

## Effects of host nutrition on virulence and fitness of entomopathogenic nematodes: Lipid- and protein-based supplements in *Tenebrio molitor* diets

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**Abstract:** Entomopathogenic nematodes, *Heterorhabditis indica* and *Steinernema riobrave*, were tested for virulence and reproductive yield in *Tenebrio molitor* that were fed wheat bran diets with varying lipid- and protein-based supplements. Lipid supplements were based on 20% canola oil, peanut, pork or salmon, or a low lipid control (5% canola). Protein treatments consisted of basic supplement ingredients plus 0, 10, or 20% egg white; a bran-only control was also included. Some diet supplements had positive effects on nematode quality, whereas others had negative or neutral effects. All supplements with 20% lipids except canola oil caused increased *T. molitor* susceptibility to *H. indica*, whereas susceptibility to *S. riobrave* was not affected. Protein supplements did not affect host susceptibility, and neither lipid nor protein diet supplements affected reproductive capacity of either nematode species. Subsequently, we determined the pest control efficacy of progeny of nematodes that had been reared through *T. molitor* from different diets against *Diaprepes abbreviatus* and *Otiorynchus sulcatus*. All nematode treatments reduced insect survival relative to the control (water only). Nematodes originating from *T. molitor* diets with the 0% or 20% protein exhibited lower efficacy versus *D. abbreviatus* than the intermediate level of protein (10%) or bran-only treatments. Nematodes originating from *T. molitor* lipid or control diets did not differ in virulence. Our research indicates that nutritional content of an insect host diet can affect host susceptibility to entomopathogenic nematodes and nematode fitness; therefore, host media could conceivably be optimized to increase in vivo nematode production efficiency.

**Key words:** diet, entomopathogenic nematode, *Heterorhabditis*, in vivo, mass production, *Steinernema*

Entomopathogenic nematodes (genera *Steinernema* and *Heterorhabditis*) are biological control agents that are used to control a variety of economically important insect pests (Shapiro-Ilan et al., 2002b; Grewal et al., 2005). These nematodes have a mutualistic symbiosis with a bacterium (*Xenorhabdus* spp. and *Photorhabdus* spp. for steinernematids and heterorhabditids, respectively) (Poinar, 1990). Infective juveniles (IJ), the only free-living stage, enter hosts through natural openings (mouth, anus and spiracles) or, in some cases, through the cuticle. After entering the host's hemocoel, nematodes release their bacterial symbionts, which are primarily responsible for killing the host within 24–48 hours, defending against secondary invaders and providing the nematodes with nutrition (Dowds and Peters, 2002). The nematodes molt and complete up to three generations within the host, after which IJ exit the cadaver to find new hosts (Kaya and Gaugler, 1993).

Entomopathogenic nematodes are cultured in vivo or in vitro for large-scale commercial production as well as for laboratory experimentation or field testing (Shapiro-Ilan and Gaugler, 2002; Ehlers and Shapiro-Ilan, 2005). In vitro production is accomplished in solid or liquid media (Shapiro-Ilan and Gaugler, 2002). In vivo culture is most commonly achieved in larvae of the greater wax moth *Galleria mellonella* (L.), but a number

of other hosts can be used, such as the yellow mealworm, *Tenebrio molitor* L. (Shapiro-Ilan and Gaugler, 2002; Shapiro-Ilan et al., 2002a). Various aspects of the culture conditions can affect the yield, quality and efficacy of nematodes produced (Shapiro-Ilan and Gaugler, 2002; Ehlers and Shapiro-Ilan, 2005). Our overall goal is to comprehensively optimize all steps of in vivo production from insect culture to nematode packaging; in this study we focus on optimization of insect media.

The quality of in vitro-produced nematodes can depend heavily on the make-up of artificial media, and therefore considerable research has been directed toward nutrient optimization (Han et al., 1992; Yang et al., 1997; Yoo et al., 2000; Abu Hatab and Gaugler, 2001; Gil et al., 2002). In contrast, research toward improving “media” in in vivo production has received little attention. Prior studies have indicated that the choice of host species is of importance in nematode production efficiency (Blinova and Ivanova, 1987; Shapiro-Ilan et al., 2005); yet the effects of media quality within a host species have not been examined. We hypothesized that nutritional components of an insect host diet can also affect efficiency of in vivo entomopathogenic nematode production and the quality of the nematodes produced. We expect that varying diet components can alter the nutritional make-up of the host and thereby affect host susceptibility as well as nematode yield and fitness of progeny. To test our hypothesis, we determined the effects of varying protein and lipid supplements in *T. molitor* diet on host susceptibility and fitness of *Heterorhabditis indica* Poinar, Karunakar, & David and *Steinernema riobrave* Cabanillas, Poinar, & Raulston. We focused on protein and lipid supplements because these components have been

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shown to be important in determining fitness of entomopathogenic nematodes cultured *in vitro* (Yang et al., 1997; Yoo et al., 2000).

#### MATERIALS AND METHODS

*Insects and nematodes:* *Tenebrio molitor* eggs or first instars were initially supplied by Southeastern Insectaries, Inc. (Perry, GA). Insects were subsequently cultured at 27°C on a wheat bran (coarse grade, Siemer Milling Co., Teutopolis, IL) diet with or without dietary supplements. Insects were introduced to the experimental diets with or without supplements as early instars (3<sup>rd</sup>-5<sup>th</sup>). Insects were then cultured in the diets for approximately 2 mon until their weights ranged from 0.08 to 0.12 g.

Insects intended for use in testing the effects of protein supplements were cultured at the Southeastern Insectaries' laboratory, and those intended for use in testing the effects of lipid supplements were reared at the USDA-ARS National Biocontrol laboratory (Stoneville, MS); insects were then sent to the USDA-ARS laboratory in Byron, GA, for experimentation.

For each experiment, nematodes *H. indica* (HOM1 strain) or *S. riobrave* (7-12 strain) were reared on last instar *Galleria mellonella* (L.) at 25°C according to procedures described in Kaya and Stock (1997). *Galleria mellonella* larvae were obtained from Webster's Waxie-Ranch (Webster, WI). Following harvest, nematodes were stored at 13°C for less than 2 wk before experimentation. All experiments were conducted at approximately 25°C.

*Host diet effects on host susceptibility:* Host susceptibility was tested separately in *T. molitor* reared in lipid- and protein-enriched diets. For testing the effects of lipid diet supplements, *T. molitor* were cultured in plastic boxes (110 × 110 × 35 mm) (uncovered) containing 100 insects. Control insects were cultured on wheat bran plus a basic supplement with low-lipid content. The basic diet supplement consisted of (by weight) 10% dry egg whites (P. No. 40586, Bulkfoods.com) and 85% dry potato (P. No. 12854, Bulkfoods.com); 5% canola oil (Kroger brand, Cincinnati, OH) was added as the lipid source. Addition of the basic supplement was based on preliminary experiments that indicated a benefit of these components to insect growth (unpublished data). All other insects received supplements containing an increased lipid:starch ratio, i.e., the basic supplement ingredients of dry potato (70%) and dry egg whites (10%) mixed with additional lipid compliments of 20% canola oil, peanut oil (Ventura Foods, LLC, Opelousas, LA), pork (as sliced bacon, Tyson, Inc., Springdale, AR), or salmon oil (Lenier Health Products, LLC, Carson, CA). Approximately 50 g of supplement was added to each box twice a week until the insects reached the desired size (80 to 120 mg). All boxes received approximately 5 ml water (added by

plastic syringe) twice a week. The experiments were repeated in time (repeated once for *H. indica* and twice for *S. riobrave*).

For testing the effects of protein diet supplements, *T. molitor* were cultured in uncovered plastic boxes (30 × 45 × 12 cm pans) containing approximately 5,000 insects. One treatment (hereafter referred as Diet 0) received two of the ingredients from the basic supplement (dry potato and canola oil at 95% and 5%, respectively) but without any protein. Other treatments received increased ratios of protein (dry egg white): starch in their supplements: Diet 10 (which was equivalent to our basic supplement) received 85% dry potato, 5% canola oil and 10% egg white, and Diet 20 received 75% dry potato, 5% canola oil and 20% egg white. An additional set of control insects received bran only with no supplement. The experiments (for *H. indica* and *S. riobrave*) were each repeated once in time.

*Tenebrio molitor* cultured on the different diets described above were tested for susceptibility to *H. indica* and *S. riobrave*. Approximately 0.8 (± 0.05) g of insects from each diet was placed in each of eight 100 mm petri dishes lined with filter paper. Four of the dishes received 1 ml of water each only as a control. The other four dishes received nematodes in 1 ml of water, i.e., 6,000 IJ of *H. indica* or 2,000 IJ of *S. riobrave* (the different rates were based on preliminary data indicating higher virulence of *S. riobrave* to *T. molitor*). Dishes were stored at 25°C and host susceptibility (insect mortality) was recorded 4 d post-inoculation.

*Host diet effects on nematode yield:* Host diet effects on nematode yield were determined by measuring progeny production in nematode-infected insects from the host susceptibility experiments. Dead *T. molitor* that showed signs of nematode infection from each replicate in the host susceptibility experiments were weighed and placed together on modified White traps (Shapiro-Ilan and Gaugler, 2002). White traps were kept at 25°C, date of first infective juvenile emergence was noted, and nematodes were collected up to 7 d post-emergence. Harvests from each White trap were pooled (four replicates per treatment) and converted to nematode yield per mg of host.

*Host diet effects on efficacy of nematode progeny:* The goal of this objective was to determine the effects of insect host diet used during *in vivo* nematode production on subsequent pest control efficacy. To address this issue, we compared the virulence of nematodes produced in *T. molitor* fed modified and unmodified diets.

Virulence trials for nematodes produced on modified diets were focused on laboratory suppression of the diaprepes root weevil, *Diaprepes abbreviatus* (L.). Laboratory trials in protected environments are well suited to detecting differences in virulence (Shapiro and McCoy, 2000; Shapiro-Ilan et al., 2002b). The virulence evaluation was conducted according to Shapiro and McCoy (2000). Briefly, experimental units consisted of

30 ml plastic cups containing 27 g of sand and one larva (40- to 60-d-old). Approximately 500 IJ were added to each cup in 0.5 ml of water; the final soil moisture was 8%. The cups were incubated at 25°C, and insect survival was recorded 7 and 14 d post-inoculation. Treatments included nematodes produced in the protein and lipid diets as well as a water-only control. Each experiment consisted of four replicates of 10 cups/treatment, and the experiments (lipid and protein effects) were each repeated once in time.

Additionally, a single trial to determine effects of insect host diet on subsequent pest control efficacy was conducted using the black vine weevil, *Otiorhynchus sulcatus* (F.), as the target pest. Treatments included nematodes produced in the lipid diets and a water-only control as described above (protein diet effects were not tested for this target pest). Sterilized sand and Metro-mix potting soil were mixed together in a 1:1 ratio and moistened to 10% w/w. This mixture was then added to plastic cups (8.25-cm-diam. × 5-cm deep) to a depth of 4 cm. Infective juveniles were applied at a rate of 25 IJ/cm<sup>2</sup>. After the IJ were added, they were given 1 hr to distribute before a single insect was added to each cup. Eight replicate late instar insects were included per treatment. Insect mortality was determined after 8 d.

**Data analysis:** Treatment effects in all experiments (except efficacy vs. *O. sulcatus*) were detected through analysis of variance (ANOVA); if the *F* value was significant ( $P \leq 0.05$ ), then treatment differences were further elucidated through the Student-Newman-Keul's test. Data from experiments repeated in time were combined, and variation among trials was accounted for as a block effect. Percentage data (survival or mortality) were arcsine transformed, and numerical data (nematode yield) were square-root transformed prior to analysis (Southwood, 1978; Steel and Torrie, 1980). All means and standard errors presented represent untransformed data.

We realized that the different insect host diets could affect natural mortality in *T. molitor*. Therefore, two analyses were conducted for the host susceptibility experiments. To account for potential variation in natural (no-nematode control) mortality of *T. molitor* fed different diets, treatment differences in host susceptibility experiments were analyzed after correction with Abbott's formula (Abbott, 1925). Additionally, for the sake of comparison among controls, we conducted a separate analysis evaluating insect mortality in the controls (no nematodes) relative to each other and to the nematode treatments.

Treatment effects in the *O. sulcatus* efficacy experiment were analyzed using a  $2 \times 2 \chi^2$  analysis ( $P \leq 0.05$ ).

## RESULTS

**Host diet effects on host susceptibility:** *Tenebrio molitor* fed supplements with 20% lipids (except canola oil) were

more susceptible to *H. indica* infection than insects fed the low-lipid supplement ( $F = 5.3$ ;  $df = 4,34$ ;  $P = 0.002$ ) (Fig. 1). This indicates that *T. molitor* that are fed diet supplements with a higher starch:lipid ratio (in addition to their normal bran diet) are more susceptible to *H. indica* infection than insects fed a lower starch:lipid ratio. In contrast, the various lipid supplements did not affect host susceptibility to *S. riobrave* ( $F = 1.2$ ;  $df = 4,53$ ;  $P = 0.359$ ) (Fig. 1). Insect diets that included protein supplement treatments did not differ in *T. molitor* susceptibility to *H. indica* ( $F = 0.79$ ;  $df = 3, 24$ ;  $P = 0.382$ ) or *S. riobrave* ( $F = 3.0$ ;  $df = 3,22$ ;  $P = 0.052$ ) relative to each other or to the bran only control diet (Fig. 2). This indicates that food supplements containing increased protein content do not affect the susceptibility of *T. molitor* to *H. indica* or *S. riobrave*.

The control mortality (i.e., in *T. molitor* with no exposure to nematodes) in the results reported above is not presented in the Figures (Figs. 1,2) because the data were corrected with Abbott's formula. Therefore,

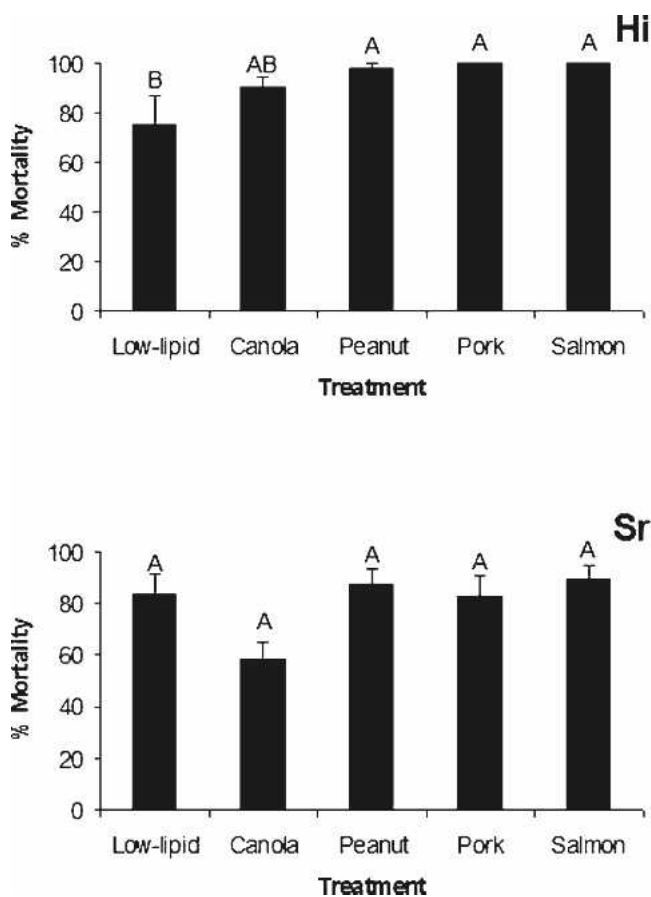


FIG. 1. Susceptibility of *Tenebrio molitor* to *Heterorhabditis indica* (Hi) or *Steinernema riobrave* (Sr). Prior to exposure to nematodes, *T. molitor* were fed diets containing different lipid-based supplements with 20% canola, peanut, pork or salmon; a low-lipid control (5% canola oil) was also included. Bars represent percentage (mean  $\pm$  SE) *T. molitor* mortality corrected for corresponding control (no-nematode) mortality in each diet based on Abbott's formula. Different letters above bars indicate statistically significant differences (SNK test,  $\alpha = 0.05$ ).

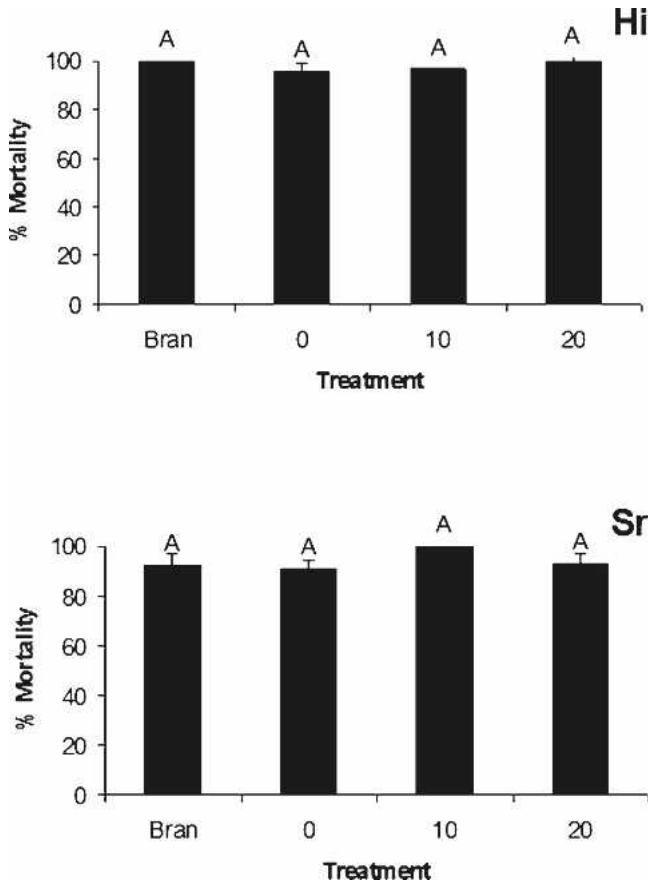


FIG. 2. Susceptibility of *Tenebrio molitor* to *Heterorhabditis indica* (Hi) or *Steinernema riobrave* (Sr). *Tenebrio molitor* were fed diets with protein supplements containing 0, 10 or 20% dry egg whites prior to exposure to nematodes. A bran-only control (no supplement) was also included. Bars represent percentage (mean ± SE) *T. molitor* mortality corrected for corresponding control (no-nematode) mortality in each diet based on Abbott's formula. Different letters above bars indicate statistically significant differences (SNK test,  $\alpha = 0.05$ ).

the control mortality is presented as follows. In the lipid supplement experiments, percentage insect mortality in the controls was (mean ± SE)  $1.6 \pm 1.6$ ,  $4.7 \pm 2.9$ ,  $1.8 \pm 1.8$ ,  $1.4 \pm 1.4$  and  $3.1 \pm 2.2$  for the *H. indica* experiment and 0,  $6.25 \pm 2.9$ ,  $8.3 \pm 7.3$ ,  $22.9 \pm 10.0$  and  $1.0 \pm 1.0$  for the *S. riobrave* experiment in the low-lipid, canola, peanut, pork and salmon diets, respectively. In the *H. indica* experiment, mortality in all lipid diet controls was lower than all treatments, and the controls were not different from each other ( $F = 138.5$ ;  $df = 9, 69$ ;  $P < 0.0001$ ). In the *S. riobrave* experiment, mortality in all lipid diet controls was lower than all treatments, and mortality in the pork diet without nematodes was higher than mortality in other controls (no other differences among controls were detected) ( $F = 90.0$ ;  $df = 9, 108$ ;  $P < 0.0001$ ).

In the protein supplement experiments, percentage insect mortality in the controls was (mean ± SE) 0, 0,  $1.6 \pm 1.6$  and  $1.6 \pm 1.6$  for the *H. indica* experiment and 0,  $1.8 \pm 1.8$ ,  $4.5 \pm 2.2$  and 0 for the *S. riobrave* experiment in the diets with bran only or supplements with 0%,

10% or 20% protein, respectively. Mortality in all protein diet controls was lower than all treatments, and the controls were not different from each other ( $F = 451.1$ ;  $df = 7, 52$ ;  $P < 0.0001$  for the *H. indica* experiment, and  $F = 185.2$ ;  $df = 7, 48$ ;  $P < 0.0001$  for the *S. riobrave* experiment).

**Host diet effects on nematode yield:** Reproductive yield of *H. indica* or *S. riobrave* in *T. molitor* was not affected by the host diets tested ( $F = 1.19$ ;  $df = 4, 12$ ;  $P = 0.365$  and  $F = 0.57$ ;  $df = 4, 39$ ;  $P = 0.689$  for the *H. indica* and *S. riobrave* lipid experiments, respectively;  $F = 0.15$ ;  $df = 3, 24$ ;  $P = 0.241$  and  $F = 1.31$ ;  $df = 4, 22$ ;  $P = 0.297$  for the *H. indica* and *S. riobrave* protein experiments, respectively) (Table 1). Nematode yields ranged from 405,026 to 879,375/mg *T. molitor* in *H. indica* and from 240,813 to 788,599/mg *T. molitor* in *S. riobrave*.

**Host diet effects on efficacy of nematode progeny:** In the experiments involving IJ progeny that emerged from *T. molitor* fed different lipid supplements, survival of *D. abbreviatus* at 7 and 14 d post-inoculation was reduced in all nematode treatments relative to the no-nematode controls; however, no differences in *D. abbreviatus* survival due to *T. molitor* diets were detected ( $F = 5.47$ ;  $df = 5, 41$ ;  $P = 0.0006$  and  $F = 4.95$ ;  $df = 5, 41$ ;  $P = 0.0012$  for the *H. indica* experiment at 7 and 14 d, and  $F = 8.31$ ;  $df = 5, 41$ ;  $P < 0.0001$  and  $F = 21.15$ ;  $df = 5, 41$ ;  $P < 0.0001$  for the *S. riobrave* experiments at 7 and 14 d, respectively) (Fig. 3).

Similar to the experiments with *D. abbreviatus*, IJ progeny arising from lipid supplement treatments did

TABLE 1. Reproductive yield of infective juvenile nematodes per mg of infected *Tenebrio molitor*<sup>a</sup>

Test	Diet <sup>b</sup>	Nematode <sup>c</sup>	Yield <sup>d</sup> ± SE
Lipid	Low-lipid	Hi	813791.74 ± 232225.70 (A)
Lipid	Canola	Hi	657305.37 ± 156587.14 (A)
Lipid	Peanut	Hi	844268.08 ± 148559.85 (A)
Lipid	Pork	Hi	457671.58 ± 190495.07 (A)
Lipid	Salmon	Hi	743305.42 ± 77839.08 (A)
Lipid	Low-lipid	Sr	240813.18 ± 41475.46 (A)
Lipid	Canola	Sr	301884.86 ± 56239.68 (A)
Lipid	Peanut	Sr	326919.57 ± 32184.77 (A)
Lipid	Port	Sr	335694.14 ± 40573.16 (A)
Lipid	Salmon	Sr	330591.21 ± 37677.73 (A)
Protein	Bran	Hi	602982.80 ± 146085.18 (A)
Protein	0	Hi	712481.72 ± 124856.45 (A)
Protein	10	Hi	879375.38 ± 199963.82 (A)
Protein	20	Hi	405026.11 ± 80530.45 (A)
Protein	Bran	Sr	509241.85 ± 150897.20 (A)
Protein	0	Sr	756488.77 ± 144695.48 (A)
Protein	10	Sr	788598.58 ± 130712.20 (A)
Protein	20	Sr	566648.38 ± 91089.58 (A)

<sup>a</sup> Prior to infection *T. molitor* were fed wheat bran diets containing differing lipid or protein supplements.

<sup>b</sup> Low-lipid = supplement with 5% canola oil (other treatments in lipid tests contained 20% lipids); bran = bran-only control (no supplement); protein treatments were 0 (basic supplement ingredients without protein) and 10 or 20% protein added.

<sup>c</sup> Hi = *Heterorhabditis indica*; Sr = *Steinernema riobrave*.

<sup>d</sup> Numbers followed by the same letters within each test indicate a lack of statistical separation ( $P \leq 0.05$ ).

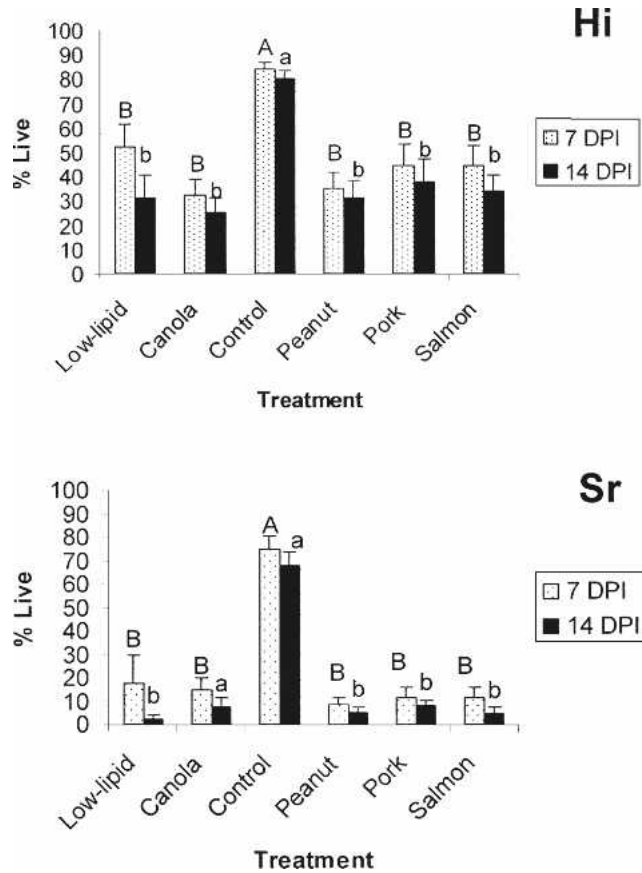


FIG. 3. Efficacy of *Heterorhabditis indica* (Hi) and *Steinernema riobrave* (Sr) in suppressing *Diaprepes abbreviatus*. Nematodes were applied to *D. abbreviatus* in all treatments except the control (which received water only). Nematodes were derived from *T. molitor* that were fed diets containing lipid-based supplements of 20% canola, peanut, pork or salmon; a low-lipid control diet (5% canola) was also included. Bars represent percentage (mean  $\pm$  SE) *D. abbreviatus* survival 7 and 14 days post-inoculation (DPI). Different upper and lower case letters above bars indicate statistically significant differences in 7 and 14 day analyses, respectively (SNK test,  $\alpha = 0.05$ ).

not differ in their affects on *O. sulcatus* suppression. All nematode treatments produced higher insect mortality than was observed in the water-only control, yet no other differences were detected (Table 2). Percentage mortality was 0% in the water-only control and 50%, 87.5%, 75%, 57.15% and 75% in the canola, low-lipid,

peanut, pork and salmon nematode treatments, respectively.

In the experiments involving IJ progeny that emerged from *T. molitor* fed different protein supplements, survival of *D. abbreviatus* at 7 and 14 d post-inoculation was reduced in all nematode treatments relative to the no-nematode controls ( $F = 26.22$ ;  $df = 4,34$ ;  $P < 0.0001$  and  $F = 16.81$ ;  $df = 4, 34$ ;  $P < 0.0001$  for the *H. indica* experiment at 7 and 14 d, and  $F = 14.28$ ;  $df = 4,34$ ;  $P < 0.0001$  and  $F = 14.87$ ;  $df = 4, 34$ ;  $P < 0.0001$  for the *S. riobrave* experiments at 7 and 14 d, respectively) (Fig. 4). In the *H. indica* experiment 7 d post-inoculation, *D. abbreviatus* survival in the 20% protein treatment was higher than the 10% treatment and the bran-only treatment (but was not different from basic supplement ingredients without protein). At 14 d post-inoculation (in the *H. indica* experiment), the 0 and 20% protein treatments both exhibited higher *D. abbreviatus* survival than the bran-only treatment (Fig. 4). No differences in *D. abbreviatus* survival due to original *T. molitor* diets (with or without protein supplements) were detected in the *S. riobrave* experiment (Fig. 4).

## DISCUSSION

Our data indicate that the nutritional content of an insect host diet can affect host susceptibility to entomopathogenic nematodes and nematode fitness. Based on findings involving in vitro production of nematodes, we know that media nutrition affects nematode fitness at a bi-trophic level (Han et al., 1992; Yang et al., 1997; Yoo et al., 2000; Abu Hatab and Gaugler, 2001; Gil et al., 2002). For example, lipid source and quantity in artificial media affected heterorhabditid development rate and yield (Yoo et al., 2000; Abu Hatab and Gaugler, 2001). Our findings indicate that during in vivo production nutritional effects on entomopathogenic nematodes can pass through at least three trophic levels. Given that tri-trophic effects from host plant chemicals, e.g., negative impact of allelochemicals such as cucurbitacins, have been observed previously (Barbercheck et al., 1995; Barbercheck and Wang, 1996; Kunkel et al., 2004), we expected nutritional effects in

TABLE 2. Results from  $2 \times 2 \chi^2$  analysis comparing efficacy of *Heterorhabditis indica* in suppressing *Otiorynchus sulcatus*<sup>a</sup>

Treatment	Pork	Salmon	Canola	Low-lipid	Peanut
Control	$\chi^2 = 6.23$ $P = 0.013$	$\chi^2 = 9.6$ $P = 0.002$	$\chi^2 = 5.33$ $P = 0.02$	$\chi^2 = 12.44$ $P < 0.001$	$\chi^2 = 9.6$ $P = 0.002$
Pork		$\chi^2 = 0.536$ $P = 0.46$	$\chi^2 = 0.077$ $P = 0.781$	$\chi^2 = 1.759$ $P = 0.185$	$\chi^2 = 0.536$ $P = 0.46$
Salmon			$\chi^2 = 1.067$ $P = 0.301$	$\chi^2 = 0.41$ $P = 0.522$	$\chi^2 = 0$ $P = 1$
Canola				$\chi^2 = 2.618$ $P = 0.106$	$\chi^2 = 1.067$ $P = 0.302$
Low-lipid					$\chi^2 = 0.41$ $P = 0.522$

<sup>a</sup> Nematodes were applied to *O. sulcatus* in all treatments except the control (which received water only). Nematodes were derived from *Tenebrio molitor* that were fed diets containing lipid-based supplements of 20% canola, peanut, pork or salmon; a low-lipid control diet (5% canola) was also included.

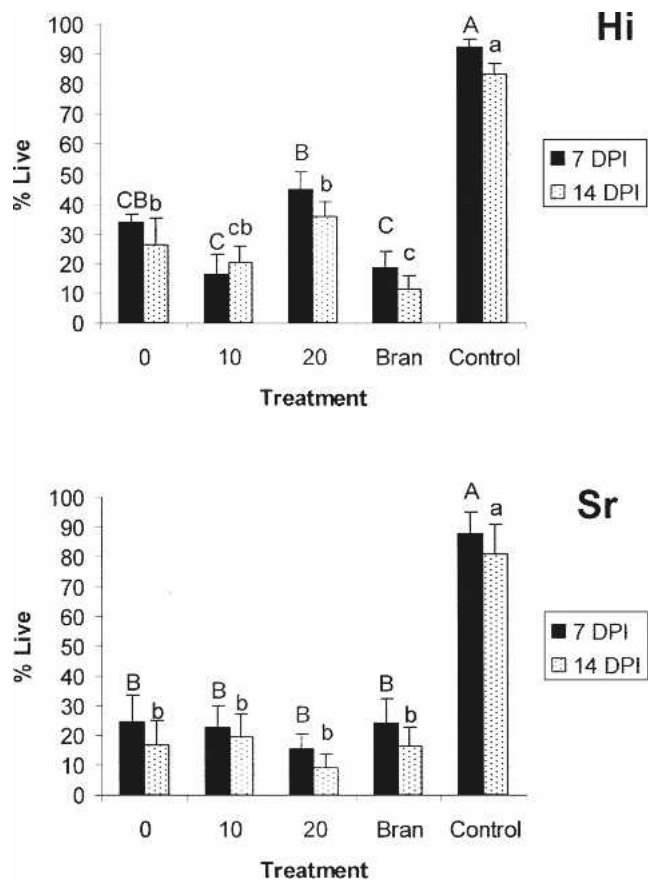


FIG. 4. Efficacy of *Heterorhabditis indica* (Hi) and *Steinernema riobrave* (Sr) in suppressing *Diaprepes abbreviatus*. Nematodes were applied to *D. abbreviatus* in all treatments except the control (which received water only). Nematodes were derived from *T. molitor* that were fed diets with differing protein supplements of 0, 10 or 20% dry egg whites. A bran-only control diet (no supplement) was also included. Bars represent percentage (mean  $\pm$  SE) *D. abbreviatus* survival 7 and 14 days post-inoculation (DPI). Different upper and lower case letters above bars indicate statistically significant differences in 7 and 14 day analyses, respectively (SNK test,  $\alpha = 0.05$ ).

an insect diet to carry through three trophic levels as well.

The lipid and protein supplements in the insect diet varied in their effects on entomopathogenic nematodes. Supplements with increased lipid:starch ratios (except canola oil) increased host susceptibility to *H. indica* but did not affect the nematode's yield or efficacy of progeny nematodes. In contrast, in in vitro media, increases in lipid content led to increased nematode yield (Yoo et al., 2000). The fact that all lipid supplements increased host susceptibility except canola oil indicates that source of lipid in the host diet is important; similarly, lipid source in in vitro media has been shown to impact nematode quality (Yoo et al., 2000; Abu Hatab and Gaugler, 2001).

Excess protein levels, i.e., supplements with protein added at the higher level (20%), were detrimental to the pest control efficacy of *H. indica* progeny, yet intermediate levels (10%) were not. Apparently, the no-protein supplement (potato and canola oil) also caused

a reduction in *H. indica* efficacy relative to the bran-only diet. Thus, it appears there may be an optimal level of protein required with too little, or too much, having a negative impact on the nematodes. Direct effects of protein in nematode media have been observed previously, e.g., Yang et al. (1997) observed differences in *S. carpocapsae* dispersal and infectivity based on protein source.

Entomopathogenic nematode media optimization, and thus sensitivity to media changes, is species-dependant (Ehlers and Shapiro-Ilan, 2005). We detected effects of *T. molitor* host diet on *H. indica* but not *S. riobrave*. Similarly, media-related effects such as differing host species or culture methods have been reported to have varying impacts on different entomopathogenic nematode species (Gaugler and Georgis, 1991; Han et al., 1992; Shapiro-Ilan et al., 2005). For example, Gaugler and Georgis (1991) reported culture methods effects (in vivo vs. in vitro) on *H. bacteriophora* efficacy but not *S. carpocapsae*.

Our results have implications for improving the efficiency of in vivo production. Based on our findings, it is conceivable that insect host media can be optimized for maximization of nematode fitness and quality. For example, *H. indica* production efficiency might be improved by supplementing insect diet with lipids (thereby enhancing host susceptibility and infection rates). Modification in host media would have to be weighed with costs and effects on insect growth. In the case of lipid supplements, we observed an added benefit in that our lipid treatments caused increased *T. molitor* growth rates relative to a bran-only or low-lipid diet (Morales-Ramos, Rojas, Tedders, and Shapiro-Ilan, unpubl. data); such increases in insect host development may also improve the economic efficiency of in vivo nematode production.

Our data are also relevant to other aspects of entomopathogenic nematode biology and biological control. When conducting laboratory or field trials on nematode fitness or efficacy, researchers have often observed variation in nematode batches, which can be due to a variety of factors during culture, storage or application (Ricci et al., 1996; Shapiro-Ilan et al., 2006). Our results indicate that another reason for variability in nematode batches or replicated laboratory or field trials could arise from host diet effects on in vivo cultured nematodes. Additionally, fitness and competitiveness of entomopathogenic nematodes in nature could stem in part from the nutritional content of their host diets.

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