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Population biology of entomopathogenic nematodes: Concepts, issues, and models

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Abstract

Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) are lethal obligatory parasites of insects and are found in soils throughout the world. The recognition that these nematodes are major natural enemies of soil insect pests has stimulated research into various aspects of their biology and enabled their use in augmentation and conservation biological control programs. Unfortunately, relatively little is known about the structure and dynamics of their populations or the factors that influence them. This knowledge is required if these nematodes are to fulfill their considerable potential as manageable components of cultivated ecosystems. The unusual life history of entomopathogenic nematodes imposes important constraints on their population biology. The host cadaver serves as the focus for many of the fundamental interactions associated with their population dynamics because feeding, development, mating, and reproduction are confined to the cadaver environment. Only non-feeding infective juveniles (dauer larvae) leave the host, but their production, dispersal, persistence, and infection potential provide critical links for the survival and proliferation of populations. Infective juveniles also carry symbiotic bacteria (Enterobacteriaceae) that are released within the host, are largely responsible for host death, and form an integral part of their life history. In this paper, we discuss the structure of entomopathogenic nematode populations, the various biotic and abiotic factors that influence them, and procedures for sampling and modeling their spatial and temporal dynamics. Environmental degradation and the economic and social realities of modern agriculture assure that entomopathogenic nematodes will remain prime subjects for continuing basic and applied ecological research.
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1. Introduction

Research on the population biology of soil organisms is a challenging aspect of modern ecology, and entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae provide a particularly salient case in point. As the fundamental importance of EPNs for the biological control of soil insect pests in both natural and managed ecosystems has gained increased recognition, research into their biology has moved forward at an increasingly ra-

pid rate. Unfortunately, investigations into the structure and dynamics of their populations have seemingly lagged behind. This slow progress is not due to lack of interest or importance but is likely associated with the inherent difficulties of sampling, manipulating, and studying organisms in soil (Brown and Gange, 1990). Nonetheless, if EPNs are to fulfill their considerable biological control potential as manageable components of cultivated ecosystems, then a comprehensive understanding of their population biology is required. Indeed, over a decade ago, Hominick and Reid (1990) noted that: "We are almost completely ignorant of the population biology of entomopathogenic nematodes, yet such information is fundamen-

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tal to understanding their persistence, distribution, effect on insect populations, and to the development of predictive models for control programs." Despite fundamental progress, this statement continues to apply today. In this paper, we assess the population biology of EPNs and the concepts, issues, and models providing the focus for current research. First, we examine the spatial distributions of EPN populations and the various biotic and abiotic factors influencing these distributions. Then we present new ideas on sampling EPNs and modeling their population dynamics.

The unusual life history of EPNs places important constraints on the structure and dynamics of their populations. The only free-living stage is the third stage infective juvenile (IJ), a non-feeding, environmentally resistant "dauer" larva that occurs in the soil and seeks out and penetrates potential insect hosts (Kaya and Gaugler, 1993; Kaya et al., 1993). Once inside the insect, the IJ releases symbiotic bacteria (Enterobacteriaceae) from its alimentary tract. The bacteria associated with the Steinernematidae are in the genus *Xenorhabdus* whereas those with the Heterorhabditidae are *Photorhabdus*, and they are largely responsible for overwhelming the insect's immune system and killing the host through septicemia (Boemare, 2002; Forst and Clarke, 2002). The bacteria proliferate rapidly and soon dominate the insect cadaver. The nematodes feed on the symbiont biomass and insect tissues, develop, mate, and reproduce, often for multiple generations, before producing another generation of IJs for release into the soil. Thus, most of the life history and population dynamics of EPNs takes place within the host cadaver. Moreover, because the IJ is the only free-living stage, the production, dispersal, persistence, and infection potential of cohorts of IJs derived from individual cadavers provide critical but perhaps tenuous links for the survival and proliferation of local populations. How these dynamics play out in diverse natural and manipulated environments is largely unknown and provides considerable scope for future research.

Current interest and advances in our knowledge of the biology of EPNs can be attributed to their great potential as effective, practical, and relatively inexpensive augmentative biological control agents for agriculture and other managed environments (Kaya and Gaugler, 1993; Kaya et al., 1993; Shapiro-Ilan et al., 2002). EPNs are efficacious against numerous insect pests in a variety of environments, and mass-produced nematodes have achieved commercial success in various niche markets and high value crops (Shapiro-Ilan et al., 2002). Consequently, a small international industry has developed around the production and merchandising of nematode products. However, the commercial production and use of EPNs raises numerous questions regarding population dynamics. How does the augmentation of natural populations with commercially produced native or exotic nematodes influence local nematode populations? What is the best strategy for augmentation to maximize control of particular insect pests in particular environments? When and where is the conserva-

tion of endemic nematodes a viable alternative to augmentation for pest management? Moreover, some methods for the laboratory culture or commercial production of EPNs rely on factitious insect hosts that have unusually high susceptibility to infection by the nematodes, whereas other commercial production methods rely on artificial media in culture flasks or bioreactors (Gaugler and Han, 2002). These circumstances raise important questions concerning the consequences of artificial rearing for the structure and dynamics of populations both during culture and after release in the field (Stuart and Gaugler, 1996; Stuart et al., 1996). Technological developments in the production and use of EPNs provide much fertile ground for population research.

2. Population distributions

2.1. Patchiness of populations

Surveys indicate that EPNs are widely distributed on all continents except Antarctica and throughout a broad range of habitats and soil types (Hominick, 2002). However, there is considerable variability across seasons, habitats, and geographic regions; and factors such as soil texture, moisture content, temperature, and availability of hosts are thought to be important in determining local distributions (Akhurst and Brooks, 1984; Hominick and Briscoe, 1990a,b; Ehlers et al., 1991; Stuart and Gaugler, 1994). Unfortunately, most surveys have merely assessed occurrence and provide little information on abundance or distribution. Studies addressing these issues have found that populations are extremely patchy both spatially and temporally, within and among sites (Cabanillas and Raulston, 1994; Campbell et al., 1998; Efron et al., 2001; Garcia Del Pino and Palomo, 1996; Glazer et al., 1996; Koppenhöfer and Kaya, 1996a; Spiridonov and Voronov, 1995; Strong et al., 1996; Stuart and Gaugler, 1994; Taylor, 1999).

In general, populations can exhibit uniform, random, or patchy distributions but the pattern observed depends upon the scale over which it is measured (Dutilleul and Legendre, 1993). Patchy distributions are often an apparent consequence of the distribution of resources or of interactions among conspecifics or heterospecifics. However, whatever the cause, patchy distributions can have important ramifications at the population and community levels by influencing gene flow and altering the dynamics of competition, predation, and parasitism (Harrison and Hastings, 1996; McCauley, 1991, 1995).

EPNs probably exhibit a patchy distribution within and among sites for various reasons including variability in the distribution and abundance of suitable habitat and susceptible hosts, the large number of IJs produced within single hosts (e.g., 30,000–400,000 IJs, Stuart et al., 1996), the limited dispersal capabilities of IJs, and variability in founding, establishment, and persistence ability under different circumstances (Efron et al., 2001; Kaya, 1990; Kaya and Gaugler, 1993; Strong, 2002; Stuart and Gaugler, 1994).

Local extinctions and reintroductions could be important aspects of the distribution of these species, and populations could be extremely transitory in space and time (Hominick and Briscoe, 1990a). Passive dispersal by water or phoretic dispersal within or upon hosts (Timper et al., 1988; Lacey et al., 1995) or other organisms (e.g., mites, Epsky et al., 1988; earthworms, Shapiro et al., 1993) could play important roles. Spiridonov and Voronov (1995) hypothesized that the field distribution pattern of Steinernema feltiae (Filipjev) IJs is a combination of a Poisson low-level background of "old" IJs and discrete narrow peaks consisting of several dozen IJs resulting from recent insect infestations. The characteristic dimension of these IJ peaks along linear transects was 15–20 cm.

All EPN species probably exhibit patchy distributions but the degree of patchiness could be characteristic for particular species and sets of conditions. In a study of spatial distribution in turfgrass in New Jersey, Campbell et al. (1995, 1996) recovered Steinernema carpocapsae (Weiser) from a larger proportion of sections along transects than Heterorhabditis bacteriophora Poinar, and concluded that S. carpocapsae populations might generally be more contiguous than those of H. bacteriophora. Moreover, S. carpocapsae was recovered primarily near the soil surface whereas H. bacteriophora was recovered uniformly throughout the soil profile. This result probably relates to differing foraging strategies: S. carpocapsae appears to function primarily as an "ambush" forager and attacks mobile insects on the soil surface whereas H. bacteriophora is a "cruise" forager and attacks more sedentary insects further down in the soil matrix (Gaugler et al., 1997). Differences in foraging strategy and host usage patterns might be the underlying cause for the difference in patchiness for these two species. However, the distribution of a potentially important sedentary host, the Japanese beetle, Popillia japonica Newman, was not related to the distribution of either nematode species (Campbell et al., 1998). Interestingly, even when H. bacteriophora is released in a uniform distribution in a turfgrass habitat, it quickly returns to the typical aggregated pattern of natural populations (Campbell et al., 1998; Wilson et al., 2003).

2.2. Metapopulations

Many organisms exhibit complex population structures in which arrays of local populations are interconnected to varying degrees by limited dispersal and gene flow to form what are referred to as metapopulations (Hanski, 1999a,b; Harrison and Taylor, 1997; McCauley, 1995). The study of metapopulation structure and dynamics has become an important theme in population ecology with natural populations being viewed as a series of transient ephemeral local populations with average lifespans that are much shorter than that of the whole network. Rates of birth, death, immigration, and emigration, influence local populations but the persistence of a metapopulation results from a balance between recurrent colonization and extinction events

with a high turnover of local populations. The extinction rate generally decreases with increasing patch size, and the colonization rate decreases with increasing distance between patches (Hanski, 1998, 2001; Hanski and Simberloff, 1997). Given the inherently patchy distribution of EPNs, concepts and models developed for metapopulations could have important applications for understanding the population dynamics of these species.

2.3. Metapopulations and genetic diversity

The patchy distribution of EPNs within and among sites is consistent with a metapopulation concept and could have various ramifications for the genetic diversity of populations (Harrison and Hastings, 1996; McCauley, 1991, 1995). Indeed, this kind of fragmented and dynamic population structure might explain the apparent rarity of mixed restriction fragment length polymorphism (RFLP) types from individual collection sites and provide for sufficient gene flow among populations or subpopulations to prevent extensive intraspecific genetic differentiation (Reid and Hominick, 1992; Stuart and Gaugler, 1994). The apparent lack of genetic differentiation within species of EPNs is consistent with the apparent overall lack of adaptive radiation within the group as evidenced by the relatively small number of described species, many of which have broad distributions (Hominick, 2002). Nonetheless, the number of undescribed species could be large (Hominick, 2002; Poinar, 1990).

For at least some species of EPNs under certain conditions, genetic diversity among patches within sites appears to be considerable. Grewal et al. (2002) found that IJ longevity and tolerance to major environmental stresses including heat, ultraviolet radiation, hypoxia, and desiccation differed significantly among isolates of H. bacteriophora taken from an apparently uniform turfgrass site of 200 m². The isolates also had different isozyme patterns for several metabolic enzymes (Jagdale and Grewal, unpublished data). Similarly, Stuart et al. (2004) found significant differences in virulence to larvae of the root weevil, Diaprepes abbreviatus (L.), for a series of isolates of Steinernema riobrave Cabanillas, Poinar, & Raulston from a Texas pecan orchard.

In contrast, within localized patches at a given site, nematodes might often exhibit very little genetic variation. If the number of individual IJs colonizing an insect host tends to be small, then all of the IJs produced by that cadaver would be very closely related genetically and, consequently, effective population sizes might often be relatively small. Heterorhabditids might be especially well adapted for this eventuality because the first generation inside the host is hermaphroditic. Thus, a study of mitochondrial DNA sequence data for H. marelatus Liu & Berry indicated relatively low genetic diversity within and among populations (Blouin et al., 1999). Nonetheless, it is unclear how general this pattern might be because the genetic diversity within a strain of H. bacteriophora (HP88 strain) appears to be

considerable, even though this strain was isolated from a single cadaver taken from the field (Glazer et al., 1991).

3. Factors influencing EPN distributions

3.1. Biotic factors

Various components of the soil biota are likely to influence the distribution and abundance of EPNs. Soils contain rich and diverse communities of flora and fauna that are interconnected in complex trophic webs (Hawksworth, 1991; Neher and Barbercheck, 1999; Strong et al., 1999; Wall and Moore, 1999). From an EPN perspective, the soil contains a broad range of host and non-host arthropods, competitors (Barbercheck and Kaya, 1990; Barbercheck and Kaya, 1991b; Kaya, 2002), predators (Baur et al., 1998; Sayre and Walter, 1991), parasites and pathogens (Bellows, 1999; Ishibashi and Kondo, 1987; Kaya, 2002; Stirling, 1991; Timper et al., 1991; Timper and Kaya, 1992). Although laboratory evidence clearly indicates that various organisms influence the survival and reproduction of EPNs, very few field studies have examined the relative importance of different factors (Strong et al., 1999). Moreover, omnivory is rampant in soil communities, and trophic webs based on detritus and primary production are linked in various ways that might often produce indirect and diffuse impacts on EPNs (Walter, 1987a,b, 1988a; Walter et al., 1989). Thus, determining the relative importance of a broad range of biotic factors for the spatial and temporal distribution of EPNs within their appropriate ecological context is a daunting task.

3.1.1. Natural enemies of EPNs

The primary biotic factor influencing the occurrence and persistence of EPNs at a particular site is probably the presence of suitable hosts (Mrácek and Webster, 1993; Mrácek et al., 1999; Peters, 1996). When hosts are abundant, predators, parasites, and pathogens could regulate populations. The widespread occurrence of nematophagous fungi, bacteria, protozoa, nematodes, mites, collembolans, and other microarthropods in soil, and the high rates of predation observed in the laboratory suggest that these organisms might have considerable impact on EPNs in nature (Kaya, 2002; Stirling, 1991). Even specialist nematophagous invertebrates will attack a variety of nematode prey (Small, 1987; Walter et al., 1987).

The potential impact of natural enemies on EPNs has generally been assessed in simplified observation chambers or sterilized soil, but there is little evidence that activity in these simple systems is correlated with effects in the field. For example, Gilmore and Raffensperger (1970) observed that collembolans consumed large numbers of plant-parasitic nematodes in charcoal–plaster of Paris observation arenas, but predatory activity was substantially reduced when a soil-vermiculite mix was added to the arenas. Similarly, the assay system and host insect used can modify the apparent impact of predation on EPNs. Predatory micro-

arthropods reduced the efficacy of EPNs against a very susceptible but not natural host (wax moth larvae, *Galleria mellonella* (L.)) in soilless assay arenas but had no effect against a natural host (Japanese beetle grubs) in an assay arena containing turf (Epsky et al., 1988; Gilmore and Potter, 1993).

The effectiveness of a natural enemy can depend on many factors, which can include voracity, specificity, survival at low prey or host densities, dispersal, and distribution in relation to the prey or host, and reproductive potential (Pianka, 1999). Under laboratory conditions, omnivorous and nematophagous predators can be voracious feeders. In assays with raw field soil, the presence of astigmatid mites in the genus *Sancassania* greatly reduced IJ production by *S. carpocapsae*, *S. riobrave*, and *H. bacteriophora* in *G. mellonella* (Barbercheck and Greenwood, unpubl. data). Many nematophagous organisms have rapid development and high reproductive rates, and many species (e.g., mesostigmatid mites) exhibit some degree of specificity towards nematodes and are capable of reproducing rapidly by parthenogenesis. Mites had faster development times, lower mortality and higher egg-laying rates when feeding on nematodes than when feeding on arthropod prey (Walter et al., 1987; Walter, 1988a,b). Furthermore, predatory nematodes typically exhibit high consumption rates with little indication of satiation (Bilgrami and Jairajpuri, 1989a,b).

Insect cadavers with EPNs are subject to predation by various scavengers (Kaya, 2002). However, the cadavers of some species are repellent to certain ants, and thereby provide protection for the developing EPNs (Baur et al., 1998; Zhou et al., 2002). However, to date, this effect has only been demonstrated for a limited number of ant and EPN species (Kaya, 2002).

3.1.2. Competition and displacement

The relative importance of competition in determining the characteristics of organisms, populations, and communities has long been a major issue in ecology (Pianka, 1999; Wootton, 1994), and there are practical reasons to examine the role of competition in the biology of EPNs. A greater understanding of competitive abilities could aid evaluation of the suitability of particular EPN species for control programs because these abilities could impact the establishment, persistence, and population dynamics of introduced EPNs. More importantly, when EPN applications are made, what are the risks of displacing non-target natural enemies, including endemic EPNs? What are the ecological consequences of augmentation?

Competition is defined as any mutually negative interaction that does not directly involve predation or parasitism (Pianka, 1999; Wootton, 1994). Competition is most obvious and dramatic when it occurs between species but also occurs and can have important consequences within species. Competition theory predicts that coexisting species that share limited resources will compete, and that competing species must diverge in resource use and reduce niche

overlap for competitive exclusion to be avoided and coexistence to continue. The resulting competitive (or character) displacement tends to produce a regular segregation of coexisting species (and their characteristics) in resource space. Variation in resource availability can influence the dynamics of this phenomenon as coexisting species respond opportunistically to superabundant resources, specialize when resources are more limiting, and converge when resources are scarce.

There has been little field research on inter- and intra-specific competition, and we can only speculate on whether observed phenomena are due to competitive interactions (i.e., "the ghost of competition past," Connell, 1980). However, numerous aspects of EPN ecology and behavior could have evolved in this context and might enable the coexistence of certain species. To conclusively demonstrate the coevolutionary divergence of competitors, one must demonstrate that changes occurred, that the changes have a genetic basis, and that competition was responsible (Pianka, 1999). These requirements have yet to be met for EPNs.

Surveys show that multiple species of EPNs can coexist with as many as four species being reported from a single site (Akhurst and Brooks, 1984; Stuart and Gaugler, 1994; Duncan et al., 2003b). Coexistence would be expected when behavioral differences and variability in environmental factors enable strong niche separation and avoidance of competition. In laboratory and greenhouse studies, differences in foraging behaviors apparently reduce competition among some EPN species and permit coexistence (Koppenhöfer and Kaya, 1996a,b). The foraging strategies of EPNs vary along a continuum with the extremes represented by "ambushers," which tend to remain relatively sedentary at or near the soil surface and attack mobile insects, and "cruisers," which actively seek out sedentary hosts deeper in the soil profile (Gaugler et al., 1997). S. carpocapsae is an ambusher (Kaya and Gaugler, 1993), H. bacteriophora is a cruiser (Grewal et al., 1995a), and S. riobrave is intermediate with characteristics of both (Cabanillas and Raulston, 1994; Grewal et al., 1995a). In studies in North Carolina cornfields, these species were able to coexist, with each moving to a different location in the soil profile (Millar and Barbercheck, 2001). The native H. bacteriophora was found deepest in the soil, introduced S. riobrave occurred at intermediate depths, and the native S. carpocapsae remained near the surface. Thus, differences in foraging behavior might explain the ability of these species to coexist.

Habitat heterogeneity can also facilitate coexistence and avoidance of competition. Habitat patches exist in a matrix within which the numbers, arrangement and size of patches influence the movements of organisms, food web interactions, and the persistence of populations (Polis et al., 1997; Wiens et al., 1997; With et al., 2002). Habitat heterogeneity and complexity can contribute to population persistence by presenting microhabitats in a mosaic that spatially and temporally separate competitors, predators, and prey (Ettema, 1998). These processes might help explain the highly aggregated distribution of many soil-dwelling species (Adl, 2003; Coleman and Crossley, 1996) and the coexistence of multiple species of EPNs within sites (Koppenhöfer and Kaya, 1996a).

3.1.3. Types of competition and other ecological interactions

Interspecific competition can be direct or indirect. Direct competition involves direct interference between two species, whereas indirect competition applies to a wide range of effects that are mediated by the presence of one or more other species or by a change in the chemical or physical environment (Pianka, 1999; Wootton, 1994). Indirect effects in ecological communities can be either positive or negative, with only the negative effects being considered competitive. Negative indirect effects promote traits that minimize the indirect effects, reduce competition, and facilitate coexistence whereas positive indirect effects tend to move species toward increased sympatry and the maximization of the indirect effects in mutualistic or commensalistic interactions. Indirect effects require strong interactions but high levels of environmental variation, stress or disturbance might keep populations at such low levels that the species do not interact strongly and effects do not occur. Because EPNs are associated with symbiotic bacteria within host cadavers, direct competition might be rare but indirect competition mediated by the bacteria could be common.

At least five types of indirect effects (both positive and negative) have been demonstrated in ecological communities (Wootton, 1994) and include: (1) exploitative competition, (2) trophic cascades, (3) apparent competition, (4) indirect mutualism and commensalism, and (5) higher order interactions. In exploitative competition one species indirectly reduces a second species by reducing the abundance of a shared resource. For example, an insect is rarely infected by more than one species of EPN (Ehlers et al., 1991), and heterorhabditids and steinernematids apparently cannot develop on each other's symbiotic bacteria (Alatorre-Rosas and Kaya, 1990, 1991). However, two steinernematids, S. carpocapsae and Steinernema glaseri (Steiner), can coinfect and produce progeny from a single G. mellonella (Koppenhöfer et al., 1995a). In this case, S. glaseri is less negatively affected by the mixed infection than S. carpocapsae, perhaps because of its faster development and ability to use the symbiont of S. carpocapsae.

A trophic cascade is an indirect effect mediated through a series of consumer–resource interactions. In coastal California, endemic H. marelatus are dynamically linked with populations of root-feeding larvae of a hepialid moth, Hepialus californicus (L.), and its bush lupine host plant, Lupinus arboreus (Sims) (Strong, 2002; Strong et al., 1995, 1996, 1999). Hepialid larvae inflict heavy root damage and can kill bush lupines but H. marelatus causes high mortality of hepialid larvae, and the spatial distribution of H. marelatus is positively correlated with long-term fluctuations in the local distribution of lupines. Any other organisms that influence the abundance of this resource, either

positively or negatively, are likely to impact *H. marelatus* through this trophic cascade. Moreover, by protecting bush lupine, a nitrogen-fixer, *H. marelatus* probably mediates additional community effects (Preisser, 2003).

Apparent competition can occur when two prey species share a common natural enemy, with an increase in one prey resulting in an increase in the natural enemy and a decline in the second prey. For example, when one EPN occurs naturally at a site and another EPN is applied then the resulting increase in the overall abundance of EPNs could cause a numerical response in predatory mites and a subsequent reduction of both EPN species. Some soil mites have demonstrated a numerical response when fed EPNs in laboratory studies (Walter et al., 1986).

Indirect mutualism and commensalism are positive effects and typically involve a consumer–resource interaction linked to either exploitative (indirect) or interference (direct) competition. For example, certain ants might preferentially prey on steinernematid rather than heterorhabditid-infected cadavers (Alatorre-Rosas and Kaya, 1990, 1991; Baur et al., 1998; Koppenhöfer et al., 1995a). Preferential predation on a competitive dominant could allow an otherwise inferior sympatric competitor to increase through indirect commensalism.

Higher order interactions refer to non-additive effects between groups of species or individuals. The interactions do not meet the assumption that the combined effect of several species on a particular species of interest can be represented by adding up all the pair-wise effects. For example, characteristics of certain food plants could modify the susceptibility of an herbivorous insect to one EPN species but not to another (Barbercheck et al., 1995).

3.1.4. Interspecific competition among EPNs

The dynamics and ramifications of interspecific competition among EPN species are of special interest because of the potential effects that biological control applications of either exotic or endemic nematodes might have on endemic nematode communities. In simple laboratory assays, when larvae were exposed to various concentrations of IJs of two EPN species, *S. carpocapsae* successfully infected and reproduced in a greater number of cadavers than *H. bacteriophora* at all concentrations of IJs tested and also displayed a competitive advantage when directly inoculated into the hemocoel (Alatorre-Rosas and Kaya, 1991). The authors concluded that the result was caused by interference competition between the nematodes within the host cadaver, a direct effect. However, because of the symbiotic bacteria, the result for the nematodes might better be interpreted as exploitative competition, an indirect effect in which one species indirectly reduces a second species by reducing the abundance of a shared resource. Nonetheless, since *Xenorhabdus* species are known to produce bacteriocins that kill *Photorhabdus* species (Boemare, 2002), the interaction between the bacteria could be direct interference competition. In other assays, *Spodoptera litura* (F.) co-infected with *S. glaseri* and *S. feltiae* produced

mixed progeny (Kondo, 1989) but *G. mellonella* co-infected with *S. glaseri* and *S. carpocapsae* depressed *S. carpocapsae* IJ production (Koppenhöfer et al., 1995b). The competitive advantage of *S. glaseri* over *S. carpocapsae* might be due to its faster development and less specific association with its symbiotic bacteria (Koppenhöfer and Kaya, 1996b).

In the field, interactions between EPN species could be mediated by various factors including differences in foraging strategies and host preferences. In paired comparisons, *S. carpocapsae*, an ambusher, was more successful at the soil surface whereas *H. bacteriophora*, a cruiser, was more successful at depths greater than 5 cm (Alatorre-Rosas and Kaya, 1990). In tests against different hosts, *S. carpocapsae* dominated *S. glaseri* against the surface dwelling black cutworm, *Agrotis ipsilon* (Hufnagel), whereas *S. glaseri* dominated *S. carpocapsae* against the soil inhabiting masked chafer, *Cyclocephala hirta* LeConte (Koppenhöfer and Kaya, 1996a). In co-infections, *S. glaseri* dominated *S. riobrave* but when *S. carpocapsae* and *S. glaseri* were co-infected, both were depressed (Koppenhöfer and Kaya, 1996a).

Millar and Barbercheck (2001) applied an exotic EPN, *S. riobrave*, to a corn field in North Carolina that contained endemic *H. bacteriophora* and *S. carpocapsae*, and monitored the outcome by baiting soil samples with *G. mellonella* larvae. One week after application, *S. riobrave* was detected in less than half of the samples. Subsequently, the distributions of the three species rarely overlapped, and multiple species were rarely found in the same soil sample. The lack of overlap was further indicated by the absence of insects co-infected by multiple species even though coinfections had been demonstrated in laboratory tests (Millar, unpublished data). Overall, the detection of *H. bacteriophora* was significantly reduced in the presence of *S. riobrave* but this endemic nematode was not completely displaced two years after the introduction. Detection of *S. carpocapsae* and *S. riobrave* was not affected by the presence of each other, and detection of *S. riobrave* was not affected by the presence of *H. bacteriophora*. *H. bacteriophora* had the strongest tendency to be detected deeper in the soil profile, followed by *S. riobrave*, and then *S. carpocapsae*. In this case, differences in environmental tolerance, foraging behavior, host usage, vertical distribution, and patchiness probably contributed to coexistence.

In a Florida citrus grove, twice yearly applications of an exotic EPN, *S. riobrave*, to control the root weevil, *D. abbreviatus*, suppressed endemic EPNs and provided levels of weevil control higher than those caused by endemic EPNs in untreated plots only during months of treatment while providing less control during non-treatment months (Duncan et al., 2003b). The endemic EPNs included *S. diaprepesi* Nguyen & Duncan, *H. bacteriophora*, *Heterorhabditis indica* Poinar, Karunakar & David, and *Heterorhabditis zealandica* Poinar, and the abundance of adult weevils was directly correlated with the proportion of sentinel weevil larvae infected by the endemic EPNs but was inversely correlated with the proportion of larvae

infected by *S. riobrave*. Apparently, *S. riobrave* partially displaced the endemic EPNs but reproduced and persisted poorly, partly because of competition for cadavers with a native bacterial-feeding nematode (see below; Duncan et al., 2003a). It is unclear whether these results would apply to other sites, nematodes, insects, or soil communities, and what other monitoring techniques might reveal.

3.1.5. Intraspecific competition among EPNs

Intraspecific competition could influence various aspects of the biology of EPNs including emergence patterns, foraging strategies, and the dynamics of host invasion, establishment, and reproduction. The emergence of IJs from the host cadaver often begins abruptly, peaks during the first few days, and involves tens or hundreds of thousands of IJs (Stuart et al., 1996). Certain traits of IJs vary predictably as the emergence progresses: early emerging IJs are typically larger and, in steinernematids, have a more male-biased sex ratio than later emerging IJs (Lewis and Gaugler, 1994; Nguyen and Smart, 1995; Stuart et al., 1996). The pattern of IJ emergence and certain characteristics of IJs could be associated with the population dynamics of the nematodes and their bacteria within the host cadaver, the availability and utilization of resources, and other conditions and cues that trigger the formation and release of IJs; and much of this could be associated with intense intraspecific competition. Moreover, the emergence pattern sets the stage for dispersal, host finding, and host colonization, and could influence potential competition and cooperation among IJs during the infection process. Little research has directly addressed intraspecific competition but Stuart et al. (1996) showed that the pattern of emergence for *S. glaseri* has a genetic component and that genetic variability for the emergence pattern occurs in natural populations.

The adaptive value of a particular emergence pattern might reflect the relative reproductive success of IJs emerging at different times (Stuart et al., 1996). For cruise foragers that exploit sedentary hosts, early emerging IJs from a particular cadaver are likely to have the best opportunity to locate, infect, and reproduce within nearby hosts. Those emerging just a few days later might be required to disperse farther before encountering additional uninfected hosts or might suffer negative fitness consequences because of their late arrival within already infected hosts where other IJs have a head start in development, mating, and reproduction. Consequently, later emerging IJs might often have lower reproductive success than early emerging IJs. This difference might not apply to ambush foragers that exploit mobile hosts. However, EPNs occur in various habitats and use a broad range of hosts (Kaya and Gaugler, 1993), and the temporal and spatial distribution of hosts might vary considerably across habitats or times of the year. Variability in the adaptive value of particular emergence patterns could maintain genetic variability for this trait in the general population. Other traits of the nematodes and their symbiotic bacteria that are correlated with the pattern of emergence (see above) might also impose trade-offs or constraints on adaptive modifications in the emergence pattern.

Competitive and cooperative interactions among IJs could form the basis for the evolution of alternative infection strategies by early and late emerging IJs (Stuart et al., 1996). When a host is colonized by EPNs, a certain number of IJs are necessary to overcome host defenses (Gaugler et al., 1994; Wang et al., 1994) and to guarantee mating for steinernematids but too many IJs impede development, survival, and reproduction (Selvan et al., 1993a; Zervos et al., 1991). Given the large number of IJs that emerge from a single cadaver, various strategies could have evolved to regulate dispersal and infectivity. Such strategies might be especially likely if the IJs emerging from a cadaver are often close relatives since kin selection could be involved (Maynard Smith, 1989). The repellency of cadavers with EPNs (Glazer, 1997) and staggered patterns of infectivity (Bohan and Hominick, 1995, 1996, 1997b; Hominick and Reid, 1990; Kaya and Koppenhöfer, 1996; but see Campbell et al., 1999) might have evolved in this context. Size differences among IJs probably correlate with lipid reserves and longevity (Lewis and Gaugler, 1994; Selvan et al., 1993b,c) but, since early emerging IJs are larger than later emerging IJs, this is not indicative of a greater potential for delayed infectivity by the latter. However, later emerging IJs are more mobile and less responsive to host cues than early emerging IJs (Lewis and Gaugler, 1994), and these traits would facilitate greater dispersal.

The first IJs to successfully invade a host and develop into adults are likely to have reproductive advantages but early host colonization is probably risky since early invaders could suffer high mortality from host defenses (Gaugler et al., 1994; Peters and Ehlers, 1994; Wang et al., 1994). Nonetheless, if IJs emerging from a cadaver and arriving at a new host are often close relatives, then kin selection might confer fitness benefits on IJs that contribute to subduing a host but die in the process if their relatives are thereby able to reproduce (Maynard Smith, 1989; Stuart et al., 1998). Thus, the optimal times for IJs to invade a host might be a function of numerous factors including host-induced mortality rates, development times, reproductive competition, and genetic relatedness.

Experiments indicate that optimal invasion times might exist for EPNs invading hosts. Initial infections by *S. feltiae* facilitate subsequent infections (Hay and Fenlon, 1997), and ongoing infections by *S. carpocapsae*, *S. feltiae*, and *S. riobrave* cause the release of a chemical that deters further infection (Fairbairn et al., 2000; Glazer, 1997). Nonetheless, it is unclear how constrained optimal invasion times might be. Glazer (1997) found that invasion into insects injected with IJs of certain steinernematid species was reduced 6–9 h after injection whereas Stuart et al. (1998) found that IJs of *S. glaseri* invade *G. mellonella* larvae up to at least 14 h after the first IJ has entered. Optimal invasion intervals could be quite plastic and depend on the rate of invasion and dynamics of the interaction between particular EPN species and their hosts.

Research indicates that either males (Grewal et al., 1993) or females (Bohan and Hominick, 1997a) might show a bias toward early host colonization, results that suggest fundamental differences in reproductive competition for the species involved. However, Stuart et al. (1998) found no gender bias in host colonization by *S. glaseri* even though this species exhibits the various behavioral differences between male and female IJs that suggest an early male colonization bias (Grewal et al., 1993).

Intraspecific competition could also be a factor in commercial production of EPNs because it might influence optimal inoculation rates and conditions for in vivo and in vitro production systems. Indeed, artificial rearing conditions themselves could have an important influence on the development and reproduction of EPNs, alter the dynamics involved, and select for an array of different traits from those that are important in nature. This kind of inadvertent selection has been documented for laboratory cultures of EPNs (Stuart and Gaugler, 1996; Wang and Grewal, 2002) and could influence the establishment and persistence abilities of mass-reared nematodes when applied in the field.

3.1.6. Competition with non-EPNs

Free-living bactivorous, fungivorous, predatory, and omnivorous nematodes constitute important components of decomposition and nutrient cycling food webs in the soil. Duncan et al. (2003a) examined interactions between introduced *S. riobrave*, native *S. diaprepesi*, and a native free-living bacterial feeding nematode, *Pellioditis* sp., with respect to mortality of *D. abbreviatus* larvae in Florida citrus. The presence of *S. riobrave* increased the number of *Pellioditis* that developed in insect cadavers, and the presence of *Pellioditis* suppressed the number of *S. riobrave* that developed. However, there was no interaction observed between *Pellioditis* and *S. diaprepesi*. Similarly, addition of *S. carpocapsae* or *S. glaseri* to soil resulted in a temporary increase in predatory and free-living rhabditid nematodes (Ishibashi and Kondo, 1986, 1987). In contrast, Grewal et al. (1997) found no effects of application of *S. carpocapsae* or *S. glaseri* on free-living nematodes. EPNs appear to interact negatively with certain plant parasitic nematodes, and can reduce their populations and associated plant damage (Ishibashi and Kondo, 1986; Jagdale et al., 2002; Lewis et al., 2001; Somasekhar et al., 2002).

Competition for insect resources can also occur between EPNs and other microbial insect pathogens. EPNs will infect certain virus-infected insects but the insect cadavers have a fragile integument that can break open and reduce IJ production (Kaya and Brayton, 1978; Kaya and Burlando, 1989). *S. carpocapsae* and *Bacillus thuringiensis* (Berliner) (Bt) can develop simultaneously in co-infected hosts, but the development of the EPNs is abnormal and the resulting IJs are smaller and have less food reserves than do IJs produced from insects that are not infected with Bt (Kaya and Burlando, 1989). Nonetheless, combinations of EPNs and Bt can additively or synergistically increase levels of mortality of scarab grubs for certain combinations of EPN and grub species (Koppenhöfer and Kaya, 1997; Koppenhöfer et al., 1999).

Environmental conditions can influence the outcome of competitive interactions. When EPNs compete with other insect pathogens for a host insect, the host usually dies but EPN progeny may not be produced from the co-infected hosts (Barbercheck and Kaya, 1990, 1991b). When insects are co-infected with the fungus, *Beauveria bassiana* (Balsamo) Vuillemin, and EPNs, the EPNs usually outcompete the fungus but this result is influenced by temperature and the relative time of infection (Barbercheck and Kaya, 1990, 1991b). If *B. bassiana* is given a head start of 3–4 days at 30 °C, 1–2 days at 22 °C and 1 day at 15 °C, then the fungus will develop to the exclusion of the EPNs.

3.2. Abiotic factors

Many abiotic factors can affect the occurrence and persistence of EPNs. These include natural physical or chemical factors (e.g., climate, soil pH, soil texture, and structure) as well as those resulting from human activities (e.g., physical or chemical disturbance). The effects of abiotic factors on EPNs have been widely studied under simplified laboratory conditions with soils or artificial substrates treated to reduce interactions with other abiotic and biotic factors (Barbercheck, 1992; Glazer, 2002). However, in nature, complex interactions are common and extrapolation from simple laboratory studies to ecosystems is problematic. Nonetheless, this research does provide some indication of the importance of various factors.

Most studies of soil effects on EPNs have focused on soil texture (i.e., the composition of soil solids by particle size range) rather than on soil structure (i.e., the arrangement of soil particles into aggregates of varying size, geometry, and porosity) (Hillel, 1982). Structural pore space is determined largely by size and arrangement of aggregates, and affects the movement of water, air, and organisms in soil. In laboratory studies, nematodes are differentially affected by soil texture and structure (Barbercheck, 1993; Barbercheck and Kaya, 1991a; Kung et al., 1990a). Movement is more restricted in soils with restrictive pore space (heavy or poorly structured soils) than in soils with a more porous structure. In laboratory experiments, the survival and movement of *H. bacteriophora*, *S. carpocapsae*, and *S. glaseri* varied with soil texture and bulk density (Portillo-Aguilar et al., 1999). All three species moved significantly more in sandy loam than in loam or silty clay loam, and movement generally decreased as bulk density increased. However, the degree to which soils of high bulk density reduced movement differed among species and soil textures: *H. bacteriophora* was least restricted, whereas *S. carpocapsae* was most restricted. Survival of *S. glaseri* was positively correlated with bulk density but survival of *H. bacteriophora* was negatively correlated, and survival of *S. carpocapsae* was unaffected. The infection rate of *G. mellonella* by *H. bacteriophora* and *S. glaseri* showed no

significant variation in relation to bulk density but the infection rate for *S. carpocapsae* increased with bulk density. In general, rates of movement and infection were strongly correlated with the amount of soil pore space having dimensions similar to or greater than the diameter of the EPNs.

In natural ecosystems, soil type might have a greater influence on heterorhabditids than on steinernematids (Hominick, 2002). However, for *H. bacteriophora* in turfgrass, edaphic factors were relatively uniform along transects and only weakly correlated with EPN recovery (Campbell et al., 1998). In no-till and conventional-till maize fields in North Carolina, no significant relationships were detected between the occurrence of endemic *S. carpocapsae* or *H. bacteriophora* and soil organic matter, pH or soil texture (Millar and Barbercheck, 2002). In Florida citrus groves, soil type was not correlated with infection of root weevils by *S. carpocapsae* (Beavers et al., 1983) but suppression of root weevils by *S. riobrave* was greater in coarse, sandy soils than in fine textured soils (Duncan et al., 2001; Shapiro et al., 2000).

Moisture is arguably the most critical abiotic factor affecting soil nematodes (Nickle, 1984). Terrestrial nematodes require water films of sufficient thickness and continuity to allow movement. In very wet or saturated soils, oxygen may be limiting and nematode movement can be restricted due to lack of surface tension forces (Wallace, 1971). Numerous laboratory studies have examined the effect of soil moisture on the efficacy and survival of EPNs (Gaugler and Kaya, 1990; Glazer, 2002; Kaya and Gaugler, 1993; Shapiro-Ilan et al., 2002). In the laboratory, virulence of *H. bacteriophora*, *S. glaseri*, *S. feltiae*, and *S. carpocapsae* increased with soil moisture content in sandy loam soils ranging in moisture content from below the permanent wilting point to near saturation (Grant and Villani, 2003). Hudson and Nguyen (1989) tested the infectivity of *Steinernema scapterisci* Nguyen and Smart to the mole crickets, *Scapteriscus vicinus* Scudder and *Scapteriscus acletus* Rehn & Hebard, under a variety of conditions in the laboratory and found that soil moisture that varied from 5 to 15% had no effect on infection. In a survey of Spanish soils for EPNs, Garcia Del Pino and Palomo (1996) concluded that soil moisture and temperature regimes are more important than other factors in determining the prevalence of EPNs in cold moist soils. In conventional-till and no-till maize in North Carolina there was a quadratic relationship between soil moisture content and numbers of sentinel *G. mellonella* infected by *S. carpocapsae* but not by *S. riobrave* or *H. bacteriophora* (Millar and Barbercheck, 2002). Many nematodes have physiological or behavioral adaptations that allow resumption of activity after quiescence induced by moisture limiting conditions (Glazer, 2002). Reduced virulence of EPNs in low moisture conditions can be increased by rehydrating the soil to simulate rainfall or irrigation (Grant and Villani, 2003). Moreover, the survival of *S. riobrave* is apparently enhanced following quiescence induced by moisture deficits (Duncan et al., 1996).

The chemistry and pH of the soil solution can affect EPNs but nematodes can tolerate a wide range of soil pH. Kung et al. (1990b) found reduced survival of steinernematid nematodes at pH 10 but no differences from pH 4 to 8. Mortality of the cotton leafworm, *Spodoptera littoralis* (Boisduval), from *H. bacteriophora* and *S. carpocapsae* was higher and more rapid at pH 6.9 and 8.0 than at pH 5.6 (Ghally, 1995). Acid deposition may be a limiting factor in some areas but we are not aware of any studies that document such effects on EPNs (Sharpe and Drohan, 1999). In laboratory experiments, acid pH reduced the efficacy of *S. carpocapsae*, *S. feltiae*, and *H. bacteriophora* against diapausing larvae of *Cephalcia abietis* (L.), and it was suggested that application of lime or magnesium fertilizers that raise soil pH might induce EPN epizootics by increasing the activity of EPNs (Jaworska, 1993). At high concentrations, NaCl, KCl, and $CaCl_2$ inhibited the ability of *S. glaseri* to move through a soil column and to locate and infect a susceptible host (Thurston et al., 1994). Calcium chloride and KCl had no effect on *H. bacteriophora* survival, infection efficiency, or movement through a soil column, but moderate concentrations of these salts enhanced *H. bacteriophora* virulence. NaCl at high salinities (>16 dS/m) adversely affected all of these parameters (Thurston et al., 1994).

Nematode activity and survival are reduced by low oxygen conditions (e.g., waterlogged soils) and can be influenced by the relative humidity of the soil atmosphere (Kung et al., 1990b; Qiu and Bedding, 1999). Under normal field conditions where moisture levels are high enough to support plant growth, the soil atmosphere is nearly always vapor saturated. Survival and pathogenicity of *S. carpocapsae* and *S. glaseri* decreased as relative humidity decreased from 100 to 25% over a 32-day test period (Kung et al., 1990b). Brown and Gaugler (1997) found that IJs could survive adverse environmental conditions by remaining in host cadavers for up to 50 days. Survival varied among species and was dependent upon environmental conditions. *S. carpocapsae*, an ambush forager, might be especially well adapted to survive in cadavers in dry soil because of its tendency to infect insects near the soil surface (Koppenhöfer et al., 1997) and *S. riobrave* might have similar adaptations because of the subtropical, semiarid climate of its area of origin in southern Texas (Koppenhöfer et al., 1995a; Koppenhöfer et al., 1997).

Temperature can be an important environmental factor for the survival of nematodes and for rates of biological processes. EPN species and strains exhibit various tolerances for survival, activity, and reproduction in different temperature ranges, and temperature tolerances have been modified through selection (Grewal et al., 1994; Grewal et al., 1996; Jagdale and Gordon, 1998; Mason and Hominick, 1995; Westerman, 1998). EPNs are usually killed at temperatures above 37 °C (Ghally, 1995; Grewal et al., 1994; Griffin, 1993; Hudson and Nguyen, 1989; Kung et al., 1991; Townsend et al., 1998).

3.3. Conservation biological control and managed ecosystems

EPNs have generally been used for short-term inundative or augmentative biological control but longer-term strategies of conservation biological control might ultimately be more practical and cost effective (Lewis et al., 1998). In the context of conservation biological control, various aspects of agricultural and other managed ecosystems can influence populations of insects and their natural enemies. Two distinct components of biodiversity, planned and associated, exist in managed ecosystems (Vandermeer and Perfecto, 1995). Planned biodiversity is associated with crops or animals intentionally included by the farmer or land manager, and varies depending on management system and practices in space and time. Associated biodiversity includes the flora and fauna that colonize the ecosystem from surrounding habitats and that establish and persist depending on management and structure. The microenvironment in a field can be altered significantly by crop species and practices such as irrigation, planting density, variety selection, tillage regime, fertility inputs, pesticide use, and various other factors. These modifications can affect the abundance and diversity of pests and their natural enemies or enhance host plant resistance to herbivores (Cook and Baker, 1983).

A goal of conservation biological control is to identify the type of biodiversity that is needed to maintain or enhance biological control. Conservation of naturally occurring EPNs through choice of production practices could improve the persistence and efficacy of endemic EPNs as insect control agents (Lewis et al., 1998). However, it is difficult to assess mechanisms or causal effects of production practices on EPNs or on biological control because of the interaction of direct and indirect biotic and abiotic effects. For example, tillage can have far reaching consequences on community composition either directly by killing pests and beneficial organisms or indirectly by changing soil temperature, moisture, and structure. Biotic interactions and their mediation by physical factors could be critical for conservation biological control with EPNs but practices that favor EPNs and soil biodiversity in general might also favor the natural enemies of EPNs (Bellows, 1999; Sayre and Walter, 1991; Stirling, 1991). In laboratory and greenhouse experiments, EPNs that give effective control of pests in depauperate planting media often show lower efficacy in native soil with more complex soil communities (Ishibashi and Kondo, 1986; Timper and Kaya, 1992; Timper et al., 1991).

The success of natural enemies of above ground herbivorous insects can often be related to plant species or variety (Barbosa and Benrey, 1998). Similarly, crop varieties directly affect the soil abiotic environment (e.g., soil temperature and moisture) through shading and water uptake, and the biotic environment through the provision of particular insect hosts associated with the crop. Root density in a system can affect the ability of EPNs to find a host insect (Choo and Kaya, 1991) and hydraulic lift associated with plant roots can create favorable conditions for EPNs and their insect hosts in otherwise dry surface soils (Duncan and McCoy, 2001). The efficacy of natural enemies of herbivorous insects can often be related to plant secondary chemistry, and this has been demonstrated for several pathogen groups, including EPNs (Barbercheck, 1993; Barbercheck et al., 1995; Epsky and Capinera, 1994; Grewal et al., 1995b).

In agriculture, tillage is especially disruptive to the soil environment and can influence the survival and persistence of EPNs. Soil faunal biomass often drops with increased agricultural usage, especially where conventional tillage is practiced (Stinner et al., 1988). Diversity and abundance of predators are greater under no-till than under conventional-till, and natural control of pest insects in soil may be enhanced in conservation tillage systems (Brust, 1991; Letourneau, 1998; Stinner and House, 1990). The greater complexity of the soil environment associated with relatively high levels of crop residue in no-till regimes might influence the abundance of EPNs through provision of a greater number and diversity of hosts. Under a conventional tillage regime, the soil surface tends to have greater fluctuations in temperature and moisture than under no-till or reduced tillage, and EPNs are often more frequently detected in reduced tillage regimes (Hsiao and All, 1998; Hummel et al., 2002; Millar and Barbercheck, 2002; Shapiro et al., 1999b). However, the effects of tillage can depend on EPN species (Millar and Barbercheck, 2002). When non-native *S. riobrave* were applied to no-till and conventional till cornfields containing native *H. bacteriophora* and *S. carpocapsae*, both *H. bacteriophora* and *S. riobrave* were favored by tillage whereas *S. carpocapsae* was favored by the no-till regime. This result might be explained by differences in EPN foraging strategies (Gaugler et al., 1997)

The application of fertilizers to soil represents a nutrient disturbance that can have profound direct and indirect effects on the abundance and community composition of soil biota (Neher and Barbercheck, 1999). High concentrations of mineral or manure-based fertilizers can be detrimental to soil biota because of toxicity (e.g., anhydrous ammonia) or high osmotic pressure from salts (Andrén and Lagerlöf, 1983). In laboratory experiments, prolonged (10- to 20-day) exposure to high inorganic fertilizer concentrations inhibited EPN infectivity and reproduction, whereas short (1-day) exposures increased infectivity (Bednarek and Gaugler, 1997). *Heterorhabditis bacteriophora* was more sensitive to adverse effects of fertilizer than were two species of *Steinernema*.

Additions of organic matter effectively change the soil environment and can increase the diversity of organisms. Organic materials can improve the physical properties of the soil that directly and indirectly affect EPNs (e.g., bulk density, porosity, and moisture-holding capacity), and enhance plant growth and health. Organic amendments can be highly variable and have been used successfully to create phytopathogen-suppressive soils, but almost no documentation exists on the effects of these amendments on popula-

tions of EPNs. The strategy for increasing the suppressiveness of soils for phytopathogens is based on stimulation of high levels of biological diversity (Windels, 1997). In the creation or restoration of disease-suppressive soils, it is rare that any single biotic or abiotic factor accounts for suppression of disease (Hoitink and Fahy, 1986).

In field studies, organic manure used as fertilizer has either increased or decreased EPN establishment and recycling. Bednarek and Gaugler (1997) found that the application of organic manure resulted in increased densities of native *S. feltiae*, whereas NPK fertilizer suppressed nematode densities. The authors concluded that inorganic fertilizers are likely to be compatible with EPNs in tank mixes and should not reduce the effectiveness of EPNs applied for short-term control as biological insecticides, but might interfere with the use of EPNs as inoculative agents for long-term control. Shapiro et al. (1999a) found that applications of *S. carpocapsae* reduced damage to seedling corn by the black cutworm, *A. ipsilon*, in soil amended with fresh cow manure, composted manure, or urea except at the higher rate of fresh manure. Black cutworm damage in EPN-treated plots was greater in plots with fresh manure than in plots without fertilizer. Amendments of urea or composted manure did not have a detrimental effect on suppression of the black cutworm by *S. carpocapsae*. In field and laboratory tests, pathogenicity of *S. carpocapsae* was reduced by poultry, swine, and beef cattle manure (Hsiao and All, 1997).

Although tolerance of EPNs to insecticides is variable (Smith, 1999), several commonly used insecticides, fungicides, herbicides, miticides, and synthetic fertilizers are not detrimental to EPNs and can be applied with EPNs in tank mixes (Georgis and Poinar, 1994; Smith, 1999). Not surprisingly, nematicides (e.g., fenamiphos) are generally not compatible with EPNs. The effect of pesticide applications on endemic EPNs has not been assessed.

4. Estimating EPN abundance

4.1. Developing an efficient and cost-effective sampling methodology

Sampling is a fundamental aspect of both basic and applied ecology and, for EPNs, is necessary to verify application efficiency and monitor introduced and endemic populations. However, sampling can be time-consuming and expensive; and the difficulties and limitations associated with sampling are primary impediments to research on the population biology of EPNs. Here, in an effort to provide a more efficient and cost-effective sampling procedure for EPNs, we examine current methods, develop a theoretical model, and validate some of the assumptions of the model with laboratory data.

4.2. Estimating EPN density

Standard methods for estimating organism abundance are straightforward (e.g., Fan and Hominick, 1991; Koppenhöfer et al., 1998). Samples are taken from a selected habitat, the organisms are extracted and counted, and the abundance is expressed in terms of habitat area or volume. For EPNs, the standard sample is usually a soil core, soil auger or golf cup cutter. A typical sampling procedure involves taking several samples from a square meter of habitat, consolidating the samples, and extracting the EPNs from a series of subsamples. For extraction, the samples are baited with wax moth larvae (*G. mellonella*) exposed for a fixed period (usually 3 days). This technique also aids in the identification of the EPNs because characteristics of the cadavers are diagnostic for particular species or species groups. Following the exposure period, dead *G. mellonella* are dissected and the number of EPNs within the cadaver is counted. To extract all EPNs in a sample requires successive rounds of baiting until no more insects are infected. However, if the number of EPNs is large, then this could involve numerous rounds of baiting, dissecting, and counting. A further problem with exhaustive baiting is nematode mortality over the baiting period, especially for short-lived EPNs (e.g., heterorhabditids) and soils containing abundant natural enemies. Koppenhöfer et al. (1998) did as many as eight baiting rounds over a 3-week period whereas Stuart and Gaugler (1994) reported heterorhabditid infections through 14 rounds of baiting during an 11-week period.

The current sampling method is accurate, reliable, and robust, but the major disadvantages are the time and expense involved for successive rounds of baiting, dissecting, and counting. Moreover, because there is a lower limit to the size of soil sample that is practical to expose to *G. mellonella*, there is also a limit to the sensitivity of the assay. Another problem derives from our inability to distinguish between cases where the bait is uninvaded because the soil sample is devoid of EPNs versus the situation where EPNs are present but unable to find or successfully attack the bait in the time allowed. At some point the decision must be made to stop baiting. A typical stopping rule is two consecutive baiting rounds without infections. This rule is practical but has never been validated theoretically or experimentally. Thus, sample precision at low densities is unknown, but theoretical considerations suggest it is lower than at high densities.

Several assumptions underlie the present approach. First, the method explicitly assumes that a successful assay is achieved when all EPNs in the sample have entered a bait insect. If one EPN fails to penetrate a bait when the sample number is high, then the error is small with precision roughly proportional to the sample number. However, if one EPN fails to invade a host when the sample number is low, the error is high and the precision correspondingly low. Second, the approach assumes implicitly that penetration rate is independent of exposure time. The time taken for an EPN to encounter a bait insect depends on how close to an EPN the insect (or insects) were placed and this is not independent of the EPN population density in the sample. While this may be acceptable at high population

densities where the time for an EPN to encounter an insect is small, at low population densities, penetration will depend on the time it takes for the EPN and insect to become spatially coincident. For this reason Koppenhöfer et al. (1998) exposed baits for three days to allow sufficient time for movement of EPNs through the soil sample. Assuming three days is long enough to ensure coincidence of EPN and bait, the penetration success rate, dS/dt, is proportional to the EPN density, P, $dP/dt = k \cdot P$. Thus, the number of penetrations is $K = k \cdot \exp(t \cdot P)$, where t is exposure time, k is a proportionality constant, and $\exp(\cdot)$ is the exponential function. In view of the fact that penetration rate requires dissection of all cadavers, what is the relationship between the death rate of the bait and the EPN density? In principle, the cause of death is the successful entry and release of symbiotic enterobacteria into the hemocoel of the host by a *single* EPN. All other EPN entries are superparasites. In this situation, host death rate is proportional to the ratio of parasites to hosts, $dK/dt = k \cdot P/N$, because the death rate must go down with the number of potential hosts due to competition between baits for EPNs. The number of deaths is therefore $K = k \cdot \exp(t \cdot P/N)$.

That superparasitism occurs is easily demonstrated. Fig. 1 shows the penetration and death rates for *G. mellonella* exposed to *S. feltiae* for periods up to 64 h. Mortality of *G. mellonella* exposed for only 4 h is almost 95%, whereas the penetration rate is only about 6%. By 16 h, exposure mortality is 100% and penetration rate has risen to 27% and continues to increase long after all bait insects are dead. Interestingly, the penetration rate increases logarithmically with time over 64 h, whereas the death rate is a log–probit relationship that reaches its asymptote between 8 and 16 h. Other EPN species probably exhibit similar levels of extreme superparasitism.

4.3. Parasite–host interactions

To understand the implications of the difference in mathematical form of penetration rate and death rate, we consider the ecological theory of parasite–host interactions. EPNs are similar to insect parasitoids except parasitoids usually sting multiple hosts and deposit a single egg on each, although there are numerous exceptions. The logic of the parasite–host interaction is unaffected by whether the EPN is an "ambusher" or "cruiser" (e.g., *H. bacteriophora*).

The mathematical ecology of host–parasitoid interactions is well developed (Hassell, 2000) and underlies the formulation of our model. In its original form, the change in the host population is related to the change in the parasite population by a pair of coupled difference equations in which the number of new parasites is equal to the number of hosts successfully parasitized (Nicholson and Bailey, 1935). The number of hosts not parasitized is the zeroth term in the Poisson series with parameter $\mu = N_e/N$, the number of successful parasitoid encounters per host. The number of encounters is proportional to the product of the number of hosts and parasites (aNP). The "area of discovery," a, is the probability that a given parasite will encounter a given host. Elaborations of this model incorporate more behavior: interference between searching parasitoid females modifies a (Hassell and Varley, 1969), and aggregation can be modeled using the negative binomial distribution instead of the Poisson (May, 1978). Another elaboration, called the functional response, describes the process of hunting and acquisition of a host by the parasite. This relates the number of hosts killed per parasite to the host density. One form of functional response, known as the "disk equation" (Holling, 1959), incorporates the time taken by a natural enemy to quell and oviposit in (or kill and eat) the host. This Type I functional response has the number of hosts attacked increasing to an asymptote as host density increases. The exact form of the equation differs slightly for predation, which removes prey from the population, and parasitism, which permits superparasitism or further attacks on the same host. The Type III parasite functional response defines the relationship between the number of hosts killed, K, and host population density, N, as a sigmoid curve

$$K = N \cdot \left[1 - \exp \left\{ \frac{-bTNP}{1 + cN + bT_h N^2} \right\} \right], \tag{1}$$

where P is the parasite density, T is the total time spent searching, T_h is the "handling time," and b and c are parameters governing the shape of the sigmoid curve.

In considering the sigmoid mortality curve in Fig. 1, we asked whether functional response could model the mortality of baits exposed to EPNs. We emphasize that we are
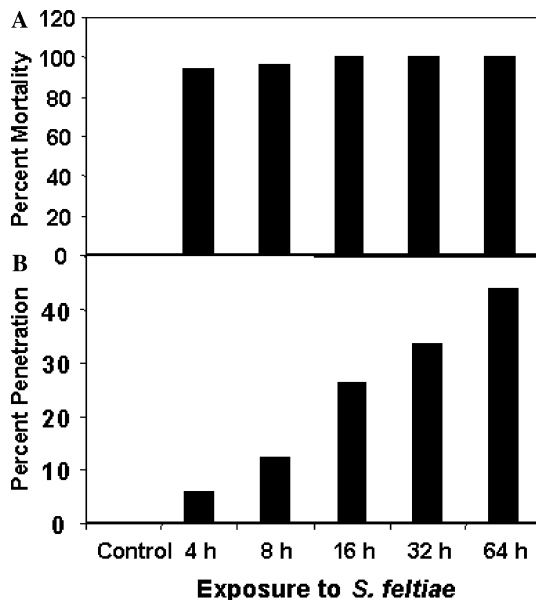

Fig. 1. Penetration of *Galleria mellonella* baits by *Steinernema feltiae* demonstrates the extreme level of superparasitism displayed by entomopathogenic nematodes. Baits exposed to *S. feltiae* for varying periods receive fatal doses within about 4 h (A), but the percentage of applied nematodes penetrating the baits continues to rise up to 64 h, long after a fatal dose has been received (B).

using functional response as a model for the relationship between host mortality and population density at the *population* level without any implications of behavioral change by an *individual* parasite in response to host population density. Clearly, individual EPNs do not exhibit a functional response because they only utilize a single host rather than a series of hosts.

4.4. Estimating the number in a sample

Koppenhöfer et al. (1998) conducted extensive experiments to determine equations to estimate EPN population density in a sample from the number of bait insects killed. Their data and analysis for *S. glaseri* are presented in the top three graphs in Fig. 2. Fig. 2A relates the logarithm of the number of EPNs that was found in baits against the logarithm of the number of baits killed. The data cover almost two orders of magnitude of prey killed and four orders of EPNs recovered. There is a very strong positive relationship suggesting a power law, $N_e = a \cdot K^b$. The coefficient of determination is $R^2 = 0.824$ and is highly significant ($p < 0.01$). In Fig. 2B, the number of EPNs recovered from baits is plotted against the known number of EPNs to which the baits were exposed, again on double log scales. This is clearly an excellent power law with $R^2 = 0.959$ ($p < 0.01$). Fig. 2C combines the previous two to relate the (known) EPN population density to the number of prey killed, again on double log scales. The fit is acceptable, $R^2 = 0.743$ ($p < 0.01$) and provides a useful equation to estimate the number of EPNs in a sample from the number of prey killed. Koppenhöfer et al. (1998) performed this experiment under a variety of conditions and with *H. bacteriophora* and *S. carpocapsae* in addition to *S. glaseri*. In each case, they obtained power laws enabling good estimates of EPN population density from bait mortality.

4.5. Sigmoid functional response

Reexamining the data on number of EPNs penetrating the bait insects against bait insects killed for the three EPN species in Figs. 2D–F, we compare the fits of power laws and logistic curves. In each case the logistic, $N_e = a + c/[1 + \exp(-b \cdot (K - \mu))]$ (where a, b, c, and μ are parameters) fits significantly better than the power law.

The coefficients of determination for the log fits are all approximately $R^2 \approx 0.8$ while the fits using the logistic model are consistently slightly better (Table 1). The curvilinear relationship between number of EPNs penetrating baits and number of baits killed is suggestive of the functional relationship relating number of prey or hosts killed to the prey or host population density. The graphs in Figs. 2G–I present the number of prey killed against prey density (on linear scales) with the Type III functional response Eq. (1) fitted. In each case the fits are significant (Table 1).

Eq. (1) has two parameters that can be interpreted, handling time $= T_h$ and area of discovery $= a'T$, where $a' =$ the instantaneous search rate which is a function of b and c and is the effective area searched by the parasite which in turn depends on the parasite and host population densities and their relative rates of movement. The theory does not depend on whether prey or parasite, or both, do the searching, so the equation (at the population level) is applicable to both cruiser and ambusher EPNs.

4.6. Finding a shortcut

By modeling, the relationship between number of prey killed, prey available, and parasite number with the Type III functional response, we can estimate the number of EPNs in a sample by rearranging Eq. (1) to make the prey number (N) and prey killed (K) a function of the parasite number

$$P = \log\left[\frac{N}{N-K}\right] \cdot \left[\frac{1 + cN + bT_h N^2}{bTN}\right]. \tag{2}$$

Here T is the time available for search by the parasites and b, c, and T_h are the parameters previously estimated. With this model, we can offer an alternative method for estimating EPN numbers using time as the independent variable.

Instead of serially exposing bait insects to a sample until no more are killed, we expose a single insect in each of n well plates for a range of times, for example 1, 2, 4, 8, 16, and 32 h. The number of bait insects exposed need not be the same for each time period; more generally the number exposed is n_t. At the end of each exposure period the bait insects are removed, incubated and evaluated after about 72 h for mortality. It is necessary to dissect only dead baits to confirm that EPNs are present. Eq. (2) is then evaluated using all times T and the corresponding N_t ($=n_t$) and K_t, a variable that will increase with T. The best estimate of number of parasites per soil sample is the average $E\{P_t\}$; the variance is $V\{P_t\}$.

We propose this method for estimating EPNs in field samples as an alternative to exhaustive baiting. Clearly it is not without its difficulties. It uses a similar number of bait insects as the older method but does not require counting the EPNs inside the cadavers, which is a significant time saving. The entire operation can be completed within 72 h. Like the earlier method, this one requires calibration and parameter estimation for all candidate species; and in this model there are three parameters to estimate instead of two. Furthermore, because both handling time and area of discovery could be influenced by soil conditions, it may have to be calibrated for several candidate media. The extra parameter also reduces the degrees of freedom and the goodness of fit, and so it might present the impression that it is a less useful model. Furthermore, the model is highly non-linear and fitting can be problematical. The next step is for field researchers to evaluate this model with new data.
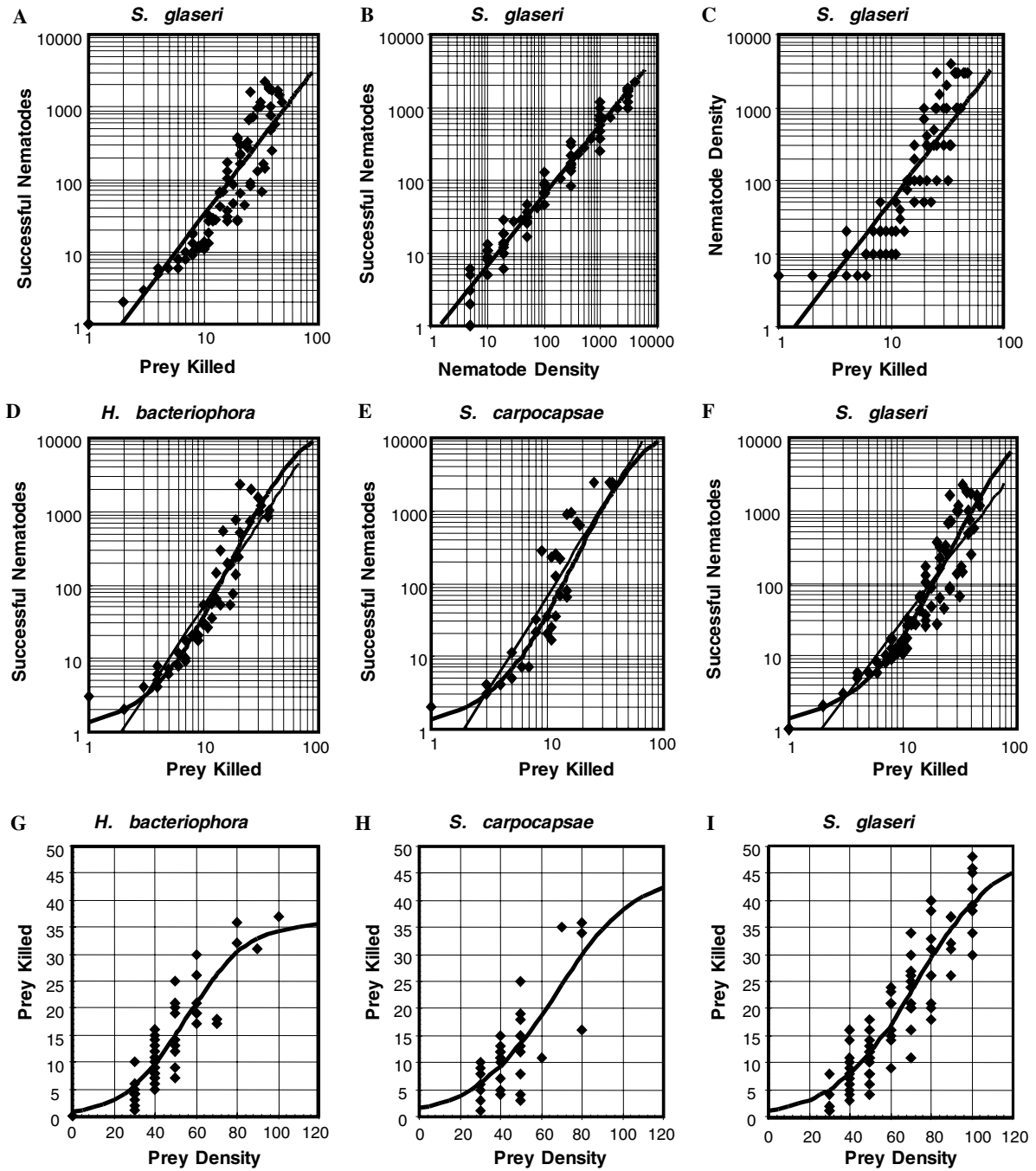
Fig. 2. (A–I). Data of Koppenhöfer et al. (1998) estimating entomopathogenic nematode population density in soil by correlation between bait insect mortality and nematode penetration reanalyzed to demonstrate the relationship between the proportion of prey killed to the prey density. See the text for details.

5. Stochastic and spatially explicit models of EPN population dynamics

5.1. Mathematical models and population dynamics

Mathematical models have been used extensively in entomology for integrating research on population dynamics but have only recently been applied to the study of EPNs. Population modeling begins with organizing information regarding the life history of the organism or organisms of interest, a process that tends to explicitly identify areas where existing data are sufficient for a quantitative, predictive understanding of population dynamics and where they are not. As described above, extensive research has been undertaken into the population dynamics of EPNs, but gaps still exist. To fill in needed information,

Table 1
Coefficients of determination for fits of power law and logistic models for the relationship between nematode penetration success and prey killed and the type III functional response model for baits killed in relation to bait density

Nematode species	Model		
	Power law (R^2)	Logistic (R^2)	Functional response (R^2)
Heterorhabditis bacteriophora	0.846	0.912	0.783
Steinernema carpocapsae	0.796	0.866	0.600
Steinernema glaseri	0.824	0.874	0.827

additional laboratory or field experimentation might be required. Once such gaps are filled, the model integrates information regarding the organism into a single package, a package that can be used to predict population dynamics and population responses to particular stimuli. Such predictions become hypotheses to guide further empirical research. An example of this is provided below.

Population dynamics refers to temporal and spatial changes in populations, and it is these changes we seek to understand and predict using mathematical models. Population dynamics can be summarized as increases through reproduction and immigration, and decreases through mortality and emigration. Although rates of reproduction, mortality, and movement can result from very complex interactions of many different factors, models provide an organized and quantitative summary for detailed analysis.

5.2. Review of mathematical modeling of EPN populations

Previous attempts at modeling EPN populations have focused on either spatial dynamics or temporal dynamics, but not both (Barbercheck and Hoy, 2005). Nonetheless, they have provided good examples of research using mathematical models that improved understanding of EPN population dynamics and biological control strategies. Briefly, changes in spatial distribution over a short period of time were considered by Van Der Werf et al. (1995) and by Westerman and Van der Werf (1998) to model the movement of released EPNs toward an insect host in a vertical soil column. Although focusing on a period short enough that reproduction and mortality were not considered, the model and its analysis did provide useful insight into strategies to improve the effectiveness of EPN application for control of black vine weevil. Changes in population density over much longer time periods were modeled by Fenton et al. (2000, 2001, 2002), but without consideration of variation in space. Because the system modeled was a relatively uniform environment in both time and space, a mushroom production facility with carefully controlled temperature and humidity, the assumptions were appropriate. Furthermore, the study again led to very useful strategic information for the number and timing of EPN applications required to control sciarid fly populations in mushroom production.

5.3. Modeling spatial and temporal changes

In both managed and unmanaged field settings, both spatial and temporal variation is typical. Spatial variation

in edaphic conditions, microclimate, topography, soil management, and plant communities all affect the survival, reproduction, and movement of EPNs. Likewise, seasonal changes in climate and soil management, and their effects on the rest of the soil ecosystem, influence EPN population dynamics. Some of the impacts of these changing conditions in the soil exert their effect through altering reproduction, either through the availability of insect hosts, their quality as hosts, or environmental effects on rates of reproduction within these hosts. Other impacts have more to do with survival of free-living IJs in the soil. In fact, effects on reproduction and mortality are likely to interact in their impacts on EPN population dynamics. To gain some insight into interaction between factors that affect EPN recycling in insect hosts (i.e., "reproduction"), and factors that influence survival in the soil and in the absence of hosts (i.e., "survival"), new simulation modeling studies were conducted.

A simple descriptive model was developed for *H. bacteriophora* population dynamics in 1 m² patches of soil (Fig. 3) that is very similar to the published models designed to simulate EPN population dynamics (Fenton et al., 2001, 2002). An novel extension, however, was the use of stochastic simulation of multiple patches. Stochastic models contain random variables, such that each run of the model produces a different quantitative result. This random variation can reflect the variation seen in the field in a heterogeneous environment, and simulating multiple patches
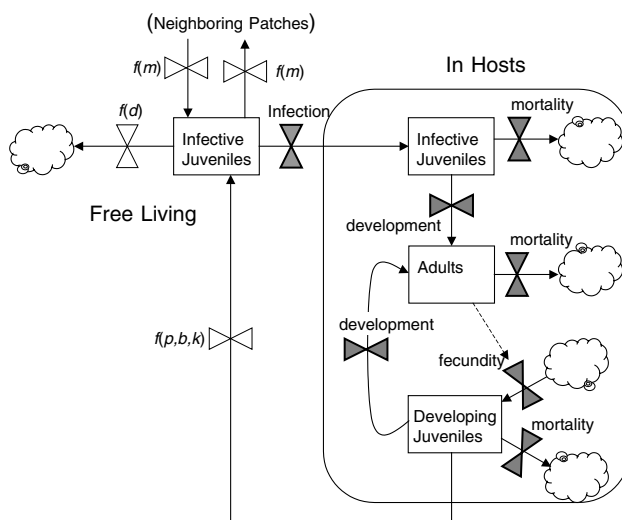

Fig. 3. A simple descriptive model for *Heterorhabditis bacteriophora* population dynamics in 1-m² patches of soil.

allows the model prediction to be compared with the results of survey sampling in multiple soil patches. In this way, the model provides a link to empirical field studies.

The model uses difference equations to describe changes in the free-living IJ EPN population, N, over time, t, in small increments of time, Δt, in a m^2 patch of soil. The free-living IJs were chosen because they can be sampled in field soil and the m^2 patch of soil is a standard sampling unit in the field. Therefore, results from this model can be compared easily with results from field samples. Births were modeled as the product of a Bernouli distributed event representing the emergence of IJs, yielding either a 0 or 1, and an Erlang distributed number of IJs emerging. The Erlang distribution was characterized with a given mean number of IJs and a shape parameter for the distribution to describe the variability in numbers of IJs produced for each infection, a function of the variability in infection and reproduction rates in the arthropod host community. The Bernouli distribution requires a parameter to describe the probability of emergence, p, which was set as a constant but was modeled as a maximum probability that was reduced according to population size by

$$p'_t = p\{1 - [10,000/(10,000 + N_t)]\}, \tag{3}$$

where p is the mean for the Bernouli distribution representing the maximum probability of emergence and p'_t is the mean adjusted for the current population size. If the population of IJs is large ($\gg 10,000/\text{m}^2$) then the probability of an emergence event will be close to the maximum but it will be reduced for smaller population sizes and tend toward zero as the population declines to very low densities. The resulting change in population due to emergence of IJs is

$$N_{t+\Delta t} = N_t + xy, \tag{4}$$

where x and y are randomly selected from the Bernouli distribution,

$$\text{probability } \{X = x\} = [p'^x_t (1 - p'_t)^{(1-x)}], \tag{5}$$

and Erlang distribution,

$$\text{probability } \{Y = y\} = [k/b\Gamma(k)][ky/b]^{k-1} e^{-ky/b}, \tag{6}$$

respectively, with mean b and shape parameter k for the Erlang distribution. Note that x can be either 0 or 1, and addition to the existing population only takes place when a 1 is generated. Mortality rates were modeled as an exponential decay with a constant rate (also giving a constant half-life, see Strong, 2002), d, estimated either from the literature or from our field studies. The entire model for a single m^2 patch of soil, assuming no immigration or emigration, is,

$$N_{t+\Delta t} = N_t + xy - dN_t. \tag{7}$$

Simulations compared the relative importance of mortality rates in the absence of hosts, the frequency of emergence events, and the average number of IJs emerging during these events. The mortality rates would be a function of the environment absent the host community,

whereas the frequency of emergence events would be a function of host abundance and susceptibility, and the average number of IJs emerging would be a function of host size and quality in terms of EPN reproduction and IJ emergence. The model was programmed and solved in the simulation package Extend (Imagine That, San Jose, CA). For each simulation, *H. bacteriophora* population dynamics were simulated in 100 soil patches for 120 days, approximately the length of the active growing season in Ohio. Patches were initialized according to the frequency of detectable EPN populations in surveys taken in Ohio (Lawrence, 2004): 20% of patches with 10,000 EPNs m^{-2} and 80% of patches with 500 EPNs m^{-2} (the limit of detection was estimated to be 1000 m^{-2}, based on a survey by Campbell et al., 1995). At least five simulations were performed for each of a number of combinations of parameter values. Mortality rates simulated were 0.05, 0.10, and 0.15. All of these are within the range of values reported for *H. bacteriophora* and 0.10 was the mortality rate estimated for an Ohio vegetable production region in previous research (Lawrence, 2004). Probabilities of emergence were varied from 0.025 to 0.05 and then from 0.05 to 0.25 in increments of 0.05, and the average number of IJs per emergence event was varied from 10,000 to 70,000 in increments of 20,000. Mean proportion of patches with detectable populations of *H. bacteriophora* was the variable of interest. Less than the initial 20% detectable patches would indicate loss over time whereas greater than 20% would indicate an increase in detectable sites, a desired result in an area that could benefit from enhanced biological control by EPNs.

To further explore the spatial dynamics of EPNs, movement was simulated among patches along the border of a soil type in which EPNs recycle naturally and another in which EPNs have not been found to naturally recycle. This situation was found in an agricultural region of Ohio in which EPNs were found in grassy field borders but not in the directly adjacent cultivated soils (Lawrence, 2004). Fifteen contiguous m^2 patches were simulated for both the grassy borders and the cultivated soils. Movement was simulated by a constant proportion of the population, m, moving out of each patch during each day of the simulation. One third of the IJs leaving each patch entered each of the 3 adjacent patches, i.e., the patches on either side and in the same soil habitat and one directly opposite the patch in the other soil habitat. Movement out of either end of the linear array of patches was added to movement into the opposite end, preserving the population in the patches being simulated. Although simplified, because it ignores such features as movement on diagonals, movement at greater distances than a single patch, and movement to and from neighboring patches further from the border being simulated, this treatment of movement captures the essential features well enough to examine its qualitative impact on EPN recycling and persistence.

Life history parameters and the model for population change within each patch were as described above. Parameters for mortality rates and probability of an emergence

event differed for the two soil habitats, with lower mortality and higher probability of an emergence event in the apparently more favorable grassy border habitat (0.075 and 0.1 for mortality rates and 0.075 and 0.05 for probabilities of emergence events in the two soil habitats, respectively) and number of IJs was set at 25,000 IJs per emergence event for both soil habitats. The simulations were initialized with 20% of the edge sites and none of the cultivated field sites having detectable populations of IJs (10,000 m^{-2}), and the remaining sites having undetectable levels (500 m^{-2}). Given a low proportion of IJs leaving the patch each day, 0.5%, these parameter values resulted in approximately 20% of sites remaining detectable over time in the favorable soil type and a very low percentage of detectable sites over time in the unfavorable soil type, as observed in the field. Proportion of IJs moving among patches per day was increased systematically according to the following progression: 0.005, 0.01, 0.025, 0.05, 0.075, 0.1, 0.15, to examine the impact of increasing rates of movement on the persistence of EPNs, as measured by the percentage of soil sampling sites with detectable ($\geqslant 1000$ IJs m^{-2}) population densities.

5.4. Model results and hypotheses generated for empirical research

Results of initial simulations of population dynamics without movement (Figs. 4A–C) were collated as the percentage of sites estimated to be detectable after 120 days given a set survival rate and various combinations of host quality (numbers of IJs per cadaver) and quantity (probability of an IJ emergence event). The model results were very sensitive to the rates of mortality of *H. bacteriophora* in the absence of insect hosts. A relatively high mortality rate (Baur and Kaya, 2001; Fenton et al., 2000; Strong, 2002), $d = 0.15$, resulted in a decreasing percentage of detectable sites under all combinations of host quality and quantity simulated (Fig. 4A). At a mortality rate of $d = 0.1$ (Fig. 4B), relatively high host quality and quantity were predicted to be required to either maintain the same

percentage of sites with detectable populations, or increase the percentage. A range of parameter values, beginning at a probability of IJ emergence greater than 0.1 emergence events per day, was evident within which small changes in either host quality or quantity were predicted to have large effects on the percentage of detectable sites. At a lower mortality rate of $d = 0.05$ (Fig. 4C), results were also very sensitive to host quantity but not particularly sensitive to host quality, at least for mean numbers of IJs per cadaver greater than 20,000.

Therefore, the simulation results predict that factors influencing survival in the absence of hosts (e.g., tillage, insecticide and fertilizer applications, soil physical, and microbial environment) are likely to interact strongly with factors influencing EPN recycling (i.e., the quality and especially the quantity of arthropod hosts), as suggested by Lewis et al. (1998). Both survival and recycling can be influenced by management practices, and understanding their impacts and interactions in a quantitative way could have very practical benefits in improving biological control. Resolving their relative importance in agroecosystems will require detailed experimental work but specific predictions from the simulations provide some guidance. For example, mortality rates $\geqslant 0.15$ are predicted to result in extinction of EPN populations regardless of host supply, and so empirical estimation of the threshold mortality rate for long-term persistence in a particular soil would be an important initial determination. At a mortality rate that we observed in the field, both host abundance and host quality (IJs produced) are important determinants of the widespread occurrence of detectable populations. If mortality rates were lower, however, then focus could shift from host quality in terms of the number of IJs produced per host to host abundance and perhaps susceptibility. Any means of manipulating the host community, such as by management of the types and quantity of vegetation and the level of broad-spectrum insecticide use, could be a means of both experimentation and eventual population management. Finally, predictions suggest that detectable sites are much more likely to remain detectable than are
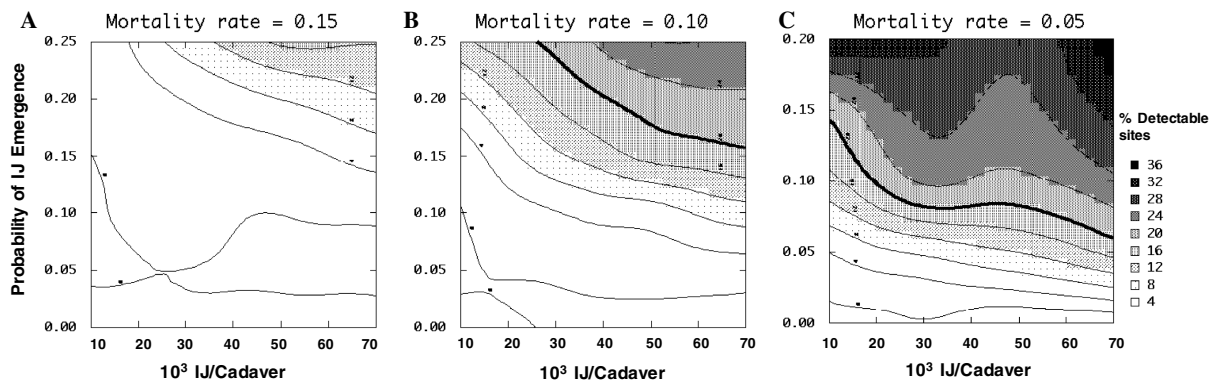

Fig. 4. Results of simulations of population dynamics without movement collated as the percentage of sites estimated to be detectable after 120 days given a set survival rate and various combinations of host quality (numbers of infective juveniles (IJs) per cadaver) and quantity (probability of an IJ emergence event) with mortality rates of $d = 0.15$ (A), $d = 0.1$ (B), and $d = 0.05$ (C).

undetectable sites to become detectable. Nonetheless, detectable populations can arise at previously undetectable sites if even a very small percentage of the population is able to persist for very long periods. Field experimentation on very low and possibly quiescent populations of EPNs would be challenging but could be important in understanding their population dynamics in natural systems.

Once movement was added to the model, the proportion of sites with detectable populations of *H. bacteriophora* was predicted to increase with increasing rates of movement in both soil habitats. Although this result might be expected, it suggests that even with greater mortality rates and lower probabilities of emergence events in cultivated soils, movement among sites would greatly increase the probability of persistence. Furthermore, as movement rates increase, the probability of detectable populations in the two soils begins to converge, without any accompanying changes in mortality or reproductive rates. This suggests that a strategy to conserve EPNs could involve facilitation of movement, in addition to decreasing mortality rates and increasing reproductive rates. Extrapolating this result to the field again leads to researchable questions. Would irrigation combined with sufficient host supply result in a more spatially uniform population density? EPNs move in soil water, and both surface and subsurface movement of water could enhance rates of EPN movement. If so, then does spatial uniformity of naturally occurring infections increase after heavy rains and flooding events? Phoresy has been reported (Timper et al., 1988), but the full impact of this form of dispersal, and how it varies with different insect host communities, is not known. Are some insect host species particularly well suited to the phoretic movement of EPNs? If so, and particularly if they are not pest species, then these could provide an additional means to increase the persistence of EPN populations.

These simulation studies provide insight into the population dynamics of EPNs. Simulation models were developed that are consistent with the known biology of these species, in concise mathematical form. The results of the simulations lend insight into how populations recycle naturally and why detectable populations are observed to be both patchy and ephemeral. Taken alone, the results are simply a more complex and complete hypothesis regarding population dynamics of EPNs than would be possible without the model that produced them. However, taken as a prediction, the same results can be a useful guide to needed and focused field research toward more rapid progress in understanding the population dynamics of EPNs.

6. Conclusions

EPNs are important natural enemies of insects in soils throughout the world and could play a fundamental role in regulating insect populations under various circumstances. Our ability to manipulate EPN populations in managed habitats through augmentation and conservation provides unique opportunities for the effective and environmentally benign control of soil insect pests. However, the extent to which such manipulations impact soil ecosystems remains largely unknown and we are only beginning to comprehend the complex trophic webs that are involved. Understanding the distribution, abundance, and dynamics of EPN populations within their broader ecological context presents interesting questions and challenges for current research. We know almost nothing about the spatial and genetic structure of populations, how populations vary over time and among sites, the various interactions that occur with the biotic and abiotic environment, and how these characteristics vary among species, strains and habitats. Past research has given us an important foundation upon which to build but might provide little more than a glimpse of the rich complexity, diversity, and variability that could be present. New ideas and methods for sampling EPNs and modeling population dynamics as described here should contribute to important advances. Meanwhile, the economic and social realities of modern agriculture, human population growth, and environmental degradation in both developed and developing countries provide an important backdrop for these studies and assure that EPNs will remain prime subjects for continuing basic and applied ecological research.

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