Fundam. appl. Nematol., 1997, **20** (4), 393-398

Inter-specific variation in entomopathogenic nematode foraging strategy: dichotomy or variation along a continuum?

James F. CAMPBELL* and Randy R. GAUGLER

Department of Entomology, Rutgers University, New Brunswick, NJ 08903-0231, USA.

Accepted for publication 24 September 1996.

Summary - The ability of six entomopathogenic nematode species with different foraging behaviors to find hosts with different levels of mobility (restrained and unrestrained *Galleria mellonella*) was investigated. *Steinernema carpocapsae* and *S. scapterisci* tend to stand on their tails in a primarily straight non-moving posture for extended periods of time (nictation). These species exhibit an ambush foraging strategy and were most effective at finding the mobile, unrestrained, larvae. *Heterorhabditis bacteriophora* and *S. glaseri* do not nictate and were most effective at finding the restrained larvae; typical of a cruise forager. Another non-nictating species, *S. feltiae*, and a species which nictates infrequently, *S. riobravis*, were able to find both types of hosts, suggesting they use an intermediate foraging strategy. The intermediate foraging strategy of *S. feltiae* may result from it raising more than 30% of its body off the substrate (body-waving) more frequently than the other non-nictating species.

Résumé - ***Variabilité interspécifique dans la stratégie de recherche d'hôte chez les nématodes entomopathogènes: dualité ou variation suivant un continuum?*** - L'aptitude de six espèces de nématodes entomopathogènes ayant des comportements différents dans la recherche d'hôtes ayant eux-mêmes des taux de mobilité variables (*Galleria mellonella* avec ou sans restriction de mouvement) a été évaluée. *Steinernema carpocapsae* et *S. scapterisci* ont tendance à se tenir droit sur leur queue sans se déplacer pendant des périodes de temps assez prolongées mais avec des mouvements de pendulation. Ces espèces utilisent une stratégie d'embuscade et sont les plus efficaces pour trouver les larves se déplaçant sans restriction de mouvement. *Heterorhabditis bacteriophora* et *S. glaseri* ne montrent pas de mouvement de pendulation et ont été les plus efficaces pour trouver les larves à déplacement restreint, ce qui est typique d'espèces cherchant leur hôte en se déplaçant. Une autre espèce sans mouvement de pendulation, *S. feltiae*, et une espèce à pendulation peu fréquente, S. *riobravis*, peuvent atteindre les deux types d'hôtes, ce qui suggère qu'elles ont un comportement intermédiaire de recherche d'hôtes. La stratégie intermédiaire de S. *feltiae* pourrait être due à sa capacité à élever de plus de 30% son corps au-dessus du support (mouvement de pendulation), et ce, plus fréquemment que les autres espèces sans mouvement pendulaire.

Key-words: entomopathogenic nematodes, foraging behavior, Heterorhabditidae, host search, host-parasite interaction, Steinernematidae.

Two broad categories of foraging behavior have been recognized (Pianka, 1966; Schoener, 1971; Eckhardt, 1979; Huey & Pianka, 1981). A cruise foraging strategy, where the organism searches while moving through the environment, is more effective at finding sedentary and cryptic resources. In contrast, an ambush strategy, where the forager waits for resources to come to it, can be more effective when resources have high mobility and resource density is high and/or forager metabolic requirements are low. It has been proposed that each foraging strategy has an array of ecological, behavioral, physiological, morphological, and life-history correlates and, therefore, identifying an organism's foraging strategy can allow generalizations about other areas of the organism's natural history (Eckhardt, 1979; Huey & Pianka, 1981). This division of foraging behavior into two strategies, cruise or ambush, has been documented for a wide range of taxa (*e.g.*, reptiles: Huey & Pianka, 1981; amphibians: Toft, 1981; birds, McLaughlin, 1989; arachnids: Janetos, 1982; insects: Inoue & Matsura, 1983). However, this dichotomous view of foraging strategies has also been criticized as arbitrary and an oversimplification of what is actually a continuum (Regal, 1978; Taigen & Pough, 1983). As a consequence, intermediate forms of foraging strategy are likely to occur (Taigen & Pough, 1983; Pietruszka, 1986; O'Brien *et al.*, 1989). The ultimate utility of the ambusher/cruiser dichotomous classification lies in how bimodal is the distribution of strategies (McLaughlin, 1989).

The applicability of foraging theory to nematode parasite infective stages, that search for only a single host, has received little investigation. However, a number of recent studies have investigated whether entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) use ambush or cruise foraging strategies (*e.g.*, Gaugler *et al.*, 1989;

*Present address: *Departments of Entomology and Nematology, University of California, Davis, CA 95616, USA.*

ISSN 1164-5571/97/04 $ 7.00 / © *Gauthier-Villars - ORSTOM*

Lewis *et al.*, 1992, 1993, 1995; Campbell & Gaugler, 1993). Entomopathogenic nematodes are lethal insect parasites that have a motile free-living infective stage: a third-stage juvenile. All other stages are parasitic within an insect host. The free-living stages' sole function is to find, penetrate, and establish in a new insect host. Although most species of entomopathogenic nematode are opportunistic generalists, differences in foraging behavior may contribute to a narrower realized host-range in the field.

Campbell and Gaugler (1993) demonstrated behavioral differences among entomopathogenic nematode species and a behavioral mechanism for nematode ambushing. A nematode's small size and low motility relative to its potential host and the strong influence of surface tension forces holding the nematode to the substrate would tend to preclude exploitation of highly mobile insects. However, by raising most of its body off the substrate, body-waving or nictating (Reed & Wallace, 1965; Ishibashi & Kondo, 1990), an infective juvenile is presumably more effective at attaching to passing insects. Campbell and Gaugler (1993) demonstrated this with nictating *Steinernema carpocapsae*. When infective juveniles body-wave, greater than 30% and less than 95% of the body is raised off the substrate, a straight posture is not assumed, and the duration is typically only a few seconds. All species of entomopathogenic nematode infective juveniles studied to date exhibit body-waving, but, some species also exhibit a specialized form of this behavior, termed nictation, in which they adopt a primarily straight posture while balancing on a bend in their tail. This posture can be maintained for extended periods of time with alternating periods of waving and motionlessness. Some species (*e.g.*, *S. carpocapsae* and *S. scapterisci*) spend a large proportion (>70%) of their foraging time exhibiting this behavior (Campbell & Gaugler, 1993). Another species, *S. riobravis*, exhibits nictation behavior (Cabanillas *et al.*, 1994), but the behavior is expressed infrequently and for a short duration (Campbell, unpubl.).

Species that do not nictate have been termed cruise foragers and have also been characterized by high mobility (Lewis *et al.*, 1992; Campbell & Gaugler 1993), ability to orientate to volatile host cues (Lewis *et al.*, 1993) and switch to localized search after host contact (Lewis *et al.*, 1992), and ability to find sedentary hosts (Alatorre-Rosas & Kaya, 1990). In contrast, species that nictate have been termed ambush foragers and have also been characterized by low motility (Lewis *et al.*, 1992; Campbell & Gaugler, 1993) and lack of response to volatile (Gaugler *et al.*, 1989; Lewis *et al.*, 1993) and contact host cues (Lewis *et al.*, 1992) unless presented in an appropriate sequence (Lewis *et al.*, 1995). This evidence has tended to support the dichotomous view of foraging strategies for this group of nematodes. However, another species (*S. feltiae*) has not been observed to nictate (Campbell & Gaugler, 1993), but also does not respond to cue hierarchies (Lewis *et al.*, 1994) or sources of host volatile cues in a manner similar to other cruise foragers (Grewal *et al.*, 1994; Lewis *et al.*, 1995). *S. riobravis* does nictate to some extent, but is also effective at orientating to host cues (Grewal *et al.*, 1994). This suggests that an intermediate type of foraging strategy may be used by some species.

Material and methods

Host-finding of mobile and non-mobile hosts

Because adoption of different foraging modes will influence the types of hosts found, we compared the ability of entomopathogenic nematodes with different foraging behavior to find hosts with different levels of motility. Grouping nematodes based on their ability to nictate, we predicted that *S. carpocapsae* (All strain) and *S. scapterisci* (Colon strain) would be more effective at finding mobile hosts compared to non-mobile hosts, *S. riobravis* would be intermediate due to its lower tendency to nictate, and *H. bacteriophora* (HB1 strain), *S. feltiae* (SN strain) and *S. glaseri* (NC strain) would be more effective at finding non-mobile compared to mobile hosts. Unlike prior studies, we provided an opportunity for the nematodes to perform either ambush or cruise search tactics and ample time for either search strategy to be effective.

One thousand infective juveniles were added in 850 µl of deionized water to filter paper (Fisher brand P8) covered with a thin layer of sand (< 0.15 mm diam) in a 90 mm Petri dish. The nematodes were added in a ring approximately half the radius of the Petri dish and a single highly susceptible host – *Galleria mellonella* L. larva, (Lepidoptera, Pyralidae) – was added to the dish. The larva was either allowed to move freely within the dish or restrained in a screen cage (nylon window screen, 1 mm openings) placed in the center of the Petri dish. The larva was exposed to nematodes for either 5, 10, 20, 30, 40, 50, 60, 120, or 180 min and five replicates were performed for each nematode species, treatment, and exposure time combination.

After the exposure period, the larva was removed from the dish. Restrained larvae were extracted from their screen cages. Each larva was individually rinsed and the number of nematodes recovered in the rinse was determined. Because some infective juveniles may have already entered the host, especially in the longer exposure times, all larvae were held for 3 days. The dead larvae were dissected, and the number of nematodes that had penetrated and established was determined. The total number of nematodes in the rinse and in the host was considered the number of host-

finding nematodes. Differences in mean number of host-finding nematodes between the restrained and unrestrained treatments were determined using analysis of variance (Anon., 1987). A significance level of $P \leq 0.05$ was used for all comparisons.

Behavioral mechanism involved in an intermediate foraging strategy

Campbell and Gaugler (1993) did not find that *S. feltiae* exhibited any behavioral differences from *H. bacteriophora* or *S. glaseri* that would explain why our results indicate that it is an intermediate forager. We propose two possible explanations why *S. feltiae* is better able to ambush mobile hosts than these other two species. First, a body-waving *S. feltiae* may be more effective at attaching to a passing insect than a body-waving *H. bacteriophora* or *S. glaseri*. *Steinernema feltiae* may accomplish this by body-waving more frequently, for longer periods of time in each bout, or by lifting a greater portion of its body off the substrate during a bout. Second, the presence of host associated cues may stimulate *S. feltiae* infective juveniles to increase either the proportion of individuals, or amount of time spent, exhibiting body-waving behavior to a greater extent than *H. bacteriophora* or *S. glaseri*.

The body-waving behavior of *S. feltiae* was compared to that of *H. bacteriophora*, *S. carpocapsae*, and *S. glaseri*. For these experiments, we used an agar and sand arena described in Campbell and Gaugler (1993). Briefly, the arena was prepared by pouring agar (2%) into a 90 mm Petri dish, the agar was air dried for 60 min, and then fine sand (< 0.15 mm diam) was scattered on the surface. Four holes were made in the lid of the Petri dish and 1000 µl plastic Eppendorf style pipet tips were inserted into the holes so that the tips were approximately 2 mm above the agar surface. One *G. mellonella* larva was added to each of the four pipet tips (treatment), whereas none were added to the four control pipet tips. This enables host volatiles to be introduced into the arena, but prevents the nematodes from contacting the host. This arena also provides adequate moisture for nematodes to exhibit nictation and body-waving behavior and enables individual infective juveniles to be observed through a dissecting microscope. At the beginning of the experiments, nematodes were added to the agar surface using a probe.

Four types of data were collected. First, the proportion of the individuals exhibiting body-waving at a given time, with or without the presence of host-associated volatiles, was determined. Nematodes were observed approximately 15 min after being added to the arena. Fields-of-view were selected arbitrarily and the behaviors currently being expressed by all individuals within the field-of-view were recorded. Approximately 500 individuals were observed for each combination of nematode species and host associated volatile cues. A more sensitive measurement of the tendency to body-wave is the duration of time until an arbitrarily selected crawling nematode exhibits the behavior. Crawling individuals closest to the center of an arbitrarily chosen field-of-view were selected for observation. The nematodes were observed for a 5 min period or until they exhibited body-waving behavior for at least 2 seconds. Twenty individuals for each nematode species/host associated volatile cues combination were observed and the proportion expressing body-waving behavior within 5 min calculated.

The length of body-waving bouts and the proportion of the body raised during a bout was also determined. Twenty individuals of each nematode species/host associated volatile cues treatment were observed. Individuals were followed from the initiation of body-waving until its transition back to crawling behavior. *S. carpocapsae* was not included because most of its body-waving bouts proceeded into nictation behavior and not back to crawling. The maximum proportion of the body raised off the substrate was estimated. The proportion of individuals with greater than 50% of the body raised off the substrate were compared among species and between treatments.

All proportional data were analyzed using contingency table analysis and the chi square statistic (Zar, 1984). Duration data were analyzed using analysis of variance (Anon., 1987). A $P \leq 0.05$ significance level was used for all comparisons.

Results

Host-finding of mobile and non-mobile hosts

There were differences in the pattern of host-finding of restrained and unrestrained hosts among nematode species (Fig. 1). The two species that exhibit nictation behavior, *S. carpocapsae* and *S. scapterisci*, were more effective at finding the unrestrained (mobile) compared with the restrained (non-mobile) hosts. The number of host-finding nematodes increased until about 60 min and then remained relatively level. Two of the species that do not exhibit nictation behavior, *H. bacteriophora* and *S. glaseri*, exhibited a host-finding response that was consistent with a cruise foraging strategy. The number of nematodes finding the restrained host was generally low until about 60 min and then increased linearly. The third non-nictating species, *S. feltiae*, as well as *S. riobravis*, which nictates infrequently, found both the restrained and unrestrained hosts in a relatively similar pattern. However, both of these species tended to be more effective at finding the restrained than the unrestrained host, *i.e.*, closer to the cruiser end of the continuum.

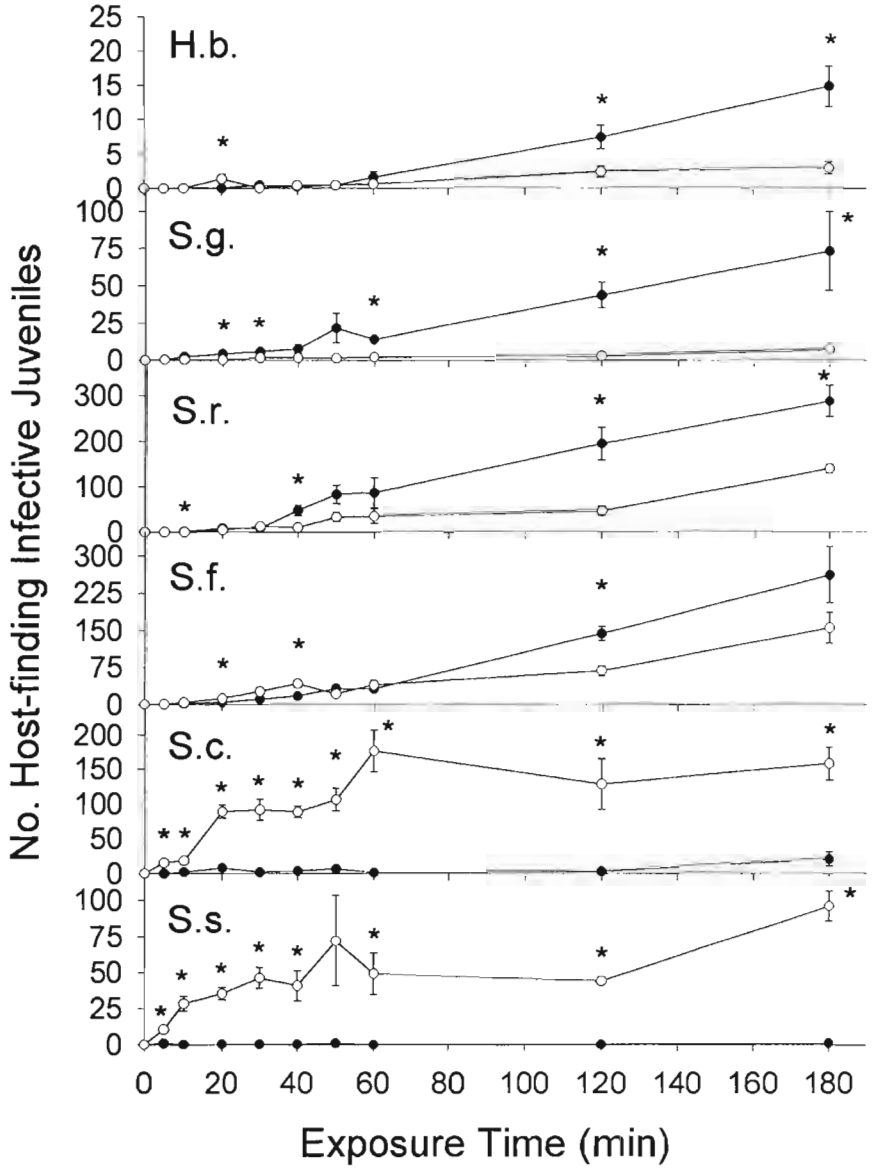

Fig. 1. *The number of* Heterorhabditis bacteriophora *(H.b.),* Steinernema glaseri *(S.g.),* S. riobravis *(S.r.),* S. feltiae *(S.f.),* S. carpocapsae *(S.c.), and* S. scapterisci *(S.s.) infective juveniles finding unrestrained (○) and restrained (●)* Galleria mellonella *larvae over time. * indicates significant differences ($P \leq 0.05$) between the number of nematodes finding unrestrained and restrained hosts.*

Behavioral mechanism involved in an intermediate foraging strategy

Host-associated volatiles did not significantly influence *H. bacteriophora*, *S. carpocapsae* or *S. glaseri* behavior, but *S. feltiae* body-waving was significantly greater when host cues were present (Table 1). However, the increase was not large and the proportion body-waving was not greater than either of the cruise foragers. No *S. feltiae*, *S. glaseri*, or *H. bacteriophora* were observed to exhibit nictation behavior during the observations.

The length of time until raising the body off the substrate (reported as the proportion body-waving within 5 min) was not significantly influenced by host volatiles, but did differ among the species (Table 1).

Most of the *S. carpocapsae* infective juveniles exhibited body-waving behavior within 5 min. This proportion is high because this nematode species progresses from body-waving into nictation behavior; during the same observation period approximately 80% of *S. carpocapsae* also exhibited nictation. More than two times as many *S. feltiae* exhibited body-waving within the observation period compared to either *S. glaseri* or *H. bacteriophora* (Table 1). The average length of individual body-waving bouts and the proportion of bouts where more than half the body was raised off the substrate were not significantly influenced by the presence of hosts and did not differ significantly among species (Table 1).

Discussion

Our results support the idea that foraging strategies of entomopathogenic nematodes lie along a continuum rather than a dichotomy based on ability to nictate. Species that spend a majority of their foraging time nictating (*S. carpocapsae* and *S. scapterisci*) appear to use primarily an ambush type of foraging strategy on surfaces. However, species that do not nictate are not necessarily strict cruise foragers and classifications based strictly on nictation behavior can be misleading. Two of the species tested (*H. bacteriophora* and *S. glaseri*) exhibit a foraging strategy at the cruiser end of the continuum; based on their ability to find sedentary and mobile hosts as well as other evidence previously described in the introduction. Another species, *S. feltiae*, does not exhibit stable nictation behavior and is more or less equally able to find mobile and sedentary hosts. *S. riobravis*, which nictates infrequently, is also able to find both mobile and sedentary hosts. However, both of these species would appear to be closer to the cruiser end of the continuum.

It is not apparent yet whether the greater proportion of infective juveniles exhibiting waving bouts is sufficient to explain the pattern of *S. feltiae* host-finding. At the population level, the proportion of body-waving *S. feltiae* individuals at a given point in time was not greater than the other two species that do not nictate. Initially, it may appear counterintuitive for *S. feltiae* body-waving to be more than twice as prevalent on an individual level, but not detectable when measured at the population level. Estimations based on the individual observations, however, predict that the proportion of the population expected to be body-waving at a given point in time is low (e.g., 1.2% for *S. feltiae*, 0.6% for *H. bacteriophora*, and 0.4% for *S. glaseri* with host cues present). This is an estimate of the number of individuals initiating body-waving in a five s interval, which is the approximate time to count a field of view, and is therefore an underestimate. However, both the predicted and observed data indicate that at a given point in time only a small proportion of the population is body-waving. Even counts of 500 individuals, as in our population observations, would be unlikely to detect these small differences. The biological significance of such small differences is not clear.

Table 1. *The influence of host associated volatiles on body-waving behavior of four species of entomopathogenic nematode*[1].

Species	% of population body-waving[2]		% of individuals that body-wave during a 5 min observation[3]		Duration (sec) of body-waving bout[4]		Percent of body waving bouts where >1/2 of body is raised[4]	
	hv	c	hv	c	hv	c	hv	c
H. bacteriophora	4.2*b*	4.6*b*	35.0*b*	25.0*b*	3.7	4.2	15.0*a*	20.0*a*
S. carpocapsae	11.8*a*	10.2*a*	95.0*a*	95.0*a*	na	na	na	na
S. feltiae	2.8*b**	0.7*c*	75.0*a*	85.0*a*	4.4 ± 0.6*a*	6.2 ± 1.0*a*	40.0*a*	35.0*a*
S. glaseri	4.6*b*	0.4*c*	25.0*b*	30.0*b*	6.4	4.4	30.0*a*	25.0*a*

[1]Percentage data was analyzed using contingency table analysis and the chi square statistic (Zar, 1984) and duration data was analyzed using analysis of variance (Anon., 1987). *: comparisons within species that are significantly different ($P \geq 0.05$) between presence of host volatiles (hv) and control (c); numbers with the same letter are not significantly different ($P \geq 0.05$) from others in the same column.

[2]Percent of individuals (n≈500) exhibiting behavior 15 min after being added to the plate.

[3]Infective juveniles crawling on the substrate were arbitrarily selected (n=20) and observed for 5 min or until they exhibited body-waving behavior for at least two sec.

[4]Infective juveniles (n=20) that were just initiating body-waving behavior were observed and the length of the body-waving bout was measured and an estimate of the maximum proportion of the body raised off the substrate was made.

Additional factors influencing *S. feltiae* host-finding need to be investigated. For example, differences in the cuticle of the nematode could influence its degree of "stickiness" to passing insects. In addition, behavioral changes associated with the close proximity of the host or sudden changes in host cues could also elicit different responses among the species. Regardless of the mechanism(s) involved, *S. feltiae* appears to be a less effective ambusher than *S. carpocapsae* and *S. scapterisci*; because it is much slower at attaching to mobile hosts. Based on response to cue hierarchies (Lewis *et al.*, 1995) and ability to orientate to host volatile cues (Grewal *et al.*, 1994; Lewis *et al.*, 1995), *S. feltiae* is also a less effective cruiser than *H. bacteriophora* and *S. glaseri.*

Although our results support the idea of a continuum of foraging strategies among entomopathogenic nematode species, it is not clear to what degree this distribution is bimodal. If most species adopt foraging strategies near the end of the spectrum and intermediate types of foraging strategy are uncommon then the dichotomous classification still has considerable utility (Pianka, 1973; McLaughlin, 1989). The foraging strategies of more entomopathogenic nematode species need to be investigated to determine if *S. feltiae* and *S. riobravis* represent variation around a relatively robust bimodal dichotomy or if there are many other species that exhibit intermediate foraging behaviors and host-finding.

Acknowledgments

We thank H. Kaya, E. Lewis, M. Bauer, and A. Koppenhöffer for helpful comments on an earlier draft of this manuscript. This is New Jersey Experiment Station Publication No. D-08255-22-95, supported by state funds and the U.S. Hatch Act.

References

ALATORRE-ROSAS, R. & KAYA, H. K. (1990). Interspecific competition between entomopathogenic nematodes in the genera *Heterorhabditis* and *Steinernema* for an insect host in sand. *J. Invert. Pathol.*, 55: 179-188.

ANON. (1987). *SAS users guide: Statistics.* Cary, USA, SAS Institute.

CABANILLAS, H. E., POINAR, G. O., JR. & RAULSTON, J. R. (1994). *Steinernema riobravis* n. sp. (Rhabditida: Steinernematidae) from Texas. *Fundam. appl. Nematol.*, 17: 123-131.

CAMPBELL, J. F. & GAUGLER, R. (1993). Nictation behavior and its ecological implications in the host search strategies of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae). *Behaviour*, 126: 155-169.

ECKHARDT, R. C. (1979). The adaptive syndromes of two guilds of insectivorous birds in the Colorado Rocky Mountains. *Ecol. Monogr.*, 49: 129-149.

GAUGLER, R., MCGUIRE, T. & CAMPBELL, J. F. (1989). Genetic variability among strains of the entomopathogenic nematode *Steinernema feltiae*. *J. Nematol.*, 21: 247-253.

GREWAL, P. S., LEWIS, E. E., GAUGLER, R. & CAMPBELL, J. F. (1994). Host finding behaviour as a predictor of foraging strategy in entomopathogenic nematodes. *Parasitology*, 108: 207-215.

HUEY, R. B. & PIANKA, E. R. (1981). Ecological consequences of foraging mode. *Ecology*, 62: 991-999.

INOUE, T. & MATSURA, T. (1983). Foraging strategy of a mantid, *Paratenodera angustipennis* S.: mechanisms of switching tactics between ambush and active search. *Oecologia*, 56: 264-271.

ISHIBASHI, N. & KONDO, E. (1990). Behavior of infective juveniles. *In* : Gaugler, R. & Kaya, H. (Eds). *Entomopathogenic nematodes in biological control.* Boca Raton, FL, USA, CRC Press: 139-150.

JANETOS, A. C. (1982). Active foragers *vs* sit-and-wait predators: a simple model. *J. theor. Biol.*, 95: 381-385.

LEWIS, E. E., GAUGLER, R. & HARRISON, R. (1992). Entomopathogenic nematode host finding: response to host contact cues by cruise and ambush foragers. *Parasitology*, 105: 309-315.

LEWIS, E. E., GAUGLER, R. & HARRISON, R. (1993). Response of cruiser and ambusher entomopathogenic nematodes (Steinernematidae) to host volatile cues. *Can. J. Zool.*, 71: 765-769.

LEWIS, E. E., GREWAL, P. S. & GAUGLER, R. (1995). Hierarchical order of host cues in parasite foraging strategies: a question of context. *Parasitology*, 110: 207-213.

O'BRIEN, W. J., EVANS, B. I. & BROWMAN, H. I. (1989). Flexible search tactics and efficient foraging in saltatory searching animals. *Oecologia*, 80:100-110.

MCLAUGHLIN, R. L. (1989). Search modes of birds and lizards: evidence for alternative movement patterns. *Am. Naturalist*, 133: 654-670.

PIANKA, E. R. (1966). Convexity, desert lizards, and spatial heterogeneity. *Ecology*, 47: 1055-1059.

PIANKA, E. R. (1973). The structure of lizard communities. *A. Rev. Ecol. Syst.*, 4: 53-74.

PIETRUSZKA, R. D. (1986). Search tactics of desert lizards: how polarized are they? *Anim. Behavior*, 34: 1742-1758.

REED, E. E. & WALLACE, H. R. (1965). Leaping locomotion by an insect-parasitic nematode. *Nature*, 206: 210-211.

REGAL, P. J. (1978). Behavioral differences between reptiles and mammals: an analysis of activity and mental capabilities. *In* : Greenberg, N. & MacLean, P.D. (Eds). *Behavior and neurology of lizards: an interdisciplinary colloquium.* Publ. 77-491, Rockville, Department of Health, Education, and Welfare : 183-202

SCHOENER, T. W. (1971). Theory of feeding strategies. *A. Rev. Ecol. Syst.*, 2: 369-404.

TAIGEN, T. L. & POUGH, F. H. (1983). Prey preference, foraging behavior, and metabolic characteristics of frogs. *Am. Naturalist*, 122: 509-520.

TOFT, C. A. (1981). Feeding ecology of Panamanian litter anurans: patterns in diet and foraging mode. *J. Herpetol.*, 15: 139-144.

ZAR, J. H. (1984). *Biostatistical analysis.* Englewood Cliffs, NJ, USA, Prentice-Hall Inc., 718 p.