn), Diaprepes root weevil, *D. abbreviatus*, and the yellow mealworm, *Tenebrio molitor* L. Additionally, we assessed environmental tolerance (to heat, desiccation, and low oxygen levels), and host-seeking ability. For each trait, *H. mexicana* was compared to several entomopathogenic nematode species or strains. For most trait comparisons, at least one species in each comparison was known to possess high capabilities for that trait (based on previous studies). For a number of traits there was no evidence to base hypotheses on whether *H. mexicana* would be superior to other nematodes. However, for ability to tolerate low oxygen levels we hypothesized that *H. mexicana* would show superiority because the nematode was isolated in heavy moist soil (Nguyen et al., 2004; Stuart et al., 2004; personal observations). Additionally, due to the climate where *H. mexicana* was isolated, we hypothesized the nematode would possess a high level of heat tolerance.

2. Materials and methods

2.1. Nematodes and insects and experimental conditions

Nematodes used in all experiments were cultured in parallel in last instar *Galleria mellonella* (L.) (obtained from Webster's Waxie Ranch, Webster, WI) according to Kaya and Stock (1997); IJs were stored at 13 °C (or at room temperature for the *D. abbreviatus* assays) for less than 3 weeks prior to use in experiments. Third instar *A. ipsilon*, reared on artificial diet, were obtained

from the USDA-ARS Corn Insects Research unit (Ames, IA). *D. abbreviatus* (5th to 10th instar) were supplied by the Florida Department of Agriculture, Division of Plant Industry (Gainesville, FL), and *T. molitor* (ca., 80 ± 5.0 mg per insect) were obtained from Southeastern Insectary (Perry, GA). *C. caryae* (4th instar) were collected from infested nuts (on the USDA-ARS Research Station, Byron, GA); the larvae were stored in sterile (autoclaved) soil at 25 °C for 2 weeks (to remove diseased individuals), and remaining larvae stored up to 6 months in sterile soil at 4–10 °C prior to experimentation (Shapiro-Ilan, 2001a). All experiments were conducted in the laboratory at approximately 25 °C and were arranged as completely randomized designs, and repeated once in entirety (i.e., two trials) unless otherwise stated.

2.2. Virulence assays

Agrotis ipsilon and *C. caryae* virulence assays were based on methods described by Shapiro et al. (1999) and Shapiro-Ilan (2001a). Experiments were conducted in plastic cups (Bioserv, Frenchtown, NJ) (3–4 cm in diameter, 3.5 cm deep) filled with 27 g of (oven-dried) soil from the USDA-ARS pecan orchard (Byron, GA), and contained one larva each. The soil was a loamy sand with the percentage sand:silt:clay = 84:10:6, pH 6.1, and organic matter = 2.8% by weight. Nematodes were pipetted onto the soil surface of each cup in 0.5 ml water so that the final moisture was standardized at field capacity (14%). Each experiment contained four replicates of 10 cups per treatment and a water only control. For the *A. ipsilon* assay 50 IJs were applied to each cup and larval mortality was assessed after 24 and 72 h; treatments included *H. mexicana*, *Heterorhabditis bacteriophora* Poinar (Hb strain), and *S. carpocapsae* (Weiser) (All strain). Approximately, 500 IJs were applied to each cup for assessment of *C. caryae* virulence, and mortality was recorded after seven days (Shapiro-Ilan, 2001a); treatments included *H. mexicana*, *Heterorhabditis megidis* Poinar, Jackson, and Klein (UK211 strain), and *S. carpocapsae* (All). The nematode species that *H. mexicana* was compared with were chosen because relatively high levels of virulence were demonstrated previously (Capinera et al., 1988; Levine and Oloumi-Sadeghi, 1992; Shapiro-Ilan, 2001a,b).

Tenebrio molitor assays were conducted in petri dishes (100 mm) lined with filter paper (Whatman No. 1) based on Kaya and Stock (1997). Approximately, 500 IJs were applied in 1 ml water to dishes containing 10 larvae each. Larval mortality was evaluated 3 days after application. Treatments included *H. mexicana*, *H. bacteriophora* (Hb), *Heterorhabditis indica* Poinar, Karunakar, and David (Hom1 strain), and a water only control. There were four replicates of each treatment.

Virulence assays with *D. abbreviatus* were based on methods described by Shapiro and McCoy (2000a). Experimental units consisted of 25 dram lidded plastic containers (3.5 cm i.d., 8.5 cm deep) filled with Candler sand (estimated soil characteristics are: percentage sand:silt:clay = 96.6%:2.0%:1.4%, pH 6.3, organic matter = 0.3%). Soil moisture was standardized within the containers at approximately 8% by weight. A single *D. abbreviatus* larva was placed on the bottom of each container prior to filling with soil, and 200 IJs were applied to the soil surface. There were 36–40 cups pertreatment including a water only control. Larval mortality was determined 10–12 days post-inoculation. Two separate trials were conducted; the first using 7–10th instar (mean = 8.5, SE = 0.15) *D. abbreviatus* larvae, that weighed 0.138–0.602 g (mean = 0.400, SE = 0.022), and the second trial using 5–8th instar (mean = 6.4, SE = 0.13), which weighed 0.023–0.177 g (mean = 0.079, SE = 0.008). The first trial included *H. mexicana*, *H. indica* (Hom1), *S. carpocapsae* (Mexican strain), *Steinernema feltiae* (Filipjev) (SN strain), and two strains of *S. riobrave* (TM and 355 strain). The same nematodes were used in the second trial except *S. riobrave* (355) and *S. feltiae* (SN) were not included and *S. riobrave* (3–7) and *H. bacteriophora* (HP88) were added. Shapiro and McCoy (2000a) observed higher virulence in *S. riobrave* (355) than eight other nematode species and 17 strains in laboratory experiments with *D. abbreviatus*. The 355 strain of *S. riobrave* is derived from the original isolate (Cabanillas et al., 1994). The 3–7 strain of *S. riobrave* is a recently collected Texas isolate; the TM strain is a mixture of 10 recently collected isolates of *S. riobrave* from Texas and Mexico, which had higher virulence to *D. abbreviatus* than the 355 and 3–7 strains in laboratory assays (Stuart et al., 2004). High levels of virulence to *D. abbreviatus* were also reported for *H. indica* (Hom1) (Shapiro et al., 1999).

2.3. Environmental tolerance

Heat tolerance was measured using procedures described by Shapiro et al. (1996). Approximately, 2000 IJs in 0.2 ml were pipetted into 5 ml tap water in a 20-ml glass scintillation vial. The vial had already been equilibrated to 40 °C prior to addition of nematodes. After incubation for 2 h in a water bath shaker (rpm 70) at 40 °C, 0.2 ml of the suspension was transferred to a 60-mm petri dish containing 9 ml tap water. The dishes were incubated at 25 °C for 24 h at which time the percentage nematode survival was determined based on movement response when probed with a dissecting needle. Treatments (replicated four times) included *H. mexicana*, *H. indica* (Hom1), *H. bacteriophora* (Hb), and *S. riobrave* (355). Prior studies indicated relatively high levels of heat tolerance in *S. riobrave* (Grewal et al., 1994a) and *H. indica* (Shapiro and McCoy, 2000a).

Desiccation tolerance was compared among nematode strains based on procedures described by Solomon et al. (1999). Approximately, 2000 IJs were pipetted onto filter paper (55 mm, Whatman No. 1) in a 60 mm petri dish. Excess moisture was removed through vacuum filtration. The filter paper containing nematodes was then placed in a plastic desiccator (23 cm maximum diameter × 24 cm height, Nalgene, Rochester, NY) that was set to 85% RH based on a saturated solution of KCl. After 48 h of incubation at 25 °C, the filter paper was removed and placed in approximately 5 ml tap water for an additional 24 h at which time percentage nematode survival was determined using procedures described above. Each treatment (strain) contained four replicates in a randomized block design (blocked by desiccator). Treatments included *H. mexicana*, *H. bacteriophora* (Hb), *H. indica* (Hom1), *S. carpocapsae* (All), and *S. feltiae* (SN). *Steinernema carpocapsae* and *S. feltiae* have been reported to be more desiccation tolerant than several other entomopathogenic nematode species (Glazer, 2002).

Survival in reduced oxygen levels was evaluated based on procedures described by Somasekhar et al. (2002). Approximately 10,000 IJs were placed in 0.5 ml water in Eppendorf tubes (0.5 ml capacity, Brinkman, Ontario, Canada). After 1 and 3 days in the tubes the nematodes suspension was diluted into 9 ml water in a 100 mm petri dish, and percentage IJ mortality was determined after an additional 24 h at 25 °C. Treatments (five replicates each) consisted of *H. mexicana*, *H. bacteriophora* (Lewiston strain), *H. indica* (Hom1), and *Steinernema glaseri* (Steiner) (NJ43), and *S. riobrave* (355).

2.4. Host-seeking

Host-seeking ability was measured on agar plate assays adapted from Schmidt and All (1978) and Barbercheck and Kaya (1991). Plastic petri dishes (100 mm) were filled to approximately 2/3 depth with 2% agar. A pipette tip (1000 μl capacity) was inserted into a hole in the lid on opposite ends of the plate (approximately 4 mm from the edge of the plate), and a nematode inoculation port (1.0 cm diameter) was created in the center of the lid and sealed with duct tape. On treatment plates, one *G. mellonella* larva was placed in one pipette tip and the other tip was left empty. In control plates both tips were left empty; all tips were sealed with parafilm. During the 1.5 that followed placement of the insect in the pipette tip, a gradient of volatile host cues was created. Subsequently, approximately 2000 IJs in 0.35 ml water were applied to an 10 mm filter paper disc (Whatman No. 1), excess water was removed by vacuum filtration, and the disc was inserted into the center of the agar dish, which was then resealed with duct tape. After 2 and 3 h, the number of IJs found in a 1 cm diameter circle under each pipette tip was recorded. Host-seeking ability was

estimated by the percentage of IJs found under the circle with the host relative to the total under the host plus those under the empty pipette tip. In control plates one side was randomly designated the host side for calculation purposes. Treatments (four replicates each) included *H. mexicana*, *H. bacteriophora* (Hb), and *S. glaseri* (NJ43). The experiment was repeated twice. Both *S. glaseri* and *H. bacteriophora* respond positively to host volatile cues (Grewal et al., 1994b; Lewis et al., 1992).

2.5. Data analysis

Treatment effects in all experiments were analyzed with ANOVA; If the ANOVA detected a significant difference ($P \leqslant 0.05$) then treatment differences were elucidated through LSD (SAS, 2001). Data were arcsine transformed (arcsine of square root) prior to analysis (Southwood, 1978) (non-transformed means are presented in figures). A single exception to the ANOVA approach was used for the *D. abbreviatus* assays. In the *D. abbreviatus* experiments, because multiple replicates were not used, the data were analyzed through contingency tables (χ^2 analysis); the two trials were analyzed separately because treatments and larval ages differed between them.

3. Results

3.1. Virulence assays

Steinernema carpocapsae caused greater mortality in *A. ipsilon* than *H. mexicana* and *H. bacteriophora* one day after exposure ($F = 9.8$; $df = 3, 27$; $P = 0.0002$) (Fig. 1). Three days after treatment, no difference was detected in virulence of *H. mexicana* and *S. carpocapsae*;

both species caused higher *A. ipsilon* mortality than *H. bacteriophora* and mortality in all treatments was higher than in the control ($F = 47.13$; $df = 3, 27$; $P < 0.0001$) (Fig. 1). Curculio caryae mortality in *S. carpocapsae* treatments was higher than *H. mexicana* treatments but not different from *H. megidis*; control mortality was lower than mortality from *S. carpocapsae* and *H. megidis* but not different from *H. mexicana* ($F = 5.74$; $df = 3, 27$; $P = 0.0036$) (Fig. 2A). In *T. molitor* assays no differences were detected among nematode treatments, all of which caused greater mortality than the control ($F = 4.96$; $df = 3, 27$; $P < 0.0001$) (Fig. 2B). In the first trial to assess *D. abbreviatus* virulence, *S. riobrave* strains (TM and 355) caused greater mortality than all other nematode treatments and were the only strains that caused greater mortality than the control (2×7 contingency table, $\chi^2 = 46.012$; $df = 6$; $P < 0.0001$) (Fig. 3A). In the second trial with *D. abbreviatus*, *S. riobrave* strains (TM and 3–7) and *H. indica* (Hom1) caused greater mortality than other nematode treatments and were the only nematodes that caused greater mortality than the control (2×7 contingency table, $\chi^2 = 73.839$; $df = 6$; $P < 0.0001$) (Fig. 3B).
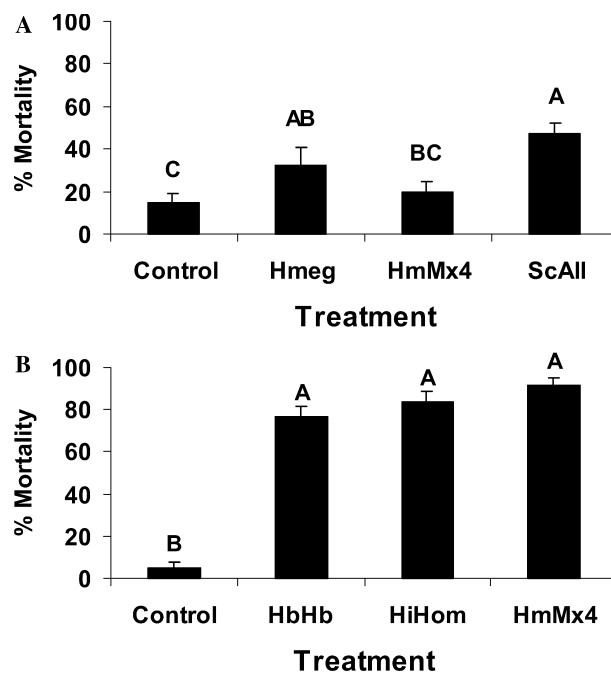
Fig. 2. Mean percentage mortality (±SEM) of *Curculio caryae* larvae seven days after treatment (A) and of *Tenebrio molitor* larvae after three days of treatment (B) with entomopathogenic nematodes: Hbhb, *Heterorhabditis bacteriophora* (Hb strain); HiHom, *Heterorhabditis indica* (Hom1 strain); Hmeg, *Tenebrio molitor* (UK211 strain); HmMx4, *H. mexicana* (Mx4 strain); ScAll, *Steinerema carpocapsae* (All strain). *Curculio caryae* assays were conducted in 30 ml plastic cups, which were filled with soil and contained one insect and 500 infective juvenile nematodes each; *T. molitor* assays were conducted in 100 mm petri dishes lined with filter paper and containing 10 insects and 500 infective juvenile nematodes each. Different letters above bars indicate statistical differences ($P \leqslant 0.05$).
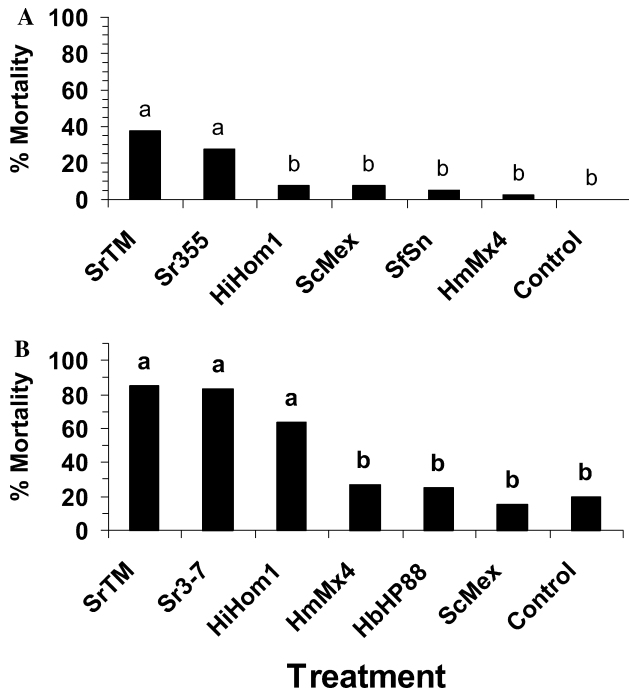
Fig. 1. Mean percentage mortality (±SEM) of *Agrotis ipsilon* larvae 1 or 3 days after treatment (DAT) with entomopathogenic nematodes: Hbhb, *Heterorhabditis bacteriophora* (Hb strain); HmMx4, *H. mexicana* (Mx4 strain); ScAll, *Steinernema carpocapsae* (All strain). Assays were conducted in 30 ml plastic cups, which were filled with soil and contained one insect and 50 infective juvenile nematodes each. Different upper and lower case letters above bars indicate statistical differences 1 and 3 DAT, respectively ($P \leqslant 0.05$).
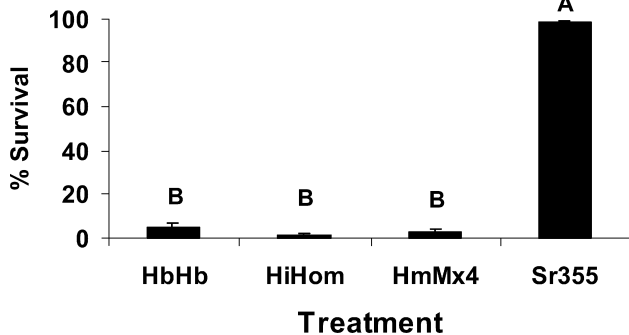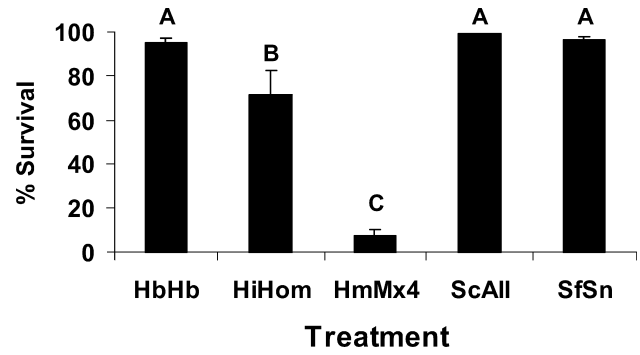
Fig. 5. Mean percentage survival (±SEM) of infective juvenile nematodes following exposure to 85% RH for 48 h. Hbhb, *Heterorhabditis bacteriophora* (Hb strain); HiHom, *H. indica* (Hom1 strain); HmMx4, *H. mexicana* (Mx4 strain); ScAll, *Steinernema carpocapsae* (All strain); SfSn, *S. feltiae* (Sn strain). Different letters above bars indicate statistical differences (*P* ⩽ 0.05).
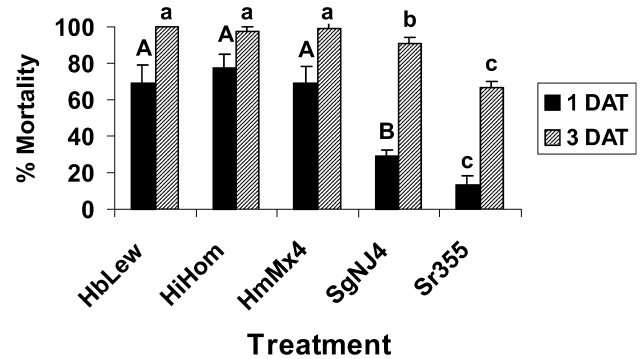
Fig. 3. Mean percentage mortality (±SEM) of *Diaprepes abbreviatus* larvae after treatment with entomopathogenic nematodes: Sr, *Steinernema riobrave* (strains 355, 3–7, and TM); HiHom, *Heterorhabditis indica* (Hom1 strain); ScMex, *S. carpocapsae* (Mexican strain); HmMx4, *H. mexicana* (Mx4 strain); SfSn, *S. feltiae* (Sn strain); HbHP88, *H. bacteriophora* (HP88 strain). Assays were conducted in 25 dram plastic containers, which were filled with soil and contained one insect and 200 infective juvenile nematodes each. Two separate trials were conducted (A and B). Different letters above bars indicate statistical differences (*P* ⩽ 0.05).

3.2. Environmental tolerance

Heat tolerance, as indicated by IJ survival, was more than five-fold greater in *S. riobrave* than the other nematode species tested (*H. mexicana, H. indica,* and *H. bacteriophora*) (*F* = 7.3; *df* = 3, 27; *P* < 0.0001), which were not different from each other (Fig. 4). Desiccation toler-

Fig. 6. Mean percentage mortality (±SEM) of infective juvenile nematodes following exposure to reduced oxygen levels. Ten thousand infective juveniles in 0.5 ml water were placed in 0.5 ml tubes for one or three days (1 or 3 DAT). HbLew, *Heterorhabditis bacteriophora* (Lewiston strain); HiHom, *H. indica* (Hom1 strain); HmMx4, *H. mexicana* (Mx4 strain); SgNJ4, *Steinernema glaseri* (NJ43 strain); Sr355, *S. riobrave* (strain 355). Different upper and lower case letters above bars indicate statistical differences 1 and 3 DAT, respectively (*P* ⩽ 0.05).

ance was greatest in *H. bacteriophora, S. carpocapsae,* and *S. feltiae* (which were not different from each other) followed by *H. indica* and least in *H. mexicana* (*F* = 5.48; *df* = 3, 27; *P* < 0.0001) (Fig. 5). Mortality of nematodes following exposure to low oxygen levels was lowest in *S. riobrave* followed by *S. glaseri* and higher in the other species (*H. mexicana, H. bacteriophora,* and *H. indica,* which were not different from each other) (*F* = 52.1; *df* = 4, 44; *P* < 0.0001; and *F* = 61.91; *df* = 4, 44, *P* < 0.0001, for 1 and 3 days post-treatment, respectively) (Fig. 6).

3.3. Host-seeking

The percentages of nematodes that moved on agar plates were combined for the 2 and 3 h readings because no interaction between time of reading and treatment

Fig. 4. Mean percentage survival (±SEM) of infective juvenile nematodes following exposure to 40 °C for 2 h. Hbhb, *Heterorhabditis bacteriophora* (Hb strain); HiHom, *H. indica* (Hom1 strain); HmMx4, *H. mexicana* (Mx4 strain); Sr355, *Steinernema. riobrave* (strain 355). Different letters above bars indicate statistical differences (*P* ⩽ 0.05).
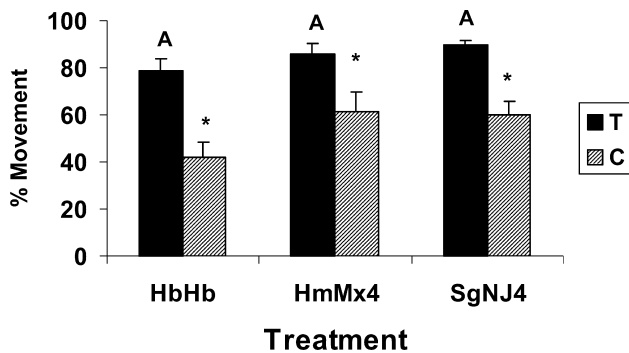
Fig. 7. Mean percentage of infective Juvenile nematodes that moved on agar-filled petri dishes toward *Galleria mellonella* host cues (T, treatment dishes), or toward a randomly selected side of a dish that did not contain any host cues (C, control dishes). HbHb, *Heterorhabditis bacteriophora* (Hb strain); HmMx4, *H. mexicana* (Mx4 strain); SgNJ4, *Steinernema glaseri* (NJ43 strain). Different letters above bars indicate statistical differences among nematode species; asterisks indicate significant differences between T and C dishes within each species ($P \leqslant 0.05$).

was detected ($F = 1.23$; $df = 2, 65$; $P = 0.30$). All nematode species tested exhibited positive attraction to the host based on significant differences in percentage movement between control and treatment plates ($F = 21.3$ for *H. bacteriophora* and *S. glaseri* and $F = 7.6$ for *H. mexicana*; $df = 1, 38$; $P < 0.009$ for all species) (Fig. 7). No difference in host-seeking ability was detected among nematode species; the percentage that moved toward *G. mellonella* cues did not differ significantly ($F = 1.17$; $df = 2, 68$; $P = 0.31$) (Fig. 7).

4. Discussion

We have characterized *H. mexicana* (MX4) for a variety of biological and ecological characteristics important to biological control. Our results indicate that *H. mexicana* exhibits a cruiser type of search strategy (Lewis, 2002) and generally possesses moderate abilities in virulence and environmental tolerance relative to other entomopathogenic nematodes for the range of hosts and conditions tested. *H. mexicana* was not superior to all other nematode species tested in any of the traits evaluated, but was only inferior to all other nematodes in desiccation tolerance. The poor desiccation tolerance observed in *H. mexicana* may have evolved due to an association with moist soils similar to the conditions where it was isolated. However, our hypotheses that *H. mexicana* might excel in heat tolerance, and tolerance to low oxygen levels based on soil conditions where the species was isolated were not supported.

Based on the results of our experiments, we consider the biocontrol potential of *H. mexicana* to be only moderate because it did not exhibit an exceptionally strong performance in any of our assays. However, further tests against other target insects or under other conditions might prove otherwise. Conceivably, the relative abilities of *H. mexicana* for the traits we evaluated under laboratory conditions may differ under the complexity of factors experienced under field conditions. Further, it is conceivable that *H. mexicana*'s virulence is exceptionally high to other pests not tested. Although our study included insects that are commonly used as hosts or are major commercial targets (Shapiro-Ilan and Gaugler, 2002; Shapiro-Ilan et al., 2002), our experiments only included two insect orders (Lepidoptera and Coleoptera), and susceptibility to nematodes among insects in different orders can vary greatly (Klein, 1990).

Some of the trait comparisons among the nematode species other than *H. mexicana* came out as expected (based on prior studies, e.g., see citations in Section 2), whereas other comparisons were novel or unexpected. For example, the superior virulence of *S. carpocapsae* to *A. ipsilon* was not unexpected (Capinera et al., 1988; Levine and Oloumi-Sadeghi, 1992), nor was superior virulence of *S. riobrave* to *D. abbreviatus* (Shapiro and McCoy, 2000a,b; Shapiro-Ilan et al., 2002). *S. riobrave* is known to be a heat tolerant nematode, and thus it is not surprising that it survived exposure to 40 °C better than *H. bacteriophora* (Grewal et al., 1994a). The substantially greater heat tolerance observed in *S. riobrave* compared with *H. indica* is of interest because the latter species has also been considered relatively heat tolerant (Shapiro and McCoy, 2000a) and differences in heat tolerance between the two species have not been previously elucidated. Additionally, comparisons of tolerance to low oxygen levels were novel and also indicated *S. riobrave* to be superior to other species tested. These novel evaluations of environmental tolerance and other traits should be taken into consideration when selecting nematode species for biocontrol programs.

Acknowledgments

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