Part II

Entomopathogenic Nematodes

2 Biology and Behaviour

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2.1. Introduction

Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae are lethal pathogens of insects. These pathogens contribute to the regulation of natural populations of insects, but the main interest in them is as an inundatively applied biocontrol agent. Their success in this role can be attributed to the unique partnership between a host-seeking nematode and a lethal insect-pathogenic bacterium. Because of their biocontrol potential, considerable attention has been directed over the past few decades to *Heterorhabditis* and *Steinernema* and their respective bacterial partners, *Photorhabdus* and *Xenorhabdus*. Landmark publications

 Nematodes as Biocontrol Agents (eds P.S. Grewal, R.-U. Ehlers and D.I. Shapiro-Ilan)

reviewing the biology and use of EPNs are Gaugler and Kaya (1990) and Gaugler (2002). A third genus of EPN, *Neosteinernema*, has received almost no attention since the first report of its association with termites by Nguyen and Smart (1994).

Although heterorhabditids and steinernematids are not closely related (Blaxter *et al.*, 1998; see also Chapter 1, this volume), they have many features in common. These similarities, including their association with insect-pathogenic bacteria, are presumed to have arisen through convergent evolution (Poinar, 1993). In both *Steinernema* and *Heterorhabditis* there is a single free-living stage, the infective juvenile (IJ), that carries in its gut bacteria of the genus *Xenorhabdus* and *Photorhabdus*, respectively (Boemare *et al.*, 1993). On encountering a suitable insect, the IJ enters through the mouth, anus or spiracles and makes its way to the haemocoel. Some species may also penetrate through the intersegmental membranes of the insect cuticle (Bedding and Molyneux, 1982; Peters and Ehlers, 1994). In *Heterorhabditis* spp. this is facilitated by the possession of an anterior tooth (Bedding and Molyneux, 1982).

In the haemocoel, the IJ releases cells of its bacterial symbiont from its intestine. The bacteria proliferate in the nutrient-rich insect haemolymph. Death of the insect ensues, normally within 24–48 h. The IJs recover from their arrested state and feed on the proliferating bacteria and digested host tissues. The nematodes develop through the fourth to the fifth (adult) stage, and then reproduce. One or more generations may occur within the host cadaver, depending on available resources.

Steinernematids and heterorhabditids differ in their mode of reproduction. In heterorhabditids, the first generation consists of self-fertile hermaphrodites, while males, females and hermaphrodites are produced in subsequent generations (Dix *et al.*, 1992). In steinernematids, all generations reproduce by amphimixis (cross-fertilization involving males and females) (Poinar, 1990). Recently, a *Steinernema* sp. was found to depart from the norm; in that species, the majority of individuals are self-fertile hermaphrodites, while a small proportion of the population in each generation are males (Griffin *et al.*, 2001). Thus, heterorhabditids and at least one *Steinernema* sp. can develop in a host when a single IJ invades, while most steinernematids require at least two individuals to colonize the host before multiplication can occur.

Initially, eggs are laid into the host medium. In older females or hermaphrodites, eggs hatch in the uterus, and the developing juveniles consume the parental tissues – a process known as 'endotokia matricida' (Johnigk and Ehlers, 1999). This use of the parental tissues results in rather efficient conversion of insect biomass to IJ biomass. Juveniles developing with adequate food supply mature to adults, while those developing in crowded conditions with limited food resources arrest as IJs. Hundreds of thousands of IJs may be produced in larger hosts. These emerge from the insect cadaver over a period of days or weeks, to begin the search for new hosts (Fig. 2.1).

Newly emerged IJs retain the moulted second-stage cuticle as a sheath. Particularly in *Heterorhabditis* spp., the sheath may help in protection against desiccation, freezing, and fungal pathogens (Timper and Kaya, 1989; Campbell and Gaugler, 1991a; Wharton and Surrey, 1994). The loose-fitting sheath of steinernematids is soon lost as the nematode moves through soil, while the tighter-fitting heterorhabditid sheath is not so easily lost (Campbell and Gaugler, 1991b; Dempsey and Griffin, 2003).

2.2. Nematode–Bacterial Symbiosis

Knowledge of the nematode–bacterial symbiosis is essential to understanding the pathogenicity of the complex for target insects, and is fundamental for successful mass production. Both partners benefit from the association: the bacteria are largely responsible for the rapid death of the insect, they provide a suitable nutritive medium for nematode growth and reproduction and suppress competing organisms by the production of antibiotics. The nematode

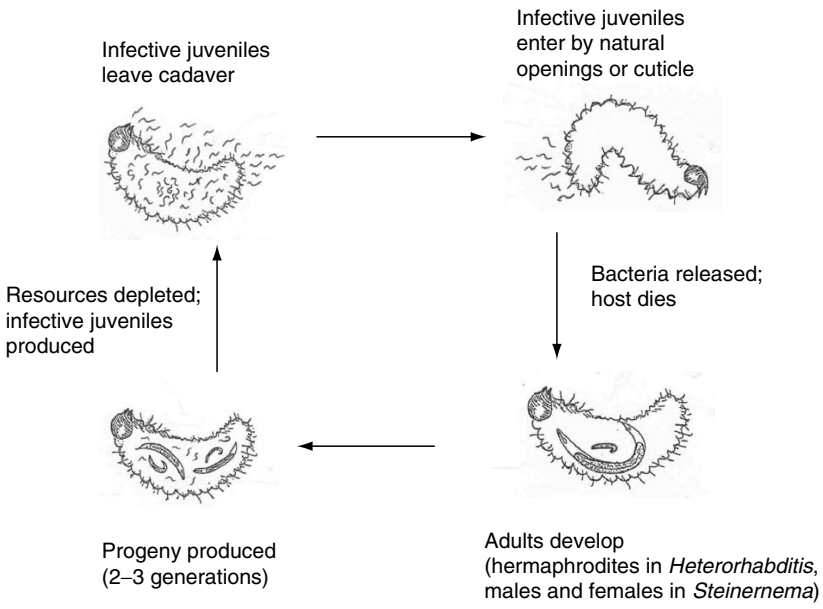

Fig. 2.1. Simplified life cycle of entomopathogenic nematodes (EPNs) (*Steinernema* spp. and *Heterorhabditis* spp.).

protects the bacteria in the external environment, vectors them into the insect haemocoel and, in some associations, inhibits the insect immune response.

The nematode–bacterial interaction is not obligate: each partner can be cultured separately, but when combined they present a high degree of specificity. The paradox of 'apparent independence and high specificity' is one of the fascinating aspects of the relationship. The symbionts occupy two different ecological niches or states in the life cycle, and thus interact with the nematode at two levels. The first is a phoretic state where the bacteria are retained in, and interact with, the intestine of the non-feeding IJ, apparently without any significant multiplication. *Xenorhabdus* occur in a special intestinal vesicle of *Steinernema* IJs (Bird and Akhurst, 1983), while *Photorhabdus* are mainly located in the anterior part of the intestine in *Heterorhabditis* (Boemare *et al.*, 1996). The second state is a vegetative one, when the bacteria overcome the insect host's defence system, allowing them to multiply unrestrained inside the infected insects.

2.2.1. Bacterial taxonomy and co-speciation with nematodes

Xenorhabdus and *Photorhabdus* are members of the γ-subclass of Proteobacteria and belong to the family Enterobacteriaceae (Boemare, 2002). Since their original description, they have been considered to be Gram-negative, facultatively anaerobic rods, as are all the Enterobacteriaceae. However, both genera are negative for nitrate reductase, and *Xenorhabdus* are negative for catalase: two major positive characters of this family. Moreover, recent results seem to indicate that some groups are strictly aerobic. These recent data, which are incompatible with the classical bacteriological canons, may result in a revision of the description of both genera (Pagès and Boemare, 2003, unpublished data).

There is a close relationship between the taxonomy of the symbiont species and of their nematode hosts. In general, for each species of nematode there is a specific association with a species or subspecies of bacteria (Fischer-Le Saux *et al.*, 1998; Boemare

and Akhurst, 2001, 2003; Akhurst and Boemare, 2003). However, some nematode species share the same species of bacterium. For example, *Xenorhabdus bovienii* is associated with four species of *Steinernema*, and *X. poinarii* is associated with two (Table 2.1). More rarely, some bacterial species share the same nematode species; for example, *Photorhabdus luminescens* and *P. temperata* are both associated with the *H. bacteriophora* group (Table 2.1). The specificity of the nematode–bacterial association can be considered to be the result of partial co-speciation, together with some recent acquisitions.

2.2.2. Phenotypic variation

Phenotypic or phase variation occurs for every strain of symbiont known so far. The

Table 2.1. Correspondence between taxonomy of the bacteria and of the nematodes.

Xenorhabdus spp.	Genotype[a]	*Steinernema* spp.[b]
X. nematophila	No 1, 2 and 3	*S. carpocapsae*
X. japonica	No 18	*S. kushidai*
X. beddingii	No 4	*Steinernema* sp.
X. bovienii	No 5 and 7	*S. feltiae*
	No 5 and 7	*S. affine*
	No 7 and 8	*S. kraussei*
	No 6	*S. intermedium*
X. poinarii	No 17	*S. cuban*
		S. glaseri
Xenorhabdus spp.	No 9	*S. karii*
		S. monticolum
	No 10	*S. serratum*
	No 10 and 11	*S. longicaudum*
	No 12	*S. siamkayai*
	No 13	*S. ceratophorum*
	No 15	*S. arenarium* (syn.: *S. anomalae*)
	No 20	*S. rarum*
	No 21	*S. puertoricense*
	No 23	*S. abbasi*
	No 24	*S. scapterisci*
	No 25	*S. riobrave*
Photorhabdus spp.	Genotype[c]	*Heterorhabditis* spp.
P. luminescens luminescens	No 10	*H. bacteriophora* group Brecon[d]
P. luminescens laumondii	No 13 and 28	*H. bacteriophora* group HP88[d]
P. luminescens akhurstii	No 12 and 27	*H. indica*
P. luminescens	No 11	*Heterorhabditis* sp.
P. temperata temperata	No 14	*H. megidis* Palaearctic group
P. temperata	No 14b	*H. downesi*
P. temperata	No 15	*H. megidis* Nearctic group
	No 16	*H. bacteriophora* group NC[d]
	No 17	*H. zealandica*

[a]New numbering using the PCR-RFLP of 16S rRNA genes methodology of Fischer Le Saux *et al.* (1998) but updated to take account of new genotypes in course of identification (Pagès, Brunel and Boemare, Montpellier, France, unpublished data).
[b]N. Boemare and P. Stock, unpublished.
[c]Numbering of the genotype follows that of Fischer-Le Saux *et al.* (1998), except for symbionts of the Irish strains of *H. downesi* that have the provisional no 14b.
[d]According to Boemare (2002), the NC strain of a nematode identified in the past as *H. bacteriophora* harbours *P. temperata* and not a subspecies of *P. luminescens* as other symbionts of *H. bacteriophora*. The re-isolation of this group in nature is required to control for possible confusion in the previous sampling.

initial isolate from the wild nematode, termed the Phase I variant, possesses two major properties: dye adsorption and antibiotic production (Akhurst, 1980). After *in vitro* subculture, there appears a variable proportion of clones, called Phase II variants, that not only have lost these two properties but are also affected in a range of other phenotypic characters, including colony and cell morphology, motility, endo- and exo-enzymatic activity, respiratory enzymes and secondary metabolites (Boemare and Akhurst, 1988; Smigielski *et al.*, 1994; Givaudan *et al.*, 1995). For every character that can be evaluated the difference between phase variants is quantitative (e.g. the emitted luminescence of the *Photorhabdus* Phase II variant is about 1% that of the Phase I variant) and is probably under the control of a genetic regulatory mechanism that is not yet understood (Forst *et al.*, 1997; Forst and Clarke, 2002). For the purposes of numerical taxonomy, any character that is recorded as positive for any variant should be considered as a positive character of that strain.

What is the ecological role of Phase II? Although such variants may also kill the insect host and are capable of colonizing the IJs, they have never been found associated with naturally occurring nematodes (Akhurst and Boemare, 1990). Moreover, some *Photorhabdus* Phase II variants may be deleterious for their original *Heterorhabditis* (Ehlers *et al.*, 1990). So far, there is no consistent ecological explanation of the significance of Phase II variants though it has been suggested that they represent a survival form (Smigielski *et al.*, 1994).

2.2.3. Pathogenicity

The pathogenic process depends on characteristics of each of the three partners of the interaction: the insect, nematode and bacteria. It is influenced by insect resistance (including humoral and cellular defences) and by virulence factors of the bacteria and of the nematode acting separately or together to overcome the defence system (reviewed by Dowds and Peters, 2002). Pathogenicity, as evaluated by injection into the insect haemocoel, varies between insects. Differences in pathogenicity among bacterial species have also been recorded, principally in larvae of the wax moth *Galleria mellonella*. Thus, most species of *Xenorhabdus* are highly pathogenic, with LD_{50} of less than 20 cells (Akhurst and Dunphy, 1993). In contrast, *X. poinarii* and the symbiont of *Serratia scapterisci* have very little pathogenicity for *G. mellonella* when injected alone ($LD_{50} > 5000$ cells), and their axenic nematode hosts, *S. glaseri* and *S. scapterisci*, are also not pathogenic when injected alone. Re-combination of both partners re-establishes the pathogenicity towards *G. mellonella* (Akhurst, 1986; Bonifassi *et al.*, 1999), illustrating the need for cooperation between both partners to kill the insect. Most *Photorhabdus* strains examined to date have been reported to be entomopathogenic, the LD_{50} usually being < 100 cells (Akhurst and Boemare, 1990). However, some non-pathogenic strains of *Photorhabdus temperata* have been found recently (Pagès, Gaudriault, 2003, unpublished data).

The recent discovery of some strains of *Photorhabdus* that are pathogenic to insects by ingestion (ffrench-Constant and Bowen, 1999) has resulted in an enhanced level of interest in these bacteria. Although development of the bacteria in the insect gut has not yet been reported, some symbionts produce a toxin that is active on the intestinal epithelium from both sides (gut, lumen as well as the haemocoel) (Blackburn *et al.*, 1998). *P. luminescens* possesses toxins, called Tc or toxin complex, that are orally active against Coleoptera and Lepidoptera (ffrench-Constant and Bowen, 2000). Such toxins have also been identified during the sequencing of the genome of another strain of *Photorhabdus* (Duchaud *et al.*, 2003), and in *S. entomophila* (Hurst *et al.*, 2000). Several other virulence factors participate in the pathogenicity of *Photorhabdus* and *Xenorhabdus* (Dowds and Peters, 2002; Forst and Clarke, 2002), including motility (Givaudan *et al.*, 1995, 1996; Givaudan and Lanois, 2000) and haemolysins (Brillard *et al.*, 2001, 2002, 2003).

2.2.4. Isolation of symbionts and maintenance of monoxeny

Only one natural symbiont species has been found in the gut of the IJs of any one nematode species, and this is true for all the species of *Steinernema* and *Heterorhabditis* collected throughout the world over the last 30 years with the exception of the *Heterorhabditis bacteriophora* group, strains of which are associated with two *Photorhabdus* spp. Some nematode species carry fewer bacterial cells, and carry them in only a proportion of the IJs. For instance, *Steinernema scapterisci* carries significantly less symbionts than *S. riobrave* and *S. carpocapsae* (Sicard *et al.*, 2003). Therefore, to be sure of isolating symbiont clones in good condition, the nematode sample from which they are isolated should contain a reasonable number of IJs (*c.*100–1000).

Sometimes bacterial strains other than the symbionts have been found associated with *Steinernema* (Aguillera *et al.*, 1993) or with *Heterorhabditis* (Jackson *et al.*, 1995; Babic *et al.*, 2000) mainly following prolonged maintenance in laboratories. It was shown that they were mostly contaminants of the cuticle (Bonifassi *et al.*, 1999) and there is no definitive evidence that any are inhabitants of the intestine. Recently, sporangia of *Paenibacillus* spp. have been noted adhering to the cuticle of *Heterorhabditis* spp. IJs, and it is suggested that the bacteria exploit the nematode as a phoretic host (Enright *et al.*, 2003).

Mechanisms involved in the specificity of the association between the nematode and its symbiont operate both in the cadaver and in the IJ. Large amounts of antimicrobial organic compounds are produced during *in vivo* multiplication of *Xenorhabdus* spp. and *Photorhabdus* spp. (Webster *et al.*, 2002), preventing global microbial contamination. Bacteriocins active against closely related bacteria such as other species of *Xenorhabdus*, *Photorhabdus* and the nearest genus, *Proteus*, are also produced (Boemare *et al.*, 1992; Thaler *et al.*, 1995). So antimicrobial barriers may play an important role in protecting the specificity of the symbiosis by eliminating microbial competitors, though some bacteria such as the *Paenibacillus* spp. mentioned above appear to be tolerant of these antimicrobials (Enright and Griffin, 2003, unpublished data). Additionally, the symbiotic bacteria must be retained in the monoxenic nematodes by an active recognition process, as illustrated by the fact that aposymbiotic (without symbiont) *Steinernema* did not retain any non-symbiotic bacterium, and rejected any symbiont that was not their natural partner (Sicard *et al.*, 2003). The nature of this recognition process has yet to be discovered, but an important step towards understanding the molecular mechanism of the association was obtained by disrupting the *rpoS* gene of *X. nematophila* (Vivas and Goodrich-Blair, 2001). This gene AQ1 encodes the sigma S factor that controls interactions with hosts in other Gram-negative bacteria. Vivas and Goodrich-Blair (2001) obtained a mutant that was able to induce pathogenesis in insects, but was unable to mutualistically colonize nematode intestines, and such a mutant should prove to be a useful tool for further studies.

2.2.5. Importance of the bacterial symbiont

Recently, Sicard *et al.* (2003) undertook gnotobiological experiments demonstrating the importance of the symbiont for the nematode. Aposymbiotic nematodes inoculated into insect hosts had reduced fitness relative to symbiotic nematodes, showing the importance of the bacteria for efficient reproduction of their corresponding nematode host. This was demonstrated for three species (*S. carpocapsae*, *S. scapterisci* and *S. riobrave*); the most extreme results were those with *S. riobrave*, which did not reproduce without its symbiotic bacteria (Sicard *et al.*, 2003). These results, together with previous ones, such as those showing that combination of *S. scapterisci* and its symbiont re-established the pathogenicity of the complex towards *G. mellonella* and gave the best yields of IJs when produced in this insect or *in vitro* on artificial diet

(Bonifassi *et al.*, 1999), demonstrate the importance of the symbiont for the nematode host. In addition, although development of non-infective stages of *S. scapterisci* occurred on all *Xenorhabdus* spp., the development of IJs to the fourth stage ('dauer recovery') was significantly delayed with *Xenorhabdus* other than the natural symbiont. This development was restored when the culture medium was supplemented with cell-free filtrates from the *Xenorhabdus* native strain (Grewal *et al.*, 1997).

Thus, apart from their pathogenicity for insects, the role played by the bacteria is possibly a nutritional one or the production of a food signal (hormonal) is apparently essential for nematode development, as the experiments of Grewal *et al.* (1997) suggest. This is also indirectly demonstrated by the fact that the symbiotic bacteria are required for successful production of nematodes in bioreactors (see Chapter 3, this volume). Like many soil-dwelling rhabditids, *Steinernema* and *Heterorhabditis* are microbivorous grazers. Nevertheless, the specific requirements provided by their specific bacteria are still unknown.

2.3. Infective Juvenile (IJ) Behaviour

The IJ is morphologically, physiologically and behaviourally adapted to its role in transmission – and hence to its acquired role as the active ingredient of a biological pesticide. A thorough understanding of the materials used is essential for predicting efficacy of any pest management product. As EPNs are active organisms that move, seek their hosts and prefer some hosts to others, a treatment of their behaviour, as it relates to efficacy, follows. IJs have a pair of sensory organs, the amphids, at their anterior end, which are used in detecting cues potentially associated with hosts, and a behavioural repertoire appropriate to their role in host-finding. Their behaviours are divided into four categories that are not mutually exclusive: dispersal, foraging strategies, host discrimination and infection.

2.3.1. Dispersal

Among the many behavioural characters that impact the biocontrol potential, the location of the IJ within the soil profile is one of the most important (Lewis, 2002). To provide control, the parasite and the host must be in the same place at the same time. The location of an IJ is dictated by how it disperses after application and by the method of application. Since application technology is covered elsewhere, we will concentrate on how the IJs disperse. The dispersal behaviours and capabilities of EPNs vary among species, strains and even among individuals emerging from the same infection (Lewis, 2002).

EPNs disperse horizontally and vertically after application.The studies that have been conducted on dispersal phenomena can be grouped into laboratory studies that measured EPN movement through various media, field studies that recorded the distribution of native EPN populations that make inferences about dispersal and field studies that re-isolated EPNs after they were applied. Different kinds of information are provided by each of these types of studies.

Laboratory studies are the easiest to conduct and have been carried out on the widest variety of species and strains; yet one must take care in extrapolating these results to field populations. Interspecies variation has been measured in several studies. *S. carpocapsae* IJs move upwards in soil columns (Georgis and Poinar, 1983; Schroeder and Beavers, 1987), whereas *S. glaseri* and *H. bacteriophora* move downwards, but they also disperse throughout the soil column. Studies of movement through soil arenas have shown that *Heterorhabditis* spp. tended to migrate farther than did *Steinernema* spp. (Westerman, 1995; Downes and Griffin, 1996). Koppenhöfer and Kaya (1996) suggested that differential distribution patterns may allow some species, such as *S. glaseri* and *S. carpocapsae*, to coexist since they would not compete for the same hosts.

While laboratory studies may be limited in their ability to predict behaviour in the field, there are aspects of dispersal behaviour that are best addressed in a small controlled environment. Variation with age, variation among IJs emerging from the same cadaver and the impact of harvesting IJs in water are three examples. Lewis *et al.* (1995) compared changes in several aspects of IJ behaviour as they aged in water and found that the behaviours of *H. bacteriophora*, including locomotory rate on agar plates, degraded at a faster rate than of *S. carpocapsae* or *S. glaseri*. They also found that the nictation rate of *S. carpocapsae* declined with age. Differences among individuals emerging from the same cadaver represent a source of variation usually not considered. IJs emerge from host cadavers for up to 3 weeks in some species, and several differences among those emerging first versus last have been shown. In *S. glaseri* male IJs emerge before females, and those males emerging first are more responsive to host cues than are females (Lewis and Gaugler, 1994). This is not the case for *S. carpocapsae* or *S. feltiae*, where males did not emerge first (Lewis, 2002). Male IJs of some EPN species are more responsive to host cues (Grewal *et al.*, 1993) and disperse quicker (Lewis and Gaugler, 1994) than females. These findings gave rise to the 'male colonization hypothesis', which suggests that males establish infections before females. In the only direct test of this hypothesis to date, however, Stuart *et al.* (1998) found no evidence of earlier invasion by male than female IJs of *S. glaseri*, despite the documented behavioural differences. *H. megidis* IJs that emerged early differed in their behaviour, but also differed in their tolerance of temperature extremes and desiccation from those that emerged later (O'Leary *et al.*, 1998). Ryder and Griffin (2003) showed that the infectivity of *H. megidis* IJs produced in the first and second generation differed, and that infectivity of juveniles was further affected by the extent of crowding in the insect cadaver in which they developed. Shapiro and Glazer (1996) compared the dispersal of EPNs emerging from their host cadaver into sand with nematodes applied in water and found that *H. bacteriophora* and *S. carpocapsae* directly moving from their host cadaver to the soil had greater movement. How these findings relate to nematodes applied as products is impossible to know, but these findings may allow development of production technologies to favour particular characteristics.

Several field studies describe the distribution of EPNs. In the vertical plane, natural populations of *S. carpocapsae* were found in the upper 1–2 cm of soil, whereas *H. bacteriophora* was distributed throughout the upper 8 cm of soil (Campbell *et al.*, 1995). Ferguson *et al.* (1995) compared the vertical distributions of three species after application. *S. carpocapsae* and an undescribed *Steinernema* sp. remained near the soil surface, while *H. bacteriophora* strains moved to greater depths. Horizontal distribution studies on natural populations show that EPNs are patchily distributed, with a variable degree of patchiness among species (Stuart and Gaugler, 1994; Campbell *et al.*, 1995; Strong *et al.*, 1996). In general *H. bacteriophora* populations are patchier than either *S. carpocapsae* or *S. feltiae* populations (Campbell *et al.*, 1998). Host distribution, nematode behaviour and soil factors will all contribute to the spatial distribution of the nematodes.

Populations of *H. bacteriophora*, which were applied in a homogeneous layer, had a patchy distribution that mirrored native populations within 2 months of application (Campbell *et al.*, 1998), but the mechanism – whether due to recycling in patchily distributed hosts or redistribution of the applied nematodes – was unknown. Wilson *et al.* (2002), while studying the possibility of using different spatial application patterns to lengthen nematode persistence, showed that *H. bacteriophora* can move up to 3 m from their point of application.

2.3.2. Foraging strategies

Understanding foraging behaviour is essential to accurate prediction of efficacy for

EPNs because foraging mode predicts where the nematodes will be located and what hosts they are likely to contact (Gaugler *et al.*, 1997). EPN foraging strategies vary along a continuum from ambush to cruise foraging (Lewis *et al.*, 1992; Grewal *et al.*, 1994a; Campbell and Gaugler, 1997). The variation in foraging behaviour among species is considerable.

The way nematodes search for hosts has a direct impact on efficacy because mobile nematodes tend to find sedentary hosts and vice versa. Ambushing nematodes nictate during foraging by raising nearly all of their bodies off the substrate (Fig. 2.2) (Campbell and Gaugler, 1993). Of the commercially available EPN species, *S. carpocapsae* and *S. scapterisci* are the most extreme ambushers and may nictate for hours at a time (Campbell and Gaugler, 1993). Ambushing nematode species are usually associated with highly mobile, surface-dwelling hosts. Cruising nematodes never nictate and probably spend most of the IJ stage moving through the soil. Commercially available cruise foraging species include the *Heterorhabditis* spp. and *S. glaseri* spp. (Lewis, 2002). These species are usually effective against relatively sedentary hosts located throughout the soil column. Some EPN species, e.g. *S. riobrave* and *S. feltiae*, adopt an intermediate foraging strategy (Table 2.2) and have been effective against pests with a range of habits from mobile to sedentary.

AQ4

Fig. 2.2. Nictating infective juvenile (IJ) of *Steinernema carpocapsae*. The nematode stands on its tail and waves from side to side. (Photo: Jim Campbell, USDA ARS GMPRC, Kansas, USA.)

2.3.3. Host discrimination

Dispersal and foraging strategy constrain the host range of EPN species indirectly. The IJs themselves discriminate directly among potential hosts. Knowledge of natural host ranges of EPNs could help predict which nematodes would be effective against a particular insect pest. When an EPN is isolated from soil, we are essentially ignorant of its natural host range because of the use of *G. mellonella* as a bait (Bedding and Akhurst, 1975). Current knowledge of natural EPN host ranges is limited to anecdotal accounts of native populations found infecting a host in the field (Peters, 1996). There is also information on potential host range to be gleaned from field trials that test EPN species against particular hosts (treated elsewhere in this volume).

AQ5

Host recognition behaviour has been studied in a few species of EPNs, and has been measured by recording changes in several behaviours in response to host-related materials. Responses of *H. bacteriophora*, *S. glaseri*, *S. carpocapsae* and *S. scapterisci* to gut contents of four host species suggested consistent host affiliations: infectivity of nematode species to hosts was correlated with their behavioural responses to those hosts (Grewal *et al.*, 1993). Grewal *et al.* (1993b) also suggested that these EPN species respond differently to excretory

AQ6

Table 2.2. Foraging strategy and summary of behavioural tests for four species of *Steinernema* (Campbell, unpublished data). AQ7

Steinernema spp.	Foraging strategy[a]	Nictation	Jumping	Dispersal decreased by sand	Ranging to localized search by host contact	Attraction increased by host contact
S. carpocapsae	Ambusher	Yes	Yes	Yes	No	Yes
S. feltiae	Intermediate	No	No	No	No	No
S. riobrave	Intermediate	No	Yes	No	No	No
S. glaseri	Cruiser	No	No	No	Yes	No

[a]Based on attachment to mobile vs. immobile host.
Note: For a more complete treatment of IJ foraging behaviour see Lewis (2002) and Campbell *et al.* (2003).

products of various natural and experimental hosts. Lewis *et al.* (1996) studied the behavioural recognition response of *S. carpocapsae* IJs by measuring their response to volatiles from *G. mellonella* larvae following exposure to contact with the cuticle of nine candidate host species. Again, the level of recognition response to different hosts was correlated with the infectivity of the nematodes for those hosts, and also with IJ production per gram of host tissue. Measures of host recognition might be useful in the characterization of new isolates from the field, and a standard testing procedure for assessment of host range could be developed.

2.3.4. Infection behaviours

Once an IJ has located a host and found it acceptable, penetration into the host haemocoel is the next step. Different species use different routes of entry into hosts: via the natural openings (mouth, anus, spiracles) or by penetration through the external cuticle. Wang and Gaugler (1999) compared the penetration behaviour of *S. glaseri* and *H. bacteriophora* into *Popillia japonica* larvae and found that *S. glaseri* penetrated primarily through the gut. *H. bacteriophora* was not efficient at penetrating the gut, presumably because of the thick peritrophic membrane, but penetrated through the intersegmental membranes of the cuticle. Cui *et al.* (1993) found that *S. glaseri* IJs would penetrate through existing holes in the gut made by previous nematodes. Renn (1999) found that *S. feltiae* IJs also followed established routes of penetration in larval houseflies.

Fan and Hominick (1991) suggested that in the 'phased infectivity hypothesis' less than 40% of *S. feltiae* IJs that emerged from a host were infectious at any time, regardless of host availability. Nematodes were assumed to be either infectious or non-infectious, and to convert from one state to the other. Bohan and Hominick (1996, 1997) described short- and long-term interactions between a cohort of IJs and potential hosts that support this idea. However, Campbell *et al.* (1999) found that *S. feltiae* IJs will infect hosts when enough are available, but they also collected data for *H. bacteriophora* that support the phased infectivity hypothesis for this species. Infectivity of *H. megidis* shows an initial increase from time of emergence from the host cadaver, before eventually declining (Griffin, 1996; Dempsey and Griffin, 2002; Ryder and Griffin, 2003), and Griffin (1996) proposed that individual infectious nematodes may have variable levels of infectivity (tendency to infect), as an alternative to the dichotomous (infectious versus non-infectious) phased infectivity hypothesis.

2.4. Ecology

Field studies show that numbers of EPNs recovered from soil decline sharply in a short period following application (Selvan *et al.*, 1993a; Gaugler *et al.*, 1997). Although

soil is a relatively buffered environment, IJs may experience stressful conditions such as desiccation and high temperatures, especially at the soil surface immediately after application, while waterlogged soils may develop anoxic conditions. Nematodes in soil also face a variety of diseases and predators. If they are not killed by antagonists or lethal levels of abiotic factors, IJs can survive for months in the soil, and have evolved a suite of adaptations such as high levels of energy reserves and a protective sheath that allow them to persist in this sometimes hostile environment. Consideration of the survival mechanisms of IJs is important for formulation also.

2.4.1. Energy reserves and starvation

The IJ does not feed, but relies, on stored energy reserves. Lipids (especially triglycerides) constitute up to 40% of the body weight (Selvan *et al.*, 1993b; Fitters *et al.*, 1999) and are the most important energy reserve, though proteins and the carbohydrates, glycogen and trehalose, also yield energy (Qiu and Bedding, 2000). It is probable that, unless subjected to other mortality factors, IJs will starve to death. Thus, the lifespan is largely determined by the quantity and quality of reserves that it has built up during its prior feeding phase and by the rate at which the reserves are depleted (Qiu and Bedding, 2000). Both the rate of activity and basal metabolic rate – and hence the rate at which reserves are utilized – are affected by ambient conditions, most notably temperature. IJs survive longer at low temperatures, with optimal temperature for survival of most species typically between 5° and 15°C (Georgis, 1990), though 20°C is optimal for storage of certain tropical strains. The tendency of IJs to become inactive in the absence of stimulation, even when temperature and other conditions permit movement, also favours energy conservation. Foraging strategies have been related to several life history characters that have an impact on survival. Lewis *et al.* (1995) found that *S. carpocapsae*, an ambush forager, had a lower metabolic rate than *H. bacteriophora*. We also find that the products with the longest shelf-life tend to comprise ambush foragers. Foraging strategy also affects the choice of appropriate formulation for species of EPNs. For example, formulation in water-dispersible granule is very successful with the ambush forager *S. carpocapsae*, while the cruise foraging *S. feltiae* and *S. riobrave* rapidly migrate out of the granules (Grewal, 2002). Before starvation reaches critical lethal levels, motility and infectivity of the IJ may have declined (Lewis *et al.*, 1995; Patel *et al.*, 1997b), with the result that viability is not the only indicator of nematode quality.

2.4.2. Abiotic stress

Desiccation and temperature extremes are the most important abiotic factors affecting survival of EPNs (reviewed by Glazer, 2002). Nematodes require free water for movement, and as it disappears they necessarily become inactive. As the environment dries further, water is lost from the nematode body. *Steinernema* and *Heterorhabditis* have relatively limited tolerance of desiccation, and are classed as partial anhydrobiotes. Even partially anhydrobiotic nematodes have lowered energy consumption and increased tolerance to temperature extremes, making induction into this state the Holy Grail of formulation technology (see Chapter 4, this volume). Most studies have concentrated on *S. carpocapsae*, which is noted as one of the more desiccation-tolerant species (Patel *et al.*, 1997a), perhaps related to its tendency to remain near the soil surface, waiting to ambush passing hosts.

Exposure to extremes of temperature is damaging for nematodes, but the extent and nature of damage depends on the duration of exposure. Steinernematids and heterorhabditids tolerate exposure to sub-zero temperatures for several days (Wharton and Surrey, 1994) and, with suitable preconditioning, IJs may be stored indefinitely in liquid nitrogen (Popiel and Vasquez, 1991). This is an important property,

allowing the maintenance of genetic stock without the need for repeated subculture with the attendant risk of inadvertant selection (Wang and Grewal, 2002). Temperatures above 30°C inhibit infection and reproduction of several species of EPNs though others such as *S. riobrave* reproduce at 32°C and infect up to 39°C (Grewal *et al.*, 1994b). In laboratory assays, IJs of *S. carpocapsae* are killed by short periods (hours) at 40°C (Somasekhar *et al.*, 2002), but an Arkansas isolate of *S. carpocapsae* survived for 2 weeks at 40°C in soil (Gray and Johnson, 1983). Indeed, the limited ability of EPNs to tolerate ultraviolet light, desiccation and high temperature undoubtedly reflects their soil-dwelling evolutionary history.

2.4.3. Biotic stress

In soil, IJs are subject to attack by a variety of microbial and invertebrate antagonists (reviewed by Kaya, 2002). The main natural enemies with the potential to affect the survival of EPNs in soil are predatory mites and collembolans (e.g. Epsky *et al.*, 1988), nematode-trapping fungi (e.g. Poinar and Jansson, 1986) and parasitic fungi that produce adhesive spores (Timper *et al.*, 1991). Little is known about the impact of such organisms on natural or applied populations of EPNs. Indirect evidence for an effect of naturally occurring antagonists on nematode survival comes from the observation that nematodes survived longer when applied to sterilized soil (Ishibashi and Kondo, 1986). Developmental stages of EPNs are also at risk from scavengers attacking the cadavers (Baur *et al.*, 1998), and the fact that some cadavers deter predation by ants (Zhou *et al.*, 2002) suggests that such predation may exert selective pressure.

2.5. Geographical Distribution of Natural Populations

EPNs are very common in cultivated and uncultivated soils, and numerous surveys have documented their occurrence throughout the world (reviewed by Hominick *et al.*, 1996; Hominick, 2002). The level of effort that has been applied to the recovery of EPNs varies, with Europe being the most intensively studied continent. Amongst the species recovered are those with a global distribution: *S. carpocapsae* and *S. feltiae* are widely distributed in temperate regions; *H. bacteriophora* is common in regions with continental and Mediterranean climates; and *H. indica* is found throughout the tropics and subtropics. For some species, the known distribution is much more restricted; e.g. *S. cubanum* and *S. kushidai* are so far known only from Cuba and Japan, respectively.

The distribution of EPNs on a global scale, like that of other taxa, is probably strongly influenced by climate and chance dispersal events, including those associated with human activities. Soil texture, vegetation and availability of suitable hosts are amongst the factors that have been implicated in affecting local distribution patterns. There is growing evidence of preferences of nematode species for certain habitats. For example, *S. affine* is found largely in arable lands and grasslands, and is virtually absent in forests, while *S. kraussei* is commonly found in forests (Hominick, 2002). It is likely that such habitat preferences are at least partly due to host preferences, and the fact that associations with habitat are rather weak probably reflects the lack of strict host specificity in most EPN species (Peters, 1996). More striking is the association of some species with soil of a particular texture, in particular sand. *H. megidis* and *H. indica* are almost exclusively found in sandy soils, resulting in a mainly coastal distribution (Hara *et al.*, 1991; Amarasinghe *et al.*, 1994; Griffin *et al.*, 1994, 2000), and there is some evidence of a similar association for tropical steinernematids (Amarasinghe *et al.*, 1994; Griffin *et al.*, 2000). While laboratory assays are useful in predicting the effect of ecological factors on the potential of inundatively applied nematodes to survive and infect, predictions of whether such applied nematodes will establish as self-renewing populations are best informed by knowledge of the factors affecting the prevalence of natural popula-

tions (see Chapter 18, this volume). For example, from the known association of *H. megidis* with sandy soils, it could be predicted that this species is highly unlikely to persist long term in peat or clay soils.

While a grower with little knowledge of the biology of EPNs can apply them in line with the supplier's instructions, even a small amount of knowledge will increase the likelihood of his or her success. Continuing advances by researchers in understanding the complex requirements and strategies of these organisms in their natural environment will lead to the much more efficient targeting and expanded use of EPNs in the future.

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