THE EFFECT OF DAY OF EMERGENCE FROM THE INSECT CADAVER ON THE BEHAVIOR AND ENVIRONMENTAL TOLERANCES OF INFECTIVE JUVENILES OF THE ENTOMOPATHOGENIC NEMATODE *HETERORHABDITIS MEGIDIS* (STRAIN UK211)

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ABSTRACT: Infective juveniles (IJs) of entomopathogenic nematodes (EPNs) are obligate parasites of insect larvae. Inside the host they develop into sexually mature adult stages and complete their life cycle. Two or 3 adult nematode generations can occur in the insect host. The increase in nematode population density in the insect cadaver, together with limiting nutrient conditions, result in the formation of IJs. These IJs emerge into the soil to search for a new host. It typically takes 7–8 days for all IJs to emerge from a parasitized insect. We have investigated the effect of the day of emergence of IJs from insect cadavers on the environmental tolerance and behavior of the EPN *Heterorhabditis megidis* strain UK211. The IJs that emerge early display good initial host-finding ability and increased temperature tolerance but disperse poorly and have poor tolerance to desiccation. Conversely, the IJs that emerge later display poor initial host-finding ability and poor temperature tolerance but they disperse well and possess increased desiccation tolerance. These phenotypic differences are likely to facilitate early-emerging IJs in locating and infecting hosts in the vicinity of the cadaver, whereas IJs that emerge late are adapted to disperse away from their natal cadaver. We hypothesize that adaptive phenotypic plasticity rather than allelic variability may provide the genetic basis for the different physiological and behavioral phenotypes of the early- and late-emerging IJs.

Entomopathogenic nematodes (EPN) of the genera *Heterorhabditis* and *Steinernema* are capable of infecting a broad range of insect species, including some important pests of commercial crops (Klien, 1990). Heterorhabditid infective juveniles (IJs) carry in their intestines bacteria of the genus *Photorhabdus* that are released into the insect hemocoel upon infection. The insect larva typically dies within 48 hr after infection. Bacterial growth in the hemocoel provides suitable nutrient conditions for nematode growth and reproduction. The third-stage IJs develop into J4 juveniles in the insect hemocoel and these give rise to a first generation of self-fertilizing hermaphrodite females. Eggs that are laid into the hemocoel by the first-generation females typically develop into amphimictic males and females (Dix et al., 1992), whereas eggs that hatch and grow inside the uterus of hermaphrodites or amphimictic females (via endotokia matricida) develop into IJs that eventually destroy their mother (Wang and Bedding, 1996). Two or 3 adult generations typically occur over a 16-day period at 23 C in the last instar larva of the wax moth *Galleria mellonella* (Wang and Bedding, 1996). Strauch et al. (1994) have shown that when food conditions are limiting, J1 juveniles develop into either IJs or hermaphrodite females. Thus both in vitro in liquid cultures and in vivo in insect cadavers the proportion of IJs increases as the cultures age, and 3 wk postinfection a *G. mellonella* cadaver infected with a single IJ and incubated at 23 C may contain as many as 150,000 IJs (Wang and Bedding, 1996). These IJs migrate from the insect host into the soil and their function is to locate and infect a new insect host. The life cycle of *Steinernema* is similar to that of *Heterorhabditis* except that upon infecting a suitable insect host, a steinernematid IJ develops into either an adult male or an amphimictic adult female. However, the similarities in life cycle between *Steinernema* and *Heterorhabditis* are considered to result not from a close phylogenetic relationship but from convergent evolution (Poinar, 1993).

EPN IJs are morphologically and physiologically adapted for survival in the soil. IJs do not feed but carry rich food reserves in the form of stored lipid and glycogen that decrease with age and determine the life span of the IJs during storage in water (Selvan et al., 1993). In their natural habitat, additional factors such as soil moisture and temperature also play important roles in determining the life span of the IJs (Hominick, 1990). Many studies have been carried out on the environmental tolerance and behavior of EPN IJs; however, few of these studies tested either the stage in the infectious cycle in which the IJs were produced or their time of emergence from the insect cadaver. Lewis and Gaugler (1994) have demonstrated a relationship between the foraging strategy of steinernematid IJs and the pattern of emergence of male and female IJs from the host. IJs of *Steinernema glaseri*, a "cruise forager" (Lewis et al., 1992), contain more males than females on their first day of emergence from the host, whereas IJs from later harvests are mostly female. *Steinernema glaseri* female IJs are less attracted to host volatile cues than are male IJs during the first week postemergence. However, these female IJs are strongly attracted to hosts already parasitized by *S. glaseri*, a strategy that would increase the chances of reproduction for these females (Lewis and Gaugler, 1994). These authors suggest that variability in infectivity between batches of *S. glaseri* IJs may be due to differences in sex ratio between the IJs that emerge first and last from a cadaver.

It is possible, as has been suggested by Womersley (1993), that changing conditions in the insect cadaver affect the physiology of IJs produced. Thus, the behavior and environmental tolerance of IJs formed in the later stages of the infection process may differ from that of IJs that develop in earlier generations. Wang and Bedding (1996) have shown that heterorhabditid IJs are produced as early as 9 days postinfection (PI) in *G. mellonella* (that had been injected with 1 IJ) even though IJs did not begin to emerge until 12 days later. Nguyen and Smart (1995) have shown that there is generally a linear relationship between the body length of heterorhabditid and steinernematid IJs and their time of harvest from the host *G. mellonella*. These authors postulate that the size reduction over time of IJs from in vivo culture is related to a reduced food

supply in the cadaver. The population size of EPN in the insect cadaver increases dramatically with time; therefore, it is likely that as the population density increases, the available food supply will decline. Fodor et al. (1990) have postulated that there could exist in *Heterorhabditis* a pheromone, similar to dauer recovery inhibiting factor (DRIF) in the free-living nematode *Caenorhabditis elegans,* that may alter the behavior and physiological status of IJs produced under stressful conditions, e.g., lack of food or crowded conditions or both. Increased levels of this postulated pheromone might be produced as the numbers of nematodes increased in the cadaver with the result that dauer juvenile or IJ formation would increase from generation to generation.

We have carried out a study of the behavior and environmental tolerances of *Heterorhabditis megidis* IJs that have emerged on different days PI from parasitized *Tenebrio molitor* larvae. Because heterorhabditid IJs are destined to develop into hermaphrodite females when they resume development in an insect host, these data are not confounded by the sex ratio of the emerging IJs. The data presented here clearly show that the environmental tolerances and behavior of the IJ varies between days of emergence from the insect host. These findings have important implications for the design of bioassays to measure environmental physiology and infectivity in *Heterorhabditis* IJs.

MATERIALS AND METHODS

Source and maintenance of *H. megidis*

Heterorhabditis megidis (strain UK211) was isolated in England by Dr. W. Hominick and a stock is maintained in the Department of Biology, National University of Ireland, Maynooth. Nematodes were cultured in vivo at 20 C in *T. molitor* (Coleoptera: Tenebrionidae) larvae (Woodring and Kaya, 1988). *Tenebrio molitor* larvae were chosen because their integument is stronger than that of *G. mellonella,* and parasitized *T. molitor* larvae remain intact during the entire course of *Heterorhabditis* infection and IJ emergence. Petri dishes (9 cm) were lined with Whatman no. 1 filter paper and then ~1 ml of water containing ~500 IJs was pipetted onto the filter paper. Twenty *T. molitor* larvae were placed in each petri dish. The petri dishes were incubated at 20 C for 5–10 days to allow the IJs to infect the larvae. The larvae exhibited a characteristic red color once successful infection had occurred. Parasitized larvae were placed on White traps (White, 1927). White taps were constructed by placing the inverted lid of a 5-cm petri dish in the center of a 9-cm petri dish. Filter paper (Whatman no. 1) cut to size (~8 cm) was positioned on the lid of the 5-cm petri dish. Tap water was added to the 9-cm petri dish until the edges of the filter paper became immersed in the water. The insect cadavers were placed on the platform formed by the lid of the 5-cm petri dish. Five cadavers were placed in each White trap for the experiment presented in Figure 1; for all other experiments each White trap contained 10 cadavers. The White traps were incubated at 20 C.

Harvesting of IJs

White traps were monitored by microscopic examination each day until IJs were observed in the water. Once IJs began to emerge, they were collected daily from the trap by pouring the trap water into 50-ml conical tubes. The water trap was rinsed out 3 times to ensure harvesting of all IJs. The IJs were concentrated by sedimentation, i.e., they were allowed to settle in conical tubes and were stored (~5,000/ml) in 9-cm petri dishes at 20 C until required. It typically takes 7–8 days for all IJs to emerge from a *T. molitor* cadaver. IJs were either tested on each day of emergence (when measuring desiccation tolerance) or the emerging IJs were combined to form 3 groups A, B, and C (for all the other assays). Group A contained IJs from day 1 to day 3, group B contained IJs from those that emerged from day 4 to day 5 and group C contained the remainder of the IJs to emerge (Fig. 1). IJs were tested

on the last day of emergence for each emergence group, i.e., day 3 for group A, day 5 for group B, and day 8 for group C. The IJs used in the different assays were collected from 5 White traps, each White trap containing 10 cadavers. Unless otherwise stated these IJs were pooled into the 3 emergence groups A, B, and C immediately prior to their use in the assays described below and aliquots from the pooled IJs were used in these assays.

Desiccation tolerance of IJs

Relative humidity (RH) was maintained at 57% by the use of sulfuric acid/water solutions as described by Solomon (1951). A 1-ml suspension of IJs in water (concentration of 200 IJs/ml) was vacuum-filtered onto a 2.5-cm Whatman no. 1 filter paper disc using a Sartorius funnel and a vacuum flask attached to an electric pump. The discs were transferred to 3-cm petri dishes that were then placed in 30-cm-diameter glass desiccators containing the appropriate concentration of sulfuric acid. After a 180-min desiccation period, the petri dishes were removed and the IJs were rehydrated at 20 C with 3 ml of tap water. Percentage survival was assessed by microscopic observation of motility and response to probing after storage at 20 C for 24 hr. The 3-cm petri dishes were placed on a grid under a dissecting microscope and the total number of living and dead IJs were counted in each dish. Five separate batches of IJs were tested, each batch derived from a single White trap containing 10 cadavers. Five replicate samples of 200 IJs were assayed for each batch for each day of IJ emergence. The mean percentage survival for each batch was calculated and then the overall mean value was calculated from 5 batch means.

Length of IJs from the 3 emergence groups

Two insect hosts were used, *G. mellonella* larvae and *T. molitor* larvae. IJs of *H. megidis* were cultured in *G. mellonella* as described previously for *T. molitor.* IJs were measured on the last day of emergence for each emergence group. The first IJs to emerge in each group were stored at 15 C prior to measurement. Live IJs were mounted in water on glass slides and covered with coverslips. The slides were passed briefly over a Bunsen flame to kill the IJs. The length of the straightened IJs was then measured using a Nikon microscope with a graticule incorporated into one of the eyepieces. Forty IJs per emergence group were measured for each insect host species.

Temperature tolerance of IJs

A 50-ml suspension containing 200 IJs was pipetted onto 5 layers of Whatman no. 1 filter paper (1.2 × 0.8 cm). The top layer of filter paper was then placed in a glass test tube that was immersed in either a cooler containing antifreeze (Maxol Coolant) at −15 C for 40 min or in a water bath at 35 C for 38 min. The test tubes were then removed and 3 ml of tap water was added. The tubes were vortexed for 10 sec, and the nematode suspension from each test tube was poured into a 3-cm petri dish. The dishes were incubated at 20 C and the percentage survival was recorded 24 hr later as described above. For temperature regimes that required longer incubation periods, i.e., 1–10 days, IJs (5,000/ml) were stored in 5-cm petri dishes and placed at either 2 C or 30 C. After the test period, the contents of the petri dishes were transferred to 50-ml conical tubes. Three replicate 0.1-ml samples were taken from each tube, transferred to 3-cm petri dishes, and adjusted to a volume of 3 ml with tap water. Percentage survival was assessed after storage at 20 C for 24 hr.

IJ longevity

IJs were stored in petri dishes (5,000/ml) at 20 C for periods of up to 14 wk. Three replicate dishes were used for each experimental observation. For each determination the contents of each petri dish were poured into a separate conical tube. One 0.2-ml sample was taken from each conical tube, transferred to a 3-cm petri dish, and adjusted to a volume of 3 ml with tap water. The 3-cm petri dishes were placed on a grid under a dissecting microscope, and the total number of living and dead IJs were counted in each dish.

IJ host-finding capability

A modification of the bioassay described by Gaugler et al. (1989) was used to compare the host-finding capabilities of IJs from the 3
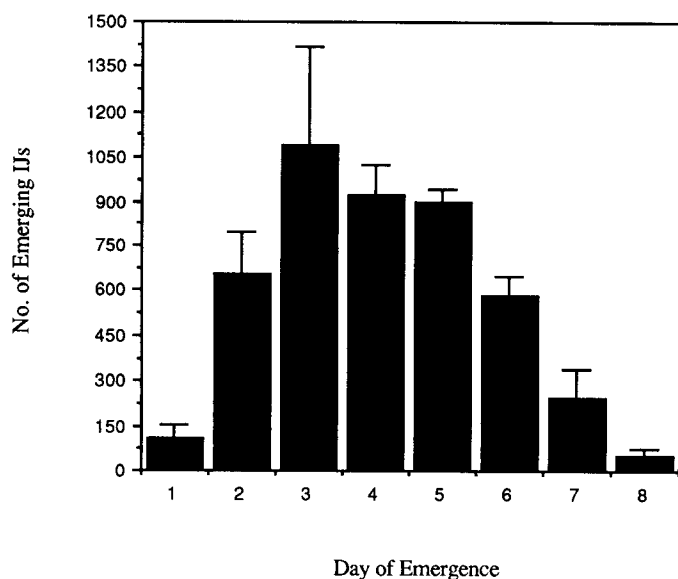
FIGURE 1.   Typical pattern of emergence of *Heterorhabditis megidis* strain UK211 IJs showing the mean number of IJs that emerge per *Tenebrio molitor* cadaver on each day of emergence. These data are based on IJ emergence from 5 White traps, each containing 5 *T. molitor* cadavers. The mean number of IJs to emerge was 35,670 ± 1,459 (SE) per *T. molitor* cadaver.

emergence groups. Fifty milliliters of 2% agar were poured into a 9-cm petri dish and allowed to set on a level surface. The lid was then placed on the petri dish, and the dish was sealed with parafilm. The center of the lid contained an access port (1 cm in diameter) which could be sealed with masking tape. The lid also contained 2 openings (4 mm in diameter) on opposite sides and of equal distance (3.5 cm) from the center of the access port. Into each of these openings was placed a 1-ml disposable pipette tip containing steel wool near the tip of each. One pipette tip (the host tip) also contained 2 *G. mellonella* larvae. The second tip did not contain insect larvae (control tip). Both tips were sealed with parafilm at their top end. Once the test dishes were prepared they were incubated for 1 hr at 20 C so that a host chemoattractant gradient could form. The IJs respond to CO_2 produced by the larvae in the host tip (Gaugler et al., 1989). IJs (~1,000) were then introduced through the access port by first vacuum filtering them onto a filter paper disc to form a clump and then applying clumps of equal size to the assay plates. The test dishes were incubated in a styrofoam box at 20 C for 1 hr to allow IJ migration in response to host cues. The response of the IJs to the test was scored by determining the percentage of IJs in the following 5 regions of 9 replicate plates, i.e., those that (1) accumulated under the host tip in a 1-cm-diameter area, (2) accumulated under the control tip in a 1-cm-diameter area, (3) displayed no net movement, i.e., IJs positioned in a strip 1 cm in width running under the access port and perpendicular to the pipette tips, (4) responded positively to the host, i.e., IJs located in that half of the plate containing the host tip (IJs at the host tip were included, but IJs in area 3 were excluded), and (5) responded negatively to the host, i.e., IJs in

that half of the plate containing the control tip (IJs at the control tip were included but IJs in area 3 were excluded). To facilitate counting of IJs in the different regions, the agar regions representing each test area were removed using a scalpel and were rinsed separately into conical tubes to recover the IJs.

When investigating the host-finding abilities of the 3 emergence groups over time, the same procedure was followed. However, not all IJs survived the storage period. In all cases, only living IJs were counted.

IJ dispersal on agar plates

IJs were applied as described for the host-finding bioassay to the center of 9-cm petri dishes containing 2% agar. The dishes were incubated in a styrofoam box at 20 C. After 60 min, the agar from 5 replicate dishes was divided into 3 concentric rings of the same width (1.5 cm), i.e., the inner, middle, and outer rings. These rings were cut out with a scalpel and were rinsed separately into conical tubes to recover the IJs.

IJ-induced host mortality

A test described by Griffin and Downes (1991) was used to compare the ability of IJs from the 3 emergence groups to induce host mortality. Three layers of Whatman no. 1 filter paper were placed in the base of a 9-cm petri dish. Three milliliters of a suspension containing 1,000 IJs was pipetted evenly onto the filter paper. Twenty *T. molitor* larvae were added to the petri dish. Three milliliters of water without IJs was added to control dishes. Following incubation at 20 C for 24 hr the *T. molitor* larvae were washed with tap water and dried carefully to remove IJs from their surface before returning them to 20 C. The number of dead *T. molitor* larvae was counted in each of 10 replicate dishes for each emergence group 3 days PI.

IJ motility

Motility of IJs was assessed by observing their number of body waves per minute at room temperature. Fifteen IJs in 100 ml tap water were transferred to a watch glass on a microscope table at room temperature and allowed to settle for 5—10 min. The number of body waves per minute of each worm was recorded in the absence of the microscope light source.

Statistical analysis

Data are presented as the mean ± SE. ANOVA was performed on normalized data. The data were normalized using an arcsine transformation for proportions. The significance of difference was tested using Duncan's multiple-range test at $P < 0.05$ significance level.

RESULTS

IJ emergence from the insect cadaver

IJs began to emerge from *T. molitor* cadavers 20 days PI. The majority of IJs emerge from day 2 to day 6. No IJs emerged after day 9 (Fig. 1). The mean number of IJs of *H. megidis* that emerged per *T. molitor* cadaver was 35,670 ± 1,459. From Table I it can be seen that IJ length is correlated to the time at which the IJ emerged from the cadaver. IJs from group A (early emerging IJs) were significantly longer than IJs from groups B

TABLE I.   Body length (μm) of IJs of *Heterorhabditis megidis* strain UK211 from 3 emergence groups when cultured in vivo in either *Tenebrio molitor* or *Galleria mellonella* larvae. IJs from the first 3 days of emergence were pooled to form group A, IJs that emerged on days 4 and 5 were pooled to form group B, and IJs from the last 3 days of emergence were combined to form group C. Data are presented as the mean body length of 40 IJs ± SE. Means within a row followed by the same letter are not significantly different (Duncan's multiple-range test, $P < 0.05$).

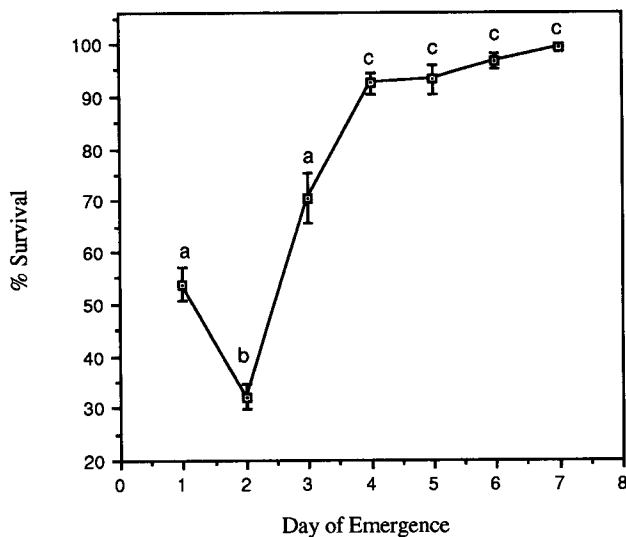| Insect host	Emergence group		
	A	B	C
T. molitor larvae	815.1 ± 15.5 (a)	752.5 ± 17.4 (b)	771.3 ± 12.2 (b)
G. mellonella larvae	845.4 ± 9.25 (a)	803.7 ± 12.5 (b)	811.6 ± 10.5 (b)

FIGURE 2. Percentage survival of *H. megidis* IJs from different days of emergence when subjected to desiccation at 57% RH for 180 min. Five separate batches of IJs were tested, each batch derived from a single White trap containing 10 cadavers. Five replicate samples of 200 IJs were assayed for each batch for each day of IJ emergence. The mean percentage survival for each batch was calculated and then the overall mean value was calculated from 5 batch means.

and C (groups that emerge later in the infection process) whether IJs were raised in vivo in *G. mellonella* or *T. molitor*.

Environmental tolerances of IJs that emerge from cadavers on different days PI

IJs of *H. megidis* were harvested daily and IJs were subjected, on their day of emergence, to desiccation at 57% RH for 180 min (Fig. 2). The results demonstrate that the desiccation tolerance of these IJs is correlated with the day of emergence and that the IJs that emerge during the last 4 days of emergence are the most tolerant to desiccation. For subsequent experiments IJs were organized into 3 groups based on their day of emergence as described in the Materials and Methods section. Figure 3 shows the survival of IJs from the 3 emergence groups when subjected to 4 different temperature regimes, i.e., −15 C for 35 min, 2 C for 168 hr, 30 C for 168 hr, and 37 C for 135 min. The tolerance of IJs from group C for 3 of the temperature regimes, i.e., 2, 30, and 37 C, is significantly lower than that of IJs from the first group to emerge (group A). However, the tolerance of IJs to subzero temperatures (−15 C) appears to be similar for all groups. Longevity tests demonstrated that IJs from group B persisted longer than IJs from groups A and C (Fig. 4).

Host-finding ability of IJs from the 3 emergence groups

Host-finding assays demonstrated that significant differences existed between IJs from the 3 emergence groups. From Figure 5A it can be seen that IJs from group A did not disperse well, i.e., ~55% of the IJs displayed no net movement, but of those that did, significantly more responded positively to the host than responded negatively. Similarly for IJs from group B, significantly more IJs responded positively to the host than responded negatively. However, a different response pattern was observed
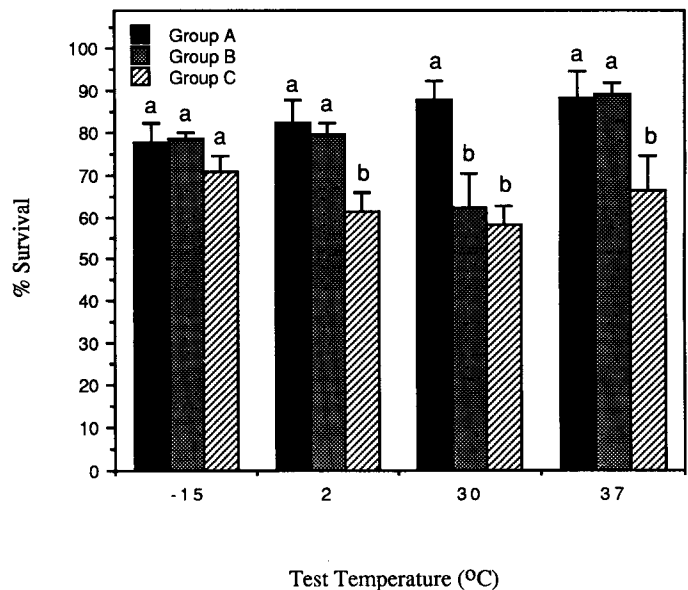

Test Temperature (°C)

FIGURE 3. Survival of *H. megidis* IJs from the different emergence groups exposed to −15 C for 35 min, 2 C for 240 hr, 30 C for 168 hr, and 37 C for 135 min. Each point is the mean ± SE of 5 replicates. For each test temperature, means followed by the same letter are not significantly different (Duncan's multiple-range test, $P < 0.05$). IJs from the first 3 days of emergence were pooled to form group A, IJs that emerged on days 4 and 5 were pooled to form group B, and IJs from the last 3 days of emergence were combined to form group C.

for IJs from group C. These late-emerging IJs appear to disperse further than those of groups A and B, but those that responded positively to the host did not significantly differ from those that responded negatively to the host. Figure 5B shows the propor-
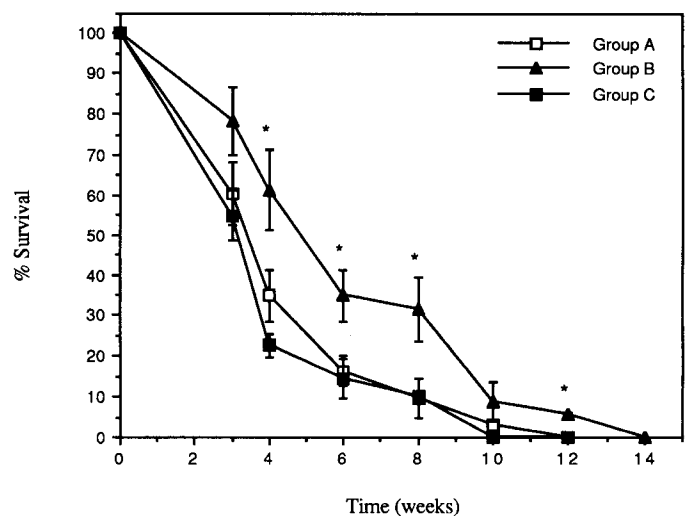

Time (weeks)

FIGURE 4. Longevity of IJs of *H. megidis* from different emergence groups during storage in petri dishes (concentration of ~5,000/ml) at 20 C. Each point is the mean ± SE of 3 replicates. For each time point, an asterisk indicates that the value for group B is significantly different from the corresponding values of both group A and group C (Duncan's multiple-range test, $P < 0.05$). IJs from the first 3 days of emergence were pooled to form group A, IJs that emerged on days 4 and 5 were pooled to form group B, and IJs from the last 3 days of emergence were combined to form group C.
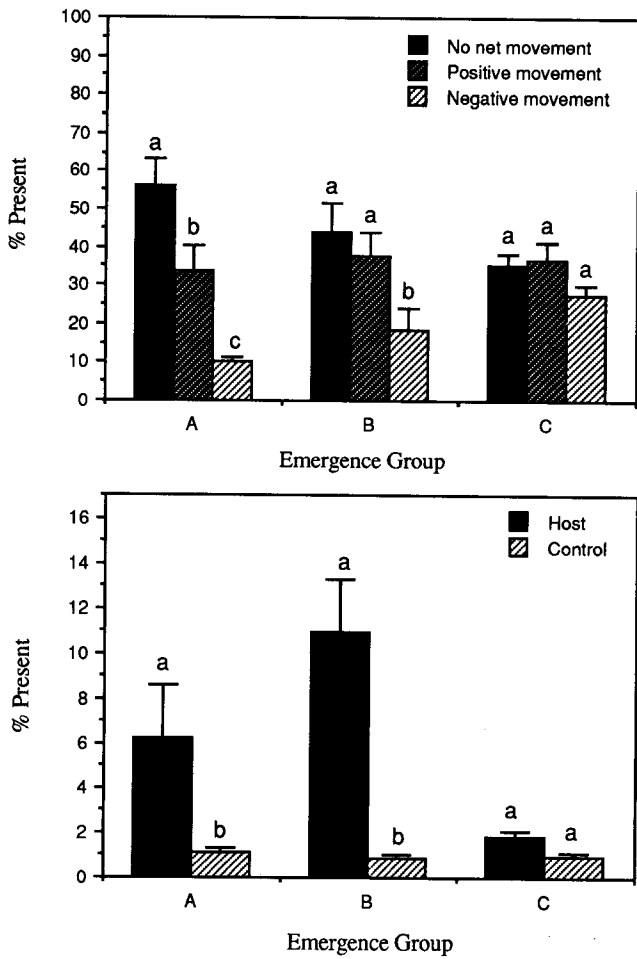
FIGURE 5.   The host-finding ability of *H. megidis* IJs from different emergence groups. **A.** The mean percentage ± SE (n = 9) of IJs from each emergence group that displayed either no net movement, migrated positively toward the host, or migrated negatively away from the host. **B.** The mean percentage ± SE (n = 9) of IJs from each emergence group located at the host and at the control tips. Statistical analysis was performed within each group. Means followed by the same letter are not significantly different (Duncan's multiple-range test, *P* < 0.05). IJs from the first 3 days of emergence were pooled to form group A, IJs that emerged on days 4 and 5 were pooled to form group B, and IJs from the last 3 days of emergence were combined to form group C.
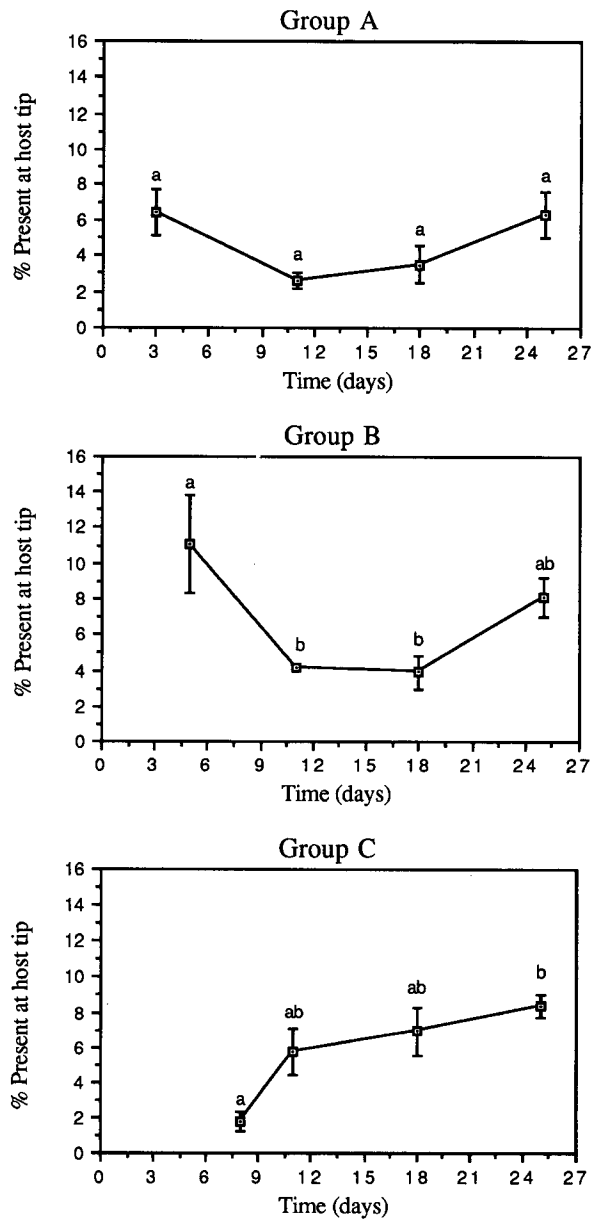

FIGURE 6.   Effect of storage at 20 C on the host-finding ability of *H. megidis* IJs from different emergence groups. The percentage of IJs found at the host tip ± SE of 5 replicates is shown. In all cases, less than 1% of IJs were found at the control tip. The time axis starts from the first day of emergence so that the day in which each batch was first tested depends on their time of emergence. IJs from the first 3 days of emergence were pooled to form group A, IJs that emerged on days 4 and 5 were pooled to form group B, and IJs from the last 3 days of emergence were combined to form group C.

tion of IJs at the host and control tips in the same bioassay. IJs from groups A and B were the best host finders with significantly more IJs being present at the host than at the control. However, IJs from group C were not good host finders, there being no significant difference between those at the host and those at the control.

Storage of IJs of the 3 emergence groups at 20 C affects their host-finding abilities (Fig. 6). The host-finding ability of IJs from group B decreases with time and then increases again during an 18-day period. The IJs from group A display a trend similar to group B in their host-finding, but the values do not differ significantly from each other, suggesting that the response of group A to the host did not change with time. IJs from group C, whereas not accumulating at the host tip upon emergence, improve in host-finding ability after a few days storage at 20 C. Eventually, after 18 days storage, a 4-fold increase in the

proportion of late-emerging IJs (group C) accumulating at the host tip was observed.

The dispersal activity of IJs from the 3 emergence groups in the absence of a host

The greater dispersal of IJs from group C observed in the host-finding assay was also observed on agar in the absence of host cues (Fig. 7). Significantly more IJs from group C were
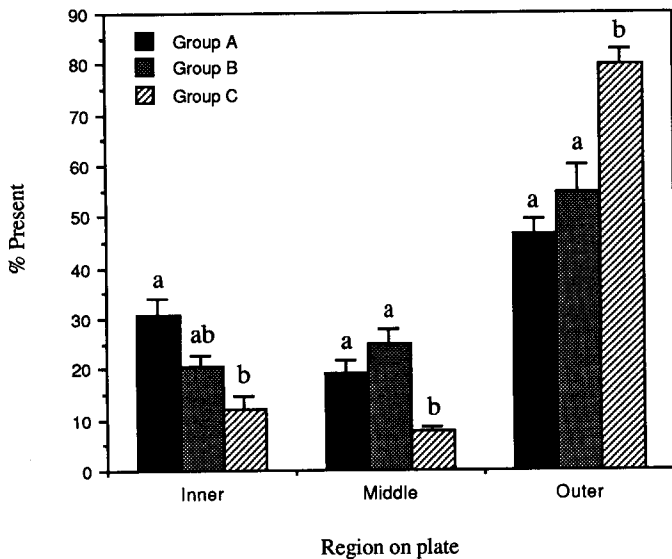
FIGURE 7. The mean dispersal activity ± SE (n = 5) of *H. megidis* IJs from different emergence groups on agar plates in the absence of host cues. IJs from the first 3 days of emergence were pooled to form group A, IJs that emerged on days 4 and 5 were pooled to form group B, and IJs from the last 3 days of emergence were combined to form group C. IJs were applied at the center of each dish and their position on the dishes was recorded after 60 min. For each plate sector, means followed by the same letter are not significantly different (Duncan's multiple-range test, $P < 0.05$).

found in the outer segment of the dish than IJs from group A and group B. The motility of IJs in water from the 3 emergence groups was also measured. IJs from group A displayed significantly fewer body waves per minute than IJs from groups B and C (group A, mean number of body waves/min = 13.53 ± 1.4 (a); group B, mean = 24.42 ± 2.9 (b); group C, mean = 27.16 ± 3.0 (b); means followed by the same letter are not significantly different, Duncan's multiple-range test, $P < 0.05$). The data for IJ motility correlate well with the data obtained for IJ dispersal. Thus, late-emerging IJs that had the greatest dispersal activity also displayed the highest number of mean body waves per minute, whereas the earliest IJs to emerge had the lowest number of mean body waves per minute and dispersed least into the outer segment of the test arena (Fig. 7).

IJ-induced host mortality

Despite the fact that significant differences in host-finding ability were recorded between each of the 3 different emergence groups (Fig. 5), no significant difference was found between the ability of IJs from the 3 emergence groups to cause insect mortality (group A, mean of dead *T. molitor* larvae = 16.83 ± 1.1 (a); group B, mean = 15.07 ± 3.6 (a); group C, mean = 17.32 ± 0.8 (a); means followed by the same letter are not significantly different, Duncan's multiple-range test, $P < 0.05$). The assay was very simple, with *T. molitor* larvae moving over moist filter paper containing IJs. Host mortality in this case is likely to depend largely on the IJs' ability to move and infect rather than the IJs' ability to host find.

DISCUSSION

IJs are the only stage of *Heterorhabditis* that occurs outside the host, and their function is to locate a new host in the soil.

IJ survival in the soil depends on their energy reserves, their metabolic rate, their environmental tolerances, and the biotic and abiotic soil environment. Womersley (1993) proposed that changing conditions within the insect cadaver as the numbers of IJs increase could affect the quality of the IJ produced. He postulated that IJs produced during the early stages of the infection process and benefiting from optimal conditions in the insect cadaver may be more robust, may be capable of surviving for long periods of time, and may possibly be adapted to remain dormant for a set period before becoming infective, whereas IJs produced later in the infection cycle and under more crowded conditions in the cadaver might not be well adapted for long-term survival but might be highly infective upon emergence from the cadaver.

The results that we present here support Womersley's (1993) hypothesis that the behavior and physiology of IJs are likely to be affected by the stage in the infection cycle at which they are formed. We show that the first IJs to emerge from the cadaver have poor desiccation tolerance, poor locomotor activity, and poor dispersal behavior, both in the presence and absence of a host. However they are responsive to host cues and accumulate at the host. Thus, the role of these IJs may be to quickly infect readily available hosts or to wait in the soil near the cadaver from which they have emerged until a suitable host comes within the range in which they can detect it. The last IJs to emerge are more desiccation tolerant, more active, and disperse well. In contrast, they are poor host finders, neither responding positively to the host nor accumulating at the host. However, their host-finding ability improves with increased duration of storage. Thus, their function may be to disperse in the soil, possibly not detecting host cues at least in the early stages. Lewis and Gaugler (1994) have also observed that later-emerging IJs of *S. glaseri* were more mobile and less responsive to host cues than early-emerging IJs and suggest that later-emerging *S. glaseri* IJs may be adapted for greater dispersal than are the early-emerging IJs. We have not demonstrated here that the IJs produced early in the infection process, i.e., the IJ progeny of first- and second-generation females, are actually the first IJs to emerge from the cadaver. However, the negative linear relationship between the body length of the emerging IJs and their time of emergence from the cadaver that was observed by Nguyen and Smart (1995) and also herein are suggestive of such a relationship. The increased length of the earlier emerging IJs that we have observed is likely to result from the quality of the nutrients available in the cadaver early in the infection process. It may be that increased IJ body length is related to increased food reserves and that the early-emerging IJs might be expected to display increased activity, dispersal, and/or environmental tolerance. However, we have observed the converse and because it is the IJs from group B (midcycle emergers) that display the longest life-span, it can be concluded that a complex association exists between longevity, adequacy of food reserves, behavior, and environmental tolerance of IJs.

From their studies on *Steinernema feltiae*, Bohan and Hominick (1995) concluded that individual IJs may be either infectious or noninfectious and thus all IJs should not be considered equivalent. Fan and Hominick (1991) demonstrated that the infectivity pattern of *Steinernema* spp. at 15 C after storage at 5 C displayed a U-shaped curve, i.e., infection levels were high initially, then infection levels decreased, but after several weeks

storage, the number of IJs infecting the *G. mellonella* larvae increased back to the original level. One explanation, based on the data for *Heterorhabditis* presented here, might be that this curve is related to the day of emergence of the IJs. The first IJs to emerge could be responsible for the initial infectivity that then decreased with increasing length of storage of the IJs. The increase in infectivity observed after several weeks storage might be due to the activation of the last IJs to emerge to host find. Our results show that the host-finding ability of the first IJs to emerge decreases with storage but then improves again and that the host-finding ability of the last IJs to emerge from the cadaver improves with time, thus giving a U-shaped curve. Similarly, Griffin (1996) observed an increase in infectivity of the UK211 strain of *H. megidis* with time during storage in tap water at 20 C using a *G. mellonella* sand bioassay. Whereas we observed differences in the host-finding ability of IJs from the 3 groups, no significant differences were found among the 3 groups as regards nematode-induced host mortality. However, this is probably related to the nature of the infectivity test used in our study (petri dish bioassay) that simply measures the ability of IJs to enter the host and cause insect mortality.

The work of Stuart et al. (1996) indicates that genetic variability for delayed emergence exists in natural populations of *S. glaseri,* and the authors note that the adaptive value of a particular IJ emergence pattern could be quite variable and may reflect the relative reproductive success of IJs emerging at different times from the parasitized cadaver. The magnitude of the relative success rate for early- and late-emerging IJs is likely to be variable and to depend on the temporal and spatial distribution of hosts in the local environment. Stuart et al. (1996) postulate that such variability in the adaptive value of a particular emergence pattern could maintain genetic variability for this trait in the general population.

In recent years, there has been a renewed interest in the phenomenon of phenotype plasticity, i.e., the ability of a single genotype to produce more than 1 alternative form of morphology, physiological state, and/or behavior in response to an environmental cue (see reviews by West-Eberhard, 1989; Stearns, 1989; Scheiner, 1993; Via et al., 1995). A number of studies have demonstrated the existence of genes that, when switched on during development in response to a particular environmental condition, trigger the expression of a particular phenotype. Animal examples include temperature-dependant sex determination in reptiles (Crews et al., 1994), spine induction in *Daphnia* in response to premediated cues (Herbert and Grewe, 1985; Parejko and Dodson, 1991), and aphid life cycles in which individuals switch between sexual and asexual reproduction (Weiser and Stadler, 1994). Moran (1992) analyzed the conditions under which polyphenism is maintained by natural selection. She concluded that a spatially or temporally variable environment that results in the reversal of the relative fitnesses of alternative phenotypes is a prerequisite for the evolution of adaptive plasticity. Allied to this is the requirement for an environmental cue that can be accurately correlated with the future selective environment. Thus, there are many examples of seasonal polyphenisms where selective factors such as a decrease in temperature or food availability can be reliably predicted by the photoperiod. Stuart et al. (1996) point out that early-emerging IJs have the best opportunity of locating and infecting nearby insect hosts, whereas IJs that emerge later from

the cadaver may have to disperse farther or wait longer before encountering additional hosts. It is likely that a density-dependent environmental cue within the insect cadaver may be a reliable predictor of these alternative states. Potential cues might be the nutrient status of the insect cadaver, the accumulation of metabolites from the breakdown of the host's tissues, or from the metabolism of the symbiont bacteria and possibly by the buildup of a dauer-inducing pheromone similar to that produced by the free-living nematode *C. elegans* (Golden and Riddle, 1984a, 1984b). Because the reproductive strategy of *Heterorhabditis* tends toward inbreeding (Downes, 1995; Wang and Bedding, 1996), phenotypic plasticity rather than allelic variability may provide the genetic basis for the different physiological and behavioral phenotypes of early- and late-emerging IJs. These alternative hypotheses could be tested using isogenic lines of *H. megidis.*

Finally, the results presented here, which clearly indicate that there are differences between IJs from different days of emergence, have important implications for the choice of IJs for bioassays. Using pooled samples from the total number of IJs to emerge from cadavers should minimize variance between batches in studies on the environmental tolerances, behavior, and infectivity of EPN IJs.

ACKNOWLEDGMENTS

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