

# Host resistance reverses the outcome of competition between microparasites

DANIEL S. GRUNER,<sup>1,2,6</sup> ARUNIMA KOLEKAR,<sup>3</sup> JOHN P. McLAUGHLIN,<sup>4</sup> AND DONALD R. STRONG<sup>1,5</sup>

<sup>1</sup>Bodega Marine Laboratory, University of California–Davis, Bodega Bay, California 94923 USA

<sup>2</sup>Department of Entomology, University of Maryland, 4112 Plant Sciences Building, College Park, Maryland 20742 USA

<sup>3</sup>Department of Neurology, University of California, Los Angeles, California 90095 USA

<sup>4</sup>Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, California 93106 USA

<sup>5</sup>Section of Evolution and Ecology, University of California, Davis, California 95616 USA

**Abstract.** Predators and parasites can control the abundance or biomass of herbivores with indirect effects on producer communities and ecosystems, but the interplay of multiple natural enemies may yield unexpected dynamics. We experimentally examined interactions between two microparasites (entomopathogenic nematodes) isolated from sandy grassland soils of coastal California: *Heterorhabditis marelatus* (Heterorhabditidae) and *Steinernema feltiae* (Steinernematidae). *Heterorhabditis marelatus* drives trophic cascades by attacking root- and stem-boring ghost moth caterpillars (*Hepialus californicus*, Hepialidae), thereby indirectly protecting bush lupine shrubs (*Lupinus arboreus*, Fabaceae). Extensive field surveys demonstrated sympatric overlap in microhabitat use under lupine canopies and similar mean prevalence of the two nematode species. Using a response-surface design in the laboratory, we varied relative and absolute microparasite densities to test for competitive outcomes within an evolutionary naïve host, larvae of the greater wax moth *Galleria mellonella* (Pyrilidae), and within the native host *Hepialus californicus*. Independent of conspecific or interspecific density, *S. feltiae* dominated as expected over *H. marelatus* within the naïve *Galleria*, but *S. feltiae* infected hosts at low frequency and showed lower reproductive fitness than *H. marelatus* within native *Hepialus* hosts. Contrary to studies that demonstrate the pairwise dominance of steinernematid over heterorhabditid species in laboratory hosts, host resistance to *S. feltiae* may provide a mechanism for coexistence of multiple microparasite species. We hypothesize that the ubiquitous field prevalence and rapid life history of *S. feltiae* imply its use of widespread, abundant but small-bodied hosts and indicate the lack of direct competition with *H. marelatus* in the *Hepialus*–*Lupinus* trophic cascade.

**Key words:** Bodega Marine Laboratory, California, USA; entomopathogenic nematodes; *Galleria mellonella*; *Hepialus californicus*; *Heterorhabditis* and *Steinernema*; intraspecific and interspecific competition; predator–prey interactions; trophic cascade.

## INTRODUCTION

Predators, parasites, and pathogens play important roles in ecosystems by directly controlling the abundance or biomass of herbivores, and can indirectly affect ecosystem functions such as primary productivity and elemental cycling through trophic cascades (Paine 1980, Schmitz 2006). These processes are clear in simplified food-chain models with a single species at each trophic level, but are less obvious in complex food webs characteristic of many natural and managed systems (Polis and Strong 1996, Gruner 2004). An emerging body of empirical evidence suggests that increasing predator species diversity can yield widely divergent effects on herbivore suppression (Sih et al. 1998, Schmitz 2007). Two or more predator species can act additively if effects are independent (e.g., Straub and Snyder 2006), or else

can interact synergistically to enhance prey suppression (e.g., Losey and Denno 1998) or antagonistically to dampen effects on herbivores if predators consume each other (intraguild predation) or compete and interfere for capture success (e.g., Finke and Denno 2003).

Multispecies interactions of parasites in food webs can be intense (Lafferty et al. 2008), and may be particularly fierce within the spatially constrained microenvironment of an individual host (Anderson and May 1981, Dobson 1985, Hochberg and Holt 1990). A combination of consumptive and interference competition, via resource depletion and intraguild predation, can decide the effects of these interactions on population dynamics of hosts and natural enemies (Borer et al. 2007). A suite of physical, behavioral, biochemical, and immunological host resistance mechanisms, imposed prior to, during, or post-infection, can mediate these interactions between multiple parasites (Cox 2001). Final outcomes depend on the relative transmission abilities and pathogenicities of infective agents, the degree of interference within host

Manuscript received 27 August 2008; revised 5 March 2009; accepted 16 March 2009. Corresponding Editor: W. E. Snyder.

<sup>6</sup> E-mail: dsgruner@umd.edu

individuals, and the resistance mechanisms of hosts (Hochberg and Holt 1990, Cox 2001).

Entomopathogenic nematodes (EPN) in the families Steinernematidae and Heterorhabditidae are pervasive natural enemies of insects in soils worldwide (Hominick et al. 1996). The pathogenicity of EPN to a wide range of arthropod orders stems from their symbiotic relationships with bacterial partners that, when transmitted by their nematode vectors, produce toxins that rapidly kill insect hosts (Ciche et al. 2006). We adopt the general terminology of "microparasite," which adheres to life-history and population-dynamical definitions of Anderson and May (1981). However, EPN variously share characteristics of pathogens, in that they undergo reproduction and multiple generations within a single host, and parasitoids, by requiring the death of an individual host to complete their life cycle (Lafferty and Kuris 2002). EPN can instigate trophic cascades by suppressing soil-dwelling, herbivorous insects in both natural systems (Preisser 2003) and in agroecosystems managed with augmentative releases (Denno et al. 2008). However, a range of antagonistic factors, such as chemical antibiosis, predation, and interspecific competition can impede the capacity of EPN to effect host-population control (Kaya and Koppenhöfer 1996).

Given the growing recognition of the ubiquity and trophic importance of these insect microparasites, there are few studies that document how coexisting EPN species partition prey resources and interact in soil ecosystems. Laboratory studies of interspecific competition between multiple EPN species within hosts document two general results: (1) competitive superiority of *Steinernema* over *Heterorhabditis* species, and (2) ultimate survival to reproduction of only one species per host individual (Kaya and Koppenhöfer 1996). However, these studies typically use cultured host insects from laboratory stocks, such as larvae of the greater wax moth *Galleria mellonella* (Lepidoptera; hereafter, *Galleria*), which are naïve to soil-borne EPN over their evolutionary history. Edaphic insects that have evolved in the presence of EPN and other pathogenic soil factors typically display structural, chemical, immunological, or behavioral resistance traits and are less susceptible to infection by EPN (Grewal et al. 2002). Additional studies need to address the contextual importance of antagonistic interactions of endemic EPN populations within host organisms that structure community and ecosystem processes. In this study, we contrast the outcome of competition between *Steinernema feltiae* and *Heterorhabditis marelatus* in two lepidopteran hosts: a naïve laboratory host (*Galleria*) and a root-boring moth caterpillar that naturally co-occurs with these EPN taxa in native habitats. We first describe surveys that demonstrate the sympatric extent of these two EPN species and detail the ecological context. Then, we report a series of single-species density and two-species response-surface experiments that document distinct competitive dynamics mediated by resistance to EPN infection by native host insects.

## METHODS

### *Study system and surveys*

We evaluated competitive hypotheses using co-occurring entomopathogenic nematodes at the University of California laboratory on the Bodega Marine Reserve (BMR), a 147-ha parcel comprised of coastal dunes and mixed annual grass and shrublands (38°19'12" N, 123°4'11" W; Barbour et al. 1973). Two species of entomopathogenic nematodes have been isolated from the soils on the BMR, one each from the families Heterorhabditidae and Steinernematidae (genetic confirmation of species by S. P. Stock using 28S ribosomal DNA; Stock et al. 2001). *Heterorhabditis marelatus* Liu & Berry is a species native to the Pacific coast of California and Oregon (USA), and *Steinernema feltiae* (Filipjev) is a cosmopolitan species often found in grassland habitats (Stock et al. 1999).

More than a decade of research has demonstrated that larval *Hepialus californicus* (Lepidoptera: Hepialidae; hereafter, "*Hepialus*"), which bore into the stems and roots of the perennial woody shrub *Lupinus arboreus* (Fabaceae), are ecologically important hosts for *H. marelatus* (Ram et al. 2008a). Independent field experiments determined that this nematode indirectly shapes landscape and ecosystem properties in coastal prairies through a top-down trophic cascade (Strong et al. 1999, Preisser 2003). However, the ecological role of *S. feltiae* in this system remains poorly understood. Like many steinernematids, *S. feltiae* can infect and reproduce within a broad phylogenetic range of host insects in the laboratory (Peters 1996). Although the two species of entomopathogenic nematodes (EPNs) coexist in sympatry in the California coastal prairie (Gruner et al. 2007), it is unclear if they compete for hosts and if multispecies interactions alter the strength of the *H. marelatus*–*Hepialus*–*Lupinus arboreus* trophic cascade.

We measured the relative incidence of the two species in targeted surveys from six sites on the reserve established for long-term monitoring for *H. marelatus* (Ram et al. 2008b). At each sample point in April 2005 we deployed four centrifuge tubes drilled with minute bottom holes, filled with 6-cc saturated soil, and stocked with two larvae of the greater wax moth *Galleria mellonella* (Lepidoptera: Pyralidae; hereafter, "*Galleria*") (see Gruner et al. [2007] for description of sampling apparatus). These sentinel traps were sunk into the soil in a 10-cm radius around focal *Lupinus arboreus* stems, incubated in the field for seven days, and scored after three additional days of incubation in the laboratory. These traps are sensitive to the presence of both species, each of which actively pursue hosts within the surface soil horizons to at least 20 cm deep (Gruner et al. 2007). Results were scored as infected by *H. marelatus*, infected by *S. feltiae*, dead by undetermined causes, or alive. Distinctive visual symptoms of host-insect cadavers characteristic of the bacterial symbionts of *H. marelatus* (red, turgid) and *S. feltiae* (brown-ochre to copper,

flaccid) allowed nematode identification without dissection. We verified these identifications using life-history traits (duration to first emergence, reproductive output) and morphometrics and behavior of emergent infective juveniles. Incidence for each EPN species was measured as the proportion of 25 sample points with at least one of eight *Galleria* hosts scored positive for infection.

In the laboratory, we tested the importance of antagonistic interactions between the two nematode species within two lepidopteran hosts. One host species, *Galleria*, is the predominant model species used for bioassays and laboratory experiments on entomopathogenic nematodes. *Hepialus*, by contrast, is native to the California coastal prairie, where it plays a prominent role in the population dynamics of *Lupinus arboreus* and indirectly alters plant community structure, biotic invasions, and ecosystem processes (Maron and Jefferies 1999, Strong et al. 1999, Maron 2001). *Galleria* individuals were obtained from commercial suppliers; *Hepialus* larvae were harvested directly from lupine stems on the BMR in July–September of 2005–2007 inclusive, and maintained on slices of organic carrot in the laboratory prior to experiments (Whipple 1998). Both EPN species were isolated from wild populations and cultured through one to two generations in *Galleria* prior to experimentation.

#### *Intraspecific-density experiment*

The first experiment was designed to understand how host infection varied as a function of intraspecific density for each of the two EPN species. We created numerical isolates of 10, 100, and 1000 infective juveniles (IJs) reared from *Galleria* larvae within three weeks of rearing. Isolates were assembled by direct count and transferred by micropipette to 1 mL distilled water in individual 1.5-mL microcentrifuge tubes. Treatment-density aliquots were transferred to 90-mm petri dishes containing pasteurized, coarse-sifted field soil moistened to 10% water content by mass and containing one larva of either *Galleria* or *Hepialus*. Host larvae were weighed individually, assigned to treatments in a random stratified design—thus eliminating host-size bias as a confounding factor with treatment levels—and stocked one day prior to IJ inoculation. Hosts were checked every two days over a period of three weeks for symptoms of infection by *H. marelatus* or *S. feltiae* (as above). The intraspecific density experiment was completed in four trials, each with five complete replicates of the host species  $\times$  IJ species  $\times$  IJ density factorial combination ( $2 \times 2 \times 3$ ), yielding 20 independent replicates per treatment for a total of 240 experimental units.

#### *Interspecific-competition experiments: density response surface*

Two-species competition experiments were run in three trials over three summers. The first trial in 2005 used a replacement-series design (Stetina et al. 1997, Jolliffe 2000) in which we held constant the total density

of nematodes while the relative frequencies of the two species varied. The total number of IJs was fixed at 100 individuals, and five treatment levels varied the relative proportions of the two species (*H. marelatus* to *S. feltiae* ratio: 100:0, 75:25, 50:50, 25:75, 0:100). In all interspecific-competition experiments, we estimated aliquot IJ densities using serial dilutions from aqueous cultures that were repeatable within  $\pm 5\%$  SD (Woodring and Kaya 1998, Gruner et al. 2007). Host arenas were prepared as above in the intraspecific-density experiments, and checked regularly for three weeks post-treatment. Each of the five treatment levels was replicated 30 times for both *Galleria* and *Hepialus* larval hosts, for a total of 300 experimental units.

Because the success of entomopathogenic nematodes (EPN) may also depend on total density (see *Methods: Intraspecific-density experiment*; Selvan et al. 1993), a replacement-series design may inadequately test the range of competitive interactions that can occur between species (Connolly 1988, Inouye 2001). Therefore, we augmented the first trial with an additive design that held the density of one species constant while varying the density of both the second species and the resulting total density. In five treatment levels in 2006, using the same relative proportions in the replacement series, we fixed the density of one species at 100 IJs while varying the density of the second species (*H. marelatus* to *S. feltiae* ratio: 100:0, 100:100, 100:300, 300:100, 0:100). The resulting full design therefore represented a response surface where both total density and the proportions of EPN species varied (Connolly et al. 2001, Inouye 2001). Because the average mass of *Hepialus* larvae differed between the first two trials (a consequence of harvest from field populations differing in phenology), in 2007 we repeated the replacement-series and additive designs in a comprehensive response-surface trial with 20 replicates for each host species in eight EPN treatment levels inclusive (320 units in 2007 trial; 920 in 3-yr total). In 2006, we also monitored the reproductive fitness of all caterpillars infected by EPN by counting the total production of infective juveniles isolated in white traps from each infection (Woodring and Kaya 1998), standardized by the body mass (in milligrams) of host larvae.

#### *Data analyses*

All analyses were run in the R package (R Development Core Team 2008). We initially compared mean incidence of the two EPN species across six survey sites using a Welch two-sample *t* test. However, low site replication limited the power of this test, so we also used generalized linear models with the logit link function on all 25 binomial responses at each site, with site and EPN species as fixed factors. Model fit was generated by iteratively reweighted least squares, using “type II” sums of squares implemented in the “car” package (each factor tested as last term to enter model, while excluding the site  $\times$  species interaction when testing an included

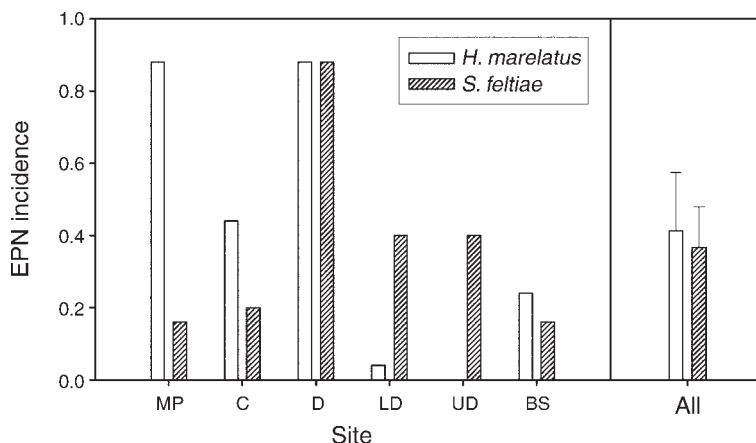


FIG. 1. Incidence of entomopathogenic nematodes (EPN) species *Steinernema feltiae* and *Heterorhabditis marelatus* at six sites on the Bodega Marine Reserve (California, USA) during April 2005, and the average incidence from all sites (mean + SE). Incidence was measured as the proportion of 25 sample points with *Galleria mellonella* hosts that were positive for EPN infection. Site abbreviations are as follows: MP = Mussel Point; C = Cove; D = Dune; LD = Lower Draw; UD = Upper Draw; BS = Bay Shore.

main effect). We began with models containing both factors and the interaction term, but proceeded with model simplification until all remaining effects were statistically significant at the  $\alpha = 0.05$  level and the Akaike information criterion was minimized. Main effects were retained when interactions were significant.

For the EPN addition experiments, we tested for systematic variation in host size as a function of discrete trial number or year (blocking term), host species, EPN species, and density or treatment level of EPN species using Gaussian GLM. For the intraspecific-density experiment, incidence of single-species infection by *H. marelatus* and *S. feltiae* data sets were analyzed in separate generalized linear models with the logit link function using the same independent terms as above—except EPN species—and all two-way interactions. We pursued a similar analytical strategy with the interspecific-competition experiments, with one difference: the binomial responses (infection failures and successes) for each EPN species were analyzed using the subset of data in which that species was included (i.e., analyses of *S. feltiae* excluded the control with 100 *H. marelatus* and zero *S. feltiae*).

#### RESULTS

Surveys across the reserve using in situ bait samples demonstrated a high incidence of *Steinernema feltiae*, relative to data observed in past soil collection samples (D. R. Strong, unpublished data). On average, the incidence of *S. feltiae* across sites was not different from *Heterorhabditis marelatus* (Welch two-sample  $t = 0.2378$ ,  $df = 8.941$ ,  $P = 0.8174$ ; Fig. 1). However, the relative importance of sites to each species varied greatly as evidenced by the highly significant interaction of site and entomopathogenic nematode (EPN) species in generalized linear models (site:  $F_{5,288} = 16.78$ ,  $P < 0.0001$ ; species:  $F_{1,288} = 0.965$ ,  $P = 0.327$ ; site  $\times$  species:  $F_{5,288} = 12.33$ ,  $P < 0.0001$ ).

In single-species EPN density manipulations, host size differed by host species ( $F_{1,235} = 26.77$ ,  $P < 0.0001$ ) and varied with the blocking factor for independent trials ( $F_{3,235} = 9.42$ ,  $P < 0.0001$ ). On average, *Galleria* larvae used in these trials ( $0.22 \pm 0.006$  g [mean  $\pm$  SE]) were larger than *Hepialus* larvae overall ( $0.18 \pm 0.008$  g), and the mean size of both hosts generally declined with each subsequent trial. However, host size was unimportant in models for infection frequency of EPNs in hosts. Host species ( $F_{1,116} = 13.227$ ,  $P = 0.0004$ ) and EPN density ( $F_{2,116} = 21.10$ ,  $P < 0.0001$ ) best predicted *H. marelatus* incidence. *H. marelatus* infection success increased with inoculated density in both hosts but overall incidence was higher in *Galleria* than in *Hepialus*, irrespective of density (Fig. 2). By contrast, density dependence was not detected for *S. feltiae* within either host species ( $F_{2,116} = 0.754$ ,  $P = 0.473$ ), but infection success was markedly higher in *Galleria* (0.90) than in *Hepialus* (0.06; Fig. 2),

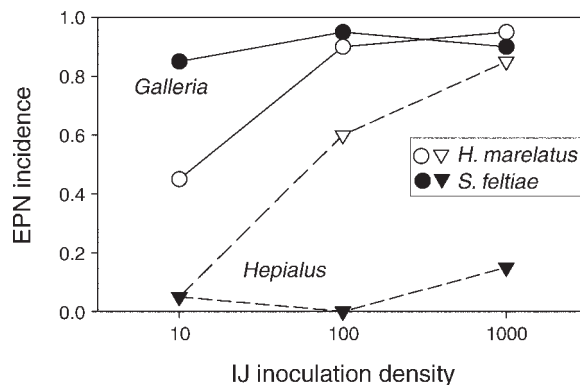


FIG. 2. Density-dependent infection of larvae of the greater wax moth *Galleria mellonella* (circles) and a root-boring caterpillar moth *Hepialus californicus* (triangles) in single-species inoculations of two EPN species, *Steinernema feltiae* (filled symbols) and *Heterorhabditis marelatus* (open symbols), at densities of 10, 100, and 1000 infective juveniles (IJs) per larva.

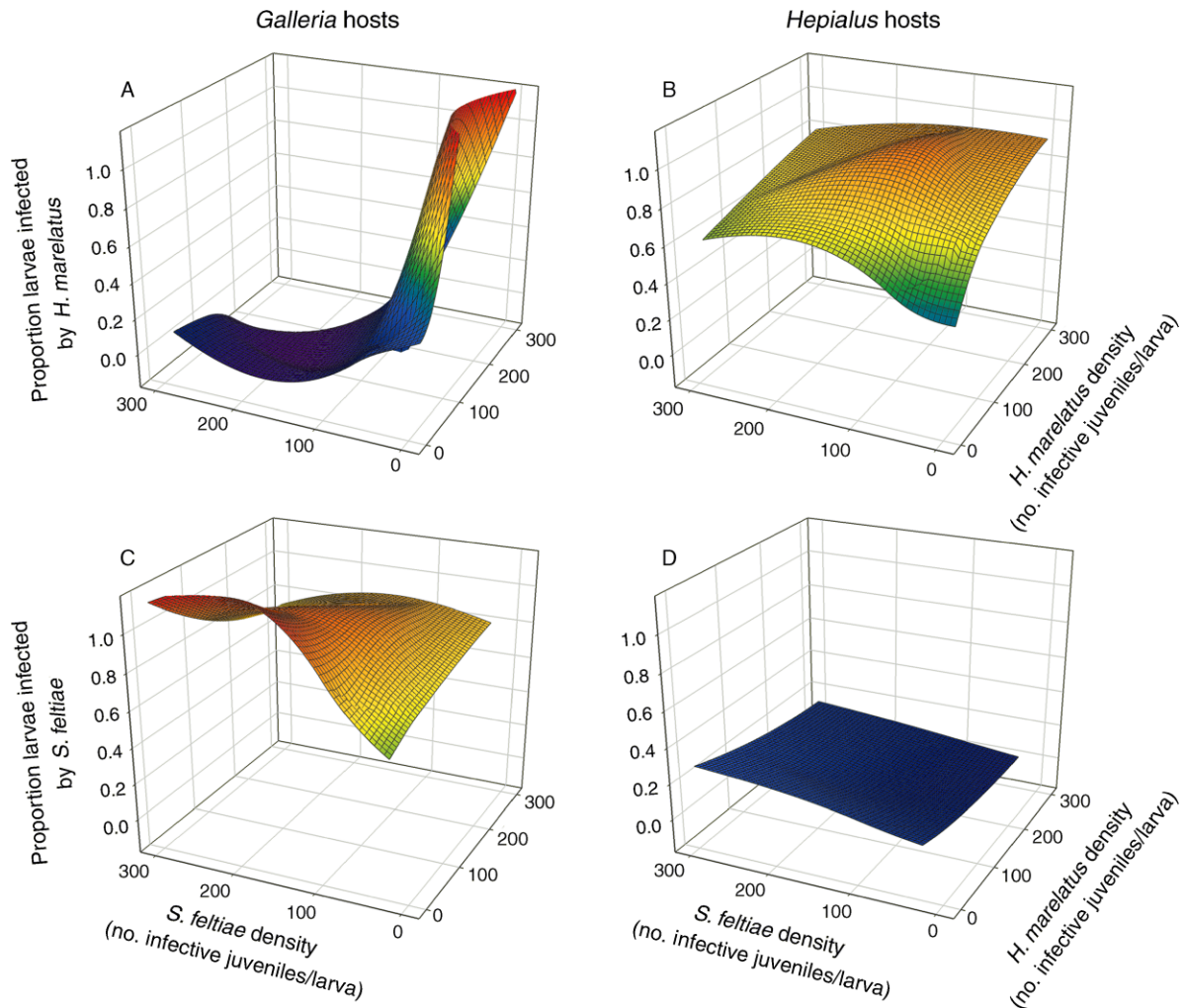


FIG. 3. Proportion of *Galleria mellonella* and *Hepialus californicus* hosts infected by two entomopathogenic nematodes (EPN), *Heterorhabditis marelatus* and *Steinernema feltiae*, at varying interspecific densities. Top panels (A and B) show the incidence of *H. marelatus*, and the bottom panels (C and D) show the incidence of *S. feltiae*; the left panels (A and C) show results from *Galleria* hosts, and the right panels (B and D) show results from *Hepialus* hosts. Wireframes interpolate among plotted points representing the total proportion of samples testing positive for each nematode species within the hosts *Galleria* and *Hepialus* but omit single-species treatments where the focal EPN species was absent.

and host species was the only term in the final model for *S. feltiae* ( $F_{1,118} = 96.19$ ,  $P < 0.0001$ ).

We developed the total competition response surface over three field seasons (see *Methods: Interspecific competition experiments: density response surface*, above), and each year the mean size of *Hepialus* larvae differed (mean  $\pm$  SE: 2005,  $0.197 \pm 0.008$  g; 2006,  $0.363$  g  $\pm$  0.012 g; 2007,  $0.125 \pm 0.005$  g). We stratified the assignment of larvae to treatments according to larval mass in such a way that there were no size differences within larval species between treatments within years. Therefore, caterpillar mass did not vary among EPN-addition treatments for either *Galleria* ( $0.226 \pm 0.007$  g; treatment,  $F_{7,450} = 1.45$ ,  $P = 0.184$ ; year,  $F_{2,450} = 31.36$ ,  $P < 0.0001$ ) or *Hepialus* ( $0.221 \pm 0.003$  g; treatment,  $F_{7,450} = 0.184$ ,  $P = 0.999$ ; year,  $F_{2,450} = 153.77$ ,  $P <$

$0.0001$ ). Because we also found that caterpillar mass explained no additional variation alongside trial year, we dropped mass from subsequent interspecific models.

Infection success of both EPN species varied across years (year: *H. marelatus*,  $F_{2,744} = 8.05$ ,  $P = 0.0003$ ; *S. feltiae*,  $F_{2,750} = 6.61$ ,  $P = 0.0014$ ), but these effects were small compared to the influence of host species (host: *H. marelatus*,  $F_{1,744} = 205.92$ ,  $P < 0.0001$ ; *S. feltiae*,  $F_{1,750} = 389.87$ ,  $P < 0.0001$ ). Infection by *H. marelatus* was strongly influenced by the presence of *S. feltiae* (treatment:  $F_{6,744} = 27.16$ ,  $P < 0.0001$ ), but this effect was visible primarily within *Galleria* and not within *Hepialus* hosts (Fig. 3, A vs. B; host  $\times$  treatment:  $F_{6,744} = 31.12$ ,  $P < 0.0001$ ). By contrast, EPN treatment ratios and the presence of *H. marelatus* only weakly affected infection by *S. feltiae* (treatment:  $F_{6,750} = 2.53$ ,  $P =$

0.0195), did not interact significantly with host species, and was eliminated from the final model (host  $\times$  treatment:  $F_{6,746} = 1.45$ ,  $P = 0.193$ ). Infection by *S. feltiae* in *Hepialus* was almost uniformly low irrespective of conspecific or heterospecific densities (Fig. 3D).

The reproductive fitness of *H. marelatus*, measured as the total production of emerging infective juveniles (IJ) per infected host, corrected for the total biomass (in milligrams) of that host, was not different in *Galleria* vs. *Hepialus* larvae (Welch two-sample  $t = 0.27$ ,  $df = 42.73$ ,  $P = 0.786$ ; grand mean  $\pm$  SE =  $622.3 \pm 25.82$  IJ/mg,  $n = 116$  larvae). However, *S. feltiae* mean reproductive fitness was 50% lower from infected *Hepialus* larvae than from *Galleria* (Welch two-sample  $t = -3.46$ ,  $df = 18.27$ ,  $P = 0.0027$ ; *Hepialus* mean  $\pm$  SE =  $189.36 \pm 45.75$  IJ/mg,  $n = 15$  larvae; *Galleria* mean  $\pm$  SE =  $358.83 \pm 17.36$  IJ/mg,  $n = 113$  larvae).

#### DISCUSSION

Entomopathogenic nematodes (EPNs) are common and widespread belowground microparasites that are thought to be polyphagous and intensely competitive (Kaya and Gaugler 1993, Kaya and Koppenhöfer 1996). Given an evolutionarily naïve host (*Galleria mellonella*) in which both EPN species successfully reproduce, *Steinernema feltiae* successfully and predictably excludes *Heterorhabditis marelatus*. We provide evidence, however, that sympatric nematode species segregate by host use, thereby reducing the likelihood of interference interactions that would dampen the strength of the *Heterorhabditis*–*Hepialus*–*Lupinus* trophic cascade. As demonstrated experimentally, *H. marelatus* kills *Hepialus* larvae in the roots of a dominant, nitrogen-fixing shrub, *Lupinus arboreus* (Strong et al. 1999, Preisser 2003) and indirectly alters plant community structure, community invasibility, and ecosystem function (Maron and Connors 1996, Maron and Jefferies 1999, Maron 2001). Resistance of *Hepialus* larvae to *S. feltiae* provides a basis for multi-species assemblages of entomopathogenic nematodes to persist in nature, and reconciles the results of field surveys (Fig. 1) with predictions arising from laboratory studies that suggest the inevitability of competitive interference.

Our results with the host *Galleria*, in accordance with predictions from the literature, showed competitive dominance of *Steinernema feltiae* over *Heterorhabditis marelatus* irrespective of relative density levels (Fig. 3). Numerous studies of EPN multispecies interactions have spurred the general conclusion that steinernematid nematodes are superior competitors to heterorhabditids (Kaya and Koppenhöfer 1996). In an example contesting *S. feltiae* and *H. bacteriophora* in three laboratory hosts (*Galleria*, *Tenebrio molitor*, and *Tribolium confusum*), the steinernematid outcompeted the heterorhabditid in every case, although both infected 100% of *Galleria* in the absence of the other species (Kreft and Skrzypek 2002). Notably, *S. feltiae* infections were rapid: infection levels reached 20% of maximum in 24 hours, whereas *H.*

*bacteriophora* infections required 48–72 hours (Kreft and Skrzypek 2002).

Beyond the rapidity of their life cycle, another proposed mechanism for the competitive superiority of steinernematids is that their symbiotic bacteria (Enterobacteriaceae: *Xenorhabdus* spp.) produce chemical bacteriocins that inhibit heterorhabditid symbionts (Enterobacteriaceae: *Photorhabdus* spp.) from multiplying (Kaya and Koppenhöfer 1996). Bacterial symbionts are central mediators of interspecific competitive performance: outcomes in *Galleria* between *Steinernema carpocapsae* and *S. scapterisci* reversed depending on whether nematodes contained their *Xenorhabdus* spp. symbionts (Sicard et al. 2006). As expected from evolutionary theory, this mutualism involves a cost-benefit trade-off whereby free-living infective juveniles (IJs) survive longer in the environment when deprived of their bacteria (aposymbiotic) but parasitic success in insect hosts improves markedly with the bacteria (Emelianoff et al. 2008). Bacterial symbionts provide enhanced access to insect food sources, modify competitive interactions, and shape host range evolution (Sicard et al. 2005).

For ecological understanding of species coexistence, however, knowledge of interactions with wild host species that support parasite populations is a critical component of inference. The steinernematid (*S. feltiae*) failed to utilize the native host *Hepialus* regardless of intraspecific density (range: 10–1000 IJ; Fig. 2) or the relative density of *H. marelatus* (Fig. 3). Within *Galleria*, *S. feltiae* also showed density independence, demonstrated in this case by its nearly complete utilization of hosts at all densities (Fig. 2). Intraspecific density dependence within a host can lead to reduced per capita host penetration, reduced production and mean size of IJs, and even reproductive failure (Selvan et al. 1993). In the current case, *H. marelatus* infection rate successively improved as inoculation densities increased from 10 to 1000 IJs; this may be evidence that additional numbers “pile on” to overcome host resistance mechanisms (Lewis et al. 2006). The lack of intra- or interspecific density dependence in *S. feltiae*, by contrast, demonstrates the central importance of categorical host resistance in these interactions.

Potential insect hosts may employ active- and passive-resistance mechanisms that impair successful penetration, infection, or development of EPN. *Steinernema feltiae*, as with all steinernematids, lacks the large, anterior, terminal tooth that heterorhabditids can use to abrade and puncture the cuticle of host insects (Bedding and Molyneux 1982) and therefore may rely on other routes of ingress into thickly cuticled insects (Poinar 1990). Hosts can reduce their vulnerability to IJ attachment through evasion behavior or by grooming (Gaugler et al. 1994), through physical attributes that deter penetration (Eidt and Thurston 1995), or through immunological responses such as encapsulation and melanization (Li et al. 2007). Following penetration and

bacterial infection, nematode infections may still fail via interactions with enteric bacteria (Blackburn et al. 2007) or with the chemical constituents of host gut contents sequestered directly from plants or from associated endophytic fungi (Barbercheck 1993, Kunkel et al. 2004, Richmond et al. 2004). The fact that few *Hepialus* larvae were infected or killed by *S. feltiae* under any single- or two-species density treatment, combined with our finding that *S. feltiae* reproductive fitness was half that of infected *Galleria*, supports the conclusion that multiple mechanisms cause poor performance of *S. feltiae*. While host choice behavior may limit host range of EPN species (Lewis et al. 2006), these data show that pre-infection resistance and developmental suppression mechanisms contribute to host range limitation.

Evidence from this study does not support the hypothesis that *S. feltiae* competes with *H. marelatus* for *Hepialus*, a large-bodied but seasonally and spatially patchy herbivore of *Lupinus arboreus* in California (USA) coastal prairie (Strong et al. 1995). Although we found comparable incidence of *S. feltiae* and *H. marelatus* from 150 *Lupinus arboreus* rhizospheres across six field sites (Fig. 1), the ecological role and natural hosts of *S. feltiae* remain unknown. The literature demonstrates a wide potential host range for *S. feltiae* across at least five insect orders (Georgis and Hague 1988, Gouge and Hague 1995, Peters 1996, Yu et al. 2006). Evolutionary theory predicts that *S. feltiae*, with its rapid exploitative life history, should compete favorably for small, relatively abundant, and well-distributed hosts (e.g., dipteran larvae) abundant in grassland soils (Crossan et al. 2007). Our preliminary studies have demonstrated infective success of *S. feltiae*, but not *H. marelatus*, in small larvae of *Tesarius* scarab beetles (~5 mg) and therevid flies (~20 mg) (D. S. Gruner, unpublished data). By contrast, we propose that “slow” infecting EPNs, such as *Heterorhabditis marelatus*, exploit hosts that are patchy in space and time (e.g., *Hepialus*), which may be more generally resistant to infection but more rewarding in reproductive output by virtue of their large size. Tests of these predictions require spatially and temporally explicit, community-level studies of potential arthropod hosts, coupled with laboratory virulence and competition studies of relative host viability. These studies will advance our understanding of the evolution of life-history strategies and host resource partitioning of multiple species of natural enemies in soil ecosystems.

#### ACKNOWLEDGMENTS

We thank Evan Preisser, Karthik Ram, the Gruner lab, and two anonymous reviewers for constructive comments on the manuscript; Betsy Harbert and Wei-Chen Hsu for technical assistance; and the National Science Foundation for financial support (DEB-0620475). This is contribution number 2451 of the Bodega Marine Laboratory, University of California–Davis.

#### LITERATURE CITED

Anderson, R. M., and R. M. May. 1981. The population dynamics of microparasites and their invertebrate hosts.

- Philosophical Transactions of the Royal Society of London B 291:451–524.
- Barbercheck, M. E. 1993. Tritrophic level effects on entomopathogenic nematodes. *Environmental Entomology* 22:1166–1171.
- Barbour, M. G., R. B. Craig, F. R. Drysdale, and M. T. Ghiselin. 1973. *Coastal ecology: Bodega Head*. University of California Press, Berkeley, California, USA.
- Bedding, R. A., and A. S. Molyneux. 1982. Penetration of insect cuticle by infective juveniles of *Heterorhabditis* spp. (Heterorhabditidae: Nematoda). *Nematologica* 28:354–359.
- Blackburn, M. B., R. R. Farrar, Jr., D. E. Gundersen-Rindal, S. D. Lawrence, and P. A. W. Martin. 2007. Reproductive failure of *Heterorhabditis marelatus* in the Colorado potato beetle: evidence of stress on the nematode symbiont *Photobacterium temperata* and potential interference from the enteric bacteria of the beetle. *Biological Control* 42:207–215.
- Borer, E. T., C. J. Briggs, and R. D. Holt. 2007. Predators, parasitoids, and pathogens: a cross-cutting examination of intraguild predation theory. *Ecology* 88:2681–2688.
- Ciche, T. A., C. Darby, R.-U. Ehlers, S. Forst, and H. Goodrich-Blair. 2006. Dangerous liaisons: the symbiosis of entomopathogenic nematodes and bacteria. *Biological Control* 38:22–46.
- Connolly, J. 1988. What is wrong with replacement series? *Trends in Ecology and Evolution* 3:24–26.
- Connolly, J., P. Wayne, and F. A. Bazzaz. 2001. Interspecific competition in plants: how well do current methods answer fundamental questions? *American Naturalist* 157:107–125.
- Cox, F. E. G. 2001. Concomitant infections, parasites and immune responses. *Parasitology* 122:S23–S38.
- Crossan, J., S. Paterson, and A. Fenton. 2007. Host availability and the evolution of parasite life-history strategies. *Evolution* 61:675–684.
- Denno, R. F., D. S. Gruner, and I. Kaplan. 2008. Potential for entomopathogenic nematodes in biological control: a meta-analytical synthesis and insights from trophic cascade theory. *Journal of Nematology* 40:61–72.
- Dobson, A. P. 1985. The population dynamics of competition between parasites. *Parasitology* 91:317–347.
- Eidt, D. C., and G. S. Thurston. 1995. Physical deterrents to infection by entomopathogenic nematodes in wireworms (Coleoptera: Elateridae) and other soil insects. *Canadian Entomologist* 127:423–429.
- Emelianoff, V., E. Chapuis, N. Le Brun, M. Chiral, C. Moullia, and J.-B. Ferdy. 2008. A survival-reproduction trade-off in entomopathogenic nematodes mediated by their bacterial symbionts. *Evolution* 62:932–942.
- Finke, D. L., and R. F. Denno. 2003. Intra-guild predation relaxes natural enemy impacts on herbivore populations. *Ecological Entomology* 28:67–73.
- Gaugler, R., Y. Wang, and J. F. Campbell. 1994. Aggressive and evasive behaviors in *Popillia japonica* (Coleoptera: Scarabaeidae) larvae: defense against entomopathogenic nematode attack. *Journal of Invertebrate Pathology* 64:193–199.
- Georgis, R., and N. G. M. Hague. 1988. Field evaluation of *Steinernema feltiae* against the web-spinning larch sawfly *Cephalcia lariciphila*. *Journal of Nematology* 20:317–320.
- Gouge, D. H., and N. G. M. Hague. 1995. The development of *Steinernema feltiae* (Nematoda: Steinernematidae) in the sciarid fly *Bradysia paupera* (Diptera: Sciaridae). *Annals of Applied Biology* 126:395–401.
- Grewal, P. S., S. K. Grewal, V. S. Malik, and M. G. Klein. 2002. Differences in susceptibility of introduced and native white grub species to entomopathogenic nematodes from various geographic localities. *Biological Control* 24:230–237.
- Gruner, D. S. 2004. Attenuation of top-down and bottom-up forces in a complex terrestrial community. *Ecology* 85:3010–3022.

- Gruner, D. S., K. Ram, and D. R. Strong. 2007. Soil mediates the interaction of coexisting entomopathogenic nematodes with an insect host. *Journal of Invertebrate Pathology* 94:12–19.
- Hochberg, M. E., and R. D. Holt. 1990. The coexistence of competing parasites. I. The role of cross-species infection. *American Naturalist* 136:517–541.
- Hominick, W. M., A. P. Reid, and D. A. Bohan. 1996. Entomopathogenic nematodes: biodiversity, geographical distribution and the convention on biological diversity. *Biocontrol Science and Technology* 6:317–331.
- Inouye, B. D. 2001. Response surface experimental designs for investigating interspecific competition. *Ecology* 82:2696–2706.
- Jolliffe, P. A. 2000. The replacement series. *Journal of Ecology* 88:371–385.
- Kaya, H. K., and R. Gaugler. 1993. Entomopathogenic nematodes. *Annual Review of Entomology* 38:181–206.
- Kaya, H. K., and A. M. Koppenhöfer. 1996. Effects of microbial and other antagonistic organism [sic] and competition on entomopathogenic nematodes. *Biocontrol Science and Technology* 6:357–371.
- Kreft, A., and H. Skrzypek. 2002. Insect infection by entomogenous nemaodes (Rhabditida: Steinernematidae and Heterorhabditidae) in the conditions of competition. *Annales Universitatis Mariae Curie-Skłodowska* 62:1–14.
- Kunkel, B. A., P. S. Grewal, and M. F. Quigley. 2004. A mechanism of acquired resistance against an entomopathogenic nematode by *Agrotis ipsilon* feeding on perennial ryegrass harboring a fungal endophyte. *Biological Control* 29:100–108.
- Lafferty, K. D., et al. 2008. Parasites in food webs: the ultimate missing links. *Ecology Letters* 11:533–546.
- Lafferty, K. D., and A. M. Kuris. 2002. Trophic strategies, animal diversity and body size. *Trends in Ecology and Evolution* 17:507–513.
- Lewis, E. E., J. Campbell, C. Griffin, H. Kaya, and A. Peters. 2006. Behavioral ecology of entomopathogenic nematodes. *Biological Control* 38:66–79.
- Li, X. Y., R. S. Cowles, E. A. Cowles, R. Gaugler, and D. L. Cox-Foster. 2007. Relationship between the successful infection by entomopathogenic nematodes and the host immune response. *International Journal for Parasitology* 37:365–374.
- Losey, J. E., and R. F. Denno. 1998. Interspecific variation in the escape responses of aphids: effect on risk of predation from foliar-foraging and ground-foraging predators. *Oecologia* 115:245–252.
- Maron, J. L. 2001. Intraspecific competition and subterranean herbivory: individual and interactive effects on bush lupine. *Oikos* 92:178–186.
- Maron, J. L., and P. G. Connors. 1996. A native nitrogen-fixing shrub facilitates weed invasion. *Oecologia* 105:302–312.
- Maron, J. L., and R. L. Jefferies. 1999. Bush lupine mortality, altered resource availability, and alternative vegetation states. *Ecology* 80:443–454.
- Paine, R. T. 1980. Food webs: linkage, interaction strength and community infrastructure. *Journal of Animal Ecology* 49: 667–685.
- Peters, A. 1996. The natural host range of *Steinernema* and *Heterorhabditis* spp. and their impact on insect populations. *Biocontrol Science and Technology* 6:389–402.
- Poinar, G. O., Jr. 1990. Taxonomy and biology of Steinernematidae and Heterorhabditidae. Pages 23–61 in R. Gaugler and H. K. Kaya, editors. *Entomopathogenic nematodes in biological control*. CRC Press, Boca Raton, Florida, USA.
- Polis, G. A., and D. R. Strong. 1996. Food web complexity and community dynamics. *American Naturalist* 147:813–846.
- Preisser, E. L. 2003. Field evidence for a rapidly cascading underground food web. *Ecology* 84:869–874.
- R Development Core Team. 2008. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (<http://www.R-project.org>)
- Ram, K., D. S. Gruner, J. P. McLaughlin, E. L. Preisser, and D. R. Strong. 2008a. Dynamics of a subterranean trophic cascade in space and time. *Journal of Nematology* 40:85–92.
- Ram, K., E. L. Preisser, D. S. Gruner, and D. R. Strong. 2008b. Metapopulation dynamics override local limits on long-term parasite persistence. *Ecology* 89:3290–3297.
- Richmond, D. S., B. A. Kunkel, N. Somasekhar, and P. S. Grewal. 2004. Top-down and bottom-up regulation of herbivores: *Spodoptera frugiperda* turns tables on endophyte-mediated plant defence and virulence of an entomopathogenic nematode. *Ecological Entomology* 29:353–360.
- Schmitz, O. J. 2006. Predators have large effects on ecosystem properties by changing plant diversity, not plant biomass. *Ecology* 87:1432–1437.
- Schmitz, O. J. 2007. Predator diversity and trophic interactions. *Ecology* 88:2415–2426.
- Selvan, S., J. F. Campbell, and R. Gaugler. 1993. Density-dependent effects on entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) within an insect host. *Journal of Invertebrate Pathology* 62:278–284.
- Sicard, M., J. Hinsinger, N. Le Brun, S. Pages, N. Boemare, and C. Moullia. 2006. Interspecific competition between entomopathogenic nematodes (*Steinernema*) is modified by their bacterial symbionts (*Xenorhabdus*). *BMC Evolutionary Biology* 6:68.
- Sicard, M., H. Ramone, N. Le Brun, S. Pagès, and C. Moullia. 2005. Specialization of the entomopathogenic nematode *Steinernema scapterisci* with its mutualistic *Xenorhabdus* symbiont. *Naturwissenschaften* 92:472–476.
- Sih, A., G. Englund, and D. Wooster. 1998. Emergent impacts of multiple predators on prey. *Trends in Ecology and Evolution* 13:350–355.
- Stetina, S. R., J. S. Russin, and E. C. McGawley. 1997. Replacement series: a tool for characterizing competition between phytoparasitic nematodes. *Journal of Nematology* 29:35–42.
- Stock, S. P., J. F. Campbell, and S. A. Nadler. 2001. Phylogeny of *Steinernema* Travassos, 1927 (Cephalobina: Steinernematidae) inferred from ribosomal DNA sequences and morphological characters. *Journal of Parasitology* 87:877–889.
- Stock, S. P., B. M. Pryor, and H. K. Kaya. 1999. Distribution of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in natural habitats in California, USA. *Biodiversity and Conservation* 8:535–549.
- Straub, C. S., and W. E. Snyder. 2006. Species identity dominates the relationship between predator biodiversity and herbivore suppression. *Ecology* 87:277–282.
- Strong, D. R., J. L. Maron, P. G. Connors, A. Whipple, S. Harrison, and R. L. Jefferies. 1995. High mortality, fluctuation in numbers, and heavy subterranean insect herbivory in bush lupine, *Lupinus arboreus*. *Oecologia* 104:85–92.
- Strong, D. R., A. V. Whipple, A. L. Child, and B. Dennis. 1999. Model selection for a subterranean trophic cascade: root-feeding caterpillars and entomopathogenic nematodes. *Ecology* 80:2750–2761.
- Whipple, A. V. 1998. Sources of variation in the interactions among the bush lupine (*Lupinus arboreus*), a microbial symbiont (*Bradyrhizobium* sp.), a root-feeding caterpillar (*Hepialus californicus*), and an entomopathogenic nematode (*Heterorhabditis marelata*). Dissertation. University of California, Davis, California, USA.
- Woodring, J. L., and H. K. Kaya. 1998. Steinernematid and Heterorhabditid Nematodes: a Handbook of Techniques. Southern Cooperative Series Bulletin, volume 331. Arkansas Agricultural Experiment Station, Fayetteville, Arkansas, USA.
- Yu, H., D. H. Gouge, and P. Baker. 2006. Parasitism of subterranean termites (Isoptera: Rhinotermitidae: Termitidae) by entomopathogenic nematodes (Rhabditida: Steinernematidae; Heterorhabditidae). *Journal of Economic Entomology* 99:1112–1119.