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Effect of insect host age and diet on the fitness of the entomopathogenic nematode-bacteria mutualism

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Abstract Insect host age and diet were evaluated as potential factors that could affect the fitness of the entomopathogenic nematode-bacterium mutualistic partnership. Two nematode species were considered: *Steinernema carpocapsae* and *Heterorhabditis sonorensis*, together with their symbionts *Xenorhabdus nematophila* and *Photorhabdus luminescens*, respectively. The tobacco hornworm, *Manduca sexta*, was used as the insect host. Insect developmental stage was a factor that impacted nematode virulence. Non-wandering 5th instar *M. sexta* were found to be more susceptible to nematode infection compared to wandering 5th instars. This was more noticeable for *S. carpocapsae* than for *H. sonorensis*. The nutritional status of the host also had an effect on the fitness of the two nematode species tested. In general, insects fed with the reduced diet content were less susceptible to nematode parasitism. The least observed mortality (0.5 %) was in those *M. sexta* larvae exposed to the low *H. sonorensis* dose. Host diet also had an effect on the production of IJ progeny in the insect cadavers. For both nematode species tested, the highest yield of emerging IJs was observed from those insect hosts fed with the low nutrient diet and exposed to the highest nematode inoculum. However, for both nematode species tested, the nutritional status of the host did not significantly affect time of emergence of IJ progeny or the reassociation with their bacterial symbionts (expressed as cfu/IJ). This is the first study on the effect of insect host physiology on both EPN and their symbiotic bacteria fitness.

Keywords *Manduca sexta* · *Steinernema carpocapsae* · *Xenorhabdus nematophila* · *Heterorhabditis sonorensis* · *Photorhabdus luminescens* · Host diet · Host age

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1 Introduction

Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) have a mutualistic relationship with Gram negative Enterobacteria (*Xenorhabdus* and *Photorhabdus*). Together they form an insecticidal alliance that kills a wide range of insects. Each partner in this mutualistic association plays a key role in important life history events for the other, including gaining access to insect host resources, dispersal, and protection against various biotic and abiotic factors (Boemare et al. 1993; Flores-Lara et al. 2007; Sicard et al. 2004, 2006; Snyder et al. 2007). For these two mutualists, the insect host is the only environment for growth and development.

The nematodes are entirely dependent on the internal environment of an insect host to complete their life cycle. The exception is the third-stage infective juvenile (IJ), the only free-living stage in the life cycle of these nematodes. Once the IJ locates a host, it can enter through any natural opening, including the mouth, anus or spiracles. Heterorhabditis species may also penetrate a host through the cuticle using a cuticular tooth-like structure they present around the lip region (Poinar 1975). Inside the host, *Heterorhabditis* IJs expel their symbiotic bacteria via regurgitation (Ciche and Ensign 2003), while the bacteria associated with *Steinernema* are defecated (Snyder et al. 2007) by the IJs. The bacterial symbionts play dual roles; they are pathogens of insects and mutualists of the nematodes. Insect death generally occurs quickly, in 24–48 h

(Poinar 1975). Once nutrients are depleted, IJs re-associate with the bacteria and depart the cadaver in search of a new host (Akhurst 1980). The IJs remains in the soil without feeding, moving through a water film (Ishibashi and Kondo 1990), searching or waiting for a suitable host to parasitize.

Because most of the nematodes life cycle occurs within its insect host, its physiology must be taken into consideration. In particular, factors such as nutrition, age, and hormone titers are known to influence insect host physiology (Eleftherianos et al. 2008; Strand et al. 1991). Insect host condition is defined here as the physiological state of the insect at the time it encounters a pathogen or parasite. Few studies have examined the fitness of either the nematodes or their bacterial partners relative to the insect host condition. For example, it has been shown that in the infection process, *Photorhabdus luminescens,* the bacterial symbiont of *Heterorhabditis bacteriophora,* proliferates more rapidly in older 5th instar of the tobacco hornworm, *Manduca sexta,* relative to younger 5th instars (Eleftherianos et al. 2006). These results indicate that the developmental stage of the insect host may play an important role in the establishment and proliferation of nematodes' bacterial symbionts. With respect to insect host diet, Barbercheck (1993) and Barbercheck et al. (1995, 2003) suggested that insects reared on a high-lipid diet are more susceptible to nematode infection relative to those reared on low-lipid diet. However, the effect of host diet on the bacterial symbionts was not assessed.

In this study, we expanded previous knowledge by considering two insect host parameters, developmental stage and diet quality, to test their effect on the fitness of both the nematodes and their bacterial symbionts. Specifically, we focused on 1) the developmental stage of the insect host as a parameter that could impact nematode virulence, and 2) the effects of the nutritional status of the insect host on the nematodes' reproductive fitness and their re-association with their symbiotic bacteria.

2 Materials and methods

2.1 Nematode cultures and rearing conditions

Two entomopathogenic nematode species, *Heterorhabditis sonorensis* (Caborca strain) and *Steinernema carpocapsae* (ALL strain) were considered. The former species behaves as a cruiser in its host-searching strategy (Stock et al. 2009), while the latter is an ambusher species (Campbell and Gaugler 1993). Both nematode species were reared in vivo with fifth instar *Galleria mellonella* (Lepidoptera: Pyralidae) (Timberline Fisheries, Marion, IL), according to procedures described by Kaya and Stock (1997). Dead insects with signs of nematode infection were placed into modified White traps (Kaya and Stock 1997) until emergence, when IJ progeny were harvested. Emerging IJs were harvested for 1 week and stored at approximately 1,000 IJs/ml in vented 250 ml tissue culture flasks at 15 °C until used for experiments. Nematodes used for all experiments were less than 2 weeks old (i.e., storage time).

2.2 Host and rearing conditions

The tobacco hornworm, *M. sexta* (Lepidoptera: Sphingidae), was the insect host selected for these studies. This choice was based on existing well-documented data on its physiology and biology (D'Amico et al. 2001; Davidowitz et al. 2004). In particular, the 5th instar larva of this insect experiences several drastic changes in physiology and behavior due to a rapid increase in prothoracicotropic hormone (Truman 1972). At this time, the larva begins to wander in search of a pupation site (Truman and Riddiford 1974) in the soil, where it may naturally encounter the nematodes. Wandering *M. sexta* were therefore about 5–6 days older than non-wandering ones.

Wandering and non-wandering fifth instar *M. sexta* were reared on artificial diet as described by Davidowitz et al. (2003), except that no antibiotics were added to avoid interference with the EPN bacterial symbionts. One-day old eggs were obtained from the J. Hildebrand laboratory (Department of Neurobiology, University of Arizona). *M. sexta* were reared in a 25 °C walk-in temperature controlled chamber set to a 16 L: 8D photoperiod. Insect diet was available ad libitum. On the last day of the fourth instar, when head capsules had slipped, indicating preparation to molt to the fifth instar, insects were placed on one of two diet treatments: a) a standard artificial diet, containing 100 % of all ingredients described by Davidowitz et al. (2003), or b) a low-nutrient diet, with a 60 % reduction in the proportion of macro-ingredients of the standard diet. Vitamin proportions remained the same for both treatment groups (Table 1). Insects were maintained in each diet treatment for approximately 70 h.

The strategy of changing insect host diet at the end of the fourth larval instar was based on preliminary studies conducted by Davidowitz et al. (2003) that showed a significant difference in the insects' physiological performance depending on the composition of the diet at this point in the lifecycle. Insects could not be reared on low-nutrient diet from hatch because of high mortality (G. Davidowitz, personal communication).

2.3 Effect of insect host developmental stage on EPN infection

Fifth instars non-wandering and wandering of *M . sexta* were used in all experiments. The experimental arena consisted of 270 ml clear plastic Solo® cups with a straw-slit lid for oxygen exchange. Containers were filled with 100 g of moistened (10 % w/v) autoclaved sand (Sakrete® All Purpose Sand,

Table 1 *Manduca sexta* artificial diet ingredients representing optimal standard diet (100 %) and low-nutrient diet (60 %)

Ingredients for 3 L Diet	Optimal standard diet—100 %	Low-nutrient diet—60 % of optimal macro ingredients
Wheat germ (g)	**327**	**196.2**
Casein (g)	**147**	**88.2**
Torula yeast (g)	**65.4**	**39.24**
Wesson salts (g)	**49.2**	**29.52**
Cholesterol (g)	**14.4**	**8.64**
Sucrose (g)	**129**	**77.4**
Alphacel (g)	**0**	**146.4**
Methylparaben (g)	4.2	4.2
Sorbic acid (g)	8.1	8.1
Ascorbic acid (g)	20.4	20.4
Streptomycin (g)	0.81	0.81
Penicillin (g)	3	3
Vandersandt vitamin mix (g)	2.01	2.01
Water (l)	1	1
Linseed Oil (ml)	15	15
Formalin (ml)	15	15
Agar (g)	63	63

The low-nutrient diet contains approximately 60 % of the ingredients in bold and filled with a non-nutritive bulk (Alphacel) in place of the lipid, protein and carbohydrate deficiency (from Davidowitz et al. 2003)

Charlotte, NC). Insect weight was recorded prior to placing them individually in each cup.

Two nematode inocula were used: a) high dose=200 IJ/ml, and b) low dose=25 IJ/ml. Within each diet group, the application of low IJ dose, high IJ dose or sterile, distilled water for the control group, was completely randomized. One ml of IJ suspension or water was applied to the surface of the sand in each cup. Once inoculated, containers were capped with lids and placed in a 25 °C dark incubator. Insects were not given food over the 5-day period to be sure that larvae were not orally ingesting IJs, which were applied directly to the sand surface. Wandering larvae immediately buried down into the bottom of the experimental arena, while non-wandering larvae were always found resting on top of the soil, not below it. Insect host mortality was recorded over a 5-day period. Insect host death was assessed based on changes observed in the color of the body and lack of movement when prodded with a needle. Fifteen insects were used in each insect stage/EPN dose treatment group and the experiments were repeated three times, with different cohorts of *M. sexta* larvae each time. *M. sexta* mortality was analyzed separately for each nematode species. Data were subjected to ANOVA with the general lineal model using JMP® v. 8.0.2 (SAS Institute 2008). Means were compared by a Student's *t*-test.

2.4 Effect of insect host diet on EPN virulence

Three-day-old 5th instar *M. sexta* larvae were used in all experiments. The experimental arena, method of treatment, and signs of infection were measured as described above. The average weight of insects fed on the reduced-nutrient diet was 0.86 g less than the insects reared on the standard diet (ANOVA, $F_{1,94}=16.31$, $P<0.005$; 4.04 g (±0.12) (mean ± SE) vs. 4.90 g (±0.18)). To ensure that the average weight of the two diet groups was represented, all insects available from each diet group were weighed and the distribution of weights was recorded. The first to third quartile ranges of larval weights were used in each experiment. The weight used for the reduced nutrient treatment group was 3–4.5 g while the standard diet group weight range used was 4–6 g. A complete randomized, block design was considered. The main plot factor was diet type and EPN dose was the subplot factor. Fifteen insects were used in each diet type/EPN dose treatment group and the experiments were repeated six times. Mortality was arcsine-transformed and EPN dose and diet type were subjected to a one-way analysis of variance (ANOVA) using JMP® v. 8.0.2 (SAS Institute 2008). When the factor was significant ($P<0.005$), differences among means were determined by a least square means comparison test (Tukey's HSD).

2.5 Effect of insect host diet on EPN time to emergence and progeny production

Once dead insects were observed, they were rinsed in distilled water and transferred to modified White traps (Kaya and Stock 1997). Insects in White traps were checked daily to record the first day of IJ emergence. Emerging IJs migrating into the water were collected in 50 ml tissue culture flasks every day for 10 days. The total number of IJs emerged per cadaver was recorded. As mortality was highly variable across treatment groups, sample size (n) for each nematode species and diet combination was different. The average time to emergence and the total number of IJ progeny were calculated for each diet/EPN dose combination for each replicate, hence $n=6$ for all analyses. Whether time to emergence or progeny production was measured, the average number per host individual per treatment group was subjected to analysis of variance (ANOVA) using JMP® v. 8.0.2 (SAS Institute 2008).

2.6 Isolation and quantification of bacterial symbionts from EPN progeny

IJs obtained from the above mentioned experiments were considered to evaluate symbiont colonization. EPN dose treatment group was compared between diet types using linear contrast analysis with diet type as the main factor. The average number of colony forming units (cfu) per IJ was measured as

follows: one ml of IJ suspension (2–3 weeks old storage time) was transferred to a 1.7 ml microcentrifuge tube. Microcentrifuge tubes with IJ suspension were spun down at 13,000 rpm for 6 min in a Sorvall® "Pico" model tabletop microcentrifuge. The IJ pellet was rinsed with 1 % sodium hypochlorite solution to remove any bacteria on the exterior of the IJ. IJs were left in this solution for 2 min at room temperature, and centrifugation (as described above) was repeated one more time. IJs were then rinsed in 1X phosphate-buffered saline (PBS) solution twice. Microcentrifuge tubes were briefly vortexed between sampling 5 ul aliquots to determine IJ concentration because IJs quickly settled out of the solution. The IJ concentration was determined by examining 5 μl droplets under a dissecting microscope. Six droplets were averaged to determine the average IJ concentration per 5 μl. After determining IJ concentration, 200 IJs were transferred to a new 1.7 ml microcentrifuge tube and brought to a 250 μl volume with LB broth. To allow release of bacterial symbionts, the LB-IJ suspension was thoroughly ground with a Kontes® motorized grinder and a sterile pestle for 2 min. For experiments involving *Xenorhabdus nematophila*, the bacterial symbiont of *S. carpocapsae*, 50 μl of the homogenate was transferred to 950 μl LB, resulting in a 1:20 dilution. For those experiments that involved *Photorhabdus luminescens* (the symbiotic bacteria of *H. sonorensis*), 25 μl of the homogenate was added to 975 μl of LB broth, which resulted in a 1:40 dilution. This difference was a direct result of the variation in the number of cfu/IJ since a range of 30–300 colonies on each plate was desired for accurate counting. Once the bacterial dilution was made, 50 μl of the sample was plated onto LB agar plates supplemented with 1 % sodium pyruvate and 30 μg/ml ampicillin salts for *P. luminescens* and 50 μg/ml ampicillin salts for *X. nematophila.* Plates were kept in a 30 °C dark incubator for 24 h. After this period, the total number of cfu on each plate was counted. The following equation was used to determine the number of cfu per IJ (Steve Forst, personal communication):

$$\text{Number of cfu} = \frac{\text{avg.\# of cfus per plate} * \text{dilution factor} * \left[\frac{\text{totalgrindvol}}{\text{vol plated}}\right]}{\text{\# IJ in total grind vol.}}$$

3 Results

3.1 Effect of insect host developmental stage on susceptibility to EPN infection

For both nematode species tested, a significant interaction between insect stage and nematode dose was observed ($F_{1,176}=52.3$; $p<0.005$)(Fig. 1a, b). For both nematode species the highest insect mortality was observed in the non-wandering stages when compared to the wandering larvae. Concurrently, mortality was higher in those larvae exposed to the high nematode dose when compare to the low dose. Insect mortality in the wandering stages was very low for both nematode species and dose tested. Based on these results, subsequent experiments were performed with non-wandering 5th instars.

3.2 Effect of insect host diet on EPN susceptibility

Host diet had a different effect depending on the nematode species considered. For example, for *S. carpocasae*, diet type and nematode dose, as well as their interaction had a significant effect on its virulence ($P<0.005$) (Fig. 2a). For this species, the highest insect mortality was achieved with the combination of high nematode dose and standard diet. Contrarily, larvae fed with low nutrient diet (60 %) and exposed to the low nematode dose were less susceptible to nematode parasitism (Fig. 2a).

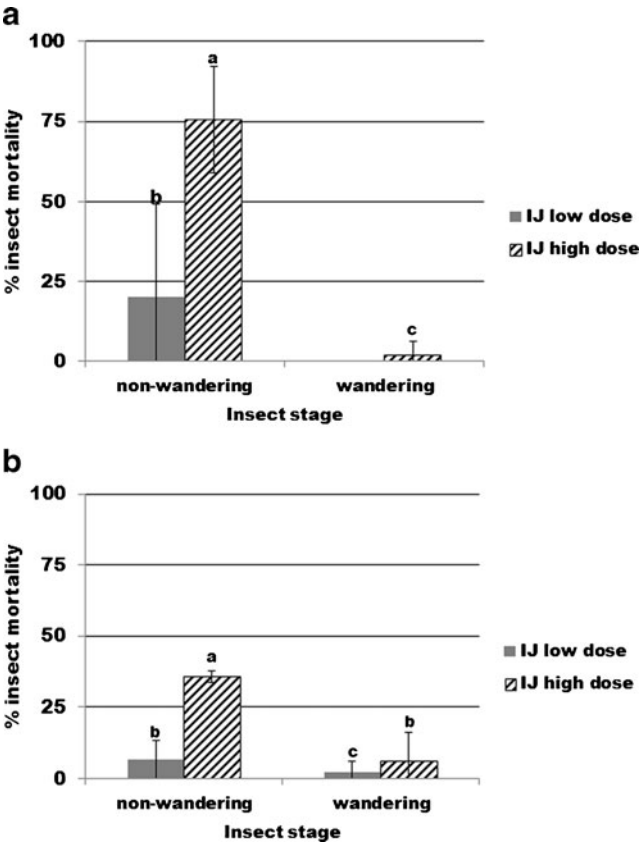

Fig. 1 Mortality of *M. sexta* wandering and non-wandering 5th instars exposed to **a** *Steinernema carpocapsae* and **b** *Heterorhabditis sonorensis.* References: S.c. = *S. carpocapsae*, H.s. = *H. sonorensis*, *LD* = low doses, *HD* = high doses

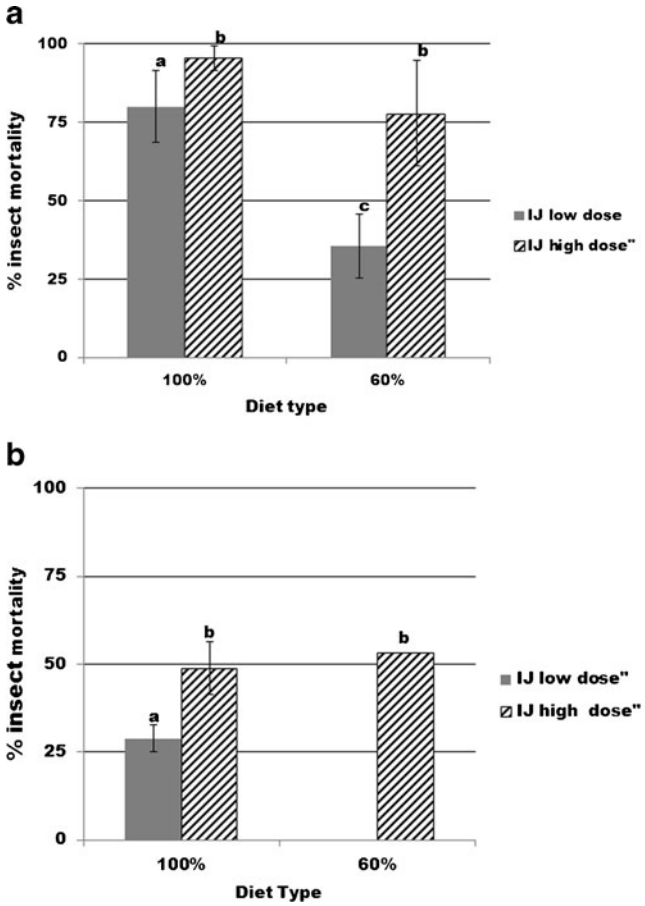

Fig. 2 Percent mortality of *M. sexta* wandering and non-wandering 5th instars reared on standard and reduced quality diets and expose to **a** *Steinernema carpocapsae* and **b** *Heterorhabditis sonorensis.* References: S.c. = *S. carpocapsae*, H.s. = *H. sonorensis*, *LD* = low doses, *HD* = high doses

For *H. sonorensis,* virulence was similar for insects fed with either diet type and exposed to the high nematode inoculum. However, insect mortality decreased to almost 50 % when larvae were fed with the standard diet and exposed to the low nematode inoculum (Fig. 2b). Moreover, insects fed with the reduced nutrient diet and exposed to the low nematode inoculum showed an extremely reduced mortality (overall mortality with all combined replicates for that treatment group was 0.5 %) (Fig. 2b).

3.3 Effect of insect host diet on EPN emergence time

Time to emergence time, the number of days required for progeny to emerge from a cadaver, was calculated from the day an insect larva was exposed to IJs (Day 1) to the first day IJs were observed emerging from the cadaver. For all experiments, time to emergence data was normally distributed. Therefore, no transformation was necessary.

For both nematode species, the number of days required for progeny emergence was not significantly affected by the interaction between nematode dose and diet type ($F_{1,\ 176}$= 42; p=0.5) or by their independent effects (nematode dose, p= 0.82; type of diet, p=0.168) (Fig. 3a, b).

For *S. carpocapsae*, the average time for progeny emergence for insects reared on standard diet ranged from 16 days (high nematode dose) to 17 days (low nematode dose). Emergence of IJs from insects fed with the reduced-nutrient diet ranged from 16 (high nematode dose) to 18 days (low nematode dose).

For *H. sonorensis*, and based on the results obtained in section 3.2, only the "high EPN dose" treatment group was compared between diet types because of the low sample size in the "reduced nutrient diet/low EPN dose" treatment group. For larvae fed on the standard diet, the average number of days it took IJs to emerge was 19.5 days, while it was 21.9 days for those larvae reared on reduced-nutrient diet.

3.4 Effect of insect host diet on nematode progeny production

Progeny production, the total number of IJs that emerged from a cadaver in a given period of time, was calculated for the first

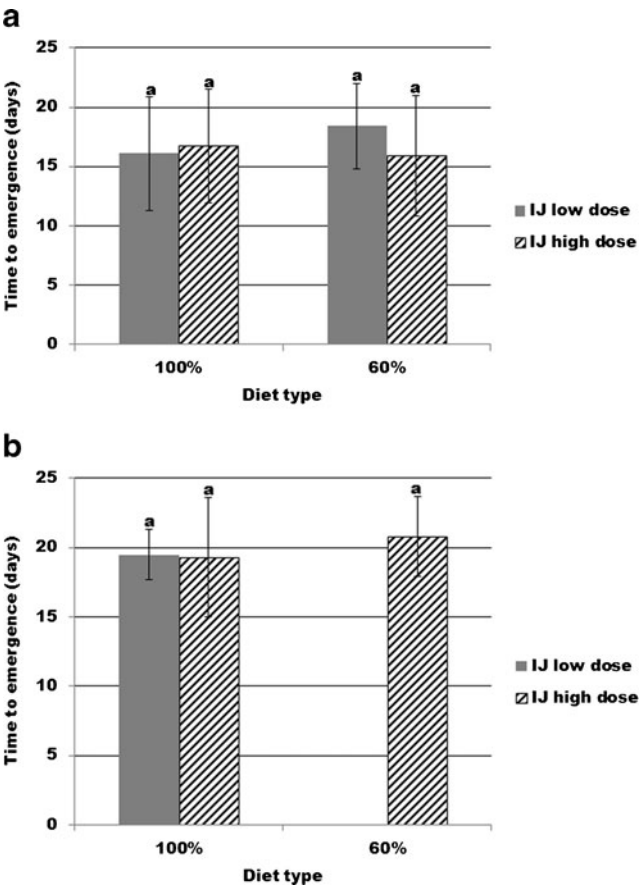

Fig. 3 Time until emergence of **a** *Steinernema carpocapsae* and **b** *Heterorhabditis sonorensis* IJs from *Manduca sexta* reared on standard and reduced quality diets. For *H. sonorensis* only the "high EPN dose" treatment group was compared between diet types

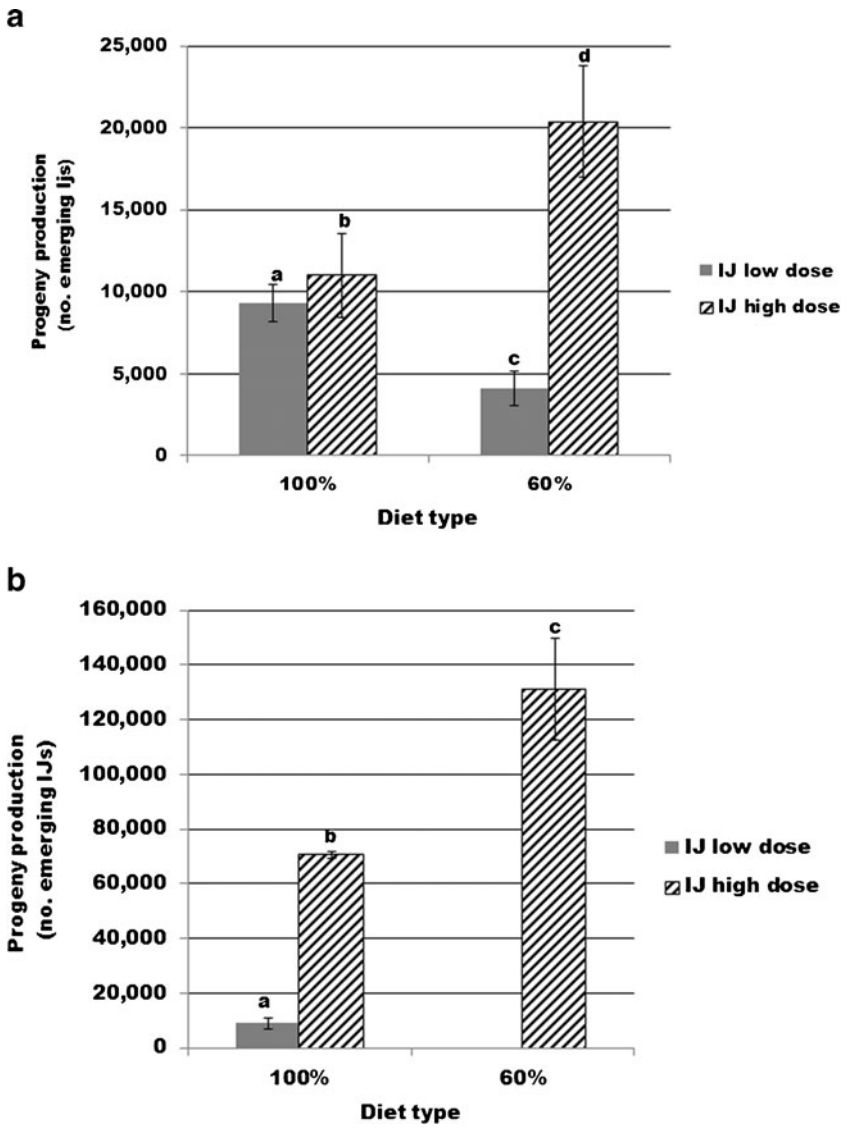

Fig. 4 Progeny production of **a** *S. carpocapsae* and **b** *H. sonorensis* from cadavers of *M. sexta* reared on standard and reduced quality diets. For *H. sonorensis* only the "high EPN dose" treatment group was compared between diet types. References: S.c. = *S. carpocapsae*, H.s. = *H.sonorensis*, *LD* = low doses, *HD* = high doses

10 days post-initial emergence. We found a significant effect of the interaction between nematode dose and type of diet on IJs emergence for both *S. carpocapsae* and *H. sonorensis* ($p<0.005$).

For *S. carpocapsae,* progeny production differed as a function of the host nutritional status (Fig. 4a). For insects fed on the standard diet (100 %), the average (Mean ± SE) number of emerging IJs ranged from 9,272±1,129 (for the low nematode inoculum) to 10,980±2,540 IJ (for the high nematode inoculum). Nematode progeny from insects fed with the low-nutrient diet ranged from 4,065±1,054 (low nematode inoculum) to 20,365±3398 IJ/host (high nematode inoculum).

Similarly, progeny production of *H. sonorensis* differed significantly as a function of insect diet type (Fig. 4b). The average (Mean ± SE) number of emerging IJs ranged from 8,993± 1,958 IJ/larva (standard diet/low nematode dose) to 70,526± 1,112 IJ/larva (standard diet/high nematode dose). Progeny production increased in those larvae fed with the reduced-nutrient diet. The average number of emerging IJs per cadaver was 131,114±18,058 for the high nematode inoculum.

3.5 Effect of insect host diet on the EPNs' bacterial symbionts

The number of bacteria colony forming units per IJ (cfu/IJ) was measured to determine the effect of the insect host diet on the symbionts population and their subsequent reassociation with the nematode IJs. For both nematode species tested, the nutritional status of the host did not significantly affect the number of colony forming units per IJ (cfu/IJ). For the *S. carpocapsae* – *X. nematophila* complex, an average of 75 cfu per IJ was observed in those nematodes that emerged from larvae fed with standard diet. While an average of 76.5 cfu per IJ was denoted from those nematodes that exited larvae fed on the reduced-nutrient ($p=0.841$) (Fig. 5a).

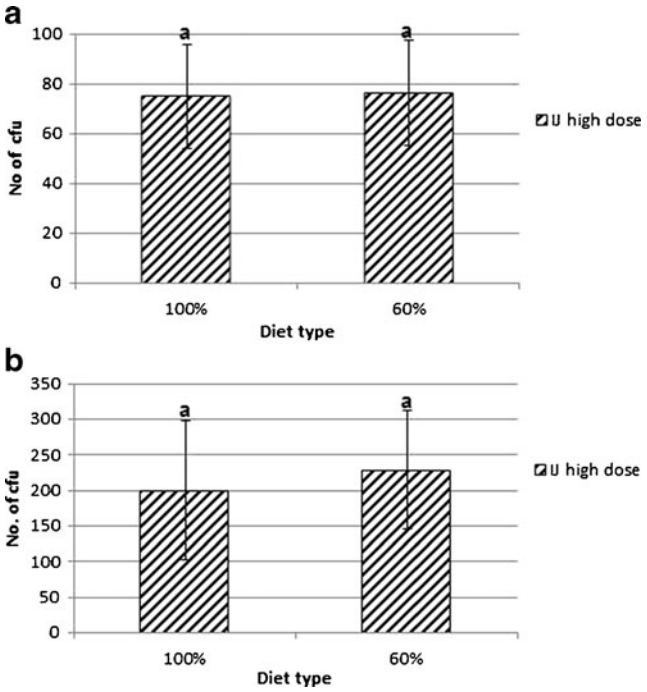

Fig. 5 Symbiont load, measured in colony forming units per infective juvenile (cfu/IJ), for (**a**) *Steinernema* and (**b**) *Heterorhabditis* IJs emerging from *Manduca sexta* larvae reared on different quality diets. For *H. sonorensis* only the "high EPN dose" treatment group was compared between diet types. References: S.c. = *S. carpocapsae*, H.s. = *H. sonorensis*, *HD* = high doses

For the *H. sonorensis* – *P. luminescens* partnership, the average number of *P. luminescens* cfu per IJ was 199.5 for those nematodes that emerged from insects reared on the standard diet, while an average of 228.5 cfu/IJ for IJ was accounted for nematodes that emerged from insects reared on the low nutrient diet (p=0.251) (Fig. 5b).

4 Discussion

Studies have shown that host plants can modulate interactions between insect herbivores and their pathogens (Agrawal 2005; Rasmann et al. 2005; Bezemer 2005; Mayer 2004). Moreover, it has been demonstrated that inter- and intraspecific differences in plant chemistry and structure can alter the susceptibility of insects to infection and to pathogen reproduction and persistence (Duffey 1995; Young et al. 1977; Raymond et al. 2005). In this respect, in this study we explored if the developmental stage and nutritional status of the host insect can affect the fitness (i.e. virulence and reproduction) of entomopathogenic nematodes and their symbiotic bacteria. Our results indicate that the developmental stage of the insect host was a factor that impacted virulence (i.e., assessed as insect mortality) of both nematode species considered in this study. In this respect, Rosa et al. (2000) found that older armyworm (*Pseudaletia unipuncta)* larvae are less susceptible to nematode parasitism. However, in a different study, Bélair et al. (2003) found no such difference in EPN susceptibility when considering other insect hosts such as the cabbage looper (*Trichoplusia ni*), diamondback moth (*Plutella xylostella*) and cabbage white butterflies (*Artogeia rapae*). We speculate that the observed decrease in the ability for *S. carpocapsase* and *H. bacteriophora* to infect older, wandering *M. sexta* may be attributed to age-dependent changes in host immunity. In this respect, there is evidence of variability in immune defense mechanisms used by *M. sexta* throughout its 5th instar stage. For example, Jiang and Wang (2004) showed that wandering 5th instar *M. sexta* have an inducible pattern recognition protein (PRP), β-1,3-Glucan recognition protein-2 (βGRP-2), that is involved in insect immune response. This specific PRP is absent in non-wandering stage, which could explain their increased susceptibility to nematodes relative to the wandering stage.

For this study we chose two entomopathogenic nematode species with different host foraging behavior: *S. carpocapsae* an ambusher and *H. sonorensis*, a cruiser. The rationale for this choice was that the cruiser species would be more efficient in infecting non-wandering larvae when compared to the ambusher species. Alternatively, the ambusher nematodes would be more successful at infecting wandering *M. sexta* larvae than the cruiser type. Observations suggest that nematodes' host search behavior did play a role in host susceptibility, but it was rather a factor of host preference as well as the developmental stage of the host as indicated above.

The other major insect host factor investigated in this study was diet. We speculated that the internal environment of an insect host, as influenced by its diet, may play a key role in the colonization and reproduction of bacterial symbionts, and consequently in nematode maturation and reproduction as well. Our results indicated that diet type and nematode dose, as well as their interaction had a significant effect on *M. sexta* mortality. Moreover, *M. sexta* susceptibility to nematode parasitism varied depending on the nematode species considered. For *H. sonorensis,* virulence was similar for insects fed with either diet type and exposed to the high nematode inoculum. Contrarily, larvae fed with the standard diet experience a reduced mortality when exposed to the low nematode inoculum. Moreover, insects fed with the reduced nutrient diet and exposed to the low nematode inoculum showed very low mortality

For *S. carpocapsae*, the highest insect mortality was observed for the combination of the standard diet and high nematode dose. Insect diet did not have an effect the time of emergence for nematode progeny, but it had an effect on progeny production. For *S. carpocapsae,* progeny production differed as a function of the host nutritional status. In particular, nematode progeny from insects fed with the low-nutrient diet varied significantly in relation to nematode inoculum.

The type of diet did not have an effect on symbiont reproduction and its consequent reassociation with the nematode IJs (expressed as cfu. load/IJ). *Steinernema carpocapsae* nematodes confine the bacterial symbionts in a specialized structure of the nematode's anterior intestine; usually refer to as the bacterial receptacle (Flores-Lara et al. 2007). Previous studies have shown that a fully colonized receptacle in *S. carpocapsae* IJ harbors about 220 cfu/IJ (Flores-Lara et al. 2007). In this study, an average of 75 cfu/IJ (for nematodes that emerged from insect fed with either diet) was accounted. This number is less than the one previously reported, but the observed differences may be related to the consideration of a different insect host.

The bacterial symbionts in *Heterorhabditis* IJ are contained along most of the intestinal lumen. On average 151 to 200 bacterial cells are present in the IJ intestinal lumen (Ciche and Ensign 2003). Our results showed that bacterial load for *Heterorhabditis* IJs was similar to previously reported data.

These results indicate that the developmental stage of the insect host may play an important role in the establishment and proliferation of nematodes' bacterial symbionts.

We speculate that *M. sexta* when presented with a low-nutrient diet, consume fewer lipids and allocate more resources toward storage rather than growth and development. This is true for other organisms (Slansky and Angelo 1984). In this respect, the potentially higher lipid content in *M. sexta* reared on the reduced-nutrient diet could explain why for both nematode species tested in this study, IJ production was higher in particular from insects exposed to the high nematode inoculum.

A few studies have investigated fitness of entomopathogenic nematodes relative to insect diet quality (Barbercheck et al. 1995; Shapiro-Ilan et al. 2008). From these studies, it is clear that lipid availability in insect hosts may play a key role for successful growth and development of certain nematode species. However, none of the aforementioned studies addressed the impact of insect host nutrition on nematodes' symbiotic bacteria. Since these bacteria are essential for the persistence of the nematodes, it is vital that they are incorporated in studies evaluating their virulence and reproductive fitness.

Finally we propose the entomopathogenic nematode-bacterium-insect interaction a model system for understanding trophic complexities and interactions of mutualists in food webs.

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