

Behavioural Ecology of Entomopathogenic Nematodes, *Steinernema* and *Heterorhabditis* for Insect Biocontrol



Tarique Hassan Askary, M. Jamal Ahmad, A. R. Wani,
Sajad Mohiddin and Muneer Ahmad Sofi

Abstract Entomopathogenic nematodes *Steinernema* and *Heterorhabditis* are excellent biocontrol agents of crop insect pests. These beneficial nematodes decrease the reliance on synthetic pesticides and assist in maintaining a desirable ecological balance. They adapt to a wide range of behavioural strategies, and their degree of efficacy varies with species and the ecological niche where nematode-insect interactions take place. Occurrence and distribution of entomopathogenic nematodes are related to certain insect groups that are considered their suitable hosts. Soil texture and presence of a host affect the nematode's direction of movement. The specific behaviours and cues used by infective juveniles of different nematode species vary while searching and finding the hosts. The process of infection is governed by several factors such as host recognition behaviour, acceptance behaviour and infection behaviour. Cues such as feces or cuticle associated with living insects affect a nematode's foraging behaviour which varies with respect to nematode species. Therefore, correct selection of nematode species is of foremost importance in the insect pest management strategies. Apart from nematodes' habitat preference and infection behaviour, survival strategy and reproductive behaviour are other important parameters that need attention.

T. H. Askary (✉) · M. Jamal Ahmad · A. R. Wani · S. Mohiddin · M. A. Sofi
Division of Entomology, Sher-e-Kashmir University of Agricultural
Sciences and Technology, Main Campus, Shalimar, Srinagar 190025,
Jammu and Kashmir, India
e-mail: tariq_askary@rediffmail.com

M. Jamal Ahmad
e-mail: ahmad_j@rediffmail.com

A. R. Wani
e-mail: rouf_wani2006@rediffmail.com

S. Mohiddin
e-mail: sajad_05@rediffmail.com

M. A. Sofi
e-mail: muneerento@yahoo.co.in

Keywords Entomopathogenic nematodes • *Steinernema* • *Heterorhabditis*
Foraging behaviour • Infection behaviour • Reproductive behaviour

1 Introduction

Entomopathogenic nematodes belonging to the families steinernematids and heterorhabditids are widespread soil nematodes that are parasitic on various insect pests. These nematodes show different behaviour which is a set of activities and responses translated by nervous system in response to internal and external stimuli and are assessed in terms of locomotion, movement, feeding, mating, migration and penetration (Gaugler and Bilgrami 2004). While searching host these nematodes behave differently (Askary 2010), as some are cruise foragers such as *Heterorhabditis bacteriophora*, *H. megidis*, *Steinernema cubanum*, *S. longicaudum* and *S. kraussei* which move actively through soil using distant volatile cues that assist them in finding the host whereas others are ambush foragers such as *S. carpocapsae*, *S. scapterisci* and *S. siamkayai* that utilize sit and wait foraging strategy, remain near the soil surface, lifting their body into the air in order to attach the passing insects. However, there are also few species that employ intermediate foraging behaviour such as *S. glaseri* and *S. feltiae* which act both as cruiser and ambusher (Campbell and Gaugler 1997; Csontos 2002).

The functional role of nematodes is generally determined by metabolic and behavioural activities (Askary and Abd-Elgawad 2017). The third stage juveniles of *Steinernema* and *Heterorhabditis* which are called infective juveniles are the only free living form found in soil. They are motile, virulent, possessing chemoreceptors, have high reproductive potential and the ability to seek out hosts, besides having the potential for long-term establishment through recycling and compatibility with other management strategies, such as chemical pesticides (Sankaranarayanan and Askary 2017). Infective juveniles emerging from a single cadaver may differ in behaviour. Males of *S. glaseri* emerge first from hosts and show responsiveness to volatile cues from an unidentified host in comparison to females. Contrary to this, males of *S. carpocapsae* and *S. feltiae* emerge later than females. The behaviour of some *Steinernema* species in relation to the soil habitat and in the presence or absence of the insect host showed that the survival of infective juveniles was for much longer periods in aerated water than in moist sand at a given set of temperature (Molyneux 1983). *Steinernema* have the ability to parasitize insects over a wide range of temperature but the temperature range of infectivity for insects differs between the species isolated from same geographical area. Therefore, in order to develop a quality entomopathogenic product, prior considerations should be the nematode host finding ability, virulence potential and reproductive ability in the target pest which demands a sound knowledge on behavioural ecology of nematodes (Askary and Ahmad 2017).

The present effort is aimed to provide comprehensive information on the behaviour of entomopathogenic nematodes in relation to their foraging strategy,

associated insect hosts, habitats, bacterial symbionts and reproductive ability so that these beneficial nematodes may be exploited efficiently when included into integrated insect pest management programme.

2 Foraging Behaviour

Foraging behaviour of nematodes is one of the most important considerations during a biological control programme as the recommendations of nematode species for the control of specific target insect pests are done on this basis. Infective juveniles of entomopathogenic nematodes find their host generally by employing three behaviours (i) Ambushing (ii) Cruising and (iii) Intermediate behaviour.

i. Ambushing behaviour: Ambushers adopt 'sit and wait' behaviour to infect insects when the later come in contact with the nematode on the surface of the soil. The nematodes acting as ambusher nictate during foraging i.e. they overcome meniscus forces, standing vertically out from the substrate and attach to a passing insect. Nictation has various forms that range from straight motionless behaviour to partial lifting from the substrate and waving back and forth. In fact, when the nictating anterior does not contact another surface projection, it bends in a loop, contacting a moist point on the posteriad body. The force generated by the subsequent opposing body wave abruptly breaks the anterior end free of surface tension at the point of contact. This can fling the anterior end upward with sufficient momentum to break the surface tension holding the posterior end to the substrate and jettison the entire body through the air (Reed and Wallace 1965; Campbell and Kaya 1999a, b; Gaugler and Bilgrami 2004). Ambushers do not respond to host released cues and they are considered suitable for mobile hosts. They are generally engage in "ranging search" on a smooth surface (Lewis et al. 1993). Species of *Steinernema* such as *S. carpocapsae* and *S. scapterisci* show ambushing behaviour i.e. standing on their tails and infect passing insect hosts easily by jumping on them.

Though, the standing and jumping behaviours of *S. carpocapsae* are suited for attaching to mobile hosts at the soil surface (Campbell and Gaugler 1993; Campbell and Kaya 2002), but this should not preclude them from use against subterranean pests. For jumping, nematodes form a loop with bodies, with the head held to the side of the body by the surface tension. Infective juveniles can leap distances of nine body lengths in the presence of a host (Campbell and Kaya 1999a, b). The frequency of jumping varies among species and increase by mechanical context, air movement and volatile host cues. Campbell and Kaya (2002) assessed the standing and jumping behaviour of the nematodes belonging to families Heterorhabditidae and Steinernematidae. Eleven species of *Steinernema* showed jumping behaviour, however jumping rate varied among the nematode species. Variation in the duration of crawling bouts, the tendency to body wave, the rate of standing, the duration of standing bouts, and the tendency to jump after standing contributed to the differences in frequency of jumping among species. The nematodes showing jumping behaviour within a species were similar in size, infectivity and sex ratio in



Fig. 1 Infective juveniles of entomopathogenic nematode, *Heterorhabditis bacteriophora*

comparison to those that did not jump. The jumpers were more infective and were found to shed their second stage cuticle.

ii. Cruising behaviour: Cruisers search their host by moving actively in soil. These nematodes are distributed throughout the soil profile and are best suited for immobile hosts, such as those which are deep in soil or in other cryptic habitats (Gaugler et al. 1997). They never nictate but respond to carbon dioxide released by insects as cues (Lewis et al. 1993). *Heterorhabditis* are generally cruisers, move actively in soil in search of their suitable host (Fig. 1). *S. glaseri* and *S. kraussei* also exhibit cruising behaviour.

iii. Intermediate behaviour: Some nematodes exhibit intermediate foraging behaviour, i.e. employing both ambush and cruise strategies to attack the mobile as well as sedentary insects present on the surface or deep in the soil. *Steinernema* are ambushers though their some species such as *S. feltiae*, *S. glaseri* and *S. riobrave* show intermediate behaviour i.e. cruising and also ambushing (raising themselves on substrate) for a short while (Grewal et al. 1994; Campbell and Gaugler 1997; Campbell and Kaya 2002; Griffin et al. 2005; Askary 2010).

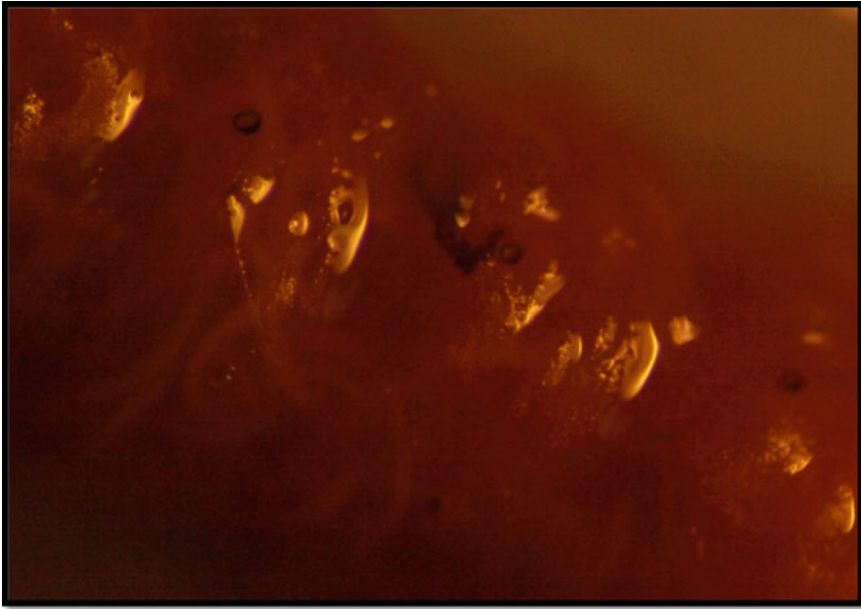


Fig. 2 Infective juveniles of *Heterorhabditis bacteriophora* inside the cadaver portion of rice case worm *Corcyra cephalonica*

3 Infection Behaviour

An important aspect which needs attention is recognition of host by infective juveniles and then entry into it. Infective juveniles of *Steinernema* and *Heterorhabditis* after entering into insect host develop there (Fig. 2). Host recognition can be measured by logging change in several types of behaviour in response to host related mortality (Ali et al. 2005). The movement parameters of entomopathogenic nematode that play an important role in host searching are duration of crawling, body waving, head waving and head thrusting. Though, infective juveniles have the ability to encounter both uninfected and infected hosts however they differ in responding. The quality of host also depends on the stage of the infection. Ramos-Rodriguez et al. (2007a) hypothesized that nematode response to infected hosts gets change over the course of an infection. They tested the attraction response of three different species of nematode i.e. *Steinernema carpocapsae*, *S. glaseri* and *S. riobrave* with different foraging strategies to infected and uninfected insects (*Galleria mellonella* and *Tenebrio molitor*). The test was performed at 24 h intervals from start of infection to emergence of infective juveniles from depleted host. *S. carpocapsae* was not very responsive to hosts but *S. glaseri* was highly responsive and *S. riobrave* was intermediate. Generally, the level of attraction did not change with time after infection and was similar between infected and

uninfected hosts. However, *T. molitor* infected with *S. glaseri* tended to be less attractive to *S. glaseri* than uninfected hosts.

The lipid reserves of infective juveniles decline with age which in turn has a negative effect in causing infection in a susceptible host. When the two foraging abilities of nematodes were compared, cruisers were found with high metabolic rate as compared to ambushers. However, the foraging ability declines with age. The ambushing behaviour (ability to stand on tail and wave the body in order to attach to a passing host) of *S. carpocapsae* has been observed decline with age (Lewis et al. 1997). The metabolic rate of *S. glaseri* is intermediate between cruisers and ambushers but they are hypothesized to contain more energy stores and are reported to survive up to 32 weeks. This is perhaps due to their larger size, approximately eight times the volume of the other species. Lewis et al. conducted a study on the three parameters i.e. locomotion, infectivity and lipid reserves of entomopathogenic nematodes, *Heterorhabditis bacteriophora*, *S. carpocapsae* and *S. glaseri*. They concluded that there was a significant effect of nematode ageing on these parameters. Locomotion, infectivity and lipid reserves declined as the age of nematodes increased. There was also a decrease in symbiotic bacteria with the increase in nematode age. Some species of *Heterorhabditis* such as *H. indica* and *H. pakistanaensis* when stored in aqueous suspension, the nematodes settle and form a precipitate on the bottom of the container. An agglomerate is formed, leaving the nematodes under stress situation in an environment of low oxygen content, which may accelerate the loss of energy reserves, such as lipids (Gaugler et al. 2002). So they get close and arrange in rosette form. A technique employed to reduce these aggregates is the use of a sodium bicarbonate solution (NaHCO_3), which aids in breaking the rosettes without causing other effects on the nematodes (Woodring and Kaya 1988).

The infection behaviour also varies among nematode species. When the infection behaviour of three nematode species, *S. feltiae*, *S. riobrave* and *S. carpocapsae* was studied, a significant decrease in infection rate was observed at 6–9 h after injecting a host with conspecifics (Glazer 1997). Grewal et al. (1997) found that infective juveniles of *S. carpocapsae* were repelled from insect hosts infected for at least 4 h with heterospecific nematodes. Infective juveniles can make their entry into a host that has already been occupied by conspecifics, even to the point of overcrowding (Lewis et al. 2006). Christen et al. (2007) reported that though the number of invading infective juveniles of *S. riobrave* declined over time, but they continued to enter the host for at least 72 h since first infection and that may be presumably after the death of the host. Dillon et al. (2006) on the basis of an experiment reported that infective juveniles of *S. carpocapsae* were repelled from host that was infected with *S. glaseri*. It is assumed that the subsequent invasion is prevented due to an inhibitory substance diffused from the host (Selvan et al. 1993). On the other hand, infective juveniles of *S. glaseri* respond no differently to a host infected with *S. carpocapsae* than to a non-infected host. Overcrowding of infective juveniles in a host which has already harbouring many competitors appears maladaptive because overcrowding results in lower reproductive output per founder (Koppenhöfer and Kaya 1995; Ryder and Griffin 2002). It is suggested that such

behaviour may be due to few alternative hosts available, in which case it is better to invade a suboptimal host than to reject it and die of starvation (Christen et al. 2007). Also infective juveniles may have limited ability to assess the quality of the host, and this may be due to lack of suitable cues from the host or suitable sensory abilities of the infective juveniles. Kunkel et al. (2006) reported that waste products from host late stage of infection repel nematodes. According to Lewis et al. (2006) there are mixed evidence available on infective juveniles, whether or not they respond differently to conspecific and heterospecific infected hosts and most studies have looked at a limited number of time-points post-infection. It has been observed that 4 days old infective juveniles of *S. glaseri* were more attracted to parasitized hosts at 4–6 h after their exposure (Lewis and Gaugler 1994) in comparison to unparasitized hosts. *S. glaseri* is reported to invade hosts that were killed 10 days previously while *H. indica* entered hosts that had been dead for three days (San-Blas and Gowen 2008). These workers have the opinion that entomopathogenic nematodes should be considered facultative scavengers rather than obligate parasites. A phenomenon known as ‘phased activation’ wherein a proportion of infective juveniles that emerged from a host cadaver are infectious at any time has also been studied by some workers. It has been noticed that there is significant variability among individuals emerging from the same host cadaver in the timing of their maximum infectivity (Campbell et al. 1999; Perez et al. 2003).

There are certain host species to which entomopathogenic nematodes cannot kill but have the ability to reproduce in them (Puza and Mracek 2010). It has also been revealed that infective juveniles of most entomopathogenic nematode species can enter the hosts killed by some other causes (e.g. freezing) (San-Blas and Gowen 2008; Puza and Mracek 2010). This has a great significance for the persistence of natural populations of entomopathogenic nematode. The recycling of applied entomopathogenic nematodes is enhanced, particularly when applied during integrated pest management programs where hosts killed by other biological agents or chemical methods may be available and suitable for development. The report of entomopathogenic nematodes developing in hosts killed by insecticides has been confirmed by several workers (Hara and Kaya 1983; Koppenhöfer et al. 2003).

Dillon et al. (2006) reported that under field condition the rate of parasitization declined as the depth of the insects in soil increased. However, the rate of decline was similar for all entomopathogenic nematode species tested, irrespective of their behaviour i.e., ambush, cruise or intermediate foragers. *S. carpocapsae* parasitized larvae and pupae of the large pine weevil (*Hylobius abietis*) which live under the bark of tree roots. When application of *S. carpocapsae* was done at the soil surface, they parasitized these insects within the roots at depths of up to 40 cm in the soil. Acid pH is reported to reduce the efficacy of *S. carpocapsae*, *S. feltiae* and *H. bacteriophora* when exposed to diapausing larvae of spruce webworm, *Cephalcia abietis* (Jaworska 1993), however at pH 6.9 and 8.0, mortality of cotton leaf worm, *Spodoptera littoralis* by *S. carpocapsae* and *H. bacteriophora* was higher and more rapid than at pH 5.6 (Ghally 1995).

4 Foraging Strategy-Host Interactions

The process of infection is governed by host recognition behaviour, acceptance behaviour and infection behaviour. Infective juveniles assess their hosts for entry by responding to cues such as feces or cuticle associated with living insects (Grewal et al. 1993; Lewis et al. 1996). The existence of a host and its chemical cues are likely to affect a nematode's foraging behaviour. The behavioural response of *S. carpocapsae* to various host species is correlated with the level of reproduction supported by the hosts (Lewis et al. 1996). The foraging strategy of nematodes while interacting with hosts is correlated with other aspects such as parasite ecology, behaviour, physiology and anatomy (Campbell and Lewis 2002). While selecting host, entomopathogenic nematodes have to follow a series of steps that include search for host habitat, host finding, host acceptance, and host suitability (Doutt 1964). The specific behaviours and cues used by infective juveniles of different nematode species vary while searching and finding the hosts. Ambushers use 'sit and wait' foraging strategy and respond to volatile chemical cues from the host when in a standing posture to enhance contact with a passing insect (Campbell and Kaya 2000), but they are not attracted to host volatile cues while crawling on the substrate, unless they have already contacted a host (Lewis et al. 1995). Cruisers readily respond to volatile chemical cues encountered by crawling toward the source of the cues (Lewis et al. 1992, 1993). Factors affecting infective juveniles behaviour include CO₂, temperature gradients, and host feces (Gaugler et al. 1980; Byers and Poinar 1982; Grewal et al. 1993; Lewis et al. 1993). Species of *Steinernema* which show nictation and jumping behaviour are stimulated by CO₂. Campbell and Lewis (2002) found *S. feltiae* showing significant infection preference for a host that was parasitized 24 h before in comparison to unparasitized host. The actual reason for these changed behaviour are unknown, however differences in CO₂ production between infected and uninfected insects have been reported (Ramos-Rodriguez et al. 2007b). Ramos-Rodriguez et al. (2007a) are of the opinion that the level of attraction would change as the quality of the host as a resource changes over time from initial infection. It is suggested that attraction of infective juveniles to host would be greatest at the time-points when the CO₂ production from infected hosts is at distinct peaks. Since the distribution of entomopathogenic nematodes in soil is often in clump (Stuart and Gaugler 1994; Glazer et al. 1996; Campbell et al. 1998), therefore, infective juveniles have the potential to encounter the hosts already infected. Infective juveniles infecting a host that has already been infected, particularly at time-points late in the infection process, may lead to increased competition for diminished resources or even the lack of sufficient nutrients in a host cadaver to complete development (Selvan et al. 1993). Besides infecting hosts by conspecifics, nematodes may also encounter hosts infected by heterospecific individuals. This ability will ease to distinguish between infected or uninfected hosts, and among hosts at different time-points after infection (Griffin 2012).

Entomopathogenic nematodes are also able to detect the presence of other pathogens of insect. Barbercheck and Kaya (1991) found accumulation of *S.*

carpocapsae and *H. bacteriophora* more around the healthy insect host than a host infected with the fungus *Beauveria bassiana*. Grewal et al. (1994) tested the foraging strategies of eight entomopathogenic nematode species for their response to host volatile cues and dispersal behaviour on 2-dimensional substrates. *H. bacteriophora*, *H. megidis*, *S. anomali* and *S. glaseri* showed positive directional response to chemical cues and travelled similar distances on smooth (agar) or nictation substrates (agar overlaid with sand grains) which suggest their cruising approach to find hosts. *S. carpocapsae* and *S. scapterisci* did not show any directional response and travelled less distance on nictation substrate, than on smooth agar. Thus, these two nematode species can be considered to have ambushing mode of foraging. *S. feltiae* and *Steinernema* sp. were intermediary in the search continuum sharing both the characteristics of ambush and cruise foragers. They responded directionally to host volatiles, but travelled less distance on the nictation substrate than on smooth agar, however neither of the two nictate. The cruisers located their hosts more effectively in the sand columns, whereas the ambushers were more effective at finding hosts on filter paper. Both *S. feltiae* and *Steinernema* sp. performed equally on filter paper as well as in the sand column. Ennis et al. (2010) reported in the case of pine weevil larvae that roots facilitated the movement of *S. carpocapsae* through media (sand or sand/peat mix), and this movement was enhanced by physical and/or chemical stimuli from weevils feeding on the roots. Susurluk (2008) compared the vertical movement of Turkish isolates of *S. feltiae* (TUR-S3) and *H. bacteriophora* (TUR-H2) at different temperatures in the presence and absence of greater wax moth, *Galleria mellonella* larvae. When the larva was placed on the bottom of the column, both the nematode species moved faster towards there. The vertical dispersal ability was found greater in *S. feltiae* as compared to *H. bacteriophora*. There was a direct relationship between nematode movement and temperature. As the temperature increased, the vertical movement of both the nematode species increased. Lower temperature depressed the movement of *H. bacteriophora* more than *S. feltiae*. The pathogenic potential of nematodes that had migrated different distances were compared with *G. mellonella*. The positive correlation between the distance travelled and infectivity indicated that there was a link between host-searching behaviour and infection behaviour in *S. feltiae* and to a lesser extent, also in *H. bacteriophora* (Susurluk 2008). *S. carpocapsae* proved much better than *H. bacteriophora* and *S. glaseri* in host finding ability, in case when the hosts were present at the soil surface but was less effective when hosts were below the surface (Alatorre-Rosas and Kaya 1990; Koppenhöfer et al. 1996).

5 Habitat Preference

Steinernematids and heterorhabditids are widespread soil nematodes which occur in various ecosystems and habitats. Their occurrence and distribution are related to certain insect groups that are considered their suitable hosts (Griffin 2012). They are

frequently available in insect rich habitats, such as tree (especially deciduous tree) habitats. Mracek et al. (1999) recovered highest numbers of entomopathogenic nematodes from apple and cherry verges along roadsides, lime and poplar hedges, and oak forests. The nematodes were recorded from 67% of habitats with high or moderate insect abundance. *S. kraussei* can be found in higher altitude in more spruce forests above 1000 m altitude. They were located at 2530 m in the Swiss Alps (Steiner 1996); 1290 m from subalpine meadows in Vitosha Mts in Bulgaria and from mountain forests at altitudes ranging from 700 to 1300 m (Shishiniova et al. 1997). Soil texture and presence of a host affect the nematode's direction of movement (Gaugler and Kaya 1993). Kruitbos et al. (2010) are of the view that *S. carpocapsae* instead of an ambush forager, is a habitat specialist, adapted to organic media such as peat or leaf litter. Their experiment showed that infective juveniles moved towards hosts more readily in peat than in sand. They suggested that *S. carpocapsae* remain near the surface because it does not move well through the mineral soils or the pure sands frequently used in experiments. The body waving characteristic of *S. carpocapsae* infective juveniles is an adaptation for bridging large pore spaces rather than attaching to passing insects at the soil surface. Natural association of *S. carpocapsae* with organic soils (e.g. peat) or soil horizons (e.g. litter layers) would support this hypothesis, however according to Griffin (2012) any such association has not been reported to date. In a study, infective juveniles of different species were observed to move into different directions when placed in the middle of the soil column. Most *H. bacteriophora* and *S. carpocapsae* move upwards rather than downwards, whereas most of the *S. glaseri* move downwards rather than upwards (Gaugler and Kaya 1990). Morton and García-Del-Pino (2009) isolated *S. feltiae* and *H. bacteriophora* from stone-fruit orchards in two Mediterranean regions of Spain. The activities of these nematodes such as tolerance to environmental heat, desiccation and hypoxia, the effect of temperature on infectivity and reproduction and nematode migration in sand columns were compared among isolates and also with a strain of *S. carpocapsae*. The results showed differences among species and a great variability within species too. Koppenhöfer et al. (1997) hypothesized that survival of entomopathogenic nematode in dry soil increased when contained within an insect cadaver. *H. bacteriophora* causing infection produced a gummy consistency in host cadaver that is supposed to help in retaining moisture.

6 Bacterial Symbionts and Nematode Behaviour

The bacterial symbionts (*Photorhabdus*, *Xenorhabdus*) of entomopathogenic nematodes play a significant role in the death of the host and nutritional requirements of nematodes (Han and Ehlers 2000; Ciche et al. 2006). These bacteria have an effective variety of toxins and antibiotics (Askary and Abd-Elgawad 2017). The behaviour of nematodes is also affected by bacterial symbionts and most of the effects on nematode behaviour occur during the growth phase of the bacteria within

the host. Few workers reported that *S. carpocapsae* infective juveniles without symbiont survived longer than those with them, and this was presumed to be due to the energetic cost of maintaining the bacteria (Mitani et al. 2004; Emelianoff et al. 2007). Also, infective juveniles of *S. carpocapsae* harbouring few bacteria survived for longer period than those with more bacteria however, the former had a lower reproductive rate upon entering into a host (Emelianoff et al. 2008). The impact of bacterial symbiont on behaviour of would-be scavengers of the insect cadaver is also reported. Some workers have reported cadaver repellent to ants due to bacterial products from both *Xenorhabdus* and *Photorhabdus* (Baur et al. 1998; Zhou et al. 2002). European robin, *Erithacus rubecula* which is an avian predator, was not attracted to the cadavers infected with *Heterorhabditis bacteriophora* due to the red colour reinforced by unpalatable taste (Fenton et al. 2011).

7 Reproductive Behaviour

Lewis et al. (2006) have the opinion that infective juveniles decide whether the reproduction is to take place within insect cadavers. The choice of invading the host by infective juveniles also affects the pool of mating partners (and competitors) available to them when mature. Pheromones have been implicated in the sexual attraction of over 30 species of nematode, but relatively few genera (Lee 2002). The females of *S. carpocapsae* produce a pheromone that has the characteristic to attract males (Neves et al. 1998). It has been reported that male *S. carpocapsae* were attracted to virgin females, but not to mated ones (Lewis et al. 2002). Thus, it may be suggested that once after mating the females do not secrete pheromone or the females do not mate repeatedly. An ethogram was developed to show that males of *S. carpocapsae* respond strongly to virgin females when present in their immediate proximity. A high percentage (80%) of males crawled to the females and among them 56.25% approached the female middle section where the vulva was located, 37.5% approached the female head while the rest i.e. 6.25% approached the tail (Lewis et al. 2002). While mating, the male of *Steinernema* coils around the female at the vulval region (Strauch et al. 1994; Lewis et al. 2002), however the male of *Heterorhabditis* has been observed to align its body approximately parallel to the female without coiling around her; male head is pointed in the opposite direction from that of the female, with much of its body away from the female (Huettel 2004), thus making copulation in liquid culture impossible (Strauch et al. 1994). The morphological reasons for the difference in the mating positions are still not clear. Ebssa et al. (2008) reported that besides attraction, the diffusible substances that originate by the females of *Steinernema* may have organizational effects on males. Presence of female nematode in close proximity is supposed to develop a male nematode for maturation. The males of *S. longicaudum* that developed alone did not mature sexually and were incapable of fertilizing eggs and no sperm were visible in their reproductive tract, but when exposed to conspecific females, though separated by a barrier allowed males to mature. This has implications for mass production as

unproductive amphimictic adults that are produced by the first generation hermaphrodites compete for resources with the hermaphrodites and the infective juveniles that develop in them (Ehlers 2001). In case of *heterorhabditis* sperm competition may occur i.e. competition between males and hermaphrodites to fertilize a given set of ova. *H. bacteriophora* hermaphrodites have been found to prefer male sperm (Dix et al. 1994). Mating or copulatory plugs are deposited over the vulva by male *Heterorhabditis* spp. (Dix et al. 1994; Strauch et al. 1994). Such plugs are assumed to have evolved in response to male-male competition, so that mating from a second male can be prevented (Barker 1994). However, plugs are not always effective (Timmermeyer et al. 2010). It is yet to be tested whether remating of *Heterorhabditis* spp. females or hermaphrodites are prevented by copulatory plug.

The juveniles of *Heterorhabditis* developing within the mother become exclusively infective juveniles, while eggs laid into the cadaver may continue to develop as adult, so it is likely that the “sacrifice” of its soma to infective juveniles is an adaptive response designed to optimize the use of a limited and ephemeral cadaver. In case of *Heterorhabditis*, only those juveniles that develop within the mother are colonized by bacteria. It is suggested that intra-uterine development is an adaptation for symbiont transmission (Ciche et al. 2008). However, this phenomenon has evolved to optimize female’s reproductive success in response to stressful or starvation conditions, and may be seen as a form of parental behaviour (Griffin 2012).

8 Conclusion

In the foregone review it is very clear that there are variations in every trait of entomopathogenic nematode. These variations may be due to differences in environmental and internal factors, including insect host, soil texture, age of infective juveniles, the conditions under which they developed, and the bacterial load carried by the infective juveniles (Griffin 2012). A better understanding of nematode behaviour and physiology will certainly lead to further development of perfect formulations and ultimately their exploitation in farmers’ field (Coupland et al. 2017). In the past two decades, the advances or breakthrough in the areas like isolation, formulation and their application against numerous economically important insect pests entail that there is much relevant information for research practitioners working in different laboratories of the world (Abd-Elgawad et al. 2017). The host-finding abilities of *Steinernema* and *Heterorhabditis* have still not been clearly understood though their some species have excellent pathogenic potential but have a narrow host range which needs special attention. Since they are applied inundatively, they may be tailored by breeding or transformation, to their intended purpose and to ecological incompetence, so as to improve their efficacy and ecological safety (Downes and Griffin 1996). It is difficult to understand precisely the functional significance either for entomopathogenic nematode fitness or biocontrol utility, since most behavioural studies are necessarily conducted in simplified conditions, that differs from natural environment where the situation is

often complex. Therefore, extensive studies are required because there are differences to what is known in the laboratory to the real lives of entomopathogenic nematodes in soil, whether as natural populations or applied for biological control. Researchers should pay attention to isolate indigenous nematodes in order to expand the genetic base of indigenous nematode strain, knowing their adaptability to exploit them in a tactful manner (Askary et al. 2017). A detail study is needed on habitat preference, host finding ability, host specificity and infection ability of entomopathogenic nematodes in soil. Another aspect that has got less attention by research workers is the reproductive behaviour of entomopathogenic nematodes. Though, as reviewed above, sex pheromones, copulation behaviour, sperm competition and sexual maturation have all received some attention but still enhanced knowledge on the subject is required so as to obtain maximum benefits from these beneficial microorganisms in sustainable agriculture.

Acknowledgements This work was supported in part by All India Coordinated Research Project (AICRP), ICAR on Biological Control of Crop Pests and Weeds running in the Biological Control Laboratory, Division of Entomology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Srinagar, Jammu and Kashmir, India.

References

- Abd-Elgawad MMM, Askary TH, Coupland J (2017) Biocontrol agents: entomopathogenic and slug parasitic nematodes. CAB International, Wallingford, UK, p 648
- Alatorre-Rosas R, Kaya HK (1990) Interspecific competition between entomopathogenic nematodes in the genera *Heterorhabditis* and *Steinernema* for an insect host in sand. *J Invertebr Pathol* 55:179–188
- Ali SS, Ahmad R, Hussain MA, Parvez R (2005) Pest management in pulses through entomopathogenic nematodes. Indian Institute of Pulse Research, Kanpur, India
- Askary TH (2010) Nematodes as biocontrol agents. In: Lichtfouse E (ed) *Sociology, organic farming climate change and soil science*. Springer, The Netherlands, pp 347–378
- Askary TH, Abd-Elgawad MMM (2017) Beneficial nematodes in agroecosystems: a global perspective. In: Abd-Elgawad MMM, Askary TH, Coupland J (eds) *Biocontrol agents: entomopathogenic and slug parasitic nematodes*. CAB International, Wallingford, UK, pp 3–25
- Askary TH, Ahmad MJ (2017) Entomopathogenic nematodes: mass production, formulation and application. In: Abd-Elgawad MMM, Askary TH, Coupland J (eds) *Biocontrol agents: entomopathogenic and slug parasitic nematodes*. CAB International, Wallingford, UK, pp 261–287
- Askary TH, Nermut J, Ahmad MJ, Ganai MA (2017) Future thrusts in expanding the use of entomopathogenic and slug parasitic nematodes in agriculture. In: Abd-Elgawad MMM, Askary TH, Coupland J (eds) *Biocontrol agents: entomopathogenic and slug parasitic nematodes*. CAB International, Wallingford, UK, pp 620–627
- Barbercheck ME, Kaya HR (1991) Effect of host condition and soil texture on host finding by the entomogenous nematodes *Heterorhabditis bacteriophora* (Rhabditida, Heterorhabditidae) and *Steinernema carpocapsae* (Rhabditida, Steinernematidae). *Environ Entomol* 20:582–589
- Barker DM (1994) Copulatory plugs and paternity assurance in the nematode *Caenorhabditis elegans*. *Anim Behav* 48:147–156
- Baur ME, Kaya HK, Strong DR (1998) Foraging ants as scavengers on entomopathogenic nematode-killed insects. *Biol Control* 12:231–236

- Byers JA, Poinar GO Jr (1982) Location of insect hosts by the nematode, *Neoplectana carpocapsae*, in response to temperature. *Behaviour* 79:1–10
- Campbell JF, Gaugler R (1993) Nictation behaviour and its ecological implications in host search strategies of entomopathogenic nematodes. *Behaviour* 126:155–169
- Campbell JF, Gaugler R (1997) Inter-specific variation in entomopathogenic nematode foraging strategy: dichotomy or variation along a continuum? *Fundam Appl Nematol* 20:393–398
- Campbell JF, Kaya HK (1999a) How and why a parasitic nematode jumps. *Nature* 397:485–486
- Campbell JF, Kaya HK (1999b) Mechanism, kinematic performance, and fitness consequences of jumping behaviour in entomopathogenic nematodes (*Steinernema* spp.). *Can J Zool* 77:1947–1955
- Campbell JF, Kaya HK (2000) Influence of insect associated cues on the jumping behavior of entomopathogenic nematodes (*Steinernema* spp.). *Behaviour* 137:591–609
- Campbell JF, Kaya HK (2002) Variation in entomopathogenic nematode (*Steinernematidae* and *Heterorhabditidae*) infective-stage jumping behaviour. *Nematology* 4:471–482
- Campbell JF, Lewis EE (2002) Entomopathogenic nematode host-search strategies. In: Lewis EE, Campbell JF, Sukhdeo MVK (eds) *The behavioural ecology of parasites*. CABI Publishing, Wallingford, UK, pp 13–38
- Campbell JF, Orza G, Yoder F, Lewis EE, Gaugler R (1998) Spatial and temporal distribution of endemic and released entomopathogenic nematode populations in turfgrass. *Entomol Exp Appl* 86:1–11
- Campbell JF, Koppenhofer AM, Kaya HK, 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
- Christen JM, Campbell JF, Lewis EE, Shapiro-Ilan DI, Ramaswamy SB (2007) Responses of the entomopathogenic nematode, *Steinernema riobrave* to its insect hosts, *Galleria mellonella* and *Tenebrio molitor*. *Parasitology* 134:889–898
- Ciche TA, Darby C, Ehlers R-U, Forst S, Goodrich-Blair H (2006) Dangerous liaisons: the symbiosis of entomopathogenic nematodes and bacteria. *Biol Control* 38:22–46
- Ciche TA, Kim KS, Kaufmann-Daszczuk B, Nguyen KCQ, Hall DH (2008) Cell invasion and matricide during *Photorhabdus luminescens* transmission by *Heterorhabditis bacteriophora* nematodes. *Appl Environ Microbiol* 74:2275–2287
- Coupland J, Abd-Elgawad MMM, Askary TH (2017) Beneficial nematodes and the changing scope of crop protection. In: Abd-Elgawad MMM, Askary TH, Coupland J (eds) *Biocontrol agents: entomopathogenic and slug parasitic nematodes*. CAB International, Wallingford, UK, pp 26–42
- Csontos AS (2002) Lateral movement of the entomopathogenic nematodes *Steinernema glaseri* and *Heterorhabditis bacteriophora* in sand at different temperatures in response to host seeking. *Biocontrol Sci Tech* 12:137–139
- Dillon AB, Ward D, Downes MJ, Griffin CT (2006) Suppression of the large pine weevil *Hylobius abietis* (L.) (Coleoptera: Curculionidae) in pine stumps by entomopathogenic nematodes with different foraging strategies. *Biol Control* 38:217–226
- Dix I, Koltai H, Glazer I, Burnell AM (1994) Sperm competition in mated 1st generation hermaphrodite females of the HP-88 strain of *Heterorhabditis* (Nematoda, Heterorhabditidae) and progeny sex-ratios in mated and unmated females. *Fundam Appl Nematol* 17:17–27
- Doutt RL (1964) Biological characteristics of entomophagous adults. In: DeBach P (ed) *Biological control of insect pests and weeds*. Reinhold, New York, USA, pp 145–167
- Downes MJ, Griffin CT (1996) Dispersal behaviour and transmission strategies of the entomopathogenic nematodes *Heterorhabditis* and *Steinernema*. *Biocontrol Sci Tech* 6:347–356
- Ebssa L, Dix I, Griffin CT (2008) Female presence is required for male sexual maturity in the nematode *Steinernema longicaudum*. *Curr Biol* 18:R997–R998
- Ehlers R-U (2001) Mass production of entomopathogenic nematodes for plant protection. *Appl Microbiol Biotechnol* 56:623–633

- Emelianoff V, Sicard M, N-le Brun, Moulia C, Ferdy JB (2007) Effect of bacterial symbionts *Xenorhabdus* on mortality of infective juveniles of two *Steinernema* species. *Parasitol Res* 100:657–659
- Emelianoff V, Chapuis E, Le Brun N, Chiral M, Moulia C, Ferdy JB (2008) A survival-reproduction trade-off in entomopathogenic nematodes mediated by their bacterial symbionts. *Evolution* 62:932–942
- Ennis DE, Dillon AB, Griffin CT (2010) Simulated roots and host feeding enhance infection of subterranean insects by the entomopathogenic nematode *Steinernema carpocapsae*. *J Invertebr Pathol* 103:140–143
- Fenton A, Magoolagan L, Kennedy Z, Spencer KA (2011) Parasite-induced warning coloration: a novel form of host manipulation. *Anim Behav* 81:417–422
- Gaugler R, Bilgrami AL (2004) Introduction and overview. In: Gaugler R, Bilgrami AL (eds) *Nematode behaviour*. CAB International, Wallingford, UK, pp XI–XXIV
- Gaugler R, Kaya HK (1990) Entomopathogenic nematodes in biological control. CRC Press, Boca Raton, Florida, USA, p 349
- Gaugler R, Kaya HK (1993) Entomopathogenic Nematodes. *Annu Rev Entomol* 38:181–206
- Gaugler R, LeBeck L, Nakagaki B, Boush GM (1980) Orientation of the entomogenous nematode, *Neoplectana carpocapsae* to carbon dioxide. *Environ Entomol* 9:649–652
- Gaugler R, Lewis EE, Stuart RJ (1997) Ecology in the service of biological control: the case of entomopathogenic nematodes. *Oecology* 109:483–489
- Gaugler R, Brown I, Shapiro-Ilan DI, Atwa A (2002) Automated technology for in vivo mass production of entomopathogenic nematodes. *Biol Control* 24:199–206
- Ghally SE (1995) Some factors affecting the activity and pathogenicity of *Heterorhabditis heliothidis* and *Steinernema carpocapsae* nematodes. *J Egypt Soc Parasitol* 25:125–135
- Glazer I (1997) Effects of infected insects on secondary invasion of steinernematid entomopathogenic nematodes. *Parasitology* 114:597–604
- Glazer I, Kozodoi E, Salame L, Nestel D (1996) Spatial and temporal occurrence of natural populations of *Heterorhabditis* spp. (Nematoda: Rhabditida) in a semiarid region. *Biol Control* 6:130–136
- Grewal PS, Gaugler R, Selvan S (1993) Host recognition by entomopathogenic nematodes: Behavioral response to contact with host feces. *J Chem Ecol* 19:1219–1231
- Grewal PS, Lewis EE, Gaugler R, Campbell JF (1994) Host finding behaviour as a predictor of foraging strategy in entomopathogenic nematodes. *Parasitology* 108:207–215
- Grewal PS, Lewis EE, Gaugler R (1997) Response of infective stage parasites (Nematoda: Steinernematidae) to volatile cues from infected hosts. *J Chem Ecol* 23:503–515
- Griffin CT (2012) Perspectives on the behavior of entomopathogenic nematodes from dispersal to reproduction: traits contributing to nematode fitness and biocontrol efficacy. *J Nematol* 44:177–184
- Griffin CT, Boemare NE, Lewis EE (2005) Biology and behaviour. In: Grewal PS, Ehlers R-U, Shapiro-Ilan DI (eds) *Nematodes as biocontrol agents*. CAB International, Wallingford, UK, pp 47–64
- Han RC, Ehlers R-U (2000) Pathogenicity, development, and reproduction of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* under axenic in vivo conditions. *J Invertebr Pathol* 75:55–58
- Hara AH, Kaya HK (1983) Development of the entomogenous nematode, *Neoplectana carpocapsae* (Rhabditida: Steinernematidae), in insecticide killed beet armyworm (Lepidoptera: Noctuidae). *J Econ Entomol* 76:423–426
- Huettel RN (2004) Reproductive behaviour. In: Gaugler R, Bilgrami AL (eds) *Nematode behaviour*. CAB International, Wallingford, UK, pp 127–149
- Jaworska M (1993) Investigations on the possibility of using entomopathogenic nematodes in reduction of *Cephalcia abietis* (L.) (Hym., Pamphilidae) population. *Polskie Pismo Entomologiczne* 62:201–213
- Koppenhöfer AM, Kaya HK (1995) Density-dependent effects on *Steinernema glaseri* (Nematoda: Steinernematidae) within an insect host. *J Parasitol* 81:797–799

- Koppenhöfer AM, Baur ME, Kaya HK (1996) Competition between two steinernematid nematode species for an insect host at different soil depths. *J Parasitol* 82:34–40
- Koppenhöfer AM, Baur ME, Stock SP, Choo HY, Chinnasri B, Kaya HK (1997) Survival of entomopathogenic nematodes within host cadavers in dry soil. *Appl Soil Ecol* 6:231–240
- Koppenhöfer AM, Cowles RS, Cowles EA, Fuzy EM, Kaya HK (2003) Effect of neonicotinoid synergists on entomopathogenic nematode fitness. *Entomol Exp Appl* 106:7–18
- Kruitbos LM, Heritage S, Hapca S, Wilson MJ (2010) The influence of habitat quality on the foraging strategies of the entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis megidis*. *Parasitology* 137:303–309
- Kunkel BA, Shapiro-Ilan DI, Campbell JF, Lewis EE (2006) Effect of *Steinernema glaseri* infected host exudates on movement of conspecific infective juveniles. *J Invertebr Pathol* 93:42–49
- Lee DL (2002) Behaviour. In: Lee DL (ed) *The biology of nematodes*. Taylor and Francis, London, UK, pp 369–387
- Lewis EE, Gaugler R (1994) Entomopathogenic nematode (Rhabditida: Steinernematidae) sex ratio relates to foraging strategy. *J Invertebr Pathol* 64:238–242
- Lewis EE, Gaugler R, Harrison R (1992) Entomopathogenic nematode host finding: response to host contact cues by cruise and ambush foragers. *Parasitology* 105:309–315
- Lewis EE, Gaugler R, Harrison R (1993) Response of cruiser and ambusher entomopathogenic nematodes (Steinernematidae) in host volatile cues. *Can J Zool* 71:765–769
- Lewis EE, Grewal PS, Gaugler R (1995) Hierarchical order of host cues in parasite foraging: a question of context. *Parasitology* 110:207–213
- Lewis EE, Ricci M, Gaugler R (1996) Host recognition behaviour predicts host suitability in the entomopathogenic nematode *Steinernema carpocapsae*. (Rhabditida: Steinernematidae). *Parasitology* 113:573–579
- Lewis EE, Campbell JF, Gaugler R (1997) The effects of ageing on the foraging behaviour of *Steinernema carpocapsae* (Rhabditida: Steinernematidae). *Nematologica* 43:355–362
- Lewis EE, Barbarosa B, Gaugler R (2002) Mating and sexual communication by *Steinernema carpocapsae* (Nematoda: Steinernematidae). *J Nematol* 34:328–331
- Lewis EE, Campbell JF, Griffin C, Kaya HK, Peters A (2006) Behavioral ecology of entomopathogenic nematodes. *Biol Control* 38:66–79
- Mitani DK, Kaya HK, Goodrich-Blair H (2004) Comparative study of the entomopathogenic nematode, *Steinernema carpocapsae*, reared on mutant and wild-type *Xenorhabdus nematophilus*. *Biol Control* 29:382–391
- Molyneux AS (1983) The biology and ecology of the entomopathogenic nematodes, *Heterorhabditis* spp. (Heterorhabditidae) and *Steinernema* spp. (Steinernematidae). Unspecified thesis, University of Tasmania
- Morton A, García-Del-Pino F (2009) Ecological characterization of entomopathogenic nematodes isolated in stone fruit orchard soils of Mediterranean areas. *J Invertebr Pathol* 102:203–213
- Mracek Z, Becvar S, Kindlmann P (1999) Survey of entomopathogenic nematodes from the families Steinernematidae and Heterorhabditidae (Nematoda: Rhabditida) in the Czech Republic. *Folia Parasitol* 46:145–148
- Neves JM, Simoes N, Mota M (1998) Evidence for a sex pheromone in *Steinernema carpocapsae*. *Nematologica* 44:95–98
- Perez EE, Lewis EE, Shapiro-Ilan DI (2003) Impact of the host cadaver on survival and infectivity of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) under desiccating conditions. *J Invertebr Pathol* 82:111–118
- Puza V, Mracek Z (2010) Does scavenging extend the host range of entomopathogenic nematodes (Nematoda: Steinernematidae)? *J Invertebr Pathol* 104:1–3
- Ramos-Rodriguez O, Campbell JF, Christen JM, Shapiro-Ilan DI, Lewis EE, Ramaswamy SB (2007a) Attraction behaviour of three entomopathogenic nematode species towards infected and uninfected hosts. *Parasitology* 134:729–738

- Ramos-Rodriguez O, Campbell JF, Lewis EE, Shapiro-Ilan DI, Ramaswamy SB (2007b) Dynamics of carbon dioxide release from insects infected with entomopathogenic nematodes. *J Invertebr Pathol* 94:64–69
- Reed EM, Wallace HR (1965) Leaping locomotion in an insect parasitic nematode. *Nature* 206:210–211
- Ryder JJ, Griffin CT (2002) Density dependent fecundity and infective juvenile production in the entomopathogenic nematode, *Heterorhabditis megidis*. *Parasitology* 125:83–92
- San-Blas E, Gowen SR (2008) Facultative scavenging as a survival strategy of entomopathogenic nematodes. *Int J Parasitol* 38:85–91
- Sankaranarayanan C, Askary TH (2017) Status of entomopathogenic nematodes in integrated pest management strategies in India. In: Abd-Elgawad MMM, Askary TH, Coupland J (eds) *Biocontrol agents: entomopathogenic and slug parasitic nematodes*. CAB International, Wallingford, UK, pp 362–382
- Selvan S, Campbell JF, Gaugler R (1993) Density-dependent effects on entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) within an insect host. *J Invertebr Pathol* 62:278–284
- Shishiniova M, Budurova I, Gradinarov D (1997) Contribution to fauna of entomopathogenic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae) from Bulgaria. *Biotechnol Biotechnol Equip* 11:45–52
- Steiner WA (1996) Distribution of entomopathogenic nematodes in the Swiss Alps. *Rev Suisse Zool* 103:439–452
- Strauch O, Stoessel S, Ehlers R-U (1994) Culture conditions define automictic or amphimictic reproduction in entomopathogenic rhabditid nematodes of the genus *Heterorhabditis*. *Fundam Appl Nematol* 17:573–582
- Stuart RJ, Gaugler R (1994) Patchiness in populations of entomopathogenic nematodes. *J Invertebr Pathol* 64:39–45
- Susurluk J (2008) Potential of the entomopathogenic nematodes *Steinernema feltiae*, *S. weiseri* and *Heterorhabditis bacteriophora* for the biological control of the sugar beet weevil *Bothynoderes punctiventris* (Coleoptera: Curculionidae). *J Pest Sci* 81:221–225
- Timmermeyer N, Gerlach T, Guempel C, Knoche J, Pfann JF, Schliessmann D, Michaels NK (2010) The function of copulatory plugs in *Caenorhabditis remanei*: hints for female benefits. *Front Zool* 7:28
- Woodring JL, Kaya HK (1988) Steinernematid and Heterorhabditid nematodes: a hand book of biology and techniques. Southern Cooperative Series Bulletin 331, Arkansas Agricultural Experimental Station, Fayetteville, Arkansas, pp 1–30
- Zhou XS, Kaya HK, Heungens K, Goodrich-Blair H (2002) Response of ants to a deterrent factor (s) produced by the symbiotic bacteria of entomopathogenic nematodes. *Appl Environ Microbiol* 68:6202–6209