

# 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 suc-

cess 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

reviewing the biology and use of EPNs are Gaugler and Kaya (1990) and Gaugler (2002). A third genus of EPN, *Neosteiner-nema*, 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 herm-

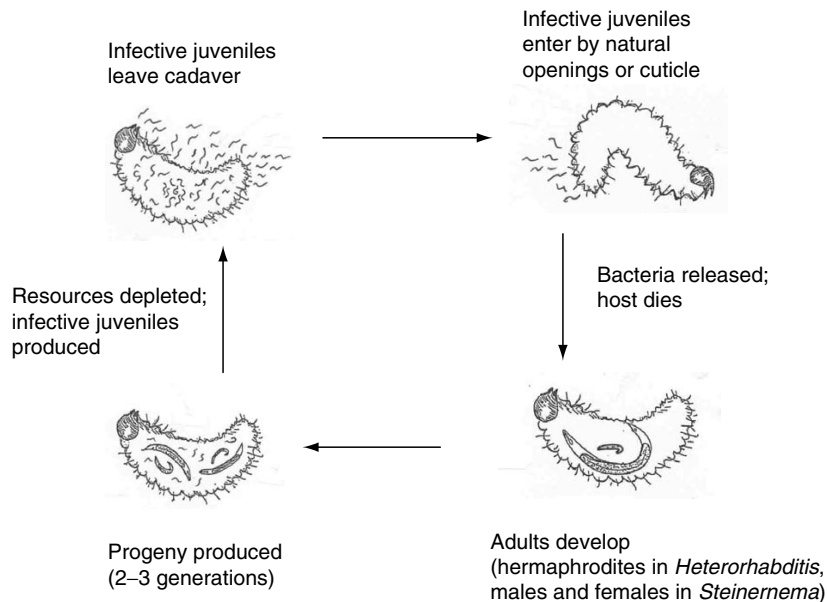
aphrodites, while a small proportion of the population in each generation are males (Griffin *et al.*, 2001). Thus, heterorhabditids and at least one *Steinernema* sp. can develop in a host when a single IJ invades, while most steinernematids require at least two individuals to colonize the host before 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 *Photobacterium* 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 *Photobacterium* are members of the  $\gamma$ -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.

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

<sup>a</sup>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).

<sup>b</sup>N. Boemare and P. Stock, unpublished.

<sup>c</sup>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.

<sup>d</sup>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<sub>50</sub> 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<sub>50</sub> > 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<sub>50</sub> 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 (French-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 (French-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 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.

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#### 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

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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.

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### 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 anec-



**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.)

dotal 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).

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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

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

<sup>a</sup>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 am-

bush 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 inadvertent 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 through-

out 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. krausei* 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.

## References

- Aguillera, M.M., Hodge, N.C., Stall, R.E. and Smart, G.C. Jr (1993) Bacterial symbionts of *Steinernema scapterisci*. *Journal of Invertebrate Pathology* 62, 68–72.
- Akhurst, R.J. (1980) Morphological and functional dimorphism in *Xenorhabdus* spp., bacteria symbiotically associated with the insect pathogenic nematodes *Neoaplectana* and *Heterorhabditis*. *Journal of General Microbiology* 121, 303–309.
- Akhurst, R.J. (1986) *Xenorhabdus nematophilus* subsp. *poinarii*: its interaction with insect pathogenic nematodes. *Systematic and Applied Microbiology* 8, 142–147.
- Akhurst, R.J. and Boemare, N.E. (1990) Biology and taxonomy of *Xenorhabdus*. In: Gaugler, R. and Kaya, H.K. (eds) *Entomopathogenic Nematodes in Biological Control*. CRC Press, Boca Raton, Florida, pp. 75–90.
- Akhurst, R.J. and Boemare, N.E. (2003) The *Xenorhabdus* genus. In: Krieg, N.R., Staley, J.T. and Brenner, D.J. (eds) *Bergey's Manual of Systematic Bacteriology*. Williams & Wilkins, Baltimore, Maryland.
- Akhurst, R.J. and Dunphy, G.B. (1993) Tripartite interactions between symbiotically associated entomopathogenic bacteria, nematodes, and their insect hosts. In: Beckage, N.E., Thompson, S.N. and Federici, B. (eds) *Parasites and Pathogens of Insects*. Academic Press, New York, pp. 1–23.
- Amarasinghe, L.D., Hominick, W.M., Briscoe, B.R. and Reid, A.P. (1994) Occurrence and distribution of entomopathogenic nematodes in Sri Lanka. *Journal of Helminthology* 68, 277–286.
- Babic, I., Fischer-Le Saux, M., Giraud, E., and Boemare, N.E. (2000) Occurrence of natural dixenic associations between the symbiont *Photorhabdus luminescens* and bacteria related to *Ochrobactrum* spp. in tropical entomopathogenic *Heterorhabditis* spp. (Nematoda: Rhabditida). *Microbiology* 146, 709–718.
- Baur, M.E., Kaya, H.K. and Strong, D.R. (1998) Foraging ants as scavengers on entomopathogenic nematode-killed insects. *Biological Control* 12, 231–236.
- Bedding, R.A. and Akhurst, R.J. (1975) A simple technique for the detection of insect parasitic rhabditid nematodes in soil. *Nematologica* 21, 109–110.
- Bedding, R.A. and Molyneux, A.S. (1982) Penetration of insect cuticle by infective juveniles of *Heterorhabditis* spp. (Heterorhabditidae: Nematoda). *Nematologica* 28, 354–359.
- Bird, A.F. and Akhurst, R.J. (1983) The nature of the intestinal vesicle in nematodes of the family Steinernematidae. *International Journal of Parasitology* 13, 599–606.
- Blackburn, M., Golubeva, E., Bowen, D. and french-Constant, R.H. (1998) A novel insecticidal toxin from *Photorhabdus luminescens*, toxin complex a (Tca), and its histopathological effects on the midgut of *Manduca sexta*. *Applied and Environmental Microbiology* 64, 3036–3041.
- Blaxter, M.L., De Ley, P., Garey, J.R., Liu, L.X., Scheldeman, P., Vierstraete, A., Vanfleteren, J.R., Mackey, L.Y., Dorris, M., Frisse, L.M., Vida, J.T. and Thomas, W.K. (1998) A molecular evolutionary framework for the phylum Nematoda. *Nature* 392, 71–75.
- Boemare, N.E. (2002) Biology, taxonomy and systematics of *Photorhabdus* and *Xenorhabdus*. In: Gaugler, R. (ed.) *Entomopathogenic Nematology*. CAB International, Oxon, UK, pp. 35–56.
- Boemare, N.E. and Akhurst, R.J. (1988) Biochemical and physiological characterization of colony form variants in *Xenorhabdus* spp. (Enterobacteriaceae). *Journal of General Microbiology* 134, 751–761.
- Boemare, N.E. and Akhurst, R.J. (2001) The genera *Photorhabdus* and *Xenorhabdus*. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H. and Stackebrandt, E. (eds) *The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community*. Springer-Verlag, NY. Available at: <http://www.prokaryotes.com>.
- Boemare, N.E. and Akhurst, R.J. (2003) The *Photorhabdus* genus. In: Krieg, N.R., Staley, J.T. and Brenner, D.J. (eds) *Bergey's Manual of Systematic Bacteriology*. Williams & Wilkins, Baltimore.

- Boemare, N.E., Boyer-Giglio, M.-H., Thaler, J.-O., Akhurst, R.J. and Brehélin, M. (1992) Lysogeny and bacteriocinogeny in *Xenorhabdus nematophila*, and other *Xenorhabdus* spp. *Applied and Environmental Microbiology* 58, 3032–3037.
- Boemare, N.E., Akhurst, R.J. and Mourant, R.G. (1993) DNA relatedness between *Xenorhabdus* spp. (Enterobacteriaceae), symbiotic bacteria of entomopathogenic nematodes, and a proposal to transfer *Xenorhabdus luminescens* to a new genus, *Photorhabdus* gen. nov. *International Journal of Systematic Bacteriology* 43, 249–255.
- Boemare, N.E., Laumond, C. and Mauléon, H. (1996) The nematode-bacterium complexes: biology, life cycle, and vertebrate safety. *Bio-control Science and Technology* 6, 333–345.
- Bohan, D.A. and Hominick, W.M. (1996) Investigations on the presence of an infectious proportion amongst populations of *Steinernema feltiae* (Site 76 Strain) infective states. *Parasitology* 112, 113–118.
- Bohan, D.A. and Hominick, W.M. (1997) Long-term dynamics of infectiousness within the infective-stage pool of the entomopathogenic nematode *Steinernema feltiae* (site 76 strain) Filipjev. *Parasitology* 114, 301–308.
- Bonifassi, E., Fischer-Le Saux, M., Boemare, N., Lanois, A., Laumond, C. and Smart, G. (1999) Gnotobiological study of infective juveniles and symbionts of *Steinernema scapterisci*: a model to clarify the concept of the natural occurrence of monoxenic associations in entomopathogenic nematodes. *Journal of Invertebrate Pathology* 74, 164–172.
- Brillard, J., Ribeiro, C., Boemare, N., Brehélin, M. and Givaudan, A. (2001) Two distinct hemolytic activities in *Xenorhabdus nematophila* are active against immunocompetent insect cells. *Applied and Environmental Microbiology* 67, 2515–2525.
- Brillard, J., Duchaud, E., Boemare, N., Kunst, F. and Givaudan, A. (2002) The PhIA hemolysin from the entomopathogenic bacterium *Photorhabdus luminescens* belongs to the two-partner secretion family of hemolysins. *Journal of Bacteriology* 184, 3871–3878.
- Brillard, J., Boyer-Giglio, M.-H., Boemare, N. and Givaudan, A. (2003) Holin locus characterisation from lysogenic *Xenorhabdus nematophila* and its involvement in *Escherichia coli* SheA haemolytic phenotype. *FEMS Microbiology Letters* 218, 107–113.
- Campbell, L.R. and Gaugler, R. (1991a) Mechanisms for exsheathment of entomopathogenic nematodes. *International Journal of Parasitology* 21, 219–224.
- Campbell, L.R. and Gaugler, R. (1991b) Role of the sheath in desiccation tolerance of two entomopathogenic nematodes. *Nematologica* 37, 324–332.
- Campbell, J.F. and Gaugler, R. (1993) Nictation behaviour and its ecological implications in the host search strategies of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae). *Behaviour* 126, 155–169.
- Campbell, J.F. and Gaugler, R. (1997) Inter-specific variation in entomopathogenic nematode foraging strategy: dichotomy or variation along a continuum? *Fundamental and Applied Nematology* 20, 393–398.
- Campbell, J.F., Lewis, E.E., Yoder, F. and Gaugler, R. (1995) Entomopathogenic nematode (Heterorhabditidae and Steinernematidae) seasonal population dynamics and impact on insect populations in turfgrass. *Biological Control* 5, 598–606.
- Campbell, J.F., Orza, G., Yoder, F., Lewis, E.E. and Gaugler, R. (1998) Entomopathogenic nematode distribution in turfgrass: variation among sites, correlation with *Popillia japonica* larvae and edaphic factors, and influence of inoculative releases. *Entomologia Experimentalis et Applicata* 86, 1–11.
- Campbell, J.F., Koppenhöfer, A.M., Kaya, H.K. and Chinnasri, B. (1999) Are there temporarily non-infectious dauer stages in entomopathogenic nematode populations: a test of the phased infectivity hypothesis. *Parasitology* 118, 499–508.
- Campbell, J.F., Lewis, E.E., Stock, S.P., Nadler, S. and Kaya, H.K. (2003) Evolution of host search strategies in entomopathogenic nematodes (Nematoda: Steinernematidae). *Journal of Nematology* 35, 142–145.
- Cui, L., Gaugler, R. and Wang, Y. (1993) Penetration of steinernematid nematodes (Nematoda: Steinernematidae) into Japanese beetle larvae, *Popillia japonica* (Coleoptera: Scarabaeidae). *Journal of Invertebrate Pathology* 62, 73–78.
- Dempsey, C.M. and Griffin, C.T. (2002) Phased activity in *Heterorhabditis megidis* infective juveniles. *Parasitology* 124, 605–613.
- Dempsey, C.M. and Griffin, C.T. (2003) The infectivity and behaviour of exsheathed and ensheathed *Heterorhabditis megidis* infective juveniles. *Nematology* 5, 49–53.
- Dix, I., Burnell, A.M., Griffin, C.T., Joyce, S.A. and Nugent, J.M. (1992) The identification of biological species in the genus *Heterorhabditis* (Nematoda: Heterorhabditidae) by cross breeding second-generation amphimictic adults. *Parasitology* 104, 509–518.



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- Dowds, B.C.A. and Peters, A. (2002) Virulence mechanisms. In: Gaugler, R. (ed.) *Entomopathogenic Nematology*. CAB International, Wallingford, UK, pp. 79–98.
- Dowds, M.J. and Griffin, C.T. (1996) Dispersal behaviour and transmission strategies of the entomopathogenic nematodes *Heterorhabditis* and *Steinernema*. *Biocontrol Science and Technology* 6, 347–356.
- Duchaud, E., Rusniok, C., Frangeul, L., Buchrieser, C., Givaudan, A., Taourit, S., Bocs, S., Bour-saux-Eude, C., Chandler, M., Charles, J.-F., Dassa, E., Derose, R., Derzelle, S., Freyssinet, G., Gaudriault, S., Médigue, C., Lanois, A., Powell, K., Siguiet, P., Vincent, R., Wingate, V., Zouine, M., Glaser, P., Boemare, N., Dan-chin, A., Kunst, F. (2003) The genome sequence of the entomopathogenic bacterium *Photorhabdus luminescens*. *Nature Biotechnology*.
- Ehlers, R.-U., Stoessel, S. and Wyss, U. (1990) The influence of phase variants of *Xenorhabdus* spp. and *Escherichia coli* (Enterobacteriaceae) on the propagation of entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis*. *Revue de Nématologie* 13, 417–424.
- Enright, M.R., McInerney, J.O. and Griffin, C.T. (2003) Characterization of endospore-forming bacteria associated with entomopathogenic nematodes, *Heterorhabditis* spp., and description of *Paenibacillus nematophilus* sp. nov. *International Journal of Systematic and Evolutionary Microbiology* 53, 435–441.
- Epsky, N.D., Walter, D.E. and Capinera, J.L. (1988) Potential role of nematophagous microarthropods as biotic mortality factors of entomogenous nematodes (Rhabditida: Steinernematidae, Heterorhabditidae). *Journal of Economic Entomology* 81, 821–825.
- Fan, X. and Hominick, W.M. (1991) Effects of low storage temperature on survival and infectivity of two *Steinernema* species (Nematoda: Steinernematidae). *Revue de Nématologie* 14, 407–412.
- Ferguson C.S., Schroeder P.C. and Shields E.J. (1995) Vertical distribution, persistence, and activity of entomopathogenic nematodes (Nematoda: Heterorhabditidae and Steinernematidae) in alfalfa snout beetle (Coleoptera: Curculionidae) infested fields. *Environmental Entomology* 24, 149–158.
- ffrench-Constant, R.H. and Bowen, D. (1999) *Photorhabdus* toxins: novel biological insecticides. *Current Opinion in Microbiology* 2, 284–288.
- ffrench-Constant, R.H. and Bowen, D.J. (2000) Novel insecticidal toxins from nematode-symbiotic bacteria. *Cellular and Molecular Life Sciences* 57, 828–833.
- Fischer-Le Saux, M., Mauleon, H., Constant, P., Brunel, B. and Boemare, N. (1998) PCR-ribotyping of *Xenorhabdus* and *Photorhabdus* isolates from the Caribbean region in relation to the taxonomy and geographic distribution of their nematode hosts. *Applied and Environmental Microbiology* 64, 4246–4254.
- Fitters, P.F.L., Patel, M.N., Griffin, C.T. and Wright, D.J. (1999) Fatty acid composition of *Heterorhabditis* sp. during storage. *Comparative Biochemistry and Physiology B* 124, 81–88.
- Forst, S. and Clarke, D.J. (2002) Bacteria-nematode symbiosis. In: Gaugler, R. (ed.) *Entomopathogenic Nematology*. CAB International, Wallingford, UK, pp. 57–77.
- Forst, S., Dowds, B., Boemare, N. and Stackebrandt, E. (1997) *Xenorhabdus* spp. and *Photorhabdus* spp.: bugs that kill bugs. *Annual Review of Microbiology* 51, 47–72.
- Gaugler, R., (ed.) (2002) *Entomopathogenic Nematology*. CAB International, Wallingford, UK, 388 pp.
- Gaugler, R. and Kaya, H.K. (eds) (1990) *Entomopathogenic Nematodes in Biological Control*. CRC Press, Boca Raton, Florida, 365 pp.
- Gaugler, R., Lewis, E.E. and Stuart, R.J. (1997) Ecology in the service of biological control: the case of entomopathogenic nematodes. *Oecologia* 109, 483–489.
- Georgis, R. (1990) Formulation and application technology. In: Gaugler, R. and Kaya, H.K. (eds) *Entomopathogenic Nematodes in Biological Control*. CRC Press, Boca Raton, Florida, pp. 173–191.
- Georgis, R. and Poinar, G.O. Jr (1983) Effect of soil texture on the distribution and infectivity of *Neoplectana carpocapsae* (Nematoda: Steinernematidae). *Journal of Nematology* 15, 308–311.
- Givaudan, A. and Lanois, A. (2000) *flhDC*, the flagellar master operon of *Xenorhabdus nematophilus*: requirement for motility, lipolysis, extracellular hemolysis, and full virulence in insects. *Journal of Bacteriology* 182, 107–115.
- Givaudan, A., Baghdiguan, S., Lanois, A. and Boemare, N. (1995) Swarming and swimming changes concomitant with phase variation in *Xenorhabdus nematophilus*. *Applied and Environmental Microbiology* 61, 1408–1413.
- Givaudan, A., Lanois, A. and Boemare, N. (1996) Cloning and nucleotide sequence of a flagellin encoding genetic locus from *Xenorhabdus nematophilus*: phase variation leads to differential transcription of two flagellar genes (*FlhCD*). *Gene* 183, 243–253.
- Glaser, I. (2002) Survival biology. In: Gaugler, R. (ed.) *Entomopathogenic Nematology*. CAB International, Wallingford, UK, pp. 169–187.

- Gray, P.A. and Johnson, D.T. (1983) Survival of the nematode *Neoplectana carpocapsae* in relation to soil temperature, moisture and time. *Journal of the Georgia Entomological Society* 18, 454–460.
- Grewal, P.S. (2002) Formulation and application technology. In: Gaugler, R. (ed.) *Entomopathogenic Nematology*. CAB International, Wallingford, UK, pp. 265–287.
- Grewal P.S., Gaugler R. and Lewis E.E. (1993a) Host recognition behavior by entomopathogenic nematodes during contact with insect gut contents. *Journal of Parasitology* 79, 495–503.
- Grewal, P.S., Gaugler, R. and Selvan, S. (1993b) Host recognition by entomopathogenic nematodes: behavioral response to contact with host feces. *Journal of Chemical Ecology* 19, 1219–1231.
- Grewal, P.S., Lewis, E.E., Campbell, J.F. and Gaugler, R. (1994a) Host finding behavior as a predictor of foraging strategy for entomopathogenic nematodes. *Parasitology* 108, 207–215.
- Grewal, P.S., Selvan, S. and Gaugler, R. (1994b) Thermal adaptation of entomopathogenic nematodes: niche breadth for infection, establishment, and reproduction. *Journal of Thermal Biology* 19, 245–253.
- Grewal, P.S., Matsuura, M. and Converse, V. (1997) Mechanisms of specificity of association between the nematode *Steinernema scapterisci* and its symbiotic bacterium. *Parasitology* 114, 483–488.
- Griffin, C.T. (1996) Effects of prior storage conditions on the infectivity of *Heterorhabditis* sp. (Nematoda: Heterorhabditidae). *Fundamental and Applied Nematology* 19, 95–102.
- Griffin, C.T., Joyce, S.A., Dix, I., Burnell, A.M. and Downes, M.J. (1994) Characterisation of the entomopathogenic nematode *Heterorhabditis* (Nematoda: Heterorhabditidae) from Ireland and Britain by molecular and cross-breeding techniques, and the occurrence of the genus in these islands. *Fundamental and Applied Nematology* 17, 245–253.
- Griffin, C.T., Chaerani, R., Fallon, D., Reid, A.P. and Downes, M.J. (2000) Occurrence and distribution of the entomopathogenic nematodes *Steinernema* spp. and *Heterorhabditis indica* in Indonesia. *Journal of Helminthology* 74, 143–150.
- Griffin, C.T., O'Callaghan, K. and Dix, I. (2001) A self-fertile species of *Steinernema* from Indonesia: further evidence of convergent evolution amongst entomopathogenic nematodes? *Parasitology* 122, 181–186.
- Hara, A.H., Gaugler, R., Kaya, H.K. and Lebeck, L.M. (1991) Natural populations of entomopathogenic nematodes (Rhabditida: Heterorhabditidae, Steinernematidae) from the Hawaiian islands. *Environmental Entomology* 20, 211–216.
- Hominick, W.M. (2002) Biogeography. In: Gaugler, R. Gaugler, (ed.) *Entomopathogenic Nematology*. CAB International, Wallingford, UK, pp. 115–143.
- Hominick, W.M., Reid, A.P., Bohan, D.A. and Briscoe, B.R. (1996) Entomopathogenic nematodes: biodiversity, geographical distribution and the convention on biological diversity. *Biocontrol Science and Technology* 6, 317–331.
- Hurst, M.R.H., Glare, T.R., Jackson, T.A. and Ronson, C.W. (2000) Plasmid-located pathogenicity determinants of *Serratia entomophila*, the causal agent of amber disease of grass grub, show similarity to the insecticidal toxins of *Photorhabdus luminescens*. *Journal of Bacteriology* 182, 5127–5138.
- Ishibashi, N. and Kondo, E. (1986) A possible quiescence of the applied entomogenous nematode, *Steinernema feltiae*, in soil. *Japanese Journal of Nematology* 16, 66–67.
- Jackson, T.J., Wang, H., Nugent, M.J., Griffin, C.T., Burnell, A.M. and Dowds, B.C.A. (1995) Isolation of insect pathogenic bacteria, *Providencia rettgeri*, from *Heterorhabditis* spp. *Journal of Applied Bacteriology* 78, 237–244.
- Johnigk, S.-A. and Ehlers, R.-U. (1999) *Endotokia matricida* in hermaphrodites of *Heterorhabditis* spp. and the effect of the food supply. *Nematology* 1, 717–726.
- Kaya, H. (2002) Natural enemies and other antagonists. In: Gaugler, R. (ed.) *Entomopathogenic Nematology*. CAB International, Wallingford, UK, pp. 189–203.
- Koppenhöfer, A.M. and Kaya, H.K. (1996) Coexistence of two steinernematid nematode species (Rhabditida: Steinernematidae) in the presence of two host species. *Applied Soil Ecology* 4, 221–230.
- Lewis, E. (2002) Behavioural ecology. In: Gaugler, R. (ed.) *Entomopathogenic Nematology*. CAB International, Wallingford, UK, pp 205–223.
- Lewis, E.E. and Gaugler, R. (1994) Entomopathogenic nematode sex ratio relates to foraging strategy. *Journal of Invertebrate Pathology* 64, 238–242.
- Lewis, E.E., Gaugler, R. and Harrison, R. (1992) Entomopathogenic nematode host finding: response to host contact cues by cruise and ambush foragers. *Parasitology* 105, 309–319.
- Lewis, E.E., Selvan, S., Campbell, J.F. and Gaugler, R. (1995) Changes in foraging behaviour during the infective juvenile stage of entomopathogenic nematodes. *Parasitology* 110, 583–590.

- Lewis, E.E., Ricci, M. and Gaugler, R. (1996) Host recognition behavior reflects host suitability for the entomopathogenic nematode, *Steinernema carpocapsae*. *Parasitology* 113, 573–579.
- Nguyen, K.B. and Smart, G.C. (1994) *Neosteiner-nema longicurvicauda* n. gen., n. sp. (Rhabditida: Steinernematidae), a parasite of the termite *Reticulitermes flavipes* (Koller). *Journal of Nematology* 26, 162–174.
- O'Leary, S.A., Stack, C.M., Chubb, M. and Burnell, A.M. (1998) The effect of day of emergence from the insect cadaver on the behavior and environmental tolerances of infective juveniles of the entomopathogenic nematode *Heterorhabditis megidis* (strain UK211). *Journal of Parasitology* 84, 665–672.
- Patel, M.N., Perry, R.N. and Wright, D.J. (1997a) Desiccation survival and water contents of entomopathogenic nematodes, *Steinernema* spp. (Rhabditida: Steinernematidae). *International Journal of Parasitology* 27, 61–70.
- Patel, M.N., Stolinski, M. and Wright, D.J. (1997b) Neutral lipids and the assessment of infectivity in entomopathogenic nematodes: observations on four *Steinernema* species. *Parasitology* 114, 489–496.
- Peters, A. (1996) The natural host range of *Steinernema* and *Heterorhabditis* spp. and their impact on insect populations. *Biocontrol Science and Technology* 6, 389–402.
- Peters, A. and Ehlers, R.-U. (1994) Susceptibility of leather jackets (*Tipula paludosa* and *Tipula oleracea*; Tipulidae: Nematocera) to the entomopathogenic nematode *Steinernema feltiae*. *Journal of Invertebrate Pathology* 63, 163–171.
- Poinar, G.O. Jr (1990) Biology and taxonomy of Steinernematidae and Heterorhabditidae. In: Gaugler, R. and Kaya, H.K. (eds) *Entomopathogenic nematodes in biological control*. CRC Press, Boca Raton, Florida, pp. 23–61.
- Poinar, G.O. Jr (1993) Origins and phylogenetic relationships of the entomophilic rhabditids, *Heterorhabditis* and *Steinernema*. *Fundamental and Applied Nematology* 16, 333–338.
- Poinar, G.O. and Jansson, H.B. (1986) Infection of *Neoplectana* and *Heterorhabditis* (Rhabditida: Nematoda) with the predatory fungi, *Monacrosporium elliposporum* and *Arthrobotrys oligospora* (Moniliales: Deuteromycetes). *Revue de Nématologie* 9, 241–244.
- Popiel, I. and Vasquez, E.M. (1991) Cryopreservation of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*. *Journal of Nematology* 23, 432–437.
- Qiu, L. and Bedding, R. (2000) Energy metabolism and its relation to survival and infectivity of infective juveniles of *Steinernema carpocapsae* under aerobic conditions. *Nematology* 2, 551–559.
- Renn, N. (1999) Routes of penetration of the entomopathogenic nematode *Steinernema feltiae* attacking larval and adult houseflies (*Musca domestica*). *Journal of Invertebrate Pathology* 72, 281–287.
- Ryder, J.J. and Griffin, C.T. (2003) Phased infectivity in *Heterorhabditis megidis*: the effects of infection density in the parental host and filial generation. *International Journal of Parasitology* 33, 1013–1018.
- Schroeder, W.J. and Beavers, J.B. (1987) Movement of the entomogenous nematodes of the families Heterorhabditidae and Steinernematidae in soil. *Journal of Nematology* 19, 257–259.
- Selvan, S., Campbell, J.F. and Gaugler, R. (1993a) Density dependent effects on entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) within an insect host. *Journal of Invertebrate Pathology* 62, 278–284.
- Selvan, S., Gaugler, R. and Lewis, E.E. (1993b) Biochemical energy reserves of entomopathogenic nematodes. *Journal of Parasitology* 79, 167–172.
- Shapiro, D.I. and Glazer, I. (1996) Comparison of entomopathogenic nematode dispersal from infected hosts versus aqueous suspension. *Environmental Entomology* 25, 1455–1461.
- Sicard, M., Le Brun, N., Pagès, S., Godelle, B., Boemare, N. and Mouliia, C. (2003) Effect of native *Xenorhabdus* on the fitness of their *Steinernema* hosts: contrasting types of interactions. *Parasitology Research* 90, .
- Smigielski, A.J., Akhurst, R.J. and Boemare, N.E. (1994) Phase variation in *Xenorhabdus nematophilus* and *Photorhabdus luminescens*: differences in respiratory activity and membrane energization. *Applied and Environmental Microbiology* 60, 120–125.
- Somasekhar, N., Grewal, P.S. and Klein, M.G. (2002) Genetic variability in stress tolerance and fitness among natural populations of *Steinernema carpocapsae*. *Biological Control* 23, 303–310.
- Strong, D.R., Kaya, H.K., Whipple, A.V., Child, A.L., Kraig, S., Bondonno, M., Dyer, K. and Maron, J.L. (1996) Entomopathogenic nematodes: natural enemies of root-feeding caterpillars on bush lupine. *Oecologia* 108, 167–173.
- Stuart, R.S. and Gaugler, R. (1994) Patchiness in populations of entomopathogenic nematodes. *Journal of Invertebrate Pathology* 64, 39–45.
- Thaler, J.-O., Baghdiguian, S. and Boemare, N. (1995) Purification and characterization of xenorhabdicolin, a phage tail-like bacteriocin, from the lysogenic strain F1 of *Xenorhabdus*

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- nematophilus*. *Applied and Environmental Microbiology* 61, 2049–2052.
- Timper, P. and Kaya, H.K. (1989) Role of the second stage cuticle of entomogenous nematodes in preventing infection by nematophagous fungi. *Journal of Invertebrate Pathology* 54, 314–321.
- Timper, P., Kaya, H.K. and Jaffee, B.A. (1991) Survival of entomogenous nematodes in soil infested with the nematode-parasitic fungus *Hirsutella rhossiliensis* (Deuteromycotina: Hyphomycetes). *Biological Control* 1, 42–50.
- Vivas, E.I. and Goodrich-Blair, H. (2001) *Xenorhabdus nematophilus* as a model for host-bacterium interactions: *rpoS* is necessary for mutualism with nematodes. *Journal of Bacteriology* 183, 4687–4693.
- Wang, Y. and Gaugler, R. (1999) Host and penetration site location by entomopathogenic nematodes against Japanese beetle larvae. *Journal of Invertebrate Pathology* 72, 313–318.
- Wang, X.D. and Grewal, P.S. (2002) Rapid genetic deterioration of environmental tolerance and reproductive potential of an entomopathogenic nematode during laboratory maintenance. *Biological Control* 23, 71–78.
- Webster, J.M., Chen, G., Hu, K. and Li, J. (2002) Bacterial metabolites. In: Gaugler, R. (ed.) *Entomopathogenic Nematology*. CAB International, Oxon, UK, pp. 99–114.
- Westerman, P.R. (1995) Comparative vertical migration of twenty one isolates of the insect parasitic nematode *Heterorhabditis* spp. in sand at 20°C. *Fundamental and Applied Nematology* 18, 149–158.
- Wharton, D.A. and Surrey, M.R. (1994) Cold tolerance mechanisms of the infective larvae of the insect parasitic nematode, *Heterorhabditis zelandica*, Poinar. *Cryo Letters* 15, 353–360.
- Wilson, M.J., Lewis, E.E., Yoder, F. and Gaugler, R. (2002) The influence of application pattern on persistence of *Heterorhabditis bacteriophora*. *Biological Control* 26, 180–188.
- Zhou, X.S., Kaya, H.K., Heungens, K. and Goodrich-Blair, H. (2002) Response of ants to a deterrent factor(s) produced by the symbiotic bacteria of entomopathogenic nematodes. *Applied and Environmental Microbiology* 68, 6202–6209.

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- AQ1: Changed according to the list.
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