Spatial Ecology of Entomopathogenic Nematodes with Contrasting Foraging Strategies

Dissertation

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By

Harit Kaur Bal

Graduate Program in Entomology

The Ohio State University

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Dissertation Committee:

Parwinder S. Grewal, Advisor

Casey W. Hoy

Luis A. Cañas

Robin A. J. Taylor

Andrew P. Michel
Abstract

Entomopathogenic nematodes (EPNs) have emerged as therapeutic biological insecticides for the management of insect pests inhabiting soil. Lack of information on population biology has created a major obstacle in establishing sustainable pest management programs and development of conservation approaches to use these biological control agents. Laboratory studies have identified a dichotomy in foraging behavior of EPN species but little is known about their dispersal patterns in the field. Therefore, the overall goal of this dissertation research was to examine the dispersal behavior of two EPN species with contrasting foraging strategies, cruiser, *Heterorhabditis bacteriophora* and ambusher, *Steinernema carpocapsae* from nematode-infected host cadavers in soil in laboratory and field conditions which will expand our knowledge of the field population dynamics of EPNs to fully utilize their biological control potential.

Specific objectives of this research were 1) to assess the rate of active lateral movement and dispersal patterns of the two EPN species, cruiser, *H. bacteriophora* and ambusher, *S. carpocapsae* with contrasting foraging strategies from nematode-infected host cadavers in soil in the absence of hosts in laboratory conditions (Chapter 2). Then, 2) to investigate the effect of vegetation (Chapter 2) and 3) mobile and non-mobile host
insects (Chapter 3) on the active lateral movement and dispersal patterns of these two EPN species from nematode-infected cadavers in soil in greenhouse conditions. Further, 4) to quantify their short-term dispersal potential from nematode-infected cadavers in the field (Chapter 4). Finally, 5) to artificially select ambusher, *S. carpocapsae* for enhanced dispersal (Chapter 5).

The results revealed that the two species differed in the spatio-temporal pattern of dispersal but showed similar average population displacement (~6 cm/day) in the absence of hosts. While a majority of *S. carpocapsae* dispersed <3.8 cm from the source cadaver, a majority of *H. bacteriophora* dispersed between 7-15 cm away from the source cadaver. However, a greater percentage of *S. carpocapsae* (~2.5 times) travelled faster than the fastest *H. bacteriophora* to larger distances, 15-61 cm. These apparent ‘sprinters’ may represent an adaptive dispersal strategy by the otherwise ambush forager *S. carpocapsae* in the absence of hosts. Vegetation enhanced dispersal of both species but more so for *H. bacteriophora*.

While mobile hosts enhanced dispersal of both species, non-mobile hosts enhanced only *H. bacteriophora* dispersal up to 24 h at the closest distance from the source cadaver. *S. carpocapsae* showed significantly greater average displacement than *H. bacteriophora* in the presence of hosts, mobile (8.06 cm/day) and non-mobile (5.07 cm/day) hosts. A greater percentage of *S. carpocapsae* (~14.5%) than *H. bacteriophora* (~0.4%) IJs dispersed the farthest distance, 11.4 cm in the presence of mobile hosts, with no difference in the presence of non-mobile hosts. Despite these differences, a
considerable proportion of IJs of both species ambushed near (< 3.8 cm) the source cadaver in the presence of hosts.

In the field, *S. carpocapsae* dispersed as far as *H. bacteriophora* with similar average displacements (27-28 cm/day) in a potato field and adjoining grassy border over a five day period. The two habitats did not differ in the abundance of surface active and soil-dwelling arthropods. *S. carpocapsae* was detected in larger numbers than *H. bacteriophora* in the pitfall traps. However, Trombidiformes (mites) were significantly correlated with percent *H. bacteriophora* dispersed to 2 m from the source cadavers.

*S. carpocapsae* responded positively to selection for dispersal with enhanced dispersal rate and reduced nictation, but also low reproduction potential. Dispersal increased significantly (13-23 fold) during the first five rounds of selection and showed only a marginal increase thereafter. The sprinters of the selected lines exhibiting greater dispersal potential than the ones in the foundation population were comprised of a greater proportion of males. The results provide quantitative understanding of EPN dispersal and suggest artificial selection as a promising approach for enhancing *S. carpocapsae* dispersal, which would have implications for designing strategies for inundative application and establishing sustainable populations of these important biological control agents.
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Vita

2005..........................................................B.S. Plant Protection, Punjab Agricultural University

2007..........................................................M.S. Entomology, Punjab Agricultural University

2007 to 2008........................................Senior Research Fellow, Department of Entomology, Punjab Agricultural University

2008 to 2009........................................Graduate Teaching Associate, Department of Entomology, The Ohio State University

2009 to 2011........................................Graduate Research Associate, Department of Entomology, The Ohio State University

2011 to 2012..........................................Presidential Fellow, The Ohio State University

Publications


**Field of Study**

**Major Field: Entomology**
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Chapter 1: Introduction

Soil insect pests cause significant problems to agriculture and urban landscapes in Ohio and around the world (Pedigo and Rice, 2009). Chemical insecticides are readily used to control these insect pests with the worldwide expenditure of $52 billion in 2009 (Bhattacharjee, 2010). In order to overcome the harmful side effects of insecticides on the environment and human health, resulting from their excessive and indiscriminate use, application of biological control agents has been encouraged. Entomopathogenic nematodes (EPNs) have emerged as one such alternative to insecticides which can effectively manage a number of insect pests in high value crops such as strawberry plantations, ornamental plants, mushrooms, orchards and turf (Grewal et al., 2005). For example, EPNs now provide the most effective control of citrus root weevil in Florida (Duncan et al., 1999) and of the black vine weevil in ornamentals in Europe (Grewal et al., 2005). In Ohio, the use of EPNs have been targeted against major insect pests such as white grubs, black vine weevil, fungus gnats, carrot weevil and grape root borer (Grewal et al., 2005; Jagdale et al., 2007; Miklasiewicz et al., 2002; Williams et al., 2002).

However, the failure to adopt them as sustainable biological control agents is due largely to our ignorance of their basic field ecology and population biology. In particular, little is known about their ability to disperse from a point of application. This information is critical for developing inoculative release and conservation strategies. A quantitative
understanding of their dispersal abilities in the presence as well as absence of insect hosts is essential for developing conservation approaches to establishing EPNs as biological control agents. Therefore, my long term goal is to expand our knowledge of the field population dynamics of EPNs to build reliable and effective sustainable approaches for utilizing these important and safe biological control agents.

The third stage juvenile, known as infective juvenile (IJ), is the only free living stage in the life cycle of the EPNs which searches for host insects, kills it in association with its symbiotic bacteria for feeding and reproduction. Laboratory studies have identified a dichotomy in foraging behavior of EPN species and have considerably contributed to the knowledge on their host finding behavior in controlled environments at relatively small spatial scales (< 30 cm). EPNs are classified as cruisers (actively searching foragers), ambushers (sit and wait foragers) and intermediate foragers based on their host finding tactics (Lewis et al., 1992; Campbell and Gaugler, 1993; Grewal et al., 1994). While, S. carpocapsae IJs actively nictate and forage near the soil surface, using an ambush-type foraging strategy (Campbell and Gaugler, 1993), H. bacteriophora IJs move actively through the soil in search of a host and directly respond to long-range host cues (Lewis et al., 1992). However, little is known about their dispersal in the absence of the hosts and the impact of these foraging strategies on the dispersal of EPNs in the field. For example, what happens in soil when host insects are not around? Are there specific conditions under which a specific type of foraging strategy is favored? Do EPNs switch their foraging strategies to maximize their host finding? Does habitat complexity and host/non-host insects affect EPN dispersal? My dissertation research was conceived to answer some of these questions with the overall goal to understand the dispersal behavior
of EPNs in their natural habitat, soil in laboratory, greenhouse and field conditions to fully utilize their biological control potential.

My central hypothesis was that neither the rate nor the pattern of dispersal will differ between the ambushing and cruising EPN species in soil in the microcosms or in the field, at a large spatial scale (in meters). My specific experimental objectives were: (1) to compare the active lateral movement and dispersal patterns of *H. bacteriophora* and *S. carpocapsae* from nematode-infected cadavers in sterilized soil in the absence of hosts in the laboratory (*Chapter 2*); (2) to assess the effect of vegetation on the active lateral movement and dispersal patterns of *H. bacteriophora* and *S. carpocapsae* from nematode-infected cadavers in sterilized soil in greenhouse conditions (*Chapter 2*); (3) to assess the effect of vegetation and host insects with different life histories on the active lateral movement and dispersal patterns of *H. bacteriophora* and *S. carpocapsae* from nematode-infected cadavers in sterilized soil in greenhouse conditions (*Chapter 3*); (4) to assess the effect of active and phoretic movement on dispersal potential of *H. bacteriophora* and *S. carpocapsae* from nematode-infected cadavers in the field with differing vegetation intensity (*Chapter 4*); and (5) to artificially select the ambusher, *S. carpocapsae* for enhanced mobility (*Chapter 5*).

**Definitions: Dispersal and Migration**

Since the overall goal of my dissertation research is to examine the dispersal behavior of two EPN species with contrasting foraging strategies from nematode-infected host cadavers in soil, I am interested in identifying the differences between the two terms
‘Dispersal’ and ‘Migration’ used in literature to explain the movement of organisms, and developing my own definitions for appropriate interpretation of results.

The Oxford English Dictionary defines “Dispersal” as “the action or process of distributing or spreading things or people over a wide area” and “Migration” as “seasonal movement of animals from one region to another.” These terms have been defined in different ways by several researchers in the past century due to which there is no single accepted definition of dispersal and migration in the scientific literature. One of the definitions of migration, proposed by Kennedy (1961) is “Migratory behavior is persistent and straightened out movement effected by the animal’s own locomotory exertions or by its active embarkation upon a vehicle. It depends on some temporary inhibition of station keeping responses but promotes their eventual distribution and recurrence.” Williams (1958) defined migration as “a continued movement in a more or less definite direction, in which both movement and direction are under the control of the animal concerned.” Johnson (1960, 1969) indicated close association of migration with reproduction, and termed this as “oogenesis-flight syndrome.” While these researchers provided the behavioral and physiological concepts of migration, ecologists pointed out its relation with habitat and considered migrants as colonizers of available habitats (Southwood, 1962; Taylor, 1986). The propensity for migration in a taxon is positively correlated with the degree of impermanence of its habitat and usually occurs at a defined stage in the life cycle (Southwood, 1962). Habitat changes result in active migration of individuals of a population leading to changes in its spatial coordinates and local density (Taylor, 1986). More recently, Enfjäll and Leimar (2009) emphasized strong unconditioned emigration in organisms that had no information about density or habitat.
quality, which could be costly in terms of mortality occurring in this new habitat with poor suitability to the organism.

Dispersal is scattering or spread of individuals such that there is an increase in the mean distance between the individuals and from their parents. It plays an important role in the life history of many species. Andrewartha and Birch (1954) and Southwood (1962) did not separate dispersive from migratory movements and concluded that migration of individuals leads to scattering of the population, that is, dispersal. Johnson (1969) stated migration as a special category of mass dispersive movements. Further, dispersal was described as a process whereby populations redistribute themselves as a result of active migration of individuals that try to keep pace with the changes in the locations of their habitats or availability of suitable host, prey or mate (Taylor, 1986). More recently, dispersal has been broadly defined as any movement between habitat patches separated in space irrespective of distance between them (Bowler and Benton, 2005). It is considered as a process that involves emigration from the current patch, inter-patch movement and immigration to a new patch. Dispersal is known to be strongly influenced by environmental heterogeneity (Hassell and Southwood, 1978; Obrien et al. 1989). Recent studies have also indicated environmental variability to play a major role in the dispersal of organisms, which results in distribution of populations at different spatial scales (Travis, 2001; Clobert et al., 2009; Bonte et al., 2012), with potential consequences for gene flow across space (Ronce, 2007).
Synthesis

Based on my understanding of dispersal and migration from the literature, I have developed my own definitions for my proposed research as follows:

**Migration**: Migration is to- and fro- or one-way movement in any direction, not necessarily seasonal, of individuals of a population, associated with behavioral, physiological, evolutionary and ecological aspects of an organism, that results in its distribution at different spatio-temporal scales.

I agree with Kennedy’s behavioral viewpoint and consider an organism to be migrating if there is a temporary inhibition to vegetative stimuli. Physiological changes may occur in the migratory individuals along with up-regulation of specific genes governing their movement from one area to another that may be triggered by variability in environmental conditions.

**Dispersal**: Dispersal is any movement of populations across a range of spatial scales resulting in an increase in the mean distance of the individuals from the source population and between the individuals.

I agree with Southwood and Taylor and believe that dispersal occurs at population level, where members of the population migrate to different spatial scales to survive and perpetuate. Dispersal is the end result of migration that involves emigration from an area, movement between one area to another, and immigration to a new area.
References


Chapter 2: Ambush Foraging Entomopathogenic Nematodes Employ ‘Sprinters’ for Long Distance Dispersal in the Absence of Hosts

Abstract

Ambush foragers may employ a long-distance dispersal strategy to maximize reproductive success in the absence of hosts. This hypothesis was tested by comparing lateral dispersal of the ambusher, Steinernema carpocapsae and cruiser, Heterorhabditis bacteriophora nematodes from infected host cadavers in sterilized silt loam soil in microcosms (0.05 m²-1.5 m²) with or without vegetation in the absence of hosts. Dispersal was estimated by taking soil cores (5 by 2 cm dia) from the microcosms at different intervals (6-240 h) and distances (3.8-61 cm) from the infected host cadavers and baiting with Galleria mellonella larvae. The numbers of baited larvae killed and the numbers of infective juveniles (IJs) penetrated in dead baits were counted to compute the percentage of IJs dispersed from the source cadavers based on the emergence potential of the two species and analyzed. Vegetation enhanced dispersal of both species but more so for H. bacteriophora. Although the pattern of dispersal differed spatio-temporally for the two species, average population displacement was similar (~6 cm/day). A majority of S. carpocapsae population (~ 46%) ambushed close to the source cadaver (< 3.8 cm),
whereas a majority of *H. bacteriophora* population (~16%) dispersed between 7-15 cm away from the source cadaver. About 5% of *S. carpocapsae* population dispersed faster than the fastest *H. bacteriophora* and reached 15-61 cm, compared to only 2% of *H. bacteriophora* population dispersing this far. This small proportion of apparent ‘sprinters’ may represent an adaptive dispersal strategy by the otherwise ambush forager *S. carpocapsae* in the absence of hosts.

**Introduction**

Dispersal is the process whereby populations redistribute themselves and generally results in an increase in mean separation of its members. Redistribution may result from active migration of individuals as they try to keep pace with the changes in the locations of their habitats or as a consequence of host, prey or mate finding (Taylor, 1986). The propensity for migration in a taxon is positively correlated with the degree of impermanence of its habitat and usually occurs at a defined stage in the life cycle (Southwood, 1962). In the case of entomopathogenic nematodes (EPNs), as with most parasitoid-like animals, migratory behavior and host-finding behavior are difficult to separate because both behaviors lead to population redistribution. As environmental heterogeneity strongly affects the foraging strategies adopted by organisms the presence or absence of host cues and other environmental stimuli strongly influences their ability to disperse (Hassell and Southwood, 1978; Obrien et al. 1989).
A key behavioral trait of EPNs in the families, Heterorhabditidae and Steinernematidae is their ability to locate suitable hosts to complete their life cycle, but information on their ability to find hosts in the soil is limited. Laboratory studies indicate a dichotomy in the host finding behavior of EPNs and have classified them as cruisers (active searchers) and ambushers (sit and wait foragers) (Lewis et al. 1992; Campbell and Gaugler, 1993). Cruisers, such as *Heterorhabditis bacteriophora*, are characterized by active mobility (Lewis et al. 1992; Campbell and Gaugler, 1993; Grewal et al. 1994a), an ability to orientate to volatile long-range host cues (Lewis et al. 1993; Grewal et al. 1994a), and an ability to find below ground sedentary hosts (Alatorre-Rosas and Kaya, 1990; Grewal et al. 1994a). On the other hand, ambushers, such as *Steinernema carpocapsae*, are known for low motility (Lewis et al. 1992; Campbell and Gaugler, 1993), the ability to nictate or tail standing (Campbell and Gaugler, 1993; Kruitbos and Wilson, 2010), and a lack of response to long-range volatile cues (Gaugler et al. 1989; Lewis et al. 1993; Grewal et al. 1994a; 1997). Ambushers respond to short-range host volatile cues either after contact with the host cuticle (Lewis et al. 1995) or during bouts of tail standing (Campbell and Kaya, 2002). These strategies in fact represent two extreme modes of a foraging continuum in which some species, such as *S. feltiae*, neither nictate like ambushers (Campbell and Gaugler, 1993) nor respond to long-range host volatile cues in a manner similar to cruisers (Grewal et al. 1994a; Lewis et al. 1995). These “intermediate foragers” (Grewal et al. 1994a) are often less effective at parasitizing hosts on the soil surface or deep in the soil profile.
Although, ambushers have generally been found to be more effective at finding mobile hosts and the cruisers have been found to be more effective at finding sedentary below ground hosts (Grewal et al. 1994a; Lewis et al. 1995; Campbell and Kaya, 2002; Lewis et al. 2006), it is not clear, how or if these foraging strategies influence EPN lateral dispersal in the soil, particularly at the population level when information about the hosts is not available. Since all motile animals have strategies for long range searching when they lack information about the location of a resource (Jander, 1975), I compared the rate of lateral movement and pattern of dispersal of the ambusher *S. carpocapsae* and cruiser *H. bacteriophora* populations in microcosms containing autoclaved field soil in the absence of host insects. My null hypothesis was that neither the rate nor the pattern of dispersal will differ between the ambushers and cruisers in the absence of host insects, host plants (host habitat) and other host/host-plant associated organisms. As habitat complexity can influence the dispersal behavior of foragers (Hendrix et al. 1986; Wiens et al. 1995; Crist and Weins, 1994; De Deyn et al. 2004; St John et al. 2006), I also compared the rate and pattern of dispersal of the two EPN species in autoclaved soil with and without vegetation. Again my null hypothesis was that vegetation will have no affect on the rate and dispersal pattern of the ambusher and cruiser species in the absence of hosts. Field soil placed in large wooden containers (up to 1.22 m x 1.22 m) was used to simulate natural conditions as most past studies on nematode movement have been conducted in Petri dishes or small containers and in artificial substrates such as sand or agar (Moyle and Kaya, 1981; Schroeder and Beavers, 1987; Alatorre-Rosas and Kaya, 1990; Grewal et al. 1993; 1997).
Currently, EPNs are used exclusively as therapeutic pest control agents with short term effect (Grewal et al. 2005). Thus, a better quantitative understanding of EPN movement, dispersal, and distribution abilities in the soil will enable the design of more efficient application strategies to better match their distribution with that of the target pests in the field to achieve cost-effective inundative biological control. This information is also critical for designing efficient inoculative release strategies for sustainable pest control with EPNs. Finally, quantitative information on movement and dispersal will also facilitate the development of ecological models for understanding spatial and temporal dynamics of inundative or endemic populations of these parasites in the soil (Stuart et al. 2006), a highly complex and heterogeneous environment.

Materials and Methods

Source of nematodes and soil

Two nematode species, *Heterorhabditis bacteriophora* GPS11 (a cruiser) and *Steinernema carpocapsae* ALL (an ambush) were obtained from our laboratory collection and new cultures were raised by infecting last instar wax moth larvae, *Galleria mellonella* (L.), obtained from Vanderhoest Canning Company, St. Mary’s, Ohio, following methods described by Kaya and Stock (1997). The two strains were obtained from their respective nematode cultures stored in liquid nitrogen and subjected to minimum sub-culturing to prevent deterioration in traits such as dispersal and virulence.
For all experiments, we used nematode infected *G. mellonella* cadavers as a source of nematodes rather than aqueous suspensions to mimic the natural conditions. Each last instar *G. mellonella* was exposed to approximately 400 freshly produced IJs of either *H. bacteriophora* or *S. carpocapsae* in a 5 cm diameter Petri dish. Twenty such Petri dishes were set up for each nematode species and held at room temperature (22°C) for 3 days. The nematode infected cadavers were then transferred to individual White traps (Kaya and Stock, 1997) to observe the initiation of the emergence of IJs. Each trap was inspected once daily to check for the initiation of IJ emergence from the cadaver. The cadavers that had just begun to release the IJs were selected for use in all experiments to minimize variation due to initiation of emergence among replications.

Wooster silt loam topsoil was collected from a corn field at The Ohio State University, Wooster, Ohio, USA. Particle size distribution of the soil, determined using methods described by Gee and Bauder (1986) and McCartney *et al.* (1997), was 26.2% clay, 2.6% sand and 61.8% silt. The pH of the soil was 7.11 and organic matter content was 3.6%. The soil, autoclaved at 121°C and 103.42 kPa pressure for 10 h, was stored at room temperature for at least 7 days before use to allow any toxic volatiles to escape. After estimating the saturation capacity of the autoclaved topsoil, its moisture level was adjusted to field capacity (i.e. 24% w/w; -10⁶ kPa) by adding tap water, in order to facilitate the movement of IJs.
Preparation of experimental microcosms

Five each of three different sized wooden microcosms (Fig. 2.1), were constructed for EPN dispersal experiments over different spatial scales (small: 22.86 cm x 22.86 cm; medium: 61 cm x 61 cm; large: 122 cm x 122 cm). Each microcosm was filled with autoclaved top soil to a depth of 5 cm and kept at room temperature (~22°C) during the course of each experiment. The microcosms were covered with black plastic sheets throughout the experiment to minimize moisture loss from the soil and to avoid exposure to light. A single, 10-day old *G. mellonella* cadaver that had just begun to release the IJs was placed 2.5 cm below the soil surface in the center of each microcosm to serve as the source of the IJs. In each microcosm, wooden sticks were inserted in the soil to mark the distances from the cadaver in six transects separated by 30° (Fig. 2.1). Two additional transects (sampled at 216 and 240 h only) were marked at 15° angle apart.

Comparing emergence patterns of *S. carpocapsae* and *H. bacteriophora* from infected cadavers in White traps

The differences between the two species in total number of IJs produced per cadaver and their temporal emergence patterns were determined by preparing individual cadavers (8 per species) as described above and counting the number of emerging nematodes in White traps daily for 10 days as described by Grewal et al. (1994b). The experiment was performed twice resulting in *N* = 16 for each species. These temporal and
Figure. 2.1. A pictorial representation of the experimental design showing three different sized microcosms (A, B and C) used for studying dispersal of infective juveniles (IJs) emerging from the source nematode-infected cadaver (black rectangle) placed in the center. Soil cores samples were collected at different distances marked by black spots and at different time intervals listed in hours outside the microcosms and then baited with live *Galleria mellonella* larvae in plastic cups to recover the moved IJs. Only the smallest microcosm (A) was used for comparing nematode dispersal in the presence of vegetation.
cumulative emergence data were used in estimating the proportion of the population dispersing in microcosms in all subsequent experiments.

*Comparing patterns of dispersal of S. carpocapsae and H. bacteriophora in sterilized soil*

The average lateral displacement and spatio-temporal dispersal patterns of IJs emerging from infected host cadavers by the two species were examined in all three different sized microcosms containing sterilized field soil. The microcosms were prepared as described above with a 10-day old infected cadaver placed in the center. One soil core sample (2 cm dia and 5 cm deep) was taken from each of the five small microcosms at 3.8, 7.6 and 11.4 cm away from the center at 6, 12, 24, 48, 72 and 96 h after placing the nematode-infected *G. mellonella* cadaver and placed separately in plastic cups (30 ml). Similarly, soil core samples were collected from the medium sized microcosms at 7.62, 15.5, 21 and 30.5 cm away from the center after 12, 24, 48, 72, 96, 120, 144 and 168 h and from the large microcosms at 15.5, 30.5, 46 and 61 cm away after 24, 48, 72, 96, 120, 144, 168, 192, 216 and 240 h for both species. Autoclaved soil was used to fill the holes left by the soil core sampler to avoid any interference with subsequent nematode movement in the microcosms. Nematodes were recovered from each soil core sample by baiting with one uninfected last instar *G. mellonella* (Bedding and Akhurst, 1975; Kaya and Stock, 1997), which was examined for nematode infection after three days. These cups were covered with lids containing five pin holes to allow for air exchange but to minimize moisture loss. Movement of at least one nematode from the cadaver to the site of the soil core
sample was inferred from the death of the baited insect showing characteristic symptoms of nematode infection. All infected larvae were dissected and the number of nematodes penetrating the bait insects was counted. Dissections were made on the third day after baiting to count the penetrated IJs before they had opportunity to reproduce. Five microcosms were used for each experiment and each experiment was performed twice resulting in $N = 10$ samples for each distance and time interval.

Comparing patterns of dispersal of $S$. carpocapsae and $H$. bacteriophora in sterilized soil with or without vegetation

The influence of vegetation on the movement of the two nematode species was studied using the small microcosms (22.86 cm x 22.86 cm). These microcosms containing autoclaved soil (5 cm deep) were seeded with tall fescue ($Festuca arundinacea$) grass @ 34 g/ m$^2$ and were held in the greenhouse (Fig. 2.1A) at ~22ºC. After the grass had grown to a height of approximately 2.5 cm, nematode dispersal in the microcosms was studied by tracking the movement of IJs emerging from infected cadavers as described earlier. Soil samples were collected as described earlier at distances of 3.8, 7.6 and 11.4 cm at after 12, 24, 48, 72 and 96 h from the center of the microcosm where 10-day-old nematode infected $G$. mellonella cadaver was buried. Empty spots were filled with the same amount of autoclaved soil to allow uninterrupted nematode movement. Similar microcosms containing soil without grass were set up as controls. The microcosms were covered with white nylon fabric to allow for air exchange and light
penetration for the grass but to prevent any insect invasion. Five microcosms were used for each experimental treatment and each experiment was performed twice resulting in $N = 10$ for each species.

Data Analysis

Three sizes of microcosms were used in these experiments in order to examine dispersal over three spatio-temporal scales: small (3.8 – 11.4 cm; 6 – 96 h), medium (7.6 - 30.5 cm; 12 – 168 h), and large (15.5 - 61.0 cm; 24 – 240 h). Because the spatial and temporal sampling intervals were nested such that observations in the small microcosm comprise a subset overlapping those in the medium and the medium a subset of the large microcosm, these data were combined into a single database with spatial range of 3.8 - 61 cm and time intervals of 6 - 240 hr.

Both the number of *G. mellonella* bait larvae killed and the number of IJs recovered from the baits were used to compare the displacements of *H. bacteriophora* and *S. carpocapsae* in sterile soil. The number of killed baits is a proxy for the displacement distance of at least one IJ. The data on killed baits was square root transformed to achieve normality and subjected to two-sample $t$-test with unequal variances to compare the displacement of the two nematode species at different distances and time points. The data on IJ emergence from 10-day old *G. mellonella* cadavers in the White traps were normalized by using square root transformation and subjected to analysis of variance (ANOVA), and means of the two EPN species were separated by
Tukey’s test. Mean percentage of IJs of both species dispersing at a time point up to a particular distance in a 2 cm wide annulus in the microcosms was calculated by equation 1.

\[ D_{di,ti} = \frac{M_{di,ti}}{C_{ti}} \times N_{di} \times 100 \]  

(1)

where, \( M_{di,ti} \) represents the mean number of IJs recovered from a soil core at a distance, \( d \) and a time point, \( t \); \( C_{ti} \) represents the mean cumulative number of IJs emerging from a 10-day old \( G. mellonella \) cadaver in the White traps at a time point, \( t \) and \( N_{di} \) represents the total number of soil cores that would be extracted from an annulus at a distance \( d \) from the source cadaver as described below.

\[ N_{di} = \frac{\text{Volume of an annulus at distance, } d}{\text{Volume of a soil core}} \]

\[ N_{di} = 4d, \text{ as radius of the soil core is } 1 \text{ cm and depth is } 5 \text{ cm.} \]

The mean proportion data obtained from the above equation were arcsine transformed to achieve normality and subjected to two-sample \( t \)-test with unequal variances to compare the two species. This emphasized the difference in numbers of dispersing IJs of the two species. Similarly, two-sample \( t \)-test with unequal variances was used to make species comparisons of the mean number of baits killed, mean percentage of dispersed IJs and average displacements (cm/day) in the presence or absence of vegetation. Alpha, the maximum probability of a type I error, was set at 0.05 for all tests. All analyses were performed using Minitab Release 16 (Minitab Inc., State College, PA).

I first analyzed the data from each repeated experiment separately and found that the two experiments produced similar results. For example, for specific distances from
the infected cadaver, significant differences were observed between the two species in the mean number of \textit{G. mellonella} baits infected at 11.4 (Experiment 1: \( t_{46} = 2.36, P = 0.02 \); Experiment 2: \( t_{46}^2 = 2.48, P = 0.01 \)) and 46 cm (Experiment 1: \( t_{85} = 2.12, P = 0.05 \); Experiment 2: \( t_{85} = 2.28, P = 0.04 \)) for both experiments. Similarly, both experiments showed similar significant differences between the two species in the mean percentage of IJs dispersed at 3.8 (Experiment 1: \( t_{24} = 2.71, P = 0.01 \); Experiment 2: \( t_{24} = 2.65, P = 0.01 \)), 7.6 (Experiment 1: \( t_{65} = 2.82, P = 0.003 \); Experiment 2: \( t_{65} = 2.75, P = 0.007 \)), 11.4 (Experiment 1: \( t_{24} = 2.32, P = 0.03 \); Experiment 2: \( t_{24} = 2.45, P = 0.02 \)), 22.8 (Experiment 1: \( t_{105} = 2.62, P = 0.01 \); Experiment 2: \( t_{105} = 2.78, P = 0.008 \)) and 30.5 cm (Experiment 1: \( t_{97} = 2.12, P = 0.04 \); Experiment 2: \( t_{97} = 2.28, P = 0.03 \)). There was no significant difference between the two experiments in the mean percentage of IJs dispersed at any distance (\( P > 0.05 \)). Therefore, the data from two repeated experiments were pooled for all further analyses and presentation.

\section*{Results}

\textit{Comparing emergence patterns of \textit{S. carpocapsae} and \textit{H. bacteriophora} from infected cadavers in White traps}

Overall, \textit{H. bacteriophora} infected cadavers released 2.25 times more IJs (Mean ± SEM) compared to \textit{S. carpocapsae} infected cadavers (264,702 ± 17,557 IJs for \textit{H. bacteriophora} and 117,986 ± 30,856 for \textit{S. carpocapsae}) over the 10-day period in the
White traps. Significantly higher number of *S. carpocapsae* IJs emerged at 24 h ($F_{1,31} = 5.38, P = 0.03$) and 48 h ($F_{1,31} = 5.03, P = 0.04$) and again at 216 h ($F_{1,31} = 4.90, P = 0.04$) and 240 h ($F_{1,31} = 5.12, P = 0.04$), whereas significantly higher number of *H. bacteriophora* IJs emerged at 72 h ($F_{31} = 29.88, P = 0.0$), 96 h ($F_{1,31} = 45.58, P = 0.0$) and 120 h ($F_{1,31} = 6.52, P = 0.02$) after placement of cadavers in the White traps (*Fig. 2.2*). Both species attained maximum emergence at 96 h.

*Comparing patterns of dispersal of S. carpocapsae and H. bacteriophora in sterilized soil*

The mean number of *G. mellonella* baits infected by *H. bacteriophora* and *S. carpocapsae* showed differences at specific times and distances from the source cadaver. At 72 h, *S. carpocapsae* killed more bait insects ($t_{217} = 2.87, P = 0.004$) whereas at 240 h, *H. bacteriophora* killed more baits ($t_{77} = 3.47, P = 0.001$) in the microcosms (*Fig. 2.3A*). At 11.4 cm from the source cadavers, *H. bacteriophora* killed more baits ($t_{80} = 2.67, P = 0.01$) whereas at 46 cm, *S. carpocapsae* killed more baits ($t_{119} = 1.98, P = 0.05$) (*Fig. 2.3B*). After the first 24h, *S. carpocapsae* was almost always detected before *H. bacteriophora* at the farthest distance (*Fig. 2.4*).

The two species differed in the percentage of IJs dispersing over time and distance (*Fig. 2.5*). The mean percentage of *H. bacteriophora* that dispersed irrespective of distance was significantly higher than *S. carpocapsae* at 24 h ($t_{119} = 2.24, P = 0.03$) and 48 h ($t_{110} = 3.50, P = 0.001$) indicating rapid early dispersal of a large fraction of *H. bacteriophora* from the source cadaver (*Fig. 2.5A*). At distances close to the source
Figure 2.2. Temporal emergence patterns of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* in last instar 10-day old *Galleria mellonella* cadavers. Data are mean (± SEM) number of infective juveniles (IJs) recovered per 10-day old *G. mellonella* cadaver, daily from 0 to 240 h in the White traps. Asterisk sign (*) indicates significant difference in the means between the two species at a time point at P <0.05.
Figure 2.3. Mean (± SEM) number of Galleria mellonella baits infected by the infective juveniles (IJs) of Heterorhabditis bacteriophora and Steinernema carpocapsae over A) time (integrated over distance) and B) distance (integrated over time). Asterisk sign (*) indicates significant difference in the means between the two species at a time point or distance at P <0.05.
Figure 2.4. Farthest distance at which infective juveniles (IJs) of *H. bacteriophora* and *S. carpocapsae* were detected first at a particular time and the total number of *G. mellonella* baits infected (out of 10) at that distance (numbers in brackets for *H. bacteriophora* and without brackets for *S. carpocapsae*).
Figure 2.5. Mean (± SEM) percentage of infective juveniles (IJs) of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* dispersed in an annulus at a certain distance in soil in the microcosm from the source cadaver over A) time (averaged over distance) and B) distance (averaged over time). Asterisk sign (*) indicates significant difference in the means between the two species at a time point or distance at P <0.05.
cadaver (< 15 cm), the mean percentage of *H. bacteriophora* dispersed was also higher than *S. carpocapsae* with significant differences at annuli, 3.8 cm (*t*$_{49}$= 2.69, *P* = 0.01), 7.6 cm (*t*$_{132}$= 2.88, *P* = 0.005) and 11.4 cm (*t*$_{49}$= 2.35, *P* = 0.02) away from the source cadaver (*Fig. 2.5B*). However, the mean percentage of *S. carpocapsae* dispersed was significantly higher than *H. bacteriophora* at 22.86 cm (*t*$_{112}$= 2.62, *P* = 0.01) and 30.5 cm (*t*$_{220}$= 2.11, *P* = 0.03) from the source cadaver, with similar percentages of both species being recovered at all the longer distances (*Fig. 2.5B*). At 3.8 cm, about 100% of *H. bacteriophora* population was detected as compared to 4% of *S. carpocapsae*. At 7.6 cm and 11.4 cm, about 13% of *H. bacteriophora* and less than 2% of *S. carpocapsae* population was detected. Overall, a greater proportion (~2.5 times) of *S. carpocapsae* (~5%) were detected at > 15 cm distance than *H. bacteriophora* (~2%) (*t*$_{836}$= 2.51, *P* = 0.01).

Despite these spatio-temporal differences in the dispersal pattern of the two species, no significant differences in their average (± SEM) population displacement were observed, with *H. bacteriophora* moving at 6.01 (± 0.10) cm/day and *S. carpocapsae* at 5.8 (± 0.12) cm/day (*t*$_{1718}$= 1.22, *P* = 0.22).

*Comparing the patterns of dispersal of S. carpocapsae and H. bacteriophora IJs in sterilized soil with or without vegetation*

The average (± SEM) displacement (cm/day) of *H. bacteriophora*, computed from all time intervals and distances, was significantly higher in microcosms containing
vegetation (7.03 ± 0.20) compared with no vegetation (6.13 ± 0.17) \( (t_{298} = 3.53, P < 0.0001) \). Higher displacement of \textit{H. bacteriophora} was observed both in case of the numbers of \textit{G. mellonella} baits infected (\textbf{Fig. 2.6}) as well as in the percentage of IJs dispersing (\textbf{Fig. 2.7}) over time (12 to 96 h) and distance (3.8 to 11.4 cm) with vegetation than without. Specifically, greater number of \textit{G. mellonella} baits was infected in the presence of vegetation than without at 12 \( (t_{32} = 2.27, P = 0.03) \), 48 \( (t_{57} = 2.11, P = 0.04) \) and 96 h \( (t_{54} = 2.12, P < 0.04) \) after placing the source cadavers in the microcosms and at 3.8 cm \( (t_{72} = 3.70, P < 0.0001) \) from the cadaver (\textbf{Fig. 2.6A}). Additionally, significantly higher mean percentage of \textit{H. bacteriophora} IJs dispersed from 3.8 to 11.4 cm in the presence of vegetation \( (t_{155} = 2.87, P = 0.005) \), with specific differences at time points, 12 \( (t_{38} = 2.63, P = 0.01) \), 24 \( (t_{29} = 2.12, P = 0.04) \), 72 \( (t_{38} = 2.45, P = 0.02) \) and 96 h \( (t_{29} = 2.61, P = 0.01) \) and at the shortest distance, 3.8 cm \( (t_{50} = 2.69, P = 0.01) \) (\textbf{Fig. 2.7A}). The effect of vegetation on \textit{S. carpocapsae} dispersal was also positive but was much less. The average (± SEM) displacement of \textit{S. carpocapsae} did not differ in soil with (5.46 ± 0.14 cm/day) or without vegetation (5.30 ± 0.13 cm/day) \( (t_{298} = 0.53, P = 0.60) \) based on the mean number of \textit{G. mellonella} baits infected in the presence and absence of vegetation over 12 to 96 h (\textbf{Fig. 2.6B}). However, a greater percentage of \textit{S. carpocapsae} IJs was observed in the presence of vegetation over time and distance \( (t_{178} = 2.17, P = 0.05) \), with significant differences at 12 \( (t_{57} = 2.11, P = 0.05) \), 24 \( (t_{48} = 1.97, P = 0.05) \) and 96 h \( (t_{41} = 1.95, P = 0.05) \) and at 7.6 cm \( (t_{58} = 2.23, P = 0.04) \) (\textbf{Fig. 2.7B}). Nevertheless, \textit{H. bacteriophora} showed significantly higher average displacement (7.03 ± 0.20 cm/day) than \textit{S. carpocapsae} (5.46 ± 0.14 cm/day) in the presence of vegetation \( (t_{298} = 4.19, P \)
Figure 2.6. Mean (± SEM) number of *Galleria mellonella* baits infected by the infective juveniles (IJJs) of *H. bacteriophora* and *S. carpocapsae* over time (averaged over distance) and distance (averaged over time) in the presence and absence of vegetation. Asterisk sign (*) indicates significant difference in the means between the presence and absence of vegetation for a nematode species at a time point or distance at P <0.05.
Figure 2.7. Mean (± SEM) percentage of infective juveniles (IJs) of *H. bacteriophora* and *S. carpocapsae* dispersed in an annulus in soil in the microcosm at a certain distance from the source cadaver over time (averaged over distance) and distance (averaged over time) in the presence and absence of vegetation. Asterisk sign (*) indicates significant difference in the means between the presence and absence of vegetation for a nematode species at a time point or distance at P < 0.05.
Discussion

This study reveals an unexpected ability of the ambush foraging EPN *S. carpocapsae* to disperse long distances over a period of ten days in the soil in the absence of hosts. Not only do a small proportion of *S. carpocapsae* IJs exiting a host cadaver leave its immediate vicinity but they also travel faster than the fastest *H. bacteriophora*. Evolutionary ecology theory suggests that there must always be some offspring that leave the natal habitat to found new populations (Hamilton and May, 1977; Taylor and Taylor, 1977; Southwood, 1977). Thus, the few *S. carpocapsae* individuals rapidly moving away from the source may be regarded as ‘sprinters’ with the ability to move faster to longer distances than the rest of the population in the same spatio-temporal scale. I observed a *H. bacteriophora* infected cadaver emitting roughly 2.25 times the number of IJs emitted by a *S. carpocapsae* cadaver during the course of the experiment. Such differences in the reproduction of the two species were also reported by the earlier studies (Grewal et al., 1994b; Wang and Grewal, 2002). Despite this difference in the reproduction potential of the two species, the percentage of *S. carpocapsae* population dispersing >15 cm was found to be nearly 2.5 times greater than *H. bacteriophora*. Such long distance movement of *S. carpocapsae* along with its occurrence at all time points up to 10 days indicates the exceptional ability of this ‘ambushing’ nematode to disperse actively apparently due to a small proportion of ‘sprinters’ in the absence of hosts.
Results of this study show that EPN species with different foraging strategies display different rates and patterns of dispersal in the soil in the absence of hosts. Although, the dispersal of IJs from the infected cadavers in terms of mean number of *G. mellonella* baits infected and the mean percentage of dispersed IJs decreased with distance in both species, there were differences in the percentage of IJs reaching specific distances. For instance, the percentage of *H. bacteriophora* IJs was nearly 8 times the percentage of *S. carpocapsae* dispersed at a short distance (7.6 cm) from the source cadaver, which is likely due to greater number of IJs emerging from the cadavers infected with *H. bacteriophora* than *S. carpocapsae* (2.25:1). Interestingly, the mean percentage of *S. carpocapsae* IJs dispersing to larger annuli (22.86 cm and 30.5 cm), computed from cumulative populations emerging from infected source cadavers was significantly higher than *H. bacteriophora*. This indicates the potential of some *S. carpocapsae* IJs to travel farther than *H. bacteriophora*. Although significantly higher number of *S. carpocapsae* emerge at early hours (24 and 48 h), the percentage of IJs moving out of that population was significantly lower than *H. bacteriophora*. This indicates that *S. carpocapsae* ambushes until 48 h after which a proportion of the population travels at almost the same speed as *H. bacteriophora* by virtue of sprinters. This is evident from greater percentage of *S. carpocapsae*, emerging from a population smaller than *H. bacteriophora*, recovered at longer distances (more than 15 cm) than *H. bacteriophora*, emerging from a larger population over a period of 10 days.

Nearly all *H. bacteriophora* and *S. carpocapsae* IJs dispersed some distance in the microcosm and some IJs of both species reached the edge (61 cm) of the largest
microcosm covering a surface area of 2.5 m² in 10 days, a minimum of 120 times their body length per day. The apparent similarity in the average displacement of the two species (~ 6.0 cm/day) is largely due to the ability of *S. carpocapsae* sprinters to travel faster and farther than *H. bacteriophora*. The emigration of few individuals from a population is an evolutionary strategy even in stable habitats (Hamilton and May, 1977). For EPNs dependent on ephemeral resources, one might expect all offspring to be dispersing. While most, if not all, *H. bacteriophora* leave the vicinity of the cadaver in search of a new host, it appears that, *S. carpocapsae* has a very different strategy for discovering new hosts. As they are sexual, *S. carpocapsae* must not only find a host, possibly distant from the natal cadaver but also must invade it with a member of the opposite sex. With few IJs migrating from the cadaver, it is difficult to see how this is achieved. In nature, the numbers of IJs in areas similar to our microcosms may be greater, increasing the chances of multiple invasions. My own experiments showed multiple invasions at distances up to 61 cm with a source population of ~118,000 IJs. These results tend to support the recruitment hypothesis in which emigrants may invade hosts first and even make them more suitable and attractive for following IJs to find and invade, enhancing the reproductive success of the entire population (Grewal *et al.* 1993; 1997; Lewis and Gaugler, 1994).

The dichotomy of migrators and non-migrators in *S. carpocapsae* populations in response to host finding on agar plates was also observed by Gaugler *et al.* (1989), where migrators aggressively searched for hosts and non-migrators followed, presumably as an energy conservation approach. Therefore, I propose that little differences occur in the
ability to disperse in soil at larger spatial scale between the two nematode species. Such similarities may evolve over time due to the evolutionary pressure to find new ways to disperse in changing habitat conditions. As nictation has been considered to have evolved as an adaptation for ambushing nematodes to find mobile hosts that are nearby (Campbell and Gaugler, 1993), a few fast moving sprinters may offer a distinct advantage for finding hosts that are further away in order to avoid intra-specific competition among siblings and/or promote out-crossing.

The results showed positive impact of habitat complexity (vegetation) on the movement of both the nematode species, however, the degree of impact varied between the species. The average displacement of *H. bacteriophora*, computed from all recoveries, was significantly higher in the presence of vegetation (7 cm/day) compared with no vegetation (6 cm/day). In contrast, *S. carpocapsae* showed nearly identical average displacement over the same time period, in soil with (5.5 cm/day) or without vegetation (5.3 cm/day). The percentage of *H. bacteriophora* IJs moving in annuli, 3.8 to 11.4 cm in the presence of vegetation was ~6 times the number with no vegetation. On the other hand, although, the percentage of *S. carpocapsae* IJs dispersing 3.8 to 11.4 cm was significantly higher in the presence of vegetation than no vegetation, the difference (~ 1.7 times) was not as high as for *H. bacteriophora*. These results indicate enhancement in the dispersal of both nematode species in the presence of vegetation but the effect being much greater in case of *H. bacteriophora*. Absence of host insects in the microcosms ensured the lack of any host-related cues and therefore, I propose that other factors such as physical properties of roots or physical alteration of the soil profile by the
roots play a role in nematode movement. For instance, the growth of plant roots in the soil profile may change soil pore size and potentially influence the movement of nematodes (Russell, 1977). Moreover, plant roots may extract water from soil and change the moisture gradient, thereby influencing nematode movement (Kaya, 1990; Duncan and McCoy, 2001). Roots may also loosen the soil and create near-linear paths for dispersing nematodes to facilitate their movement in the soil, providing a sort of nematode ‘motorway’. Improved dispersal of both EPN species in the presence of vegetation is an interesting outcome of this study, which indicates that habitat complexity may influence the dispersal behavior of EPNs and the efficacy of therapeutic application. Earlier studies of *S. carpocapsae* dispersal have shown it to be greater than *H. bacteriophora* in peaty soils (Dillon *et al.* 2006; Torr *et al.* 2007; Lacey and Unruh, 1998; Kruitbos *et al.* 2010). However, in our study *H. bacteriophora* showed significantly higher average displacement than *S. carpocapsae* in the presence of vegetation in the silt loam soil. Therefore, I conclude that the cruising ability of *H. bacteriophora* gets enhanced in silt loam soil in the presence of vegetation more so than *S. carpocapsae*.

In conclusion, this comparative study has identified differences in the rate and pattern of dispersal between ambush and cruise foraging EPN species in the soil in large microcosms in a host-free environment with or without vegetation. The use of infected cadavers as the source of nematodes and the soil medium are important features of this study that closely mimic the natural conditions for these soil dwelling invertebrates. Earlier laboratory studies have reported greater dispersal, survival and infectivity of IJs exiting infected cadavers than those applied in aqueous solution (Shapiro and Glazer,
1996; Shapiro and Lewis, 1999; Perez et al. 2003). Moreover, the emergence of IJs in large numbers as a population from the infected cadavers makes this study of lateral dispersal of nematodes at the population level. Such information could be critical to understanding spatial and temporal dynamics of both inundatively released and endemic EPN populations in the field. With nematode distribution in the field being one of the major concerns of the growers, such quantification of nematode dispersal from infected host cadavers in the absence of hosts and vegetation will open new opportunities for designing improved application strategies for EPNs, such as following an appropriate pattern for placing an optimal number of nematode infected cadavers for inundative field application or inoculative release strategies for sustainable pest control. The quantitative information on the dispersal ability of EPNs in soil provided by this study will also serve as a foundation for developing population models for understanding spatial and temporal dynamics of naturally occurring populations of these beneficial nematodes in soil, which would further enable their utilization in conservation biological control.

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References


Chapter 3: Dispersal of entomopathogenic nematodes with different foraging strategies in the presence and absence of mobile and non-mobile hosts

Abstract

Lateral dispersal of the ambush foraging nematode, Steinernema carpocapsae ALL and cruise foraging nematode, Heterorhabditis bacteriophora GPS11 from infected host cadavers was compared in sterilized silt loam soil in microcosms (0.05 m$^2$) containing vegetation in the presence and absence of mobile or non-mobile hosts, Galleria mellonella larvae. Dispersal was estimated by taking soil cores (5 by 2 cm dia) from the microcosms at different intervals (12-72 h) and distances (3.8-11.4 cm) from the infected host cadavers and baiting with G. mellonella larvae. The numbers of infective juveniles (IJ$s$) were counted by dissecting the killed baited larvae to compute the percentage of IJ$s$ dispersed from the source cadavers based on the emergence potential of the two species. Both EPN species showed greater dispersal in the presence of mobile hosts compared to no hosts. Non-mobile hosts enhanced $H$. bacteriophora dispersal up to 24 h with no significant impact on $S$. carpocapsae dispersal. Significantly lower percentages of IJ$s$ were recovered in the microcosms containing mobile hosts than no hosts at later intervals (72 h). $S$. carpocapsae showed significantly greater average displacement (cm/day) than $H$. bacteriophora in the presence of hosts, mobile (8.06 and 5.30, respectively) and non-
mobile (5.07 and 3.60, respectively) hosts. The proportion of IJs reaching the farthest distance was higher (~14.5%) in *S. carpocapsae* than *H. bacteriophora* (~0.4%) in the presence of mobile hosts but was the same in the presence of non-mobile hosts (~1.06% in *S. carpocapsae* ; ~1.4% in *H. bacteriophora*). A considerable proportion of IJs of both species ambushed near (< 3.8 cm) the source cadaver both in the presence of mobile (*H. bacteriophora*: 79%; *S. carpocapsae*: 65%) and non-mobile (*H. bacteriophora*: 67%; *S. carpocapsae*: 93%) hosts.

**Introduction**

Dispersal is a one-way movement of individuals of a population moving collectively or alone, that results in variation in local density and spatial distribution (Taylor, 1986). Dispersal is not only essential for individual fitness but it also has implications for population dynamics, population genetics and species distribution (Bowler and Benton, 2005). Environmental variability plays a major role in the dispersal of organisms, which further reduces inbreeding and resource competition, resulting in distribution of populations at different spatial scales (Travis, 2001; Clobert *et al*., 2009; Bonte *et al*., 2012). In the case of entomopathogenic nematodes (EPNs) in the families, Heterorhabditidae and Steinernematidae, presence or absence of hosts and differences in host life histories may contribute to the environmental heterogeneity, which would strongly influence the dispersal ability of EPNs, as observed in several predators (Hassell and Southwood, 1978; O'Brien *et al*. 1989).
A key behavioral trait of EPNs is their ability to locate suitable hosts to complete their life cycle, but information on their ability to find hosts in the soil is limited. Laboratory studies indicate a dichotomy in the host finding behavior of EPNs and have classified them as cruisers (active searchers) and ambushers (sit and wait foragers) (Lewis et al. 1992; Campbell and Gaugler, 1993). Crusiers, such as *Heterorhabditis bacteriophora*, are characterized by active mobility (Lewis et al. 1992; Campbell and Gaugler, 1993), an ability to orientate to volatile long-range host cues (Lewis et al. 1993; Grewal et al. 1994a), and an ability to find below ground sedentary hosts (Alatorre-Rosas and Kaya, 1990; Grewal et al. 1994a). On the other hand, ambushers, such as *Steinernema carpocapsae*, are known for low mobility (Lewis et al. 1992; Campbell and Gaugler, 1993), the ability to nictate or tail standing (Campbell and Gaugler, 1993; Kruitbos and Wilson, 2010), and a lack of response to long-range volatile cues (Gaugler et al. 1989; Lewis et al. 1993; Grewal et al. 1994a; 1997). Ambushers respond to short-range host volatile cues either after contact with the host cuticle (Lewis et al. 1995) or during bouts of tail standing (Campbell and Kaya, 2002). These strategies in fact represent two extreme modes of the foraging continuum in which some species, such as *S. feltiae*, neither nictate like ambushers (Campbell and Gaugler, 1993) nor respond to long-range host volatile cues in a manner similar to cruisers (Grewal et al. 1994a; Lewis et al. 1995). These “intermediate foragers” (Grewal et al. 1994a) are often less effective than ambushers and cruisers at parasitizing hosts on the soil surface or deep in the soil profile, respectively.

Since host cues, CO$_2$ and other host related odorants have been shown to attract or repel EPNs causing directional movement in laboratory studies (Schmidt and All, 1979;
Gaugler et al. 1980; Lewis et al. 1993; Grewal et al. 1993; Grewal et al. 1994a; Grewal et al. 1997; Glazer, 1997; O’Halloran and Burnell, 2003; Hallem et al. 2011; Dillman et al. 2012), here I compared differences in the dispersal of *H. bacteriophora* and *S. carpocapsae* in the absence or presence of hosts which differed in mobility. Ambushers have generally been found to be more effective at finding mobile hosts and cruisers have been found to be more effective at finding sedentary below ground hosts (Grewal et al. 1994a; Lewis et al. 1995; Campbell and Kaya, 2002; Lewis et al. 2006). My recent research has discovered that in the absence of hosts, a small proportion of ‘sprinters’ of the ambusher, *S. carpocapsae* travel long distances in the soil resulting in the same average daily dispersal at the population level as the cruiser, *H. bacteriophora* (Chapter 2). Therefore, my null hypothesis for this study was that there will be no difference in the rate and dispersal pattern of the two species in the presence of hosts, mobile or non-mobile.

**Materials and Methods**

*Source of nematodes and soil*

Frozen (in liquid nitrogen) stocks of two nematode species, *Heterorhabditis bacteriophora* GPS11 strain (a cruiser) and *Steinernema carpocapsae* ALL strain (an ambusher) were obtained from our laboratory collection and new cultures were raised by infecting final instar wax moth larvae, *Galleria mellonella* (L.), obtained from Vanderhoest Canning Company, St. Mary’s, Ohio, following methods described by Kaya
and Stock (1997). For all experiments, we used nematode infected *G. mellonella* cadavers as a source of nematodes rather than aqueous suspensions to mimic the natural conditions. These cadavers were prepared using the methods detailed in Chapter 2. Briefly, 20 separate 5 cm diameter Petri dishes were set up and each last instar *G. mellonella* was exposed to approximately 400 freshly produced IJs of either *H. bacteriophora* or *S. carpocapsae* at room temperature (22°C) for 3 days. The nematode infected cadavers were then transferred to individual White traps (Kaya and Stock, 1997) and observed once daily to check for the initiation of IJ emergence. Cadavers that had just begun to release the IJs were selected for use in all experiments to minimize variation due to initiation of emergence among replicates.

Wooster silt loam topsoil was collected from a corn field at The Ohio State University, Wooster, Ohio, USA. Particle size distribution of the soil, determined using methods described by McCartney *et al.* (1997), was 26.2% clay, 2.6% sand and 61.8% silt. The pH of the soil was 7.11 and organic matter content was 3.6%. The soil, autoclaved at 121°C and 103.42 kPa pressure for 10 h, was stored at room temperature for at least 7 days before use to allow any toxic volatiles to escape. After estimating the saturation capacity of the autoclaved topsoil, its moisture level was adjusted to field capacity (i.e. 24% w/w; -10^6 kPa) by adding tap water, in order to facilitate the movement of IJs.
Preparation of experimental microcosms

EPN dispersal was examined in five plastic microcosms (22.86 cm x 22.86 cm). Each microcosm was filled with autoclaved top soil to a depth of 5 cm and seeded with tall fescue (*Festuca arundinacea*) grass @ 34 g/m². The microcosms were covered with white nylon fabric to allow for air exchange and light penetration for the grass but to prevent any insect invasion and were held in the greenhouse at ~22°C during the course of each experiment. After the grass had grown to a height of approximately 2.5 cm, single 10-day old *G. mellonella* cadaver that had just begun to release the IJs was placed 2.5 cm below the soil surface in the center of each microcosm to serve as the source of the IJs. In each microcosm, wooden sticks were inserted in the soil to mark the distances from the cadaver in four transects separated by 10º angles (Fig. 3.1).

Lateral dispersal in the presence or absence of non-mobile hosts

To study the influence of non-mobile host on the lateral movement of the two species, each microcosm was divided into four quadrants. One live *G. mellonella* larva enclosed in a wire mesh cage (1 x 1 x 1 cm; mesh size = 16) was buried 2.5 cm below the soil surface at 9 cm from the center in one of the quadrants (Fig. 3.1A), at the same time when the 10-day old nematode infected *G. mellonella* cadaver was placed in the center of the microcosm (see above). One soil core sample (2 cm in diameter and 5 cm deep) was removed from each microcosm at 3.8, 7.6 and 11.4 cm from the center in the quadrant containing the non-mobile host and from the quadrant directly opposite at 12, 24, 48 and
Figure 3.1. A pictorial representation of the experimental design showing 22.86 cm x 22.86 cm microcosm used for studying dispersal of infective juveniles (IJs) emerging from the source nematode-infected cadaver (black rectangle) placed in the center in the presence of a non-mobile host (A) and two mobile hosts (B). Each microcosm was divided into four quadrants. Host insects released in the microcosm are represented by empty circles. Soil cores samples were collected from 2 cm wide arcs (dotted lines) in the microcosms containing non-mobile host at different distances marked by black spots and at different time intervals listed in hours outside the microcosms and then baited with live *Galleria mellonella* larvae in plastic cups to recover the moved IJs. Similarly, soil core samples were collected from 2 cm wide annuli (dotted circles) at different distances starting from the outer edge of the wire-mesh cylinder (bold circle) going outwards and at different time intervals in the microcosms with and without mobile hosts.
72 h after placing the infected *G. mellonella* cadaver in the center and transferred to a plastic cup. The holes left by the soil core samples were filled with the autoclaved soil to avoid any interference with subsequent nematode movement in the microcosms. Nematodes were recovered from the soil core samples using the insect baiting technique (Bedding and Akhurst, 1975; Kaya and Stock, 1997), whereby core samples were baited with one uninfected last instar *G. mellonella* in each plastic cup, which was examined for nematode infection after three days. These cups were covered with lids containing five pin holes to allow for air exchange but to minimize moisture loss. Movement of at least one nematode from the cadaver to the site of the soil sample was inferred from the death of the baited insect showing characteristic symptoms of nematode infection. All infected larvae were dissected and the number of nematodes penetrating the bait insects was counted. Dissections were made on the third day after baiting to count the penetrated IJs before they had opportunity to reproduce. Five microcosms were used for each treatment and each experiment was performed twice resulting in $N = 10$ for each species.

*Lateral dispersal in the presence or absence of mobile hosts*

The influence of mobile host on the average lateral displacement and spatio-temporal patterns of the two species was compared in the microcosms containing sterilized field soil with vegetation. After preparing the microcosms as described above, a wire mesh cylinder (7.6 cm dia, 10 cm height; mesh size = 16) was inserted in the center of each microcosm (Fig. 3.1B) to prevent the mobile host *G. mellonella* larvae to enter the center of the microcosm. After the grass had grown to a height of approximately 2.5
cm, one 10-day old nematode infected *G. mellonella* cadaver was buried in the center of the microcosm. At the same time, two last instar *G. mellonella* larvae were placed on the soil surface outside the wire mesh cylinder in two opposite quadrants, one in each quadrant (Fig. 3.1B) to keep them away from the immediate vicinity of the nematode infected source cadaver. Since *G. mellonella* larvae could move anywhere in the microcosm in the area outside the wire mesh cylinder, nematode dispersal was tracked by collecting soil samples as described earlier at distances of 3.8, 7.6 and 11.4 cm starting at the external edge of the wire mesh cylinder outwards in all four quadrants of the microcosm at 12, 24, 48 and 72 h after placing a 10-day-old nematode infected *G. mellonella* cadaver in the center of the microcosm (Fig. 3.1B). Holes left by core sample removal were filled with the same amount of autoclaved soil to allow uninterrupted nematode movement. Similar microcosms containing soil with grass with no *G. mellonella* larvae were set up as controls. Five microcosms were used for each treatment and each experiment was performed twice resulting in *N* = 10 for each species.

*Data Analysis*

The number of killed baits is a proxy for the displacement distance of at least one IJ. These data were normalized by using log_{10} transformation and subjected to analysis of variance (ANOVA), and means were separated by Tukey’s test. Mean percentage of IJs of both species dispersing at a time point up to a particular distance in a 2 cm wide arc in the microcosms in the presence and absence of non-mobile host was calculated by using the following equation.
\[ D_{dt_ii} = (M_{dt_ii} / C_{dt}) \cdot N_{dt} \cdot 100 \]  

where, \( N_{dt} \) represents the total number of cores that would be extracted from an arc at a particular distance, while other parameters remain the same as in equation 1 (Chapter 2).

In the presence and absence of mobile hosts, the mean percentage of IJs of both species dispersing at a time point up to a particular distance in a 2 cm wide annulus was calculated by equation 3 obtained by modifying equation 1 from Chapter 2.

\[ D_{dt_ii} = (M_{dt_ii}^o / C_{dt}) \cdot N_{dt} \cdot 100 \]  

where, \( M_{dt_ii}^o \) represents the mean number of IJs recovered from four soil cores collected at a distance, \( d \) and a time point, \( t \) from all four quadrants in the microcosms. All other parameters are similar to equation 1 (Chapter 2). The mean proportion data were arcsine transformed to achieve normality and subjected to analysis of variance (ANOVA), where Tukey’s test was used for means separation to compare the nematode dispersal in the presence and absence of mobile or non-mobile host for both the species. Two-sample \( t \)-test with assumed unequal variances was used to compare the mean proportion of IJs of the two species dispersed at different time points (24-72 h) and distances (3.8-11.4 cm) in the presence of mobile and non-mobile hosts. Comparisons in nematode dispersal in the presence of mobile and non-mobile hosts were made using the mean number of infected \( G. mellonella \) baits as well as mean percentage of IJs of both species dispersing at a time point up to a particular distance in a 2 cm wide annulus, calculated by equation 1 as in Chapter 2. Additionally, two-sample \( t \)-test with assumed unequal variances was used to make between and within species comparisons of the average displacements (cm/day) in the presence or absence of mobile and non-mobile host. A probability value <0.05 was considered indicative of a significant difference for the entire analysis (\( t \)-test and
ANOVA). All analyses were performed using Minitab Release 16 (Minitab Inc., State College, PA).

Separate analysis of results from each repeated experiment produced similar results. For example, significant differences were observed between the two species in the mean proportion IJs at 3.8 (Experiment 1: $t_{19} = 4.68, P = 0.001$; Experiment 2: $t_{19} = 4.72, P = 0.001$), 7.6 (Experiment 1: $t_{19} = 3.78, P = 0.04$; Experiment 2: $t_{19} = 3.54, P = 0.003$) and 11.4 cm (Experiment 1: $t_{28} = 2.24, P = 0.04$; Experiment 2: $t_{28} = 2.13, P = 0.04$) from the source cadaver in the presence of mobile hosts for both experiments. The two experiments did not differ significantly in the mean percentage of IJs dispersed at any of the three distances in the presence of mobile hosts ($P > 0.05$). Therefore, the data from two repeated experiments were pooled for all further analyses and presentation.

**Results**

*Lateral dispersal in the presence or absence of non-mobile hosts*

The presence of the non-mobile host insect had a significant effect on *H. bacteriophora* dispersal compared to the absence of the non-mobile host (Table 3.1). Significantly higher mean percentage of IJs as well as mean number of infected *G. mellonella* baits was found in the quadrant containing one live *G. mellonella* larva than the opposite quadrant without the host, at the closest distance of 3.8 cm from the source cadaver, at 12 and 24 h. The quadrant containing the host also had more mean number of infected baits and mean percentage of IJs, though not significantly higher, at 7.6 cm and
Table 3.1 Analysis of variance table showing mean (± SEM) number of dead *Galleria mellonella* baits from the collected soil core samples and mean (± SEM) percentage of infective juveniles (IJVs) of *Heterorhabditis bacteriophora* dispersed to a 2 cm wide arc at distances, 3.8, 7.6 and 11.4 cm over a period of 12 to 72 h, in the presence of a live non-mobile host, *G. mellonella* larva contained in a wire mesh cage buried in one of the quadrants in the microcosms containing autoclaved field soil with vegetation in comparison with the opposite quadrant of the same microcosm containing no host. Data from the two repeated experiments were pooled for each species giving a sample size of N = 10. A P <0.05 was considered indicative of a significant difference.

<table>
<thead>
<tr>
<th>Distance from cadaver (cm)</th>
<th>Time after placing the cadaver (h)</th>
<th>Habitat complexity (Grass + Host insect)</th>
<th>Analysis of Variance</th>
<th>Habitat complexity (Grass + Host insect)</th>
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<td>Host</td>
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<td>P</td>
</tr>
<tr>
<td>3.8</td>
<td>12</td>
<td>0.01 ± 0.07</td>
<td>0.00 ± 0.00</td>
<td>1</td>
<td>6.50</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.20 ± 0.09</td>
<td>0.05 ± 0.05</td>
<td>1</td>
<td>20.2</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>0.50 ± 0.10</td>
<td>0.35 ± 0.11</td>
<td>1</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>0.50 ± 0.11</td>
<td>0.35 ± 0.11</td>
<td>1</td>
<td>1.20</td>
</tr>
<tr>
<td>7.6</td>
<td>12</td>
<td>0.10 ± 0.07</td>
<td>0.05 ± 0.05</td>
<td>1</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.05 ± 0.05</td>
<td>0.00 ± 0.00</td>
<td>1</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>0.20 ± 0.09</td>
<td>0.25 ± 0.10</td>
<td>1</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>0.25 ± 0.10</td>
<td>0.25 ± 0.10</td>
<td>1</td>
<td>0.61</td>
</tr>
<tr>
<td>11.43</td>
<td>48</td>
<td>0.10 ± 0.07</td>
<td>0.10 ± 0.07</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>0.15 ± 0.08</td>
<td>0.05 ± 0.05</td>
<td>1</td>
<td>1.09</td>
</tr>
</tbody>
</table>

* No Dead *Galleria* and infective juveniles were recorded at 11.43 cm distance from the infected cadaver at 12 and 24 h, both in the presence and absence of the host
11.43 cm from the cadaver than the quadrant without the host at all time points (12-72 h). There was no significant difference in the average (± SEM) displacement (cm/day) of *H. bacteriophora* IJs, computed from all time intervals and distances, in the presence (3.60 ± 0.12) or absence (3.37 ± 0.08) of the host insect (*t*\textsubscript{215} = 1.54, *P* = 0.12).

The presence of the non-mobile host did not significantly affect *S. carpocapsae* dispersal (Table 3.2). Numerically higher mean percentage of IJs moved up to the shortest distance of 3.8 cm from the source cadaver, at early time points of 12 h and 24 h. A drop was observed at later time points, 48 h and 72 h. Lower percentage of IJs, though not significant in the presence and absence of hosts, detected at distances, 7.6 cm and 11.4 cm from the source cadaver indicated a small population of *S. carpocapsae* IJs travelling longer distances. Overall, there was no significant difference in the average (± SEM) displacement (cm/day) of *S. carpocapsae* IJs in the presence (5.07 ± 0.41) or absence (5.60 ± 0.45) of the non-mobile host (*t*\textsubscript{235} = 0.88, *P* = 0.38). Although greater percentage (~2 times) of IJs reached the farthest distance in the presence of non-mobile hosts (1.06 ± 0.32%) than no hosts (0.5 ± 0.21%), this difference was not significant (*t*\textsubscript{45} = 2.05, *P* = 0.16). There was no significant increase in the percentage of IJs dispersing the farthest distance from 24 (1.8 ± 2.2) to 72 h (20.0 ± 13.0) in the presence of non-mobile hosts (*t*\textsubscript{10} = 1.58, *P* = 0.17) and in the absence of hosts (0.6 ± 0.2% at 24 h and 19.0 ± 12.2% at 72 h (*t*\textsubscript{10} = 2.03, *P* = 0.18).

The mean percentage of IJs dispersing from the source cadavers decreased with distance and time in both EPN species in the presence of a non-mobile host (Fig. 3.2). The mean percentage of *S. carpocapsae* dispersed up to 11.4 cm over a period of 72 h was significantly higher than *H. bacteriophora* (Table 3.5) with significant differences at
Table 3.2 Analysis of variance table showing mean (± SEM) number of dead *Galleria mellonella* baits from the collected soil core samples and mean (± SEM) number of infective juveniles (IJ$s$) of *Steinernema carpocapsae* dispersed to a 2 cm wide arc at distances, 3.8, 7.6 and 11.4 cm over a period of 12 to 72 h, in the presence of a live non-mobile host, *G. mellonella* larva contained in a wire mesh cage buried in one of the quadrants in the microcosms containing autoclaved field soil with vegetation in comparison with the opposite quadrant of the same microcosm containing no host. Data from the two repeated experiments were pooled for each species giving a sample size of N = 10. A P <0.05 was considered indicative of a significant difference.

<table>
<thead>
<tr>
<th>Distance from cadaver (cm)</th>
<th>Time after placing the cadaver (h)</th>
<th>Habitat complexity (Grass + Host insect)</th>
<th>Analysis of Variance</th>
<th>Habitat complexity (Grass + Host insect)</th>
<th>Analysis of Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Host</td>
<td>No host</td>
<td>DF</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>3.8</td>
<td>12</td>
<td>0.50 ± 0.22 0.83 ± 0.17</td>
<td>1.43</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>1.00 ± 0.00 1.00 ± 0.00</td>
<td>0.00</td>
<td>1.00</td>
<td></td>
<td>24.67 ± 12.67 24.81 ± 5.79</td>
</tr>
<tr>
<td>48</td>
<td>0.83 ± 0.17 0.33 ± 0.21</td>
<td>3.46</td>
<td>0.09</td>
<td></td>
<td>0.03 ± 0.01 0.06 ± 0.05</td>
</tr>
<tr>
<td>72</td>
<td>0.33 ± 0.21 0.33 ± 0.21</td>
<td>0.00</td>
<td>1.00</td>
<td></td>
<td>0.02 ± 0.01 0.02 ± 0.01</td>
</tr>
<tr>
<td>7.6</td>
<td>12</td>
<td>0.33 ± 0.21 0.33 ± 0.21</td>
<td>0.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0.83 ± 0.17 0.50 ± 0.22</td>
<td>1.43</td>
<td>0.26</td>
<td></td>
<td>2.00 ± 0.87 0.77 ± 0.43</td>
</tr>
<tr>
<td>48</td>
<td>0.50 ± 0.22 0.33 ± 0.21</td>
<td>0.29</td>
<td>0.60</td>
<td></td>
<td>0.05 ± 0.05 0.02 ± 0.01</td>
</tr>
<tr>
<td>72</td>
<td>0.33 ± 0.21 0.33 ± 0.21</td>
<td>0.00</td>
<td>1.00</td>
<td></td>
<td>0.02 ± 0.01 0.02 ± 0.01</td>
</tr>
<tr>
<td>11.43</td>
<td>24</td>
<td>0.17 ± 0.17 0.33 ± 0.21</td>
<td>0.38</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>0.17 ± 0.17 0.00 ± 0.00</td>
<td>1.00</td>
<td>0.34</td>
<td></td>
<td>0.02 ± 0.01 0.00 ± 0.00</td>
</tr>
<tr>
<td>72</td>
<td>0.33 ± 0.21 0.33 ± 0.21</td>
<td>0.00</td>
<td>1.00</td>
<td></td>
<td>0.008 ± 0.005 0.008 ± 0.005</td>
</tr>
</tbody>
</table>

* No Dead *Galleria* and infective juveniles were recorded at 11.43 cm distance from the infected cadaver at 12 h, both in the presence and absence of the host
Figure 3.2. Mean (± SEM) percentage of infective juveniles of Heterorhabditis bacteriophora and Steinernema carpocapsae dispersed to a 2 cm wide annulus in soil in the microcosm at different distances from the source cadaver over time (integrated over distance) and distance (integrated over time) in the presence of mobile and non-mobile host insect Galleria mellonella larva. Asterisk sign (*) indicates significant difference in the means between the two species at a time point or distance at $P < 0.05$. 
arcs, 3.8 cm ($t_{39} = 1.93, P = 0.05$) and 7.6 cm ($t_{55} = 2.86, P = 0.01$), and at time points 12 h ($t_{29} = 1.95, P = 0.05$) and 24 h ($t_{29} = 2.24, P = 0.04$) after placing the source cadaver in the center of the microcosms (Fig. 3.2). While higher percentage of *S. carpocapsae* (93.0 ± 13.2) than *H. bacteriophora* (67.0 ± 10.34) were detected at the closest annulus (3.8 cm) from the source cadaver ($t_{74} = 3.07, P = 0.005$), there was no significant difference in the percentage of the two species (1.06 ± 0.32 in *S. carpocapsae*; 1.4 ± 0.25 in *H. bacteriophora*) moved to the farthest annulus (11.4 cm) in the presence of non-mobile host ($t_{74} = 1.53, P = 0.21$). *S. carpocapsae* showed significantly greater average (± SEM) displacement (5.07 ± 0.41 cm/day) than *H. bacteriophora* (3.60 ± 0.12 cm/day) in the presence of the non-mobile host ($t_{184} = 3.43, P = 0.001$). In addition, significantly higher mean number of *G. mellonella* baits were found infected by *S. carpocapsae* than *H. bacteriophora* in the presence of non-mobile host over 12 to 72 h (Table 3.5). *S. carpocapsae* also showed significantly greater average (± SEM) displacement (5.60 ± 0.45 cm/day) than *H. bacteriophora* (3.30 ± 0.08 cm/day) in the absence of the host ($t_{184} = 4.80, P < 0.0001$). This is evident from significantly higher mean number of *G. mellonella* baits ($t_{205} = 4.96, P < 0.0001$) infected by *S. carpocapsae* than *H. bacteriophora* as well as mean percentage of IJs dispersed in the quadrant containing no host over a period of 72 h ($t_{119} = 2.19, P = 0.03$).

$Lateral dispersal in the presence or absence of mobile hosts$

*H. bacteriophora* dispersal was not significantly affected by the presence or absence of mobile hosts in the microcosms, up to 48 h at all distances from the source.
cadaver (Table 3.3). However, significantly lower mean numbers of infected *G. mellonella* baits as well as mean percentage of dispersed *H. bacteriophora* IJs were detected in the presence of mobile hosts than in the absence of hosts at the longest time point of 72 h at all distances. *H. bacteriophora* did not differ in the average (± SEM) displacement in soil with (5.30 ± 0.36 cm/day) or without (5.40 ± 0.25 cm/day) (*t*\textsubscript{230} = 0.23, *P* = 0.82) mobile hosts.

The presence of mobile hosts enhanced *S. carpocapsae* dispersal as there was a significant increase in both the mean numbers of *G. mellonella* baits infected as well as the mean percentage of IJs dispersed at 12 h for the shortest distance of 3.8 cm and up to 48 h for the two farther distances, 7.6 cm and 11.4 cm, from the source cadaver (Table 3.4). Overall, the average (± SEM) displacement (cm/day) of *S. carpocapsae*, computed from all time intervals and distances was significantly higher in microcosms containing mobile hosts (8.06 ± 0.59) compared with no hosts (4.20 ± 0.17) (*t*\textsubscript{148} = 6.30, *P* < 0.0001). The percentage of IJs dispersing the farthest distance was greater in the presence of mobile hosts (14.5 ± 2.4) than no hosts (2.0 ± 1.2), though the differences were not significant (*t*\textsubscript{42} = 1.43, *P* = 0.16). Although the proportion of *S. carpocapsae* IJs reaching the farthest distance increased from 13.4% (± 1.3) at 24 h to 32% (± 16.3) at 72 h in the presence of mobile hosts, it was not statistically significant (*t*\textsubscript{9} = 1.48, *P* = 0.19). Similarly, there was no significant difference in the percentage of IJs recovered at 24 (0%) and 72 h (7.0 ± 2.3) in the absence of hosts at the farthest distance (*t*\textsubscript{9} = 1.26, *P* = 0.24).

The mean percentage of *S. carpocapsae* dispersed over a period of 72 h was significantly higher than *H. bacteriophora* in the presence of mobile hosts (Table 3.5).
Table 3.3 Analysis of variance table showing mean (± SEM) number of dead *Galleria mellonella* baits from the collected soil core samples and mean (± SEM) number of infective juveniles (IJ$s$) of *Heterorhabditis bacteriophora* dispersed to a 2 cm wide annulus at distances, 3.8, 7.6 and 11.4 cm over a period of 12 to 72 h, in the presence of two mobile hosts, *G. mellonella* larvae placed outside the wire mesh cylinder buried at 3.8 cm distance from the source cadaver in the microcosms containing autoclaved field soil with vegetation as opposed to similar microcosms containing no hosts. Data from the two repeated experiments were pooled for each species giving a sample size of N = 10. A $P < 0.05$ was considered indicative of a significant difference.

<table>
<thead>
<tr>
<th>Distance from cadaver (cm)</th>
<th>Time after placing the cadaver (h)</th>
<th>Habitat complexity (Grass + Host insect)</th>
<th>Analysis of Variance</th>
<th>Habitat complexity (Grass + Host insect)</th>
<th>Analysis of Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Host</td>
<td>No host</td>
<td>DF</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>3.8</td>
<td>12</td>
<td></td>
<td>0.64 ± 0.11</td>
<td>0.90 ± 0.10</td>
<td>1  2.95</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td></td>
<td>0.71 ± 0.16</td>
<td>0.90 ± 0.10</td>
<td>1  1.09</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td></td>
<td>0.68 ± 0.13</td>
<td>0.90 ± 0.10</td>
<td>1  1.88</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td></td>
<td>0.32 ± 0.09</td>
<td>1.00 ± 0.00</td>
<td>1  83.8</td>
</tr>
<tr>
<td>7.6</td>
<td>12</td>
<td></td>
<td>0.07 ± 0.04</td>
<td>0.00 ± 0.00</td>
<td>1  3.53</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td></td>
<td>0.24 ± 0.11</td>
<td>0.70 ± 0.15</td>
<td>1  6.22</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td></td>
<td>0.46 ± 0.16</td>
<td>0.80 ± 0.13</td>
<td>1  2.62</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td></td>
<td>0.28 ± 0.15</td>
<td>0.80 ± 0.13</td>
<td>1  6.46</td>
</tr>
<tr>
<td>11.43</td>
<td>24</td>
<td></td>
<td>0.00 ± 0.00</td>
<td>0.10 ± 0.10</td>
<td>1  0.69</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td></td>
<td>0.07 ± 0.04</td>
<td>0.30 ± 0.15</td>
<td>1  1.47</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td></td>
<td>0.07 ± 0.04</td>
<td>0.60 ± 0.16</td>
<td>1  6.53</td>
</tr>
</tbody>
</table>

* No Dead *Galleria* and infective juveniles were recorded at 11.43 cm distance from the infected cadaver at 12 h, both in the presence and absence of the host
Table 3.4 Analysis of variance table showing mean (± SEM) number of dead *Galleria mellonella* baits from the collected soil core samples and mean (± SEM) number of infective juveniles (IJ) of *Steinernema carpocapsae* dispersed to a 2 cm wide annulus at distances, 3.8, 7.6 and 11.4 cm over a period of 12 to 72 h, in the presence of two mobile hosts, *G. mellonella* larvae placed outside the wire mesh cylinder buried at 3.8 cm distance from the source cadaver in the microcosms containing autoclaved field soil with vegetation as opposed to similar microcosms containing no hosts. Data from the two repeated experiments were pooled for each species giving a sample size of N = 10. A P <0.05 was considered indicative of a significant difference.

<table>
<thead>
<tr>
<th>Distance from cadaver (cm)</th>
<th>Time after placing the cadaver (h)</th>
<th>Habitat complexity (Grass + Host insect)</th>
<th>Analysis of Variance</th>
<th>Habitat complexity (Grass + Host insect)</th>
<th>Analysis of Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Host</td>
<td>No host</td>
<td>DF</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Dead <em>Galleria</em></td>
<td>(Mean ± SEM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.8</td>
<td>12</td>
<td>0.93 ± 0.04</td>
<td>0.30 ± 0.15</td>
<td>1</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>1.00 ± 0.08</td>
<td>0.60 ± 0.16</td>
<td>1</td>
<td>4.12</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>0.68 ± 0.07</td>
<td>0.90 ± 0.10</td>
<td>1</td>
<td>2.72</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>0.46 ± 0.11</td>
<td>0.90 ± 0.10</td>
<td>1</td>
<td>8.07</td>
</tr>
<tr>
<td>7.6</td>
<td>12</td>
<td>0.43 ± 0.10</td>
<td>0.00 ± 0.00</td>
<td>1</td>
<td>24.4</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.61 ± 0.04</td>
<td>0.20 ± 0.13</td>
<td>1</td>
<td>4.69</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>0.60 ± 0.05</td>
<td>0.20 ± 0.13</td>
<td>1</td>
<td>4.00</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>0.50 ± 0.13</td>
<td>0.80 ± 0.13</td>
<td>1</td>
<td>2.37</td>
</tr>
<tr>
<td>11.43</td>
<td>12</td>
<td>0.14 ± 0.07</td>
<td>0.00 ± 0.00</td>
<td>1</td>
<td>5.43</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.21 ± 0.07</td>
<td>0.00 ± 0.00</td>
<td>1</td>
<td>4.51</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>0.32 ± 0.07</td>
<td>0.00 ± 0.00</td>
<td>1</td>
<td>29.8</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>0.25 ± 0.14</td>
<td>0.70 ± 0.15</td>
<td>1</td>
<td>4.20</td>
</tr>
</tbody>
</table>
Table 3.5 Comparison of mean (± SEM) number of dead *Galleria mellonella* baits from the collected soil core samples and mean (± SEM) percentage of infective juveniles (IJs) of *H. bacteriophora* and *S. carpocapsae* dispersed to 2 cm wide annuli at all three distances, 3.8, 7.6 and 11.4 cm over a period of 12 to 72 h in the presence of two mobile hosts and a non-mobile host in the microcosms containing autoclaved field soil with vegetation. Data were pooled from the two repeated experiments for each species giving a sample size of n = 10. A P <0.05 was considered indicative of a significant difference for the two-sample T-test comparing the two species and a species for two types of hosts in horizontal and vertical directions, respectively.

<table>
<thead>
<tr>
<th>Species</th>
<th><em>H. bacteriophora</em></th>
<th><em>S. carpocapsae</em></th>
<th>Two-sample T-test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Host insect</strong></td>
<td>Dead <em>Galleria</em> (Mean ± SEM)</td>
<td></td>
<td>DF</td>
</tr>
<tr>
<td>Mobile</td>
<td>0.29 ± 0.04</td>
<td>0.49 ± 0.04</td>
<td>238</td>
</tr>
<tr>
<td>Non-mobile</td>
<td>0.18 ± 0.03</td>
<td>0.40 ± 0.05</td>
<td>238</td>
</tr>
<tr>
<td><strong>Two-sample T-test</strong></td>
<td>DF</td>
<td>t</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>209</td>
<td>2.03</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Percentage of infective juveniles (Mean ± SEM)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mobile</td>
<td>1.10 ± 0.30</td>
<td>26.38 ± 4.21</td>
<td>120</td>
</tr>
<tr>
<td>Non-mobile</td>
<td>0.44 ± 0.18</td>
<td>16.39 ± 9.55</td>
<td>120</td>
</tr>
<tr>
<td><strong>Two-sample T-test</strong></td>
<td>DF</td>
<td>t</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>152</td>
<td>2.22</td>
<td>0.03</td>
</tr>
</tbody>
</table>
with significant differences at all annuli, 3.8 cm \( (t_{39} = 4.78, P < 0.0001) \), 7.6 cm \( (t_{39} = 3.28, P = 0.003) \) and 11.4 cm \( (t_{55} = 1.93, P = 0.05) \), and at all the time points, 12 h \( (t_{29} = 2.72, P = 0.01) \), 24 h \( (t_{29} = 2.12, P = 0.04) \), 48 h \( (t_{32} = 2.52, P = 0.02) \) and 72 h \( (t_{29} = 3.32, P = 0.003) \) after placing the infected cadaver in the center of the microcosms (Fig. 3.2).

Significantly higher percentage of \( S.\ carpocapsae \) (14.5 ± 2.4) than \( H.\ bacteriophora \) (0.4 ± 0.27) reached the farthest annulus at 11.4 cm from the source cadaver in the presence of mobile hosts \( (t_{40} = 2.32, P = 0.05) \). There was no significant difference in the percentage of \( S.\ carpocapsae \) (65.0 ± 8.5) and \( H.\ bacteriophora \) (79.0 ± 11.67) remaining within the closest annulus (3.8cm) from the source cadaver \( (t_{72} = 1.82, P = 0.22) \). \( S.\ carpocapsae \) showed significantly higher average \((±\ SEM)\) displacement (8.06 ± 0.59 cm/day) than \( H.\ bacteriophora \) (5.30 ± 0.36 cm/day) in the presence of mobile hosts \( (t_{165} = 4.09, P < 0.0001) \). Additionally, there was significantly higher mean number of \( G.\ mellonella \) baits infected by \( S.\ carpocapsae \) than \( H.\ bacteriophora \) in the presence of mobile hosts over a period of 72 h (Table 3.5). However, in the absence of hosts, \( H.\ bacteriophora \) showed significantly greater average \((±\ SEM)\) displacement (5.40 ± 0.24 cm/day) than \( S.\ carpocapsae \) (4.20 ± 0.17 cm/day) \( (t_{212} = 3.93, P < 0.0001) \). Significantly higher mean number of baits \( (t_{237} = 3.15, P = 0.002) \) were infected by \( H.\ bacteriophora \) than \( S.\ carpocapsae \); however, the two species did not differ significantly \( (t_{237} = 0.26, P = 0.80) \) in the mean percentage of IJs dispersed over a period of 72 h in the absence of hosts.
In case of *H. bacteriophora*, the mean number of infected *G. mellonella* baits and the mean percentage of IJs dispersed over a period of 72 h were significantly higher in the presence of mobile hosts than non-mobile hosts (*Table 3.5*). Also, *H. bacteriophora* showed significantly higher average (± SEM) displacement in the presence of mobile hosts (5.30 ± 0.36 cm/day) than non-mobile hosts (3.60 ± 0.12 cm/day) \(t_{189} = 4.47, P < 0.0001\). This is evident from a higher percentage of *H. bacteriophora* IJs remaining at distance ≤ 3.8 cm in the presence of mobile (79.0 ± 11.67) than non-mobile hosts (67.0 ± 10.34); however it was not significantly different \(t_{77} = 1.87, P = 0.12\). Also, there was no significant difference in the percentage of IJs reaching the farthest distance, 11.4 cm, in the presence of mobile (0.4 ± 0.27) as opposed to non-mobile (1.4 ± 0.25) hosts \(t_{77} = 1.74, P = 0.11\). Although *S. carpocapsae* showed no significant differences either in the mean number of infected *G. mellonella* baits or in the mean percentage of IJs in the presence of mobile and non-mobile host (*Table 3.5*), it showed significantly higher average (± SEM) displacement in the presence of mobile host (8.06 ± 0.59 cm/day) than non-mobile host (5.07 ± 0.41 cm/day, respectively) \(t_{202} = 4.23, P < 0.0001\). A higher percentage of *S. carpocapsae* IJs reached the farthest distance in the presence of mobile (14.5 ± 2.4) than non-mobile (1.06 ± 0.32) hosts; however this difference was not statistically significant \(t_{40} = 2.03, P = 0.16\). Further, there was no significant difference in the percentage of IJs remaining at distances ≤ 3.8 cm from the source cadaver in the presence of mobile (65.0 ± 8.5) as opposed to non-mobile (93.0 ± 13.12) hosts \(t_{40} = 1.83, P = 0.24\).
Discussion

The presence of mobile hosts causing greater average displacement of both *H. bacteriophora* and *S. carpocapsae* with different foraging strategies is an interesting outcome of the study. Greater average displacement of both EPN species in response to mobile hosts may be explained by likely greater dispersal of EPNs towards higher amounts of chemical cues emitted by actively moving mobile hosts respiring at a higher rate than non-mobile hosts. Earlier studies have shown CO$_2$ emissions to cause EPN orientation and aggregations (Gaugler *et al*. 1980; Lewis *et al*. 1993; O’Halloran and Burnell, 2003; Hallem *et al*. 2011). CO$_2$ has also been recently reported to be a critical host-seeking cue for EPNs regardless of their host-seeking strategy and also an essential cue for attraction to *G. mellonella* (Dillman *et al*. 2012). Greater EPN attraction to the vibrations created by host movement in the soil (Torr *et al*. 2004) could also be one of the reasons of greater average displacement of both EPN species in the presence of mobile hosts. Insects are known to transmit acoustic stimuli in soil up to 20 cm (Mankin *et al*. 2002) and EPNs could have responded to such stimuli, however, it needs to be tested.

Significantly greater average displacement of *H. bacteriophora* in the presence of mobile (5.3 cm/day) than non-mobile hosts (3.6 cm/day) most likely resulted from greater percentage (~1.2 times) of IJs remaining at distances ≤ 3.8 cm in the presence of mobile hosts. Higher percentage of IJs (~3.5 times) dispersing to the farthest distance in the presence of non-mobile hosts as compared to mobile hosts over a period of 72 h indicates the characteristic cruising ability of *H. bacteriophora* to find sedentary hosts. However, there is still a large proportion of *H. bacteriophora* IJs ambushing near the source.
cadaver at a distance ≤ 3.8 cm, in the presence of mobile (79%) and non-mobile hosts (67%). On the other hand, it is likely that significantly higher average displacement (cm/day) of *S. carpocapsae* in the presence of mobile (8.06) than non-mobile hosts (5.07) could have occurred due to greater percentage (~14.5 times) of IJs dispersing to the farthest distance in the presence of mobile than non-mobile hosts. This provides further support for the conclusion that a small proportion of *S. carpocapsae* IJs emigrate farther and faster than *H. bacteriophora* in search of hosts.

Interestingly, the proportion of *S. carpocapsae* IJs (~14.5%) reaching the farthest distance over a period of 72 h in the presence of mobile hosts was ~34 times the cruising *H. bacteriophora* IJs (~0.4%). These proportions were nearly similar (~1% *S. carpocapsae*; ~1.4% *H. bacteriophora*) in the presence of non-mobile hosts. The percentage of *H. bacteriophora* IJs reaching the farthest distance increased ~3 times in the presence of non-mobile hosts (~1.4%) than no hosts (~0.3%), but decreased ~12.5 times in the presence of mobile hosts (~0.4%) than no hosts (~5%). Such low percentage of *H. bacteriophora* IJs reaching the farthest distance in the presence of mobile hosts indicates host infection by most dispersing IJs resulting in fewer being detected in collected soil samples. In case of *S. carpocapsae*, the presence of hosts increased the proportion of apparently ‘sprinters’ that reached the farthest distance from the source. These sprinters could be considered emigrants due to their apparent temporary inhibition to the available vegetative stimuli, that is, hosts, found alive up to 24 h, and movement to a larger distance from the source (see Chapter 1). However, further investigation is required to test such temporary inhibition of the sprinters to the host cues. The proportion of sprinters was ~7 times and ~2 times higher in the presence of mobile and non-mobile
hosts, respectively as opposed to the respective no-host controls. It also increased with time, from ~13.4% at 24 h to ~32% at 72 h in the presence of mobile hosts and, from ~1.8% at 24 h to ~20% at 72 h in the presence of non-mobile hosts. A greater proportion (~7.5 times) of sprinters dispersed immediately (24 h) after placing mobile (~13.4%) than non-mobile (~1.8%) hosts. However, these differences were not statistically significant but could not be ignored as they may hold some biological significance for EPN dispersal. Therefore, the presence of hosts influenced the dispersal of *S. carpocapsae*, most likely due to greater proportion of sprinters than in the absence of hosts. The impact was stronger in the presence of mobile than non-mobile hosts. *S. carpocapsae* seems to have a distinct strategy for discovering new hosts, whereby a small proportion of sprinters travel long distances in search for hosts and then increase their number once the host is detected. These results tend to support the recruitment hypothesis in which sprinters may invade hosts first and even make them more suitable and attractive for following IJs to find and invade, enhancing the reproductive success of the entire population (Grewal *et al*. 1993; 1997).

Furthermore, greater dispersal of *S. carpocapase* in the presence of hosts, irrespective of host mobility is evident from significantly greater average displacement of *S. carpocapsae* (8.06 cm/day and 5.07 cm/day) than *H. bacteriophora* (5.3 cm/day and 3.6 cm/day) in response to mobile and non-mobile host, respectively. Additionally, the mean percentage of *S. carpocapsae* IJs dispersing at all distances (3.8-11.4 cm) during the course of the experiment (12-72 h), was found ~37 and ~24 times higher than *H. bacteriophora* in the presence of non-mobile and mobile hosts, respectively. These differences could be due to temporal differences in the numbers of IJs emerging from the
source cadavers in the two species when the cadavers are prepared in the similar manner (see Chapter 2). In the previous study, we found significantly higher number of *S. carpocapsae* IJs emerging from 10-day old infected *G. mellonella* cadavers up to the first 48 h than *H. bacteriophora*, which showed significantly higher IJ emergence at 72 h as compared to *S. carpocapsae*. Therefore, higher *S. carpocapsae* IJ emergence up to 48 h could result in higher percentage of IJs, ~24 times and ~37 times, than *H. bacteriophora* being dispersed up to the same time period in the presence of mobile and non-mobile hosts, respectively. However, at 72h, the mean percentage of *S. carpocapsae* was again higher than *H. bacteriophora* in the presence of both, mobile (~3 times) and non-mobile hosts (~35 times). This could be due to greater percentage of *S. carpocapsae* sprinters (~32% and ~20% in the presence of mobile and non-mobile hosts, respectively) than *H. bacteriophora* IJs (~19.5% and ~10%, respectively) reaching the farthest distance at 72 h. Therefore, availability of hosts, mobile or non-mobile enhanced *S. carpocapsae* dispersal, which could be due to the increase in the proportion of sprinters that make the hosts attractive for the recruitment of the following IJs to find and invade the available hosts (Grewal *et al.* 1993; 1997).

Although the presence of non-mobile hosts does not affect the average displacement of *H. bacteriophora*, its dispersal was enhanced during the first 24 h period, after which it exhibited ranging search most likely due to reduced attraction to the infected host as reported by Grewal *et al.* (1997). Significantly higher percentage of IJs as well as infected *G. mellonella* baits were found in the quadrant containing the live non-mobile host than the opposite quadrant without any host at the closest distance (3.8 cm) within first 24 h. Twice as many IJs moved to 7.6 cm in the quadrant containing live non-
mobile host as opposed to the IJs moving the same distance in the opposite quadrant. The percentage of IJs reaching 7.6 cm and 11.43 cm from the cadaver did not differ significantly in the two quadrants which indicated that the IJs were initially attracted and dispersed towards the live host but this attraction lasted only for 24 hr, after which the IJs began to move elsewhere in the microcosm. We speculate that, after the host was infected by the IJs reaching during the first 24 h, the cadaver may have become less attractive or even repellent to the IJs arriving subsequently as has been demonstrated by Grewal et al. (1997). This may have resulted in IJs switching to a ranging search, whereby IJs move elsewhere in search for another suitable host in the area. Glazer (1997) also demonstrated such deterrence in subsequent invasion of an infected host after the initial infection by Steinernema spp. due to the release of a chemical in Petri dish assays. This decrease in attraction to the parasitized hosts could also be explained as an adaptive strategy of IJs to reduce intra-specific competition (Grewal et al. 1997). The non-mobile hosts placed in the microcosms were retrieved and dissected to confirm infection by H. bacteriophora IJs. Mean (± SEM) number of IJs infecting each larva was found to be 52 ± 1.21. In the presence of mobile hosts, H. bacteriophora showed greater dispersal, though not significant, only at the earliest time point (12 h) indicating greater activity of IJs in response to mobile hosts immediately after its placement in the microcosms. However, as time progressed, lower numbers of infected G. mellonella baits and IJs were found with increasing distance in the microcosms containing mobile hosts as compared to no hosts. Interestingly, significantly lower number of infected G. mellonella baits and IJs in the presence of mobile hosts as compared to the absence of hosts at longest time point, 72 h suggests rejection by infected hosts. The mobile host might have become non-mobile by
72 h, following infection (Mean ± SEM number of IJs: 22 ± 0.52 per host larva) by dispersing IJs and subsequent repulsion or unattractiveness to other IJs which would then disperse randomly to other parts of the microcosm.

Our results did not show significant impact of non-mobile host on *S. carpocapsae* dispersal as opposed to no host. However, the quadrant containing the host received higher number of infected baits and IJs than the one without any host insect at most time points, though the pattern is not consistent. Shortest distance (3.8 cm) from the source cadaver had higher percentage of IJs in the quadrant containing the host than the one without any host, soon (12 h) after placing the infected source cadaver and the non-mobile host insect. As IJs continued to disperse, these numbers dropped over time, which could be due to the penetration of dispersing IJs in the embedded host insect which contained an average of 139 ± 4.21 IJs per larva. However, the quadrant containing live non-mobile host received two times as many IJs at 7.6 cm as opposed to the percentage of IJs moving the same distance in the opposite quadrant. A small proportion (1%) of IJs travelled longer distance, 11.4 cm in the presence of non-mobile hosts is indicative of sprinting population of otherwise ambushing *S. carpocapsae*. Nevertheless, this population is higher than the proportion of IJs (0.5%) reaching the same distance in the opposite quadrant containing no host indicating some positive impact, not significant though, of the non-mobile host insect on the dispersal of *S. carpocapsae* sprinters. On the other hand, mobile hosts had significant positive impact on the average displacement of *S. carpocapsae* which is evident from significantly higher numbers of *G. mellonella* baits infected as well as percentage of IJs dispersed at most time points, particularly at longer distances, 7.6 cm and 11.4 cm, from the source cadaver, where mobile hosts were
released. Also, significantly higher percentage of IJs could reach the farthest distance, 11.4 cm, from the source cadaver soon (12 h) after releasing the mobile hosts in the microcosms as compared to the percentage of IJs dispersing in host-free microcosms. However, lower percentage of IJs dispersed at the longest time point, 72 h, at all distances (3.8-11.4 cm) in the presence of mobile hosts as compared to no hosts again supports the contention that infected hosts (containing an average of 735 ± 8.24 IJs per larva) may be repulsive or less attractive to the following IJs (see Grewal et al. 1997; Glazer, 1997) as discussed earlier.

In conclusion, this study has shown greater dispersal ability of the two EPN species, *H. bacteriophora* and *S. carpocapsae* with distinct foraging strategies in their natural habitat, soil, in the presence of mobile host insect, *G. mellonella*. There is evidence of nematode attraction to indirect cues of host location such as herbivore induced plant volatiles (Boff *et al.* 2001, 2002; van Tol *et al.* 2001; Rasmann *et al.* 2005; Ali *et al.* 2010, 2011), however our study used a host insect that does not feed on any plant parts. Consequently, there is little scope for the release of plant volatiles other than CO₂. The use of infected cadavers as the source of nematodes releasing IJs in large numbers as a population in the soil medium with vegetation and host insects brings the experimental conditions close to the natural conditions for these soil dwelling nematodes. Such quantitative information on nematode dispersal from infected host cadavers in the presence of vegetation and hosts could be critical to understanding spatial and temporal dynamics of both inundatively released and endemic EPN populations in the field and further useful for designing improved application strategies for EPNs for sustainable pest control with these beneficial organisms.
This is a comparative study that has identified differences in the rate and pattern of lateral dispersal between the two EPN species in response to mobile vs non-mobile host insects in the soil containing vegetation. Interestingly, the results suggest that there is a proportion of *H. bacteriophora* population that remains near the source cadaver behaving more like ambushers, while the rest cruise in the soil in the presence of hosts. Similarly, there is a small proportion of *S. carpocapsae* sprinters travelling longer distances in search for hosts, while the rest sit in the immediate vicinity of the source cadaver as ambushers. *S. carpocapsae* appeared to follow a unique dispersal strategy to locate hosts, whereby more number of sprinters travel longer distances to locate mobile hosts than sedentary or no hosts. The evolutionary mechanisms behind such strategy needs to be addressed in further studies to understand the dispersal behavior of this species, otherwise known as an ambusher, and design its appropriate application strategies for pest control.

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References


Chapter 4: Dispersal of entomopathogenic nematodes with different foraging strategies in the field

Abstract

Laboratory studies have identified dichotomy in foraging behavior of EPN species but little is known about the impact of these strategies on their dispersal and distribution in the field. I compared the short-term dispersal potential of the ambusher, *Steinernema carpocapsae* and cruiser, *Heterorhabditis bacteriophora* emerging from infected host cadavers in two different habitats, a potato field and the adjoining grassy border, both of which were sampled earlier to assure the absence of naturally occurring populations of EPNs. Ten-day old *H. bacteriophora* and *S. carpocapsae* infected *Galleria mellonella* cadavers were placed at the edge of each 9 m² grassy border plot adjoining the potato field and EPN dispersal was tracked in both habitats using in-situ bait and pitfall traps. One in-situ bait trap containing 5 final instar *G. mellonella* larvae was placed 10 cm deep in the soil at 30 cm intervals up to 210 cm from the source cadavers in both habitats. Bait traps were implanted at 24 h intervals from 24 to 96 h and pitfall traps were placed at 24 and 48 h after placing the cadavers. Pitfall traps and bait traps were removed after 24 h and examined in the laboratory. The phoretic transport of EPNs by surface active arthropods was assessed by examining their presence in the pitfall traps. Abundance and
composition of surface-dwelling and below ground arthropods was determined from
pitfall trap catches and soil core samples placed in Tullgren funnels, respectively. The
insects collected were identified to family level. There were no differences in the
abundance of surface active and soil-dwelling arthropods in the two habitats. *S.
carpocapsae* dispersed as far as *H. bacteriophora* with similar average displacements
(27-28 cm/day) in the two habitats over 120 h. The percentage of *S. carpocapsae*
reaching up to 2 m from the source cadavers was lower than *H. bacteriophora* at 72 h but
higher at 120 h. *S. carpocapsae* was detected in larger numbers than *H. bacteriophora* in
the pitfall traps. However, abundance of Trombiformes (mites) was significantly
positively correlated with the percentage of *H. bacteriophora* dispersed to 2 m at 96 h.
Further research on quantification of EPN dispersal over larger spatio-temporal scale is
warranted.

**Introduction**

Although entomopathogenic nematodes (EPNs) are currently exclusively used as
therapeutic agents with short-term effect, their natural widespread occurrence in diverse
ecosystems (Hominick, 2002; Alumai et al., 2006) and persistence beyond a season in
some cases (e.g. Klein and Georgis, 1992) support the feasibility of a conservation
approach. Development and successful application of a cost effective conservation
approach requires basic information on biology and ecology of both the nematode and the
target pest along with good understanding of population biology and spatial ecology of
EPNs. Specifically, knowledge of dispersal behavior of EPNs is needed to maintain a uniform distribution of otherwise patchily distributed EPN populations (Stuart and Gaugler, 1994; Glazer et al., 1996; Campbell et al., 1995, 1996, 1998; Wilson et al., 2003; Stuart et al., 2006) in the field to obtain consistent biological control against target insect pests.

Laboratory studies have identified dichotomy in foraging behavior of EPN species and have considerably contributed to the knowledge on host finding behavior of EPNs in controlled environments. The third stage juvenile, known as infective juvenile (IJ), is the only free living stage in the life cycle of the EPNs which searches for host insects in soil. EPNs are classified as cruisers (actively searching foragers), ambushers (sit and wait foragers) and intermediate foragers based on their host finding tactics (Lewis et al., 1992; Campbell and Gaugler, 1993; Grewal et al., 1994). While, S. carpocapsae IJs actively nictate and forage near the soil surface, using an ambush-type foraging strategy (Campbell and Gaugler, 1993), H. bacteriophora IJs move actively through the soil in search of a host and directly respond to the long-range host cues (Lewis et al., 1992). However, little is known about the impact of these foraging strategies on the dispersal and distribution patterns of EPNs in the field.

With few studies examining short-term EPN dispersal in the field (Poinar and Hom, 1986; Hsiao and All, 1998), there is little information on dispersal behavior of EPNs applied as nematode infected host cadavers (Del Valle et al., 2008; Jabbour and Barbercheck, 2008). In laboratory studies, IJs emerging from host cadavers have been reported to show better ability to disperse, infect and persist in soil, compared to IJs
applied in aqueous suspension (Shapiro and Glazer 1996; Shapiro and Lewis, 1999; Perez et al. 2003). Therefore, I compared the short-term dispersal potential of two EPN species, *H. bacteriophora* and *S. carpocapsae* with different foraging strategies, applied as nematode infected host cadavers in two different habitats. Since the detection of endemic populations of EPNs have been reported to be more frequent in grassy areas than in cultivated crops (Alumai et al., 2006; Lawrence et al., 2006), I was interested in assessing the potential of EPN species movement from the grassy strips to the adjacent cultivated fields where pest control is desired. Therefore, the active movement of EPNs was assessed using an in-situ bait trap method. Passive movement was assessed by determining the presence of EPNs in Pitfall traps installed in the ground to catch populations of surface active arthropods.

Apart from habitat type and edaphic factors such as soil texture and moisture (Georgis and Poinar, 1983; Kung et al., 1991; Campbell et al., 1998; Efron et al., 2001; Lawrence et al., 2006; Alumai et al., 2006), presence or absence of hosts or non-hosts and their abundance and spatial distribution in the soil (Mráček, 1980, 1982; Campbell et al., 1995; Půža and Mráček, 2005; Chapter 3) will influence EPN dispersal in the field. These studies have investigated the interactions between EPN and insect host dynamics with variable results; however little is known about the impact of host and non-host insects and other arthropods on EPN dispersal in the field. Given the potential influence of insects and microarthropods on EPN distribution in the field by virtue of phoretic dispersal (Kaya, 1990, Epsky et al. 1988; Timper et al., 1988; Parkman et al. 1993; Mráček and Bečvář, 2000; Kruitbos et al., 2009), I hypothesized that the dispersal of the
two EPN species will differ possibly due to the habitat variation in the arthropod
abundance. Since my previous research discovered the unexpected ability of S.
carpocapsae sprinters to disperse farther and faster than *H. bacteriophora* in the absence
as well as presence of host insects (*Chapter 2; 3*), I hypothesized greater dispersal of S.
carpocapsae due to the combination of both active movement facilitated by sprinters and
passive movement likely resulting from arthropod phoresy occurring due to its
characteristic nictation ability.

**Materials and Methods**

**Source of nematodes**

Two nematode species, *Heterorhabditis bacteriophora* GPS11 (a cruiser) and
*Steinernema carpocapsae* ALL (an ambusher) were obtained from our laboratory
collection and new cultures were raised by infecting final instar wax moth larvae,
*Galleria mellonella* (L.), obtained from Vanderhoest Canning Company, St. Mary’s,
Ohio, following methods described by Kaya and Stock (1997). Nematode infected *G.
mellonella* cadavers were used as a source of nematodes rather than aqueous suspensions
to mimic natural conditions. The nematode-infected cadavers were prepared using the
methods detailed in *Chapter 2*. The cadavers from which IJ emergence had just initiated
were selected for release in the field.
Field site

Research was conducted in a potato field adjoining a grassy border strip at an agricultural farm in Shreve, OH (Latitude: 40°: 40', Longitude: -83°: 58'). The land was managed without chemical inputs and the potato field was covered with mulch during the experiment. Since the plots in the grassy border and potato field were already set up and could not be randomized, I conducted a split-plot experiment with EPN species (H. bacteriophora vs. S. carpocapsae) as the main factor and habitat nested within the main factor. The experiment consisted of 76 m long and 3 m wide potato field and adjoining grassy border, both divided into four 15 m sections (Fig 4.1A). The experiment was set up in two 15 m x 3 m sections during the first week of August (Aug 15th to 20th, 2011), whereby H. bacteriophora dispersal was studied in one section and S. carpocapsae dispersal in the other section, 3.8 m apart, to avoid the inter-specific competition between the two EPN species. The experiment was repeated once in the other two 15 m x 3 m sections of the field strip for both H. bacteriophora and S. carpocapsae in the last week of August (Aug 29th to Sep 3rd, 2011) keeping a buffer zone of 7.6 m x 3 m between the two experimental field strips. Each 15 m x 3 m section was divided into five 3 m x 3 m plots for each nematode species. Nematode infected cadavers were placed in 1 m x 1 m area in the center of each 3 m x 3 m plot at the edge of the grassy border adjoining the potato field strip. This field location (grassy border and adjoining potato field) was selected after preliminary sampling to assure the absence of naturally occurring populations of either H. bacteriophora or S. carpocapsae at the site.
Figure 4.1. Experimental plot design. A) Experiment location comprising 76 m long and 3 m wide potato field and adjoining grassy border, both divided into four 15 m sections. The first experiment was set up in two 15 m x 3 m sections during the first week of August (Aug 15\textsuperscript{th} to 20\textsuperscript{th}, 2011), whereby \textit{H. bacteriophora} dispersal was studied in one section and \textit{S. carpocapsae} dispersal in the other section, 3.8 m apart, to avoid the interference of one EPN species with the other. The experiment was repeated in the other two 15 m x 3 m sections of the field strip for both \textit{H. bacteriophora} and \textit{S. carpocapsae} in the last week of August (Aug 29\textsuperscript{th} to Sep 3\textsuperscript{rd}, 2011) keeping a buffer zone of 7.6 m x 3 m between the two experimental field strips. Each 15 m x 3 m section was divided into five 3 m x 3 m plots for each nematode species. B) 3 m x 3 m plots of potato field and adjoining grassy border. Nematode infected cadavers were placed at the edge of the grassy border adjoining the potato field. In-situ bait traps (solid circles) containing 5 last instar \textit{Galleria mellonella} larvae per trap were buried 10 cm deep in the soil at distances, 30 to 210 cm (30 cm apart) from the point where cadavers were placed along two transects, 0.5 m apart, towards the grassy border and the potato field. Arthropod and EPN abundance was estimated in Pitfall traps, 3 per plot in each habitat.
Assessment of EPN dispersal using in-situ baiting technique

*H. bacteriophora* and *S. carpocapsae* populations were established according to field application standards (~500,000 IJs per m²) by releasing two 10-day old *H. bacteriophora* and three *S. carpocapsae* infected *G. mellonella* cadavers, separately in each plot in 1 m x 1 m area in the grassy border on August 15\textsuperscript{th}, 2011 for experiment 1 and August 28\textsuperscript{th}, 2011 for experiment 2. Different numbers of nematode-infected cadavers were used for the two species due to differences in their total reproductive and emergence potential (see Chapter 2). The cadavers were released early in the morning to avoid nematode damage from sunlight and high temperature.

Particle size distribution of the soil in grassy border and potato field, determined using methods described by McCartney *et al.* (1997), was 43.5% clay, 11.5% sand, 45% silt and 24.7% clay, 11% sand, 64.4% silt, respectively. The soil moisture content was 30% in grassy border and 33% in potato field.

An in-situ soil baiting technique was used to assess EPN dispersal over time and distance in the grassy border as well as potato field after placing the nematode-infected cadavers in the grassy border. This technique had been successfully used earlier for sampling field populations of EPNs (Ferguson *et al.*, 1995; Yadav *et al.*, 2012). In this technique, five last instar *G. mellonella* larvae were placed in approximately 100 g of autoclaved field soil in the bait trap (a cylinder made of 24 mesh fiberglass window screen approximately 3 cm in diameter and 10 cm tall). This soil was originally collected from the grassy border and potato field near the experimental plots, autoclaved at 121\degree C.
and 103.42 kPa for 10 h, and stored at room temperature for at least 7 days before use to allow any toxic volatiles to escape. The open end of the bait trap was sealed with a plastic cap after adding *G. mellonella* larvae to the soil, to prevent the larvae to escape. The bait traps were placed in the holes dug in the ground using a soil corer (10 cm deep and 3 cm internal diameter) and the surface of the traps was covered with the field soil. Single bait trap was placed at each distance, 30 to 210 cm (30 cm apart), marked with flags, starting from the point where cadavers were placed along two transects, 0.5 m apart, towards the grassy border and the potato strip (*Fig. 4.1B*, N = 70 bait traps per habitat type for every time point for each species). Bait traps were implanted 24 h, 48 h, 72 h and 96 h in both habitats after placing the cadavers in the grassy border. All baited larvae were removed from the trap after 24 h, placed in a labeled Petri dish lined with a moist filter paper and brought to the laboratory to examine EPN infection. Therefore, the infected baits trapped the nematodes moving up to 48, 72, 96 and 120 h. Larvae killed by EPNs (i.e. showing characteristic smell, color and texture) were dissected 48 h after removing the baits from the soil before the nematodes had enough time to reproduce and the number of nematodes penetrated were recorded. Live larvae were incubated for additional three days after which they were examined for nematode infection and the numbers of nematodes penetrated were counted following dissection.
Assessment of EPN dispersal using pitfall traps

The phoretic transport of nematodes was assessed by setting up 3 pitfall traps in each plot. Pitfall traps (473 ml plastic cups) containing one inch of water were embedded into the ground with open top of the cup positioned at the soil surface. These were set up two times during an experiment, 24 h and 72 h after the release of the cadavers and removed after 24 h, therefore, collecting the arthropods at 48 h and 96 h. After 24 h, all 3 cups/plot were combined, covered and transported to the laboratory (N = 5 per habitat type for each EPN species). The traps were kept undisturbed at 4°C to allow the nematodes to settle in the water inside the traps. These were then examined for the presence of EPNs, which were subsequently counted. Later, last instar *G. mellonella* were infected in Petri dishes with these nematodes to confirm the presence of an EPN species in the traps.

Arthropod abundance

All plots were sampled for surface dwelling soil arthropods using pitfall traps, set up as described above. After 24 h, the traps were covered and transported to the laboratory for insect identification (N = 5 per habitat type for each EPN species). Below ground arthropods were sampled once, at 96 h, during each experiment. Three below ground soil samples per plot were taken using a soil corer, 5 cm dia and 5 cm deep, and combined in a plastic bag (N = 5 per habitat type for each EPN species). Arthropods were
extracted from these soil samples using a heat-gradient apparatus (Tullgren funnel) as described by Edwards (1991). Arthropods collected using pitfall traps and Tullgren funnels were preserved in 70% ethanol until identification to family level using keys from Triplehorn et al. (2005).

Statistical Analyses

EPN dispersal was assessed in the two habitats in terms of the mean maximum distance at which the IJs of both species were detected and the mean percentage of IJs reaching that distance at 24-72 h after placing the source cadavers. The mean percentage of IJs of both species dispersing at a time point was calculated by equation 4.

\[
D_{\text{di ti}} = \left( \frac{M_{\text{di ti}}}{nC_{\text{ti}}} \right) \cdot 100
\]  

(4)

where, \( M_{\text{di ti}} \) represents the mean number of IJs recovered from five \( G. mellonella \) larvae in a bait trap at the maximum distance traveled by IJs at a time point, \( t \); \( n \) represents number of respective nematode infected cadavers placed in the soil; \( C_{\text{ti}} \) represents the mean cumulative number of IJs emerging from each 10-day old \( G. mellonella \) cadaver at a time point, \( t \) (Chapter 2). Multivariate repeated measures analysis of variance (PROC GLM) was performed on mean maximum distance and mean proportion of IJs reaching the maximum distance to compare the dispersal of the two species over time using Wilks’ lambda \( F \) statistic (SAS Release 9.2, SAS Institute Inc., 2008). The data on mean EPN abundance and mean arthropod abundance in the pitfall traps at 48 h and 96 h were analyzed by univariate analysis of variance (ANOVA) for species and habitat
comparisons using SAS 9.2. Distance and abundance (EPN and arthropod) data were transformed as $\log_{10}(x+1)$ and proportion data were arcsine transformed prior to statistical analysis to normalize the variance. Habitat was considered nested within the species for all the above analyses. One-way analysis of variance (ANOVA) was used to make species comparisons of the average displacements (cm/day) in the two habitats, where means were separated by Tukey’s test using Minitab Release 16 (Minitab Inc., State College, PA). We used linear regression to describe 3 parameters, mean EPN abundance in the pitfall traps, mean maximum distance traveled by each EPN species and mean percentage of IJs reaching the maximum distance, as predicted by mean arthropod abundance in the pitfall traps at 48 h and 96 h after placing the nematode infected cadavers in the grassy border (Minitab 16). Similarly, linear regression was used to describe mean maximum distance traveled by an EPN species and mean percentage of IJs dispersing to the maximum distance as predicted by mean arthropod abundance (below ground and surface dwelling), 96 h after placing the source cadavers. Additionally, linear regression analysis was conducted for all three parameters based on the mean number of each arthropod taxon found in the pitfall traps at 48 h and 96 h. Analysis of variance (ANOVA) was used to compare the slopes and intercepts of the regression equations between the two EPN species and habitats using Minitab 16. EPN and arthropod abundance data were normalized by $\log_{10}$ transformation. A probability value $\leq 0.05$ was considered indicative of a significant difference for the entire analysis (ANOVA, Repeated Measures, Regression). The combination of arthropod taxa obtained from the pitfall traps that contributed to the differences between the habitats and EPN species, was
determined with principal component analysis (PCA) based on the correlation matrix using CANOCO version 4.5 (ter Braak and Smilauer, 2002). Arthropod abundance data were transformed by ln (x+1) prior to analysis to stabilize the variance and achieve normality in the data. Since experiment 2 was the true replicate of experiment 1, the data from the two experiments were pooled for all the analyses with block as a factor.

Results

Assessment of EPN dispersal using in-situ baiting technique

The two EPN species did not differ in the mean maximum distance traveled over time ($F_{6,2} = 0.16, P = 0.96$) (Fig. 4.2). *S. carpocapsae* dispersed to a slightly greater distance (cm) than *H. bacteriophora* at 72 h (171 ± 7.94 and 136 ± 18.40, respectively) and 96 h (166.5 ± 11.32 and 148.5 ± 18.40, respectively) after the placement of the source cadavers, but this difference was not significant (72 h: $F_{1,7} = 1.74, P = 0.28$; 96 h: $F_{1,7} = 2.20, P = 0.23$) (Fig. 4.2). There was no significant difference in the mean maximum distance traveled by the two species, between the two habitats at any time point, 48-120 h ($P > 0.05$). Additionally, there was no significant difference in the average displacement (cm/day) of the two species (*H. bacteriophora*: 28.18 ± 1.75; *S. carpocapsae*: 27.03 ± 1.73) ($F_{1,7} = 1.58, P = 0.21$). Furthermore, there was no difference in their average displacement among the two habitats ($F_{2,7} = 0.16, P = 0.85$). The average displacement (cm/day) of *H. bacteriophora* was 28.08 ± 2.36 and 28.32 ± 2.57 in grassy
Figure 4.2. Mean (± SEM) maximum distance traveled by the infective juveniles of *Heterorhabditis bacteriophora* (H) and *Steinernema carpocapsae* (S) in the two habitats, grassy border (G) and potato field (P) at 48, 72, 96 and 120 h after the placement of the source cadavers at the edge of the grassy border.
Figure 4.3. Mean (± SEM) percentage of infective juveniles (IJs) of *Heterorhabditis bacteriophora* (H) and *Steinernema carpocapsae* (S) dispersing to the maximum distance in the two habitats, grassy border (G) and potato field (P) at 48, 72, 96 and 120 h after the placement of the source cadavers at the edge of the grassy border. Asterisk sign (*) indicates significant difference in the means between the two species at a time point at $P < 0.05$. 
border and potato field, respectively and of *S. carpocapsae* was 26.15 ± 2.37 and 27.90 ± 2.53, respectively.

The pattern of change in the mean percentage of IJs of both EPN species reaching the maximum distance did not differ significantly over time (*F*₆,₂ = 0.36, *P* = 0.86) (**Fig. 4.3**). However, the mean percentage of *H. bacteriophora* (0.05 ± 0.01) reaching the maximum distance was significantly higher than *S. carpocapsae* (0.006 ± 0.002) at 72h (*F*₁,₇ = 17.49, *P* = 0.02), but significantly lower at 120 h (*H. bacteriophora*: 0.002 ± 1.38E-04; *S. carpocapsae*: 0.003 ± 2.62E-04) (*F*₁,₇ = 53.96, *P* = 0.005) (**Fig. 4.3**). These differences were not due to variability in EPN dispersal among the two habitats (72 h: *F*₂,₇ = 0.03, *P* = 0.96; 120 h: *F*₂,₇ = 0.72, *P* = 0.55).

**Arthropod composition**

A total of 36 families of insects and 6 taxa of other arthropods were recorded at the grassy border and the adjoining potato field (**Table 4.1**). Due to the low numbers of individuals recovered for several families, insects were grouped by order and non-insect orders were grouped by class for analysis. The most abundant arthropods were Collembola, followed by mites (Acari), spiders (Araneae) and Coleoptera. However, the arthropod composition varied between pitfall trap catches and below ground soil samples (**Fig. 4.4**). While Collembola and Araneae were the most abundant arthropods in the pitfall traps, mites and Collembolans were most abundant in the below ground soil samples (**Fig. 4.4**). Principal component analysis showed a distinct separation in
Table 4.1 Composition and abundance of arthropod populations obtained from the pitfall traps, and below ground soil samples extracted using Tullgren funnels from the field location comprising potato field and adjoining grassy border in Shreve, OH.

<table>
<thead>
<tr>
<th>Order</th>
<th>Family</th>
<th>Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collembola (Springtails)</td>
<td>Entomobryidae</td>
<td>1342</td>
</tr>
<tr>
<td></td>
<td>Sminthuridae</td>
<td>36</td>
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<tr>
<td></td>
<td>Isotomitisidae</td>
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<td>Diplura</td>
<td>Campodeidae</td>
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<td>Orthoptera</td>
<td>Gryllidae</td>
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<tr>
<td></td>
<td>Acrididae</td>
<td>8</td>
</tr>
<tr>
<td>Hemiptera</td>
<td>Miridae</td>
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</tr>
<tr>
<td></td>
<td>Pentatomidae</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Cicadellidae</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Sciaridae</td>
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</tr>
<tr>
<td></td>
<td>Aphididae</td>
<td>27</td>
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<tr>
<td></td>
<td>Cercopidae</td>
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<tr>
<td></td>
<td>Enicocephalidae</td>
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</tr>
<tr>
<td></td>
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</tr>
<tr>
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<td>3</td>
</tr>
<tr>
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<td>Elateridae</td>
<td>12</td>
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<tr>
<td></td>
<td>Chrysomelidae</td>
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<tr>
<td></td>
<td>Scaptopsidae</td>
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</tr>
<tr>
<td></td>
<td>Ciidae</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Nitidulidae</td>
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<td>Staphylinidae</td>
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<td>Passalidae</td>
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<td></td>
<td>Mordellidae</td>
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<tr>
<td></td>
<td>Carabidae</td>
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<tr>
<td></td>
<td>Phalacridae</td>
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<tr>
<td></td>
<td>Ptilidae</td>
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<td>Cantharidae</td>
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<td>Diptera</td>
<td>Agromyzidae</td>
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</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>Chironomidae</td>
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<td>Araneae (Spiders)</td>
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<tr>
<td></td>
<td>Diplopoda (Polydesmida)</td>
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<tr>
<td></td>
<td>Isopoda</td>
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</tr>
<tr>
<td></td>
<td>Chilopoda</td>
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<tr>
<td></td>
<td>Myriapoda</td>
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</tr>
<tr>
<td></td>
<td>Arachnida (Mites)</td>
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</tr>
<tr>
<td></td>
<td>Oribatida</td>
<td>582</td>
</tr>
<tr>
<td></td>
<td>Trombidiformes</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4.4. Composition and abundance of arthropod populations obtained from pitfall traps and below ground soil samples at the potato field and adjoining grassy border in Shreve, OH.
arthropod composition collected in the pitfall traps, between species as well as between habitats along the first and second PC axis, which together explained 85.5% of the total variation (Fig. 4.5). Location along the first PC axis was primarily determined by Collembola (EV: 0.99), which was associated with potato field plots where *H. bacteriophora* was released, and Hymenoptera (EV: -0.74) and Oribatida (EV: -0.48), which were associated with grassy border plots where *S. carpocapsae* was released. Araneae (EV: -0.97), Coleoptera (EV: -0.56), Isopoda (EV: -0.52) and Orthoptera (EV: -0.51) determined the location along the second PC axis and were associated with grassy border plots where *H. bacteriophora* was released. In addition, Polydesmida (EV: -0.13) was found associated with potato field plots containing *S. carpocapsae* (Fig. 4.5).

**EPN and arthropod abundance**

Significantly higher numbers of *S. carpocapsae* than *H. bacteriophora* were detected in the pitfall traps at 48 h (*F*₁,⁷ = 66.28, *P* = 0.004) and 96 h (*F*₁,⁷ = 7.54, *P* = 0.05) after the placement of the source cadavers; the mean numbers were 0.75 ± 0.10 and 0.30 ± 0.00, respectively at 48 h and 4.85 ± 0.67 and 2.25 ± 0.57, respectively at 96 h (Fig. 4.6). The two habitats did not differ in EPN abundance at 48 h (*F*₂,⁷ = 5.50, *P* = 0.10) and 96 h (*F*₂,⁷ = 1.26, *P* = 0.40) in the pitfall traps.

The mean arthropod abundance in the pitfall traps was greater in the plots where *H. bacteriophora* was released than the plots where *S. carpocapsae* was released at both, 48h (31.40 ± 8.50 and 23.95 ± 9.65, respectively) and 96 h (13.65 ± 1.56 and 6.75 ± 1.02,
Figure 4.5. Variation in A) arthropod composition between B) habitat, grassy border (solid circles) and potato field (empty squares), C) EPN species, *H. bacteriophora* (empty squares) and *S. carpocapsae* (solid circles) and, D) combination of habitat and EPN species dispersing in that habitat, *H. bacteriophora* and *S. carpocapsae* dispersing in the potato field (empty squares and circles, respectively) and the adjoining grassy border (solid squares and circles, respectively). Percentage of total variability explained by the first two axes is shown on each analysis.
Figure 4.6. Bubble graphs showing mean (± SEM) arthropod abundance and mean number of *H. bacteriophora* and *S. carpocapsae* in the pitfall traps in the potato field and the adjoining grassy border, at 48 h and 96 h after the placement of the source cadavers at the edge of the grassy border. The center of the circle and the error bars indicate mean arthropod abundance; the area of the bubble represents the mean number of respective EPN species.
respectively) (Fig. 4.6). However, this difference was statistically significant only at 96h 
\((F_{1,7} = 30.55, P = 0.01)\) and was not due to the difference in mean arthropod abundance 
between the two habitats \((F_{2,7} = 4.55, P = 0.12)\). Similar results were obtained when the 
two most abundant arthropod taxa, Collembola and Araneae were removed from the 
arthropod abundance data in the pitfall traps. Significantly greater mean arthropod 
abundance was observed in the plots where \(H.\) bacteriophora was released than the 
plots where \(S.\) carpocapsae was released at 96 h \((F_{1,7} = 10.31, P = 0.05)\); the mean 
population densities were 4.05 ± 1.04 and 2.45 ± 0.83, respectively. Habitat did not 
contribute to this difference \((F_{2,7} = 6.34, P = 0.08)\). When the arthropod data collected at 
96 h from pitfall traps and below ground soil samples were combined, no significant 
difference was observed in the mean arthropod abundance between the two habitats \((P > 
0.05)\) and between the plots where either of the two EPN species were released \((P > 
0.05)\). Similar non-significant results in the mean arthropod abundance between the two 
EPN species \((P > 0.05)\) and the two habitats \((P > 0.05)\) were obtained when the most 
abundant taxa were removed from the pitfall trap catches (Collembola and spiders) and 
below ground samples (mites and Collembola) at 96 h.

Regression analysis showed that mean EPN abundance, mean maximum distance 
traveled by the two EPN species and mean percentage of IJs reaching the maximum 
distance did not increase significantly with the increase in the mean arthropod abundance 
in the pitfall traps for either the two EPN species \((P > 0.05)\) or the two habitats \((P > 0.05)\) 
at 48 h and 96 h. Additionally there were no significant differences in slopes of the 
regression lines between the two species \((P > 0.05)\) and between the two habitats \((P >
for any of the three parameters (abundance, distance, percentage of IJs) being tested at both the time points. Similar results were obtained for all three parameters when the two EPN species and the two habitats were compared with mean numbers of each of the ten arthropod taxa found in the pitfall traps at 48 h \((P > 0.05)\) and 96 h \((P > 0.05)\). This change was non-significant between the two species \((P > 0.05)\) and between the two habitats \((P > 0.05)\) at both the time points. However, when the two most abundant taxa, Collembola and Araneae were removed from the pitfall trap arthropod abundance data at 48 h, the maximum distance travelled by \textit{S. carpocapsae} was found to increase significantly as the arthropod abundance increased \((F_{1,3} = 18.70, P = 0.05)\), though not significantly different from \textit{H. bacteriophora} \((F_{1,7} = 1.12, P = 0.35)\). When the mean maximum distance traveled by the two EPN species was compared with the total number of arthropods obtained from the pitfall traps and below ground samples at 96 h, no significant change was observed for either of the two species \((P > 0.05)\) and habitats \((P > 0.05)\) as well as between the two species \((P > 0.05)\) and between the two habitats \((P > 0.05)\). However, when the most abundant taxa were removed from the data (Collembola, Araneae and mites), there was significant increase in the mean maximum distance traveled by \textit{S. carpocapsae} than \textit{H. bacteriophora} with the increase in the arthropod abundance \((F_{1,7} = 7.06, P = 0.05)\) at 96 h. In addition, the mean percentage of \textit{H. bacteriophora} decreased as the arthropod abundance increased \((F_{1,3} = 40.07, P = 0.02)\). Among the excluded taxa, Trombidiformes (mites) was found to have a significant relationship with the percentage of \textit{H. bacteriophora} dispersing to the maximum distance at 96h \((F_{1,3} = 12.47, P = 0.05)\).
Discussion

This study is focused on short-term dispersal of two EPN species with distinct foraging strategies from infected source cadavers over a period of five days. We found both nematode species dispersing the same maximum distance from the source cadavers, though the percentage of IJs dispersing up to the maximum distance differed between the two species. *S. carpocapsae* was found to disperse to the same distance as *H. bacteriophora*, which could be due to active dispersal by sprinters (*Chapter 2*) or passive dispersal (phoresy) by arthropods (Campos-Herrera *et al.*, 2006; Lacey *et al.*, 1995; Parkman *et al.*, 1993; Timper *et al.*, 1988). This study has shown the potential of the two EPN species to disperse the same distances in two different habitats likely utilizing both active and passive dispersal mechanisms.

The ambusher, *S. carpocapsae* dispersed as far as the cruiser, *H. bacteriophora* with similar average displacement in two different habitats over five days. However, significantly lower mean percentage of *S. carpocapsae* (0.006%) than *H. bacteriophora* (0.05%) dispersed to the maximum distance of 2 m at 72 h, thus, supported our contention that a small proportion of *S. carpocapsae* IJs exiting the source cadavers disperse faster and farther than *H. bacteriophora* (*Chapter 2*). Larger percentage of *H. bacteriophora* reaching the maximum distance at 72 h could be due to greater IJ emergence from the source cadavers (*Chapter 2*). At 120 h, although the percentage of both EPN species reaching the similar maximum distance from the source cadavers was low, this percentage was significantly higher (~1.5 times) in case of *S. carpocapsae* than
*H. bacteriophora*. This again shows the ability of *S. carpocapsae* emerging from a population smaller than *H. bacteriophora*, to disperse faster than the fastest *H. bacteriophora* (Chapter 2).

Despite greater arthropod abundance in the plots where *H. bacteriophora* was released, significantly higher number of *S. carpocapsae* than *H. bacteriophora* was detected in the pitfall traps. This indicates passive dispersal of *S. carpocapsae*, which characteristically nictates, attaches to the nearby mobile host or non-host arthropods and eventually gets dispersed phoretically (Lacey et al., 1995; Parkman et al., 1993; Timper et al., 1988). Greater arthropod abundance in the pitfall traps in the plots where *H. bacteriophora* was released could be due to little infectivity of *H. bacteriophora* against the surface active arthropods resulting from the cruising behavior of *H. bacteriophora* and greater movement in deeper soil profiles. However, the regression analysis did not show a significant increase in *S. carpocapsae* abundance with an increase in arthropod abundance in the pitfall traps. Nevertheless, its phoretic transport was most likely found to be provided by certain groups of arthropods comprising Hymenoptera, Diptera, Hemiptera, Coleoptera, Orthoptera, Isopoda, mites (Arachnida) and Polydesmida. This is evident from the significant increase in the maximum distance dispersed by *S. carpocapsae* with the increase in the number of arthropods excluding the most abundant arthropod taxa from pitfall trap (Collembola and Araneae) catches at 48 h and pitfall and below ground soil samples (Collembola, mites and Araneae) at 96 h after placing the source cadavers. This increase in the maximum distance traveled by *S. carpocapsae* with the increase in arthropod abundance was significantly greater than *H. bacteriophora* only.
at 96 h. However, further investigation on the direct association of *S. carpocapsae* with these groups of arthropods is required to confirm arthropod phoresy.

Apart from its cruising ability, *H. bacteriophora* also dispersed phoretically, which is evident from its detection in the pitfall traps along with the surface active arthropods. However, there was no significant relation between arthropod abundance and *H. bacteriophora* abundance in the pitfall traps or maximum distance dispersed at 48 and 96 h. Interestingly the mean percentage of *H. bacteriophora* dispersed to the maximum distance from the source cadavers decreased as the arthropod abundance, excluding the most abundant taxa (Collembola, Araneae and mites), increased. This explained the role of these arthropod taxa in *H. bacteriophora* dispersal, which was found significant in case of Trombidiformes (mites), collected in below ground soil samples at 96 h. Since EPNs have been previously found to be phoretically dispersed by below ground arthropods such as mites (Epsky *et al.*, 1988) and isopods (Kruitbos *et al.*, 2009), I could speculate *H. bacteriophora* phoresy on mites, however, the mechanism requires further investigation.

While several arthropod taxa such as Hymenoptera, Coleoptera, Orthoptera, Arachnida, Araneae and Isopoda were associated with the grassy border, only Collembola and Diplopoda were primarily found in the potato field. Despite these differences in the arthropod composition, the mean arthropod abundance did not vary between the two habitats. This was true even when most abundant arthropod taxa were excluded from the analyses and also when each taxon was analyzed separately. Habitat variability did not contribute to differences between the two EPN species in abundance,
maximum distance dispersed and percentage of IJs reaching the maximum distance at any
time point during the experiment. This is further evident from nearly similar average
displacements (27-28 cm/day) of the two EPN species in the two habitats. This could be
due to optimum soil moisture conditions (~30%) essential for EPN survival and
movement (Lawrence et al., 2006; Millar and Barbercheck, 2002), resulting from
sufficient amount of ground cover in both habitats; wild grass and weeds in the grassy
border and mulch in the potato field.

In conclusion, this study quantified the short-term dispersal potential of two EPN
species, *H. bacteriophora* and *S. carpocapsae* with contrasting foraging strategies,
released as nematode infected host cadavers in the grassy strips to the adjacent cultivated
fields. Both species showed equivalent potential to disperse to distances up to 2 m,
actively or passively in both the habitats despite no differences in the arthropod
abundance in the two habitats. Such similar dispersal potential of the two EPN species at
larger spatial scale was proposed in my recent study, where the two species dispersed in
soil up to 60 cm with similar average displacement (*Chapter 2*). Greater EPN dispersal in
the field than in the laboratory is evident from much higher average displacement of the
two species in the field (~28 cm/day) than in my previous study in the laboratory (~6
cm/day). Since there was no rainfall during the course of the experiment, there was little
possibility for soil likely containing EPNs to get displaced in to the pitfall traps.
Therefore, such higher average displacement in the field could be most likely due to
phoretic transport of EPNs by host and/or non-host arthropods as opposed to the
laboratory conditions where EPN dispersal was studied in the absence of hosts (*Chapter*
2) or in the presence of only one host species (Chapter 3). While the cruiser, *H. bacteriophora* was found associated with mites, the ambusher, *S. carpocapsae* was detected in larger numbers in the pitfall traps likely taking a ride on diverse arthropods entering the traps.

Therefore, this study showing equivalent dispersal of two distinct EPN species in the potato field over a short period could be further tested in other cultivated field crops at different spatial and temporal scales. Given the short time frame in the current study, there was little scope for EPN reproduction in the insect hosts. However, recycling of EPNs would be a continuous process at a larger time frame resulting in significant amount of dispersal at greater spatial scales. This could be utilized in an effective biological control program by designing appropriate application methods for nematode infected cadavers to facilitate uniform distribution of otherwise patchily distributed EPN populations (Stuart and Gaugler, 1994, Campbell *et al*., 1995, 1996, 1998). Appropriate conservation approaches could improve the survival and persistence of endemic EPN populations that frequently occur in the grassy border strips (Alumai *et al*., 2006; Lawrence *et al*., 2006) and subsequently enhance their dispersal to the adjoining cultivated field crops for the provision of biological control service.

**Acknowledgements**

I thank Mr Marcelo Altamirano Goyzueta for assistance in field work. I thank Dr Casey Hoy and Dr Zhiqiang Cheng for statistical guidance. I also thank Miss Claudia Kuniyoshi
for her help in running statistical analyses in SAS. I thank Miss Nuris Acosta for helping in insect identifications.


Chapter 5: Artificial selection of the ambush foraging entomopathogenic nematode, 

*Steinernema carpocapsae* for dispersal

Abstract

The ambush foraging entomopathogenic nematode, *Steinernema carpocapsae* ALL strain was genetically selected for enhanced dispersal in the absence of hosts. Selection was performed by capturing the farthest reaching population of nematode infective juveniles (IJs) emanating from a nematode-infected source *Galleria mellonella* cadaver in microcosms (0.05 m²) containing autoclaved field soil. The farthest reaching IJs were captured by taking soil core samples at 11.4 cm from the source cadaver between 48-84 h at 12 h intervals and baiting with *G. mellonella* larvae. The infected baits were placed in White traps to obtain next generation IJs. The procedure was repeated for a total of 10 rounds of selection. The foundation population was simultaneously subjected to 10 passages through *G. mellonella* larvae in Petri dishes. The rate of emergence of the foundation population and the selected lines was estimated to calculate the percentage of IJs dispersing different distances from the source cadavers over time. The effect of selection on dispersal was evaluated by comparing the dispersal rates and patterns, sex ratios and nictation abilities of the selected lines with the foundation population, after five
and ten rounds of selection. *S. carpocapsae* showed positive response to selection for dispersal. There was 13-23 fold and 8-14 fold increase in the mean percentage of IJs dispersed to the farthest distance from the source cadaver, after five and ten rounds of selection, respectively. The average displacement of the selected lines varied from 6.85 to 7.54 cm/day and was significantly greater than the foundation population (5.54 cm/day). The overall mean realized heritability for mobility was 0.60. *S. carpocapsae* reproduction and nictation ability showed a negative response to selection for dispersal. Significantly higher proportion (~40 times) of a selected sub-line with least reproduction potential, 1/20th of the foundation population, dispersed to the farthest distance. The percentage of nictating IJs was significantly lower in the selected lines (~11%) than the foundation population (~90%). The sprinters of the selected lines comprised significantly more males (72%) than the foundation population (44%) at most time points. There was no significant change in the mean number of *G. mellonella* baits killed in the microcosms, mean percentage of dispersed IJs and males between five and ten rounds of selection. Enhancement in dispersal along with high realized heritability (> 0.5) in the selected lines suggested that artificial selection could be an effective approach for enhancing *S. carpocapsae* dispersal despite trade-offs.

**Introduction**

Dispersal is an important trait for any organism with implications for individual fitness, population dynamics and species distributions (Bowler and Benton, 2005). In
case of entomopathogenic nematodes (EPNs), dispersal plays a significant role in their efficacy against target insect pests in the field. Therefore, improvement of the dispersal ability of EPNs through selection would prove beneficial for enhancing their biological control potential. Dispersal has been artificially selected in two-spotted spider mite, *Tetranychus urticae* with variable results (Yano and Takafuji, 2002; Tien et al., 2011; Bitume et al., 2011). Although selection has been reported to successfully improve the host-finding ability of EPN, *Steinernema carpocapsae* in the presence of host cues (Gaugler et al., 1989; Gaugler and Campbell, 1991; Gaugler et al., 1990, 1991), little efforts were made to improve its dispersal ability in the absence of hosts.

Although *S. carpocapsae* is characterized as an ambush forager, it has been recently found to possess a small proportion of ‘sprinters’ that disperse faster and farther than even the cruise forager, *H. bacteriophora* (*Chapter 2*). This small population of sprinters gets dispersed much faster while the rest of the population remains near the source host cadaver in the absence of hosts. Given such distinct phenotypic dichotomy in the dispersal ability of the two populations, the present study was designed to explore the effect of artificial selection on dispersal ability of *S. carpocapsae* in sterilized soil in a host-free environment. It has been previously demonstrated that the *S. carpocapsae* IJs that are destined to become males tend to disperse farther on agar plates and are likely to find and invade hosts in sand columns before those that are destined to become females (Grewal et al., 1993). I hypothesized that *S. carpocapsae* will respond to artificial selection by increasing both the proportion of sprinters and the speed with which they disperse. I also hypothesized that there will be trade-offs in the selected population
showing reduced nictation ability, a trait associated with ambush foraging, male biased sex ratio, and reduced reproduction capacity.

**Materials and Methods**

*Source of nematodes*

New cultures of *Steinernema carpocapsae* ALL strain, obtained from the nematode cultures stored in liquid nitrogen in our laboratory, were raised by infecting final instar wax moth larvae, *Galleria mellonella* (L.), purchased from Vanderhoest Canning Company, St. Mary’s, Ohio, following methods described by Kaya and Stock (1997). These new cultures served as the ‘foundation population’, designated as ‘F’ hereafter. Nematode infected host cadavers were prepared by exposing each last instar *G. mellonella* to approximately 400 freshly produced IJs of *S. carpocapsae* in a 5 cm diameter Petri dish containing a filter paper, at room temperature (22°C). These cadavers were used as a source of nematodes mimicking the natural emergence of nematodes as opposed to using aqueous suspension. White traps containing the cadavers were inspected once daily to check for the initiation of IJ emergence. The cadavers that had just begun to release the IJs were used in all experiments. This was done to minimize variation due to differences in initiation of IJ emergence among replications.
**Experimental microcosms**

Experiments were conducted in microcosms (22.86 cm x 22.86 cm; 8 cm deep) (*Chapter 2*). Each microcosm was filled with autoclaved top soil to a depth of 5 cm and kept at room temperature (~22°C). Wooster silt loam topsoil (26.2% clay, 2.6% sand and 61.8% silt) collected from a corn field at The Ohio State University, Wooster, Ohio, USA was autoclaved at 121°C and 103.42 kPa pressure for 10 h and stored at room temperature for at least 7 days before use to allow any toxic volatiles to escape. Soil moisture level was adjusted to field capacity of this soil (i.e. 24% w/w; \(-10^6\) kPa) by adding tap water, in order to facilitate the movement of IJs. The microcosms were covered with black plastic sheets throughout the experiment to minimize moisture loss from the soil and to avoid exposure to light. Single, 10-day old nematode infected *G. mellonella* cadaver that had just begun to release the IJs was placed 2.5 cm below the soil surface in the center of each microcosm to serve as the source of IJs.

*Selection procedure*

Selection was performed by capturing the farthest reaching population of *S. carpocapsae* infective juveniles (IJs) emanating from a nematode-infected source *G. mellonella* cadaver in microcosms (0.05 m²) containing autoclaved field soil. The farthest reaching IJs were captured by taking four soil core samples at 90° angle at 11.4 cm away from the source cadaver between 48-84 h at 12 h intervals using a 2 cm diameter soil
corer and baiting with *G. mellonella* larvae. Five replications were run in 5 separate microcosms. All infected larvae obtained from all 5 microcosms were pooled and transferred to a White trap. IJs emerging from the infected larvae in the White trap within 10 days of first emergence were used for further infection to prepare the source cadavers as discussed earlier for additional rounds of selection. Five rounds of selection were carried out as described above and the selected sprinting population was designated as ‘S1’. Another sprinting population of *F* was similarly subjected to five rounds of selection as described above and designated as ‘S2’. Five sub-lines, *S1 BA-E* and *S2 BA-E* were then created from the *S1* and *S2* lines, respectively after the first five rounds of selection. These ten sub-lines were further subjected to five rounds of selection, resulting in a total of ten rounds of selection, equivalent to 20-30 nematode generations (Poinar, 1979). On the other hand, the foundation population was passed ten times through five last instar *G. mellonella* larvae separately in Petri dishes lined with moist filter paper. The infected larvae were transferred to individual White traps to produce inoculum for the next passage.

*Reproduction potential*

The differences between the selected lines (*S1, S2*), sub-lines (*S1 BA-E, S2 BA-E*) and the foundation population *F* in total number of IJs produced per cadaver were determined by preparing individual cadavers (4 per line) as described above and counting the number of emerging nematodes in White traps daily for 10 days as described by Grewal *et al.*
(1994b). The experiment was performed twice resulting in $N = 8$ for each line. The temporal and cumulative emergence data were used in estimating the proportion of the population dispersing in microcosms in all subsequent experiments.

*Dispersal patterns*

The average lateral displacement and spatio-temporal dispersal patterns of selected lines, $S1$, $S2$ and sub-lines, $S1_{A-E}$, $S2_{A-E}$ was compared with the foundation population $F$ to examine their response to selection. Nematode dispersal was studied in 22.86 cm x 22.86 cm microcosms by collecting soil core samples at distances of 3.8, 7.6 and 11.4 cm away from the source cadaver at 12, 24, 48, 72, 96 and 120 h after placing the cadaver in the center of the microcosm containing sterilized field soil. Each soil core sample was placed in a plastic cup (30 ml) and baited with one uninfected *G. mellonella* larva which was inspected for nematode infection and dissected on the third day after baiting to count the number of penetrated IJs. Five microcosms were used for each line. The entire experiment was repeated once, resulting in $N = 10$ for each line at each distance and time point.

*Sex ratio*

Sex of IJs of all the selected lines ($S1$, $S2$, $S1_{A-E}$, $S2_{A-E}$) and the foundation population $F$, reaching the farthest distance in the soil in the microcosms was determined.
by collecting four soil core samples at 90° angle at 11.4 cm from the source cadaver between 60-120 h at 12 h intervals as described earlier. The bait insects placed in the soil core samples were dissected on the third day after baiting to allow the IJs to become adults, and the numbers of males and females were counted. Each line was replicated five times using five microcosms. The experiment was repeated once with same sampling distance and time intervals (i.e. N = 10 for each line at a time point).

*Nictation behavior*

The effect of selection on nictation was determined by quantifying the proportion of IJs of lines, $S_{1A,E}$, $S_{2A,E}$ and $F$ nictating on agar plates covered with sand grains as described by Campbell and Gaugler (1993). Three circles (1 cm diameter) were marked on the bottom of each 9 cm diameter Petri dish containing 2% water agar and 0.14 g of sand grains (< 0.1 µm diameter) sprinkled uniformly. One hundred IJs of each line were released in the center of each Petri dish with a fine probe and allowed to settle for 30 min, after which the number of nictating IJs were counted in all three circles thrice at one hour intervals to obtain the proportion of nictating IJs in each dish. There were three replications for each line and the entire experiment was repeated once (i.e. N = 6 for each line).
The effect of selection on the rate of movement and average displacement of *S. carpocapsae* was determined by analyzing both the number of *G. mellonella* bait larvae killed and the number of IJs recovered from the baits in the microcosms. Mean percentage of IJs of each line dispersing at a time point up to a particular distance in a 2 cm wide annulus in the microcosms was calculated by equation 1 (see Chapter 2). The data of selected and unselected lines on total IJ emergence from 10-day old nematode infected *G. mellonella* cadavers in the White traps, mean number of killed baits, mean proportion of IJs reaching different distances, average displacements (cm/day), and mean proportion of males and nictating IJs were analyzed using analysis of variance (ANOVA), after applying appropriate transformations when needed to achieve normality. The means were separated using Tukey’s test to make comparisons after five rounds of selection. The comparisons were made between each selected line (*SI_A, SI_B, SI_C, SI_D, SI_E, S2_A, S2_B, S2_C, S2_D* and *S2_E*) and the foundation population (*F*) using Dunnett’s *t*-test after ten rounds of selection. Data on total IJ emergence and mean number of killed baits were square root transformed. Data on mean proportion of IJs, males and nictating IJs were arcsine transformed. Contrast analysis was done to make comparisons between the sub-lines as a group and the foundation population. Alpha, the maximum probability of a type I error, was set at 0.05 for all tests. All analyses were performed using SAS Release 9.2 (SAS Institute Inc., 2008). Realized heritability based on the mean number of IJs recovered from the infected *G. mellonella* baits was calculated to account for genetic
variation in the selected lines after five and ten rounds of selection using the formula, $h^2 = R/S$, where $R$ is the response to selection and $S$ is the selection differential calculated as described below (Falconer, 1981).

$R = (\text{Mean phenotype of the offspring of selected lines} - \text{Mean phenotype of the foundation population})$

$S = (\text{Mean phenotype of the selected parents} - \text{Mean phenotype of the foundation population})$

All the repeated experiments yielded same results. For example, the average (± SEM) displacement (cm/day) of the selected lines, $S1$ and $S2$ was significantly higher than the foundation population, $F$ (Experiment 1: $F_{2,269} = 12.45, P < 0.0001$; Experiment 1: $F_{2,269} = 14.12, P < 0.001$) for both experiments. Therefore, the data from all the repeated experiments were pooled for all further analyses.

**Results**

*Reproduction potential*

The total reproduction potential of selected lines declined significantly after five ($F_{2,23} = 5.83, P = 0.02$) and ten ($F_{10,87} = 5.02, P = 0.001$) rounds of selection (Fig. 5.1). After five rounds of selection, the selected line, $S1$ produced significantly lower mean number of IJs per *G. mellonella* cadaver than the foundation population, $F$ over a period of 10 days; however the two selected lines did not differ significantly (Fig. 5.1A).
Figure 5.1. Total reproduction potential of infective juveniles (IJs) of foundation population (F) of *Steinernema carpocapsae* ALL and its selected lines, A) S1 and S2 after five rounds of selection and B) S1_A, S1_B, S1_C, S1_D, S1_E, S2_A, S2_B, S2_C, S2_D, S2_E after ten rounds of selection over a period of 240 h. Asterisk sign indicate significant difference between each selected line or sub-line and the foundation population using Dunnett’s t test at $P < 0.05$. Different letters indicate separation of means of the two groups of selected lines or sub-lines and the foundation population by contrast analysis.
Additionally, contrast analysis showed significantly lower reproduction potential of the selected lines, taken together than the foundation population \((F_{1,23} = 3.34, P = 0.007)\).

After ten rounds of selection, the mean number of IJs emerging from selected lines, \(S1_{C,D,E}\) and \(S2_A\) was significantly lower than the foundation population, \(F\) over a period of 10 days (Fig. 5.1B). Contrast analysis did not show significant difference between the two groups of selected sub-lines \(S1_{A-E}\) and \(S2_{A-E}\) \((F_{1,79} = 0.25, P = 0.80\) (Fig. 5.1B).

Overall, the reproduction potential of the foundation population was \(~9\) times and \(~6\) times greater than the selected lines after five and ten rounds of selection, respectively. However, additional five rounds of selection produced no further change in the reproduction potential, which is evident from no differences between \(S1\) and \(S1_{A-E}\) \((P > 0.05)\), and \(S2\) and \(S2_{A-E}\) \((P > 0.05)\).

**Dispersal patterns**

After five rounds of selection, the mean numbers of infected \(G.\ mellonella\) baits and the mean percentages of IJs of the selected lines, \(S1\) \((\sim 13\) times) and \(S2\) \((\sim 23\) times) were significantly higher than the foundation population, \(F\) at the farthest distance of 11.4 cm; the two selected lines did not differ significantly from each other (Table 5.1).

However, the selected lines did not differ from the foundation population in the mean number of infected \(G.\ mellonella\) baits and mean percentage of IJs reaching the closest distance (3.8 cm) from the source cadaver in the microcosms (Table. 5.1). Additionally, the average (± SEM) displacement (cm/day) of the selected lines, \(S1\) \((6.85 ± 0.39)\) and \(S2\)
Table 5.1 Analysis of variance table showing mean (± SEM) number of dead *Galleria mellonella* baits from the collected soil core samples and mean (± SEM) percentage of infective juveniles (IJ$s$) of foundation population (*F*) and selected lines (*S1*, *S2*) of *Steinernema carpocapsae* ALL dispersed to a 2 cm wide annulus over a period of 12 to 120 h at the closest, 3.8 cm and farthest, 11.4 cm distances from the source cadaver in the microcosms containing autoclaved field soil. Data from the two repeated experiments were pooled for each line giving a sample size of *N* = 180, after five rounds of selection. A *P* < 0.05 was considered indicative of a significant difference for the analysis of variance comparing the selected and unselected lines at different distances after five rounds of selection, in horizontal direction. Different letters indicate the separation of means by Tukey’s test.

<table>
<thead>
<tr>
<th>Line from cadaver (cm)</th>
<th>Dead <em>Galleria</em> (Mean ± SEM)</th>
<th>DF (n, d)</th>
<th><em>f</em></th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>3.8 cm</td>
<td>0.70 ± 0.07a</td>
<td>2, 179</td>
<td>1.50</td>
<td>0.23</td>
</tr>
<tr>
<td>11.4 cm</td>
<td>0.10 ± 0.05b</td>
<td>2, 179</td>
<td>37.49</td>
<td>&lt;0.000</td>
</tr>
</tbody>
</table>

Percentage of infective juveniles (Mean ± SEM)

<table>
<thead>
<tr>
<th>Line from cadaver (cm)</th>
<th>Percentage of infective juveniles (Mean ± SEM)</th>
<th>DF (n, d)</th>
<th><em>f</em></th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>3.8 cm</td>
<td>3.73 ± 1.12a</td>
<td>2, 179</td>
<td>1.89</td>
<td>0.16</td>
</tr>
<tr>
<td>11.4 cm</td>
<td>0.34 ± 0.09b</td>
<td>2, 179</td>
<td>5.27</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Figure 5.2. Mean (± SEM) number of dead *Galleria mellonella* baits infected by the infective juveniles (IJs) of foundation population (*F*) and selected sub-lines (*S1*A, *S1*B, *S1*C, *S1*D, *S1*E, *S2*A, *S2*B, *S2*C, *S2*D, *S2*E) of *Steinernema carpocapsae* ALL dispersed to a 2 cm wide annulus over a period of 12 to 120 h at the A) closest, 3.8 cm and B) farthest, 11.4 cm distances from the source cadaver in the microcosms containing autoclaved field soil. Data from the two repeated experiments were pooled for each sub-line giving a sample size of \( N = 660 \) after ten rounds of selection. Asterisk sign indicate significant difference between each selected line and the foundation population using Dunnett’s *t* test at \( P < 0.05 \) for the analysis of variance. Different letters indicate separation of means of the two groups of selected sub-lines and the foundation population by contrast analysis.
(7.46 ± 0.54) was significantly higher than the foundation population (5.54 ± 0.17) \((F_{2,539} = 13.39, \ P < 0.0001)\).

After ten rounds of selection, significantly greater number of \textit{G. mellonella} baits were infected by sub-lines, \(S2_{A,B,D}\) and \(S2_{A,D,E}\) than the foundation population at the closest distance, 3.8 cm \((F_{10,559} = 2.14, \ P = 0.03)\) and at farthest distance, 11.4 cm \((F_{10,559} = 2.81, \ P = 0.05)\) from the source cadaver (Fig. 5.2). Based on contrast analysis, no significant difference was found in the mean number of infected \textit{G. mellonella} baits among the selected sub-lines, \(SI_{A-E}\) and \(S2_{A-E}\) \((P > 0.05)\) and between sub-lines and foundation population \((P > 0.05)\), at the closest distance of 3.8 cm (Fig. 5.2A). However, at the farthest distance, significantly higher numbers of \textit{G. mellonella} baits were infected by \(S2_{A-E}\) than the foundation population \((F_{1,559} = 7.69, \ P = 0.006)\); there was no significant difference among the selected sub-lines, \(SI_{A-E}\) and \(S2_{A-E}\) \((P > 0.05)\) and between \(SI_{A-E}\) and the foundation population \((P > 0.05)\) (Fig. 5.2B).

Furthermore, significantly higher mean percentage of \(S2_{A}\) than \(F\) dispersed to the closest, 3.8 cm \((F_{10,559} = 8.19, \ P = 0.008)\) and farthest, 11.4 cm \((F_{1,559} = 2.11, \ P = 0.03)\) distance (Fig. 5.3). While, significantly greater percentage (~3 times) of \(S2_{A-E}\) (6.46 ± 1.57) than \(SI_{A-E}\) (2.00 ± 0.41) dispersed to the closest distance \((F_{1,559} = 11.27, \ P = 0.03)\) (Fig. 5.3A), there was no significant difference between the two groups of selected sub-lines at the farthest distance \((P > 0.05)\) (Fig. 5.3B). However, greater percentage of \(SI_{A-E}\) (~ 8 times) and \(S2_{A-E}\) (~ 14.5 times) than \(F\) dispersed at the farthest distance; \(S2_{A-E}\) differed significantly from \(F\) \((F_{1,559} = 4.08, \ P = 0.04)\). The average \((± SEM)\) displacement (cm/day) of the selected lines, \(S2_{A}\) (7.95 ± 0.32) and \(S2_{D}\) (7.70 ± 0.31) was significantly
Figure 5.3. Mean (± SEM) percentage of infective juveniles (IJ$s$) of foundation population ($F$) and selected sub-lines ($S1_A$, $S1_B$, $S1_C$, $S1_D$, $S1_E$, $S2_A$, $S2_B$, $S2_C$, $S2_D$, $S2_E$) of *Steinernema carpocapsae* ALL dispersed to a 2 cm wide annulus over a period of 12 to 120 h at the A) closest, 3.8 cm and B) farthest, 11.4 cm distances from the source cadaver in the microcosms containing autoclaved field soil. Data from the two repeated experiments were pooled for each line giving a sample size of $N = 660$ after ten rounds of selection. Asterisk sign indicate significant difference between each selected sub-line and the foundation population using Dunnett’s $t$ test at $P < 0.05$ for the analysis of variance. Different letters indicate separation of means of the two groups of selected sub-lines and the foundation population by contrast analysis.
higher than the foundation population, $F (5.54 \pm 0.17)$ ($F_{10,1979} = 2.07, \ P = 0.03$). The average displacement of $SI_{A-E} (7.11 \pm 0.07)$ did not differ from $S2_{A-E} (7.54 \pm 0.13)$ ($P > 0.05$). The two groups of selected lines, $SI_{A-E}$ and $S2_{A-E}$ also did not differ from $F$, though their average displacement was numerically higher than $F$ ($P > 0.05$).

There was no significant difference between $SI$ and $SI_{A-E}$ ($P > 0.05$), and $S2$ and $S2_{A-E}$ ($P > 0.05$), in the mean number of infected $G. mellonella$ baits, mean percentage of IJs dispersed to distances, 3.8 and 11.4 cm and average displacement, indicating no change in dispersal from five to ten rounds of selection.

The realized heritability for dispersal was 0.65 for $SI$ and 0.51 for $S2$, after five rounds of selection. It was 0.65 for $SI_{A-E}$ and 0.55 for $S2_{A-E}$ after ten rounds of selection. The overall mean realized heritability for dispersal was 0.60.

**Sex ratio**

After five rounds of selection, the foundation population and the selected lines did not differ in the mean proportion of males in the population reaching the farthest distance of 11.4 cm from the source cadaver, over time ($F_{10,46} = 1.90, \ P = 0.13$) (Table 5.2). However, at all time points between 60-120h greater proportion of males were found in the population dispersed to the farthest distance in case of both the selected lines compared with the foundation population with significantly greater proportion males in $SI$ than $S2$ and $F$ only at the earliest time point, 60h (Table 5.2).
**Table 5.2** Analysis of variance table showing mean (± SEM) proportion of males of foundation population \((F)\) and selected lines \((S1, S2)\) of *Steinernema carpocapsae* ALL dispersed to a 2 cm wide annulus at the farthest distance of 11.4 cm from the source cadaver over a period of 60 to 120 h in the microcosms containing autoclaved field soil. Data from the two repeated experiments were pooled for each line giving a sample size of \(N = 30\), after five rounds of selection. A \(P < 0.05\) was considered indicative of a significant difference for the analysis of variance comparing the selected and unselected lines at different time points after five rounds of selection, in horizontal direction. Different letters indicate the separation of means by Tukey’s test.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>(F)</th>
<th>(S1)</th>
<th>(S2)</th>
<th>Analysis of variance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DF (n,d)</td>
<td>(f)</td>
<td>(P)</td>
</tr>
<tr>
<td>60</td>
<td>0.36 ± 0.07b</td>
<td>2,29</td>
<td>9.39</td>
<td>0.004</td>
</tr>
<tr>
<td>72</td>
<td>0.67 ± 0.08a</td>
<td>2,29</td>
<td>2.51</td>
<td>0.11</td>
</tr>
<tr>
<td>84</td>
<td>0.41 ± 0.08a</td>
<td>2,29</td>
<td>2.06</td>
<td>0.16</td>
</tr>
<tr>
<td>96</td>
<td>0.30 ± 0.09a</td>
<td>2,29</td>
<td>3.37</td>
<td>0.06</td>
</tr>
<tr>
<td>108</td>
<td>0.38 ± 0.11a</td>
<td>2,29</td>
<td>0.23</td>
<td>0.80</td>
</tr>
<tr>
<td>120</td>
<td>0.52 ± 0.16a</td>
<td>2,29</td>
<td>1.18</td>
<td>0.32</td>
</tr>
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</table>
Table 5.3 Analysis of variance table showing mean (± SEM) proportion of males of foundation population (F) and selected sub-lines (S1_A, S1_B, S1_C, S1_D, S1_E, S2_A, S2_B, S2_C, S2_D, S2_E) of *Steinernema carpocapsae* ALL dispersed to a 2 cm wide annulus at the farthest distance of 11.4 cm from the source cadaver over a period of 60 to 120 h in the microcosms containing autoclaved field soil. Data from the two repeated experiments were pooled for each line giving a sample size of N = 110, after ten rounds of selection. Asterisk sign indicate significant difference between each selected sub-line and the foundation population using Dunnett’s *t* test at *P* < 0.05 for the analysis of variance at different time points after ten rounds of selection, in horizontal direction.

<table>
<thead>
<tr>
<th>Line</th>
<th>F</th>
<th>S1_A</th>
<th>S1_B</th>
<th>S1_C</th>
<th>S1_D</th>
<th>S1_E</th>
<th>S2_A</th>
<th>S2_B</th>
<th>S2_C</th>
<th>S2_D</th>
<th>S2_E</th>
<th>Analysis of variance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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After ten rounds of selection, the pattern of change in the mean proportion of males in the population reaching the farthest distance, 11.4 cm differed significantly over time for different lines \((F_{50,409} = 2.09, P = 0.002)\) (Table 5.3). The selected sub-lines contained greater proportion of males than the foundation population reaching the farthest distance at most time points. However, the proportion of males was significantly higher in case of \(S1_{B,E}\) at 60 h, \(S1_B\) and \(S2_C\) at 72 h, \(S1_D\) at 84 h, \(S1_B\) at 96 h and \(S1_{C,D}\) at 108 h compared with \(F\) (Table 5.3). There was no significant difference in the mean proportion of males between the two groups of selected sub-lines \((P > 0.05)\) and between either of the two groups and the foundation population \((P > 0.05)\) at any time point. The mean proportion of males of \(S1\) and \(S2\) did not differ from \(S1_{A-E}\) and \(S2_{A-E}\), respectively \((P > 0.05)\).

**Nictation behavior**

The mean proportion of nictating IJs of the selected lines, \(S1\) and \(S2\) was significantly lower than the foundation population, \(F\) after five rounds of selection \((F_{2,17} = 17.21, P < 0.0001)\) (Fig. 5.4A). Also all selected sub-lines, \(S1_{A-E}\) and \(S2_{A-E}\) showed significant reduction in the mean proportion of nictating IJs than the foundation population, \(F\) after ten rounds of selection \((F_{10,65} = 32.19, P < 0.0001)\) (Fig. 5.4B). Contrast analysis showed significantly lower proportion of \(S1_{A-E}\) \((F_{1,65} = 230.58, P < 0.0001)\) and \(S2_{A-E}\) \((F_{1,65} = 228.09, P < 0.0001)\) IJs nictating than \(F\). However, there was no significant difference between the two groups of selected sub-lines \((P > 0.05)\). There
Figure 5.4. Mean (± SEM) proportion of infective juveniles (IJs) of foundation population (F) and selected A) lines (S1, S2) and B) sub-lines (S1A, S1B, S1C, S1D, S1E, S2A, S2B, S2C, S2D, S2E) of Steinernema carpocapsae ALL nictating after five and ten rounds of selection, respectively. This mean proportion was computed out of 100 IJs of the respective lines, released in the center of the separate agar (2%) plates covered with 0.14 g of sand grains (< 0.1 µm dia) sprinkled uniformly. Data from the two repeated experiments were pooled for each line giving a sample size of N = 18 and N = 66, after five and ten rounds of selection, respectively. In Fig. A (in a box), different letters indicate separation of means by Tukey's test at $P < 0.05$, which was considered indicative of a significant difference for the analysis of variance comparing the unselected and selected lines. In Fig. B, asterisk sign indicate significant difference between each selected sub-line and the foundation population using Dunnett’s $t$ test at $P < 0.05$. Different letters indicate separation of means of the two groups of selected sub-lines and the foundation population by contrast analysis.
was significant difference in the mean proportion of nictating IJs between $S1$ and $S1_{A-E}$ ($P > 0.05$), and $S2$ and $S2_{A-E}$ ($P > 0.05$).

**Discussion**

Results of this study showed that dispersal ability of an ambush foraging nematode, *S. carpocapsae* can be substantially enhanced through artificial selection of sprinters. Unlike previous studies using phenotypic variability in different strains or populations of EPN species to improve a trait (Tomalak, 1994; Selvan *et al*., 1994; Glazer *et al*., 1996; Stuart *et al*., 2004; Salame *et al*., 2010), I selected upon the phenotypic variability in dispersal existing within a population of a single strain of *S. carpocapsae*. At the closest distance from the source cadaver, the selected lines did not differ significantly from the foundation population in the mean number of *G. mellonella* baits infected and mean percentage of IJs, after either five or ten rounds of selection. However, at the farthest distance in the microcosms, the mean number of *G. mellonella* baits infected and mean percentage of IJs of the selected lines, dispersed significantly higher than the foundation population. This increase in the percentage of sprinters was 13-23 fold and 8-14 fold from the foundation population, after five and ten rounds of selection, respectively. In addition, the average displacements of the selected lines were greater than the foundation population. Although the reproduction potential of the selected sub-line, $S2_A$ was 1/20th of the foundation population, its dispersal potential was much higher than $F$ with greater percentage of IJs reaching the closest ($\sim 3$ times) and
farthest (~ 40 times) distance from the source cadaver. Furthermore, high realized heritability value in the selected lines (> 0.5) suggests that selection could be an effective approach for enhancing *S. carpocapsae* dispersal, despite some trade-offs such as reduced reproduction potential.

The reproduction potential of the selected lines reduced significantly after five and ten rounds of selection. The selected lines produced $1/9^{th}$ and $1/6^{th}$ of the number of IJs emitted by the foundation population over 10-day period, after five and ten rounds of selection, respectively. Such alteration in other traits while selecting for host finding has been reported in *S. carpocapsae* (Gaugler et al., 1990). Phenotypic correlation between different traits could occur by chance, or as a result of physical linkage of their genes, or genetic correlation between the traits, or inadvertent selection (Gaugler et al., 1990).

The sprinters of the selected lines exhibiting greater dispersal potential than the ones in the foundation population comprised more males. While, the proportion of males was < 0.5 in the foundation population at most time points, it was > 0.5 in the selected lines at all time points after five and ten rounds of selection. This is indicative of males reaching out first in search for food or mates, thus supporting the male colonization hypothesis proposed by Grewal et al (1993). I could speculate that after most males left the immediate vicinity of the source cadaver, females would start dispersing. Such kind of dispersal related with asymmetrical sex ratio at spatial scales occurs due to the differences in the number of available mates (Bowler and Benton, 2005). This could be one of the reasons of positive response to selection for dispersal. However, the lack of continuous response to selection for dispersal is evident from no significant change in the
mean number of dead *G. mellonella* baits, mean percentage of dispersed IJs and males from five to ten rounds of selection. These results correspond with a study by Gaugler *et al.* (1989), where host-finding of *S. carpocapsae* increased significantly during the first eight rounds of selection and showed only a marginal improvement thereafter.

Although the degree of nictation reduced considerably in selected lines as expected, it was not eliminated completely. This indicated that the selected lines with improved dispersal did retain the ambushing behavior. Sprinting alone could be a risky life history for *S. carpocapsae* for two reasons. One, the sprinters may not be able to find a host and eventually die. Second, they may not find a mate at greater distances from the source resulting in poor reproduction. Therefore, having ambushing behavior along with sprinting ability in the selected populations would prove beneficial for *S. carpocapsae* survival and reproduction. The sprinters would disperse away from the source in search of hosts when few or no hosts would be available in the immediate vicinity of the source. When sufficient number of hosts would be available around the source, *S. carpocapsae* would perhaps stick to its ambushing behavior and exhibit nictation to infect the hosts and reproduce successfully.

In conclusion, *S. carpocapsae* responded positively to selection for dispersal with enhanced dispersal rate, altered sex ratio and reduced nictation and fecundity. This is the first step to select an ambusher for dispersal which is the pre-requisite for successful host finding and invasion. It would have important implications on using this otherwise ambushing EPN species as a successful inundative biocontrol agent. However, the field efficacy of the selected populations needs to be tested. The selected populations with
improved dispersal could reach sedentary host insect-pests and also enhance connectivity between patchily distributed populations. This would not only distribute the nematodes uniformly in the field, thus providing sufficient biocontrol but could also enhance gene flow between different local populations, thus affecting the metapopulation dynamics (McCauley et al. 1995; Harrison and Taylor, 1997; Hanski, 1999). Moreover, selection could be a continuous phenomenon in the natural field conditions, where the sprinters may encounter hosts at larger distances and spread the sprinting alleles to future generations. Therefore, further work on *S. carpocapsae* dispersal is essential, since dispersal is related to many fundamental aspects of ecology and evolution, such as persistence of metapopulations and evolution of life history traits (Clobert et al., 2001; Ronce, 2007). This kind of work would be a future step where genes related to greater dispersal of the sprinters could be annotated to find the genetic basis of this trait. Further investigation will have to assess trait stability and possible trade-off effects. Cross breeding of the selected sprinters could be another step to attain improved strain with enhanced mobility. An understanding of the spatial variation in both population density and genetic composition are essential for population monitoring and estimation, and will be constructive in population conservation and management.
Acknowledgements

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Chapter 6: Summary and suggestions for future work

My thesis is that neither the rate nor the pattern of dispersal will differ between the ambushing and cruising EPN species in soil in the microcosms or in the field, at a large spatial scale (in meters). My dissertation research has revealed the unexpected ability of ambush foraging EPN *S. carpocapsae* to disperse faster and farther than cruiser *H. bacteriophora* in soil in the microcosms as well as in the field. *S. carpocapsae* appeared to exhibit a distinct dispersal strategy at larger spatial scales, whereby a small proportion of fast moving ‘sprinters’ leave the source in search for hosts or mate. Another important insight this research has provided is the potential of artificial selection to enhance *S. carpocapsae* dispersal to fully utilize its biological control potential.

Dispersal is an important behavioral attribute of entomopathogenic nematodes (EPNs) which enables them to locate and procure resources required for growth, development, maintenance and reproduction. Since all the resources are provided by hosts, successful host location holds prime importance for reproductive success of EPNs in the families Heterorhabditidae and Steinernematidae. The ability to disperse actively through the soil and locate a host is also a key element for successful use of EPNs against target pests (Cutler and Webster, 2003). The overall goal of my dissertation research was to enhance our understanding of the dispersal behavior of two EPN species with
contrasting foraging strategies, a cruiser, *Heterorhabditis bacteriophora* and an ambusher, *Steinernema carpocapsae* in soil in the presence and absence of hosts. Enhancement of dispersal of the ambusher species in soil through artificial selection is another significant component of this research.

The host finding behavior of EPNs based upon their response to host cues has been be classified along a continuum, with one extreme being a cruiser and the other being an ambusher (Lewis *et al*., 1992; Campbell and Gaugler, 1993; Grewal *et al*., 1994). Three classes of EPNs are recognized: cruisers (actively searching foragers), ambushers (sit and wait foragers) and intermediates. While *H. bacteriophora* is a cruiser, is highly mobile, responds to long-range volatile cues, and is generally effective against sedentary hosts, *S. carpocapsae* is an ambusher, characterized by its ability to nictate and response to only short-range host volatile cues (Alatorre-Rosas and Kaya, 1990; Lewis *et al*. 1992; 1993; 1995; Campbell and Gaugler, 1993; Grewal *et al*. 1994; Campbell and Kaya, 2002). Since most past studies on EPN movement have been conducted in Petri dishes or small containers and in artificial substrates such as sand or agar (Moyle and Kaya, 1981; Schroeder and Beavers, 1987; Alatorre-Rosas and Kaya, 1990; Grewal *et al*. 1993; 1997), it is not clear, how or if their foraging strategies will influence dispersal in the soil, particularly at the population level. Therefore, we compared the rate of lateral movement and pattern of dispersal of the ambusher, *S. carpocapsae* and cruiser, *H. bacteriophora* populations emerging from nematode-infected cadavers in field soil placed in large wooden microcosms (up to 1.22 m x 1.22 m), thus simulating natural conditions. The dispersal potential of the two species was first compared in the absence of hosts or
host plants, and then in the complex habitat comprising vegetation, and finally with vegetation and hosts with different life histories.

In the absence of hosts, a majority of *S. carpocapsae* ambushed whereas a majority of *H. bacteriophora* cruised at spatial scale of less than 15 cm from the nematode-infected source cadavers. However, little differences were observed between the two species at larger spatial scales over a period of ten days. Despite its lower reproduction potential, greater proportion of *S. carpocapsae* (~5%) dispersed longer distances (15-61 cm) than *H. bacteriophora* (~2%), resulting in similar average population displacement (~6 cm/day) of the two species. This study revealed the ability of both the EPN species to disperse to distances about 120 times their body length per day. However, the unexpected ability of few individuals of the ambusher, *S. carpocapsae* to disperse faster and farther than the cruiser, *H. bacteriophora* is the most interesting outcome of this study. Thus, the few *S. carpocapsae* individuals rapidly moving away from their source with the ability to disperse faster to longer distances than the rest of the population in the same spatio-temporal scale may be regarded as ‘sprinters’. The emigration of few individuals from a population is an evolutionary strategy even in stable habitats (Hamilton and May, 1977) and may result in changes in the local density and spatial distribution of the population (Taylor, 1986). Such distinct dispersal strategy of the otherwise ambush forager *S. carpocapsae* may evolve over time due to the evolutionary pressure to find new ways to disperse in changing habitat conditions such as lack of host availability or population density. Like nictation, it may offer a distinct advantage for finding hosts but at greater distances in order to avoid intra-specific
competition among siblings or promote out-crossing. It may also enhance the reproductive success of the entire population by increasing the host infection after the initial invasion by these sprinters (Grewal et al. 1993; 1997; Lewis and Gaugler, 1994).

Habitat complexity (vegetation) had positive impact on the lateral movement of both the nematode species in soil in the absence of hosts. However, the impact was greater in *H. bacteriophora* with greater percentage (~6 times) of IJs dispersing up to 11.4 cm than *S. carpocapsae* (~1.7 times) in the presence of vegetation than no vegetation over a period of 96 h. In the absence of host insects, physical alteration of the soil profile by the roots is likely to play a role in nematode movement. The growth of plant roots may loosen the soil and facilitate nematode movement by providing ‘motorway’ for the dispersing nematodes, apart from altering the soil pore size and moisture content that may also influence nematode dispersal (Russell, 1977; Kaya, 1990; Duncan and McCoy, 2001). With significantly higher average displacement of *H. bacteriophora* than *S. carpocapsae* in the presence of vegetation in the silt loam soil, we could conclude that habitat complexity may influence the dispersal behavior of EPNs and consequently influence the efficacy of therapeutic application.

Further, the average displacement of both, *H. bacteriophora* and *S. carpocapsae* was found greater in the presence of mobile than non-mobile hosts in the microcosms containing vegetation. This could be likely due to greater nematode dispersal towards the chemical cues, particularly CO₂ emitted by the mobile hosts respiring at a higher rate due to greater activity than the non-mobile hosts (Gaugler et al. 1980; Lewis et al. 1993; O’Halloran and Burnell, 2003; Hallem et al. 2011; Dillman et al. 2012). The presence of
hosts, mobile and non-mobile, strongly influenced the dispersal of *S. carpocapsae* as compared to *H. bacteriophora*, most likely due to greater proportion of *S. carpocapsae* sprinters than *H. bacteriophora* IJs that reached the farthest distance from the source cadaver in the presence of hosts than in the absence of hosts. The impact of hosts on *S. carpocapsae* dispersal was stronger in the presence of mobile than non-mobile hosts with much higher proportion of *S. carpocapsae* (~14.5%) than *H. bacteriophora* (~0.4%) dispersing to the farthest distance over a period of 24 h, in the presence of mobile hosts as opposed to the nearly similar proportions in the presence of non-mobile hosts. While there was a considerable population of IJs of both species ambushing near the source cadavers, few *S. carpocapsae* sprinters managed to disperse long distances in search for hosts. This proportion increased once the host was detected, thus supporting the recruitment hypothesis indicating enhanced reproductive success of the entire population (Grewal et al. 1993; 1997). On the other hand, significant reduction in the percentage of *H. bacteriophora* IJs dispersing at the longest time point (72 h) in the presence of mobile hosts and after 24 h in the presence of non-mobile hosts indicated host mortality and subsequent repulsion or reduced attraction of the infected hosts to the rest of the population (Glazer, 1997; Grewal et al. 1997). This could be explained as an adaptive strategy of IJs to reduce intra-specific competition (Grewal et al. 1997).

These comparative microcosm studies have quantified the rate of lateral movement and identified the differences in the pattern of dispersal between ambush and cruise foraging EPN species in the soil in the laboratory conditions. The use of infected cadavers as the source of nematodes releasing IJs in large numbers as a population in the
soil medium with or without vegetation and host insects brings our experimental conditions close to the natural conditions for these soil dwelling nematodes. Such quantitative information on nematode dispersal from infected host cadavers in soil could be critical to understanding spatial and temporal dynamics of both inundatively released and endemic EPN populations in the field and could be useful for designing improved application strategies for EPNs for sustainable pest control with these beneficial organisms.

In addition to the laboratory studies, the dispersal of the two EPN species was also examined in two different habitats, a potato field and the adjoining grassy border in order to understand their dispersal behavior in the natural field conditions. This short-term study conducted over a period of 5 days revealed the potential of *S. carpocapsae* to disperse as far as *H. bacteriophora* with similar average displacements (27-28 cm/day) in the two habitats. The average displacement of both species was much higher in the field than in the laboratory (~6 cm/day) conditions. Since there was no rainfall during the course of the experiment, there was little possibility for soil likely containing EPNs to get displaced in to the pitfall traps. Therefore, such higher average displacement in the field could be most likely due to phoretic transport of EPNs by diverse host and/or non-host arthropods as opposed to the laboratory conditions where EPN dispersal was studied in the absence of hosts or in the presence of only one host species. Despite differences in the arthropod composition, the mean surface-active and below ground arthropod abundance did not vary between the two habitats. Habitat variability did not contribute to differences between the two EPN species in abundance, maximum distance dispersed and percentage
of IJs reaching the maximum distance at any time point during the experiment. Occurrence of both EPN species in the pitfall traps along with other arthropods indicated arthropod phoresy for dispersal. Although no specific arthropod taxa had a significant impact on *S. carpocapsae* dispersal, it was detected in significantly larger numbers than *H. bacteriophora* in the pitfall traps. However, a group of arthropod taxa comprising Hymenoptera, Diptera, Hemiptera, Coleoptera, Orthoptera, Isopoda, Mites and Polydesmida, excluding the most abundant ones (Collembola and spiders) enhanced the mean maximum distance traveled by *S. carpocapsae* at 48 and 96 h after the placement of the source cadavers in the grassy border. On the other hand, Trombidiformes (mites) had a significant relationship with the mean percentage of *H. bacteriophora* dispersing to the maximum distance of 2 m from the source cadavers. *H. bacteriophora* phoresy on mites was speculated based on previous studies (Epsky *et al.*, 1988; Kruitbos *et al.*, 2009).

With little information on short-term EPN dispersal in the field (Poinar and Hom, 1986; Hsiao and All, 1998), this study quantified the dispersal potential of EPNs with different foraging strategies, applied as nematode-infected host cadavers and showed their similar dispersal potential in two different habitats at a larger spatial scale, thus supporting the conclusions drawn from our laboratory studies. Such quantitative information on field dispersal would have implications on designing appropriate application methods for nematode-infected cadavers to facilitate uniform distribution of otherwise patchily distributed EPN populations (Stuart and Gaugler, 1994, Campbell *et al.*, 1995, 1996, 1998) and utilize them in an effective biological control program in vegetable, orchard, ornamental and field crops.
Since ambusher, *S. carpocapsae* showed similar dispersal potential as the cruiser, *H. bacteriophora* in both laboratory and field conditions, we conducted artificial selection of *S. carpocapsae* to further enhance its dispersal. The phenotypic variability in dispersal existing within a population of *S. carpocapsae* ALL strain was used to conduct the artificial selection. *S. carpocapsae* responded positively to selection for dispersal with enhanced dispersal rate. However, this enhanced dispersal had trade-offs with selected population having poor nictation ability and reduced reproduction potential. Dispersal increased significantly (13-23 fold) during the first five rounds of selection and caused only a marginal improvement thereafter until ten rounds of selection. Furthermore, high realized heritability value in the selected lines (> 0.5) suggested that selection could be an effective approach for enhancing *S. carpocapsae* dispersal. Enhancement of dispersal did not completely eliminate its nictation ability. This would prove beneficial for *S. carpocapsae* survival and reproduction in natural field conditions. It may disperse away from the source in search for hosts but still ambush near the source if hosts are available. The sprinters of the selected lines contained more males than the foundation population, indicating males reaching out first in search for food or mates (Grewal *et al.*, 1993). Such asymmetry in *S. carpocapsae* sex ratio at different spatial scales may contribute to positive response to selection.

Artificial selection appeared to be a promising approach for enhancing *S. carpocapsae* dispersal, which would have implications for inundative biological control utilizing selected lines with greater dispersal potential for pest management. Further, artificial selection would also have implications for designing strategies for establishing
sustainable populations of this important biological control agent. Enhanced *S. carpocapsae* dispersal could improve its biocontrol potential by reaching the sedentary host insect-pests and increasing connectivity between patchily distributed populations, further resulting in uniform distribution in the field. Continuous recycling of the released populations inside the hosts available in the field facilitates greater survival and sustainability of EPNs, which along with appropriate habitat management strategies would play a significant role in conservation biological control. This calls for future work on trait stability and field evaluations of selected lines against target pests. This study also opens opportunities for designing future studies on the genetic basis of dispersal and its ecological and evolutionary consequences.

In conclusion, my dissertation research has quantified the rate of lateral movement and identified differences in the pattern of dispersal between ambush and cruise foraging EPN species in the soil in laboratory and field conditions. The use of infected cadavers as the source of nematodes and the soil medium are important features of this research that closely mimic the natural conditions for these soil-dwelling invertebrates. The advantages of using infected cadavers such as greater IJ dispersal, survival and infectivity over aqueous solutions have been reported earlier (Shapiro and Glazer, 1996; Shapiro and Lewis, 1999; Perez *et al.* 2003). Moreover, the emergence of IJs in large numbers as a population from the infected cadavers offers the opportunity to study EPN dispersal at the population level. Such information could be critical to understanding spatial and temporal dynamics of both inundatively released and endemic EPN populations in the field. With nematode delivery to the field being one of its major concerns of the growers,
quantification of nematode dispersal from infected host cadavers will open new arenas for developing delivery systems for them using cadavers in the field. This would further help in designing appropriate inundative field application or inoculative release strategies for sustainable pest control.

The quantitative ecology of EPNs provided by this research contributes significantly to the *four key frontiers* of ecology defined by Thompson *et al.* (2001) and illustrated in EPNs by Campos-Herrera *et al.* (2012). Influence of biotic factors, host insects and habitat complexity on EPN dispersal contributed to the *first frontier* that explains the dynamics of coalescence of complex communities. Artificial selection of *S. carpocapsae* for dispersal resulting in high realized heritability of the desired trait in the selected lines contributed to the *second frontier* suggesting underlying evolutionary mechanisms of dispersal strategies of EPNs. The integration of EPN dispersal behavior from the laboratory and field investigations contributed to the *third frontier* that relates to the emergent properties of complex systems. Finally, the quantitative information on EPN dispersal in soil and in different habitats contributed to the *fourth frontier* of ecology as this quantitative information will serve as a foundation for developing population models for understanding spatial and temporal dynamics of both inundatively released and naturally occurring populations of these beneficial nematodes in soil. These models along with more research on EPN field ecology would further enable their utilization in conservation biological control (Stuart *et al.*, 2006). This would involve development of strategies to enhance the movement, survival and persistence of EPNs, in addition to maintenance of uniform distributions of otherwise patchily distributed EPN populations.
(Campbell et al., 1998; Wilson et al., 2003; Stuart et al., 2006) in the field with the ultimate goal of providing effective biological control against target insect pests.
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