Phased activity in *Heterorhabditis megidis* infective juveniles

C. M. DEMPSEY* *and* C. T. GRIFFIN

Institute of Bioengineering and Agroecology, and Department of Biology, National University of Ireland, Maynooth, Co. Kildare, Ireland

(*Received 5 July 2001; revised 26 November 2001 and 14 January 2002; accepted 14 January 2002*)

SUMMARY

The infectivity of *Heterorhabditis megidis* infective juveniles (IJs) increases during storage in water. We investigated whether this change can be related to other features of the IJs' behaviour. IJs were stored in water for 4 weeks at 20 °C, and the following parameters were assessed at intervals: infectivity for *Galleria mellonella*, dispersal in sand, host-finding on agar, and the percentage of IJs active in water. In addition, the behaviour of the IJs in water was described using 7 categories. Immediately after emerging from the host cadaver, IJs were highly active (99 % of IJs in water were active and 65 % displayed 'waving', the normal method of forward movement). Maximum responsiveness to host volatiles in an agar plate assay was recorded on day 2 (69 % of IJs moved from the point of application and 44 % of all IJs in the agar arena moved towards a host) and maximum dispersal in sand (5·8 cm) on day 0. These tendencies declined gradually with age, while infectivity underwent a significant increase from 11 nematodes per insect on day 0 to 38 nematodes per insect on day 9. Three phases could be distinguished in the behaviour of *H. megidis* IJs: an initial dispersal phase, during which infectivity was low; an infective phase, during which dispersal tendency was declining, and a third phase during which all behaviours (dispersal, infectivity and activity) were declining. Over the 4-week storage period, infectivity of *H. megidis* IJs was correlated ($R^2 = 0{\cdot}83$) with the percentage time IJs engaged in 'head thrusting' (a behaviour that resembles penetration). There is no evidence that the observed increase in infectivity of *H. megidis* strain UK211 could be accounted for by a generally greater level of motor activity, nor by an increase in responsiveness to volatile host cues, and it is suggested that it is due to an increased tendency to attempt penetration.

Key words: behaviour, ageing, entomopathogenic nematodes, *Heterorhabditis megidis*, infectivity, foraging.

INTRODUCTION

The infectivity of non-feeding infective juveniles (IJs) of parasitic nematodes typically declines over time due to a reduction in energy stores, principally the lipid and carbohydrate reserves (Croll & Matthews, 1973; Storey, 1984; Patel, Stolinski & Wright, 1997). However, for the entomopathogenic nematodes, both heterorhabditids and steinernematids, changes in infectivity occur prior to the eventual decline associated with starvation (Fan & Hominick, 1991; Curran, 1993; Ishibashi, Wang & Kondo, 1994; Griffin, 1996; Bohan & Hominick, 1997). Two hypotheses have been proposed to explain this so-called 'phased infectivity'. The first hypothesis is based on the assumption that there are two subsets of the IJ population: infectious and non-infectious (Hominick & Reid, 1990; Bohan & Hominick, 1994, 1995*a*, *b*, 1996, 1997). A non-infectious IJ is one which, at the time of testing, will not parasitize a host even when given unlimited access. According to this model, fluctuations in infectivity result from changes in the proportion of non-infectious IJs (Bohan & Hominick, 1997). The presence of a non-infectious proportion can be detected by the continued failure of IJs to infect no matter how the test conditions are manipulated (e.g. by successive exposure to several hosts or by simultaneous exposure to high host densities). Campbell *et al.* (1999) found evidence of a temporarily non-infectious proportion in *Heterorhabditis bacteriophora*, but not in 3 *Steinernema* species. An alternative hypothesis of phased infectivity proposed by Griffin (1995, 1996) assumes that the infectious individuals are not all physiologically and behaviourally identical, but that they vary in their infectivity (probability of penetrating a host per unit time). Fluctuations in the infectivity of a population may reflect underlying changes in the infectivity of individuals – either their ability or motivation to infect an insect. Phased infectivity *sensu* Griffin will not be detected where the nematodes have unlimited access to hosts, as in such circumstances all infectious individuals, even those with low infectivity, will eventually penetrate an insect.

For *H. megidis*, an increase in infectivity occurred during the second or third week after emergence from the host cadaver when IJs were maintained at

* Corresponding author. Tel: +353 1 6285222 ext. 3136. Fax: +353 1 7083845. E-mail: catherinedempsey@hotmail.com

20 °C and tested in an assay with restricted access to hosts (Griffin, 1996; Boff, Wiegers & Smits, 2000). Griffin (1996) proposed that low infectivity of these nematodes was associated with a lower likelihood of each IJ infecting within the time-period of the assay, and that the observed increase in infectivity of the population represented an increase in infective capabilities rather than a switch from non-infectious to infectious status. This might entail an increase in general locomotor activity or improved responsiveness to the host (Griffin, 1996). The objectives of the present study were to test whether either of these parameters was correlated with infectivity of ageing *H. megidis* IJs, and to see how the changes in infectivity fit into the overall behavioural strategy of the species. To do this we examine changes in the behaviour of the IJs following emergence from the host cadaver before, during and after the increase in infectivity. The infectivity assay employs the same host- and time-limited conditions as previously described (Griffin, 1996), and hence cannot distinguish between changes in the non-infectious proportion and changes in infectivity (infective tendency) of individuals. The free-living IJ is the dispersal and host-finding stage for entomopathogenic nematodes: the migration rate both in the presence and absence of a host is therefore ecologically important and assessments of migration were included in this study. In addition, direct observations were made of the behaviour of IJs.

A wide variety of behavioural actions has been described for nematodes. With very few exceptions, nematodes move by sinusoidal or undulatory propulsion: waves are propagated in the anterior end of the worm and move backwards along the length of its body causing the nematode to move forward (Croll, 1970). Nematodes can stop during forward movement and move backward using waves that are propagated at the tail. These waves are often more rapid than the ones that propel the nematode forward and are of short duration (Croll & Matthews, 1977). Other forms of activity include head lifting, coiling, nictation and penetration behaviours. Nematodes, particularly infective juveniles, may spend periods of time inactive. Inactivity can result from lack of stimulation, lack of energy and sensory habituation. Environmental factors such as dehydration, cold, lack of oxygen and high osmotic pressure may also inactivate nematodes (Van Gundy, 1965; Croll, 1970). Inactivity in some nematodes is characterized by distinct postures or assumed shapes. Steinernematid and heterorhabditid IJs tend to become straight when inactive (Ishibashi & Kondo, 1990).

To investigate behavioural changes in ageing *H. megidis* IJs, they were stored for up to 4 weeks after emergence from the host cadaver and, at intervals, their infectivity, migration rates and responses to host volatiles were measured and their behaviour (including activity levels) in water was recorded.

MATERIALS AND METHODS

H. megidis isolate UK211 IJs were cultured in last instar larvae of the greater wax moth (*Galleria mellonella*) at 20 °C (Woodring & Kaya, 1988). IJs began to emerge approximately 14 days after infection. Those emerging prior to day 21 were harvested and discarded. Those emerging over the following 24 h were harvested and washed 3 times by sedimentation in tap water to obtain a batch of uniform age. An SDS (sodium dodecyl sulphate) test (Cassada & Russell, 1975) was carried out on freshly harvested nematodes (on day 0) to confirm that they were IJs. Nematodes were incubated in 1 % SDS at 20 °C for 24 h and the numbers dead and alive were counted; 97 % survived this treatment. Washed IJs were stored at a concentration of 1000 IJs/ml in 8 ml of water in 5·5 cm Petri dishes at 20 °C. Tests were carried out on IJs as they aged over a 4-week period. Tests were conducted twice weekly from the day of harvest (day 0). On each test day the contents of 3 dishes were combined and the concentration adjusted to 1000 live IJs/ml. IJ mortality was monitored during this time and never fell below 86 % in 4 populations tested.

Infectivity assay

The assay arena consisted of a plastic vial (40 mm height × 45 mm diam.) packed with moist sand (Griffin, 1996). The sand was sieved and washed, and the fraction retained between sieves of 160 μm and 425 μm pore size was used. This was heat sterilized at 120 °C overnight, and then moistened with 8 % (w/w) tapwater. One late instar *G. mellonella* larva was placed at the bottom of the vial which was then packed with sand. Vials were capped and thermoequilibrated at 20 °C for 2 h. Following thermoequilibration, each vial received 100 nematodes in 100 μl of tap water pipetted to an indentation in the sand. On each test date, 15 replicate vials received nematodes and 5 vials received 100 μl of tap water. The vials were incubated at 20 °C for 16·5 h. After this time, the insects were removed from the vials and washed in 3 changes of tap water to remove any nematodes adhering to the outside of the insect, and then dried in paper towelling. The insects were incubated at 20 °C for 24 h. After this time, they were placed onto moist towelling paper and incubated for a further 4 days. Any insects still alive after this time were discarded and the number of infective juveniles entering them was assumed to be zero. Mortality of insects which received water only was always zero. The number of first generation adults, assumed to represent the number of IJs that had entered each insect, was determined by dissection of the insects 5 days after their removal from the sand. This experiment was repeated 7 times.

http://dx.doi.org/10.1017/S0031182002001609

Table 1. Designated behaviour categories with a brief description of each

Behaviour	Description
Immobility	No movement.
Waving	A sine wave passes backward along the nematode's body as the anterior end swings through 180° causing the nematode to be propelled forward, usually a smooth sigmoid movement.
Body movements	Movements involving the nematode's whole body, including twitching and curving into a C-shape; no fluid forward motion is achieved.
Coiling	IJs actively coil and uncoil.
Headlifting	The anterior end of the nematode is lifted off the substrate.
Reversewave	A sine wave passes forward along the body causing the IJ to reverse away from its previous position.
Head thrusting	Repeated pushing or probing by the anterior end of the nematode against the substrate. The posterior half of the body may be raised from the substrate.

Behaviour in water

A suspension of IJs (17·5 μl of 1000 IJs/ml) was placed in a 16 mm^2 well in a 24-well cloning plate (92 × 136 mm, Sterilin). The plate was placed on the stage of a binocular microscope and allowed to acclimatize for 5 min. Nematodes were filmed using a MTI series 68 (Dage MTI Inc., Michigan, USA) camera mounted on a Nikon SMZ-V binocular microscope (Nikon Ltd, Japan) at a combined magnification of 30×. Illumination was from an Intralux 4000-1 fibre optic (cold) light (Volpi, Switzerland). Nematodes were videotaped for 10 min for subsequent analysis.

Two measures of behaviour were made. Firstly, the proportion of nematodes which were active was recorded at the beginning of each recording (10 replicates per experiment). Secondly, the behaviour of individual IJs was analysed for 1 min each (30 IJs per experiment). Seven different categories of behaviour were designated (Table 1). The duration of each behavioural category was recorded with the aid of a computer programme written using the True Basic package (True Basic Inc., 1986). Each bout length was timed to within 0·001 sec. Analysis was carried out 'blind' i.e. the observer did not know the identity of the treatment. This experiment was repeated 7 times.

Host finding on agar

The bioassay described by Gaugler, Campbell & McGuire (1989) was used to test the host-finding capabilities of IJs, with the exception that agar (2%) was poured into 5 replicate 9 cm diameter plastic Petri dishes and allowed to set on a level surface to a depth of approx. 3 mm. Dishes were incubated for 30 min at 20 °C to allow a gradient of host volatiles to form. A clump containing approximately 1000 IJs was then introduced through the access port. The test dishes were incubated at 20 °C for 30 min. Following this, the response of the IJs was scored by determining the percentage of IJs in each of the following sections on the plate: (1) a central strip 1 cm in width (containing IJs that displayed no net movement in either direction); (2) the half of the dish containing the host tip but excluding area (1) (IJs that responded positively to the host); (3) the half of the dish containing the control tip but excluding area (1) (IJs that responded negatively to the host); (4) the area (1 cm diam.) directly under the host tip, and (5) the area (1 cm diam.) directly under the control tip. Each section of agar from the assay plate was removed using a scalpel and rinsed into a 5·5 cm diam. Petri dish using 10 ml of tapwater, and the total number of IJs in each section was determined.

Nematode migration in sand

Sand columns were used to assess migration through sand as described by Westerman (1992), with the following modifications. Each column (12 cm in height) consisted of 3 rings (4 cm height, 4·5 cm diam.). The tops of the columns were wrapped in Parafilm, whereas the bottom was covered with both Parafilm and aluminium foil. Washed sea sand (the fraction retained between sieves of 160 μm and 425 μm pore size) was used. Columns were thermoequilibrated for 2 h at the test temperature of 20 °C. After thermoequilibration each of 3 replicate columns received a dose of 2000 IJs in 0·5 ml of tapwater. The duration of the test was 4 h. The sand from each ring, the ring itself and any Parafilm used were washed with 50 ml of tapwater and the rinse water passed through a sieve (32 μm mesh). The number of IJs in the rinse water was counted. The mean migration distance of the IJs was calculated using a modified method of Westerman (1992). This experiment was repeated twice.

Statistics

Statistical analysis was performed using Minitab 12.1 (Minitab Inc., 1998). When the data were not normally distributed they were transformed using an arcsine transformation. Results of tests performed on different dates were compared using ANOVA (for agar assay and behaviour) or General linear model ANOVA (for infectivity). Where a significant ($P < 0·05$) F value was obtained, this was followed by a pair-wise multi-comparison procedure, Tukey's test ($P < 0·05$). If data could not be normalized a

http://dx.doi.org/10.1017/S0031182002001609

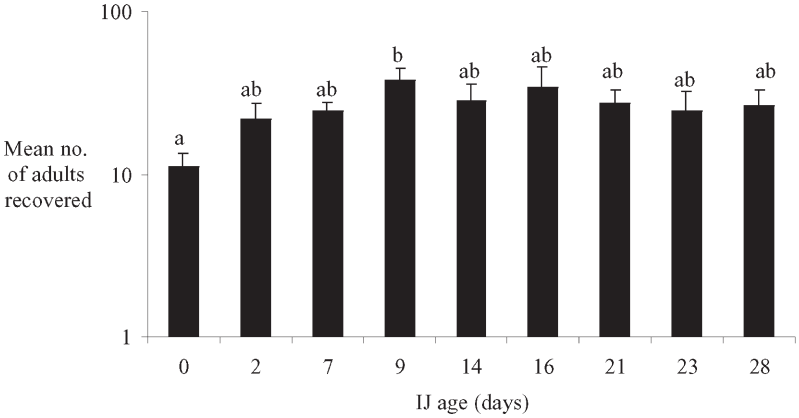

Fig. 1. The mean number (out of 100) of IJs of *Heterorhabditis megidis* of different ages infecting *Galleria mellonella* following exposure at 20 °C for 16·5 h. The same letters indicate no significant differences. Each bar represents the mean of 7 experiments ± S.E.M.

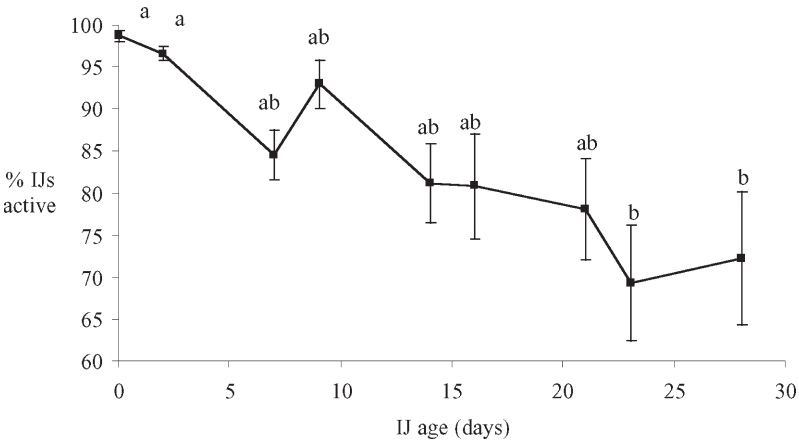

Fig. 2. Percentage of *Heterorhabditis megidis* IJs active at different ages. The same letters indicate no significant differences. Each point represents the mean of 4 experiments ± S.E.M.

non-parametric alternative (Kruskal-Wallis) was carried out. For comparisons between 2 test groups Student's *t*-test was carried out. Correlations between 2 sets of data were performed using the Pearson product moment.

RESULTS

Infectivity

The percentage of IJs establishing in insects rose from 11 on day 0 to 38 on day 9, a 3·5-fold increase in infectivity (Fig. 1). The difference between infectivity on days 0 and 9 was significant ($P < 0·05$). The percentage of IJs infecting fell thereafter, reaching 27 on day 28.

Behaviour in water

Nearly all of the IJs (99 and 97 % respectively) were active on day 0 and day 2 (Fig. 2). Activity rates decreased thereafter to approximately 70 % of IJs active on days 23 and 28. There was a significant ($P < 0·05$) difference between the percentage IJs active on the first two test days (0 and 2) and the last two test days (23 and 28).

The main behavioural categories expressed by the IJs were waving, body movements and immobility. Together they accounted for 72–85 % of the observation time (Fig. 3). The categories coiling, headlifting and reversewave remained at low level throughout the 4 weeks of storage, each of them typically below 5 % of the observation time. Head thrusting was also expressed at quite a low level, averaging less than 8 % of the total observation time.

Statistical analysis was carried out separately on each behaviour. Waving was initially the dominant behaviour but decreased ($P < 0·05$) from 66 and 68 % observation time on days 0 and 2, respectively, to 13–27 % observation time from day 14 onwards (Fig. 3). Waving was gradually replaced by immobility and less active behaviours particularly body movements. Time spent immobile increased significantly (Kruskal-Wallis, $P < 0·05$) from less than 1 % of the observation time on day 0 to over 25 % on day 28 (Fig. 3), and body movements increased ($P < 0·05$) from 10 and 12 % on days 0 and 2 respectively to 34–44 % from day 14 onwards (Fig. 3). Time spent headlifting remained the same ($P > 0·05$) throughout the test period. Coiling was at its highest level (7 %) on day 0, differing ($P < 0·05$) from day 2, day 14 and

http://dx.doi.org/10.1017/S0031182002001609

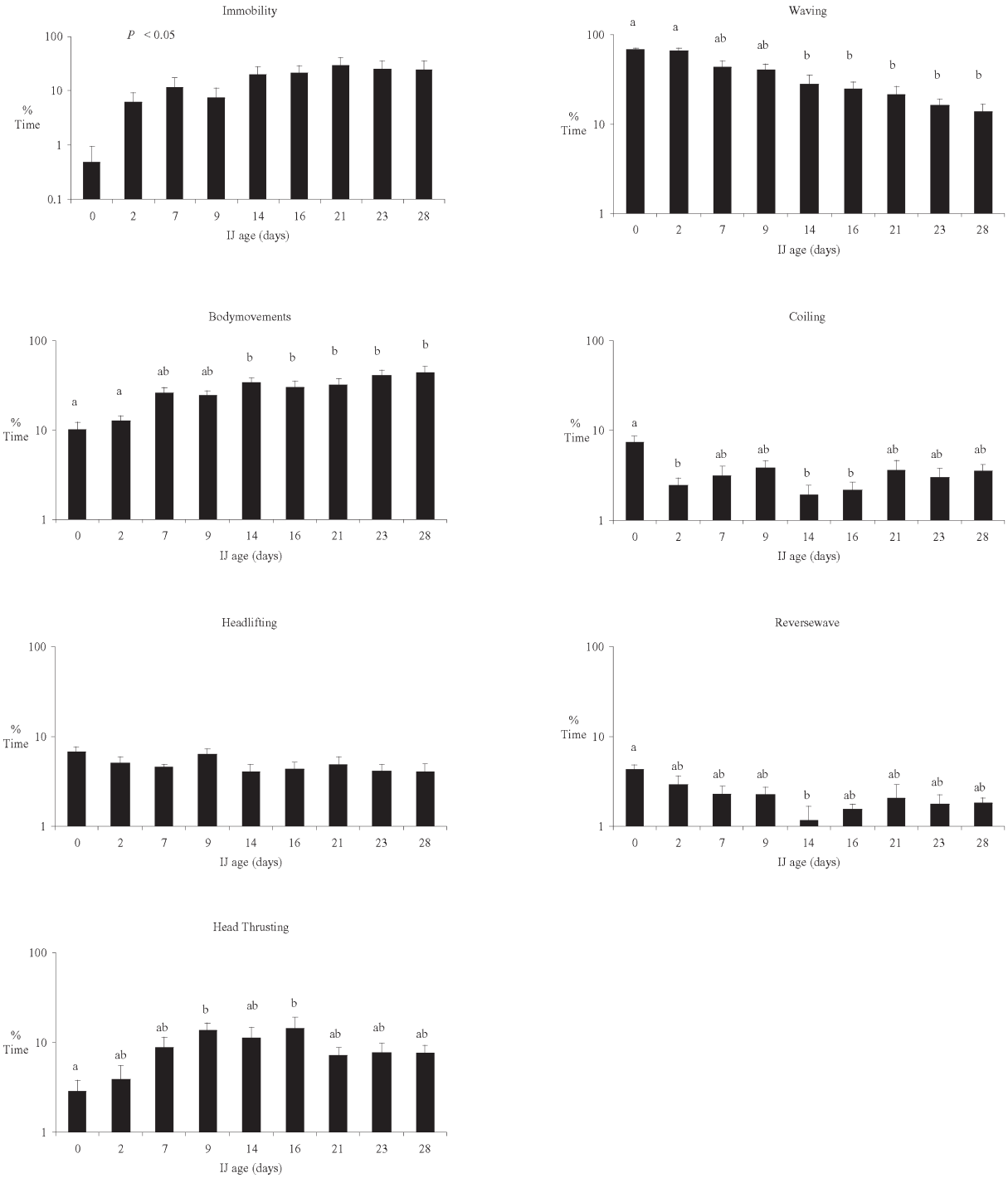

Fig. 3. Percentage of observation time IJs of *Heterorhabditis megidis* of different ages spent in designated behaviours: waving, immobility, body movements, coiling, reversewave, headlifting and head thrusting in water at 20 °C. The same or no letters indicate no significant differences. $P < 0{\cdot}05$ indicates a significant difference overall using a non-parametric test (Kruskal-Wallis). Each bar represents the mean of 7 experiments ± S.E.M.

day 16 (2–3 %). Reversewave declined ($P < 0{\cdot}05$) from 4 % on day 0 to 1 % on day 14.

Time spent head thrusting increased from 3 % of observation period on day 0 to 13·5 % on day 9 and 15 % on day 16 (Fig. 1). The difference between day 0 and day 9 was significant ($P < 0{\cdot}05$). Time spent head thrusting then declined to below 8 % from day 21 onwards. Time spent head thrusting followed a similar pattern to infectivity (Fig. 1). The two parameters were strongly correlated ($R^2 = 0{\cdot}83$, $P = 0{\cdot}001$). Both were at a maximum 9 and 16 days after emergence from the host.

Host finding on agar

Host finding on agar tended to decrease as the IJs of *H. megidis* aged (Fig. 4A and B). The total percentage of IJs moving in any direction from the

http://dx.doi.org/10.1017/S0031182002001609

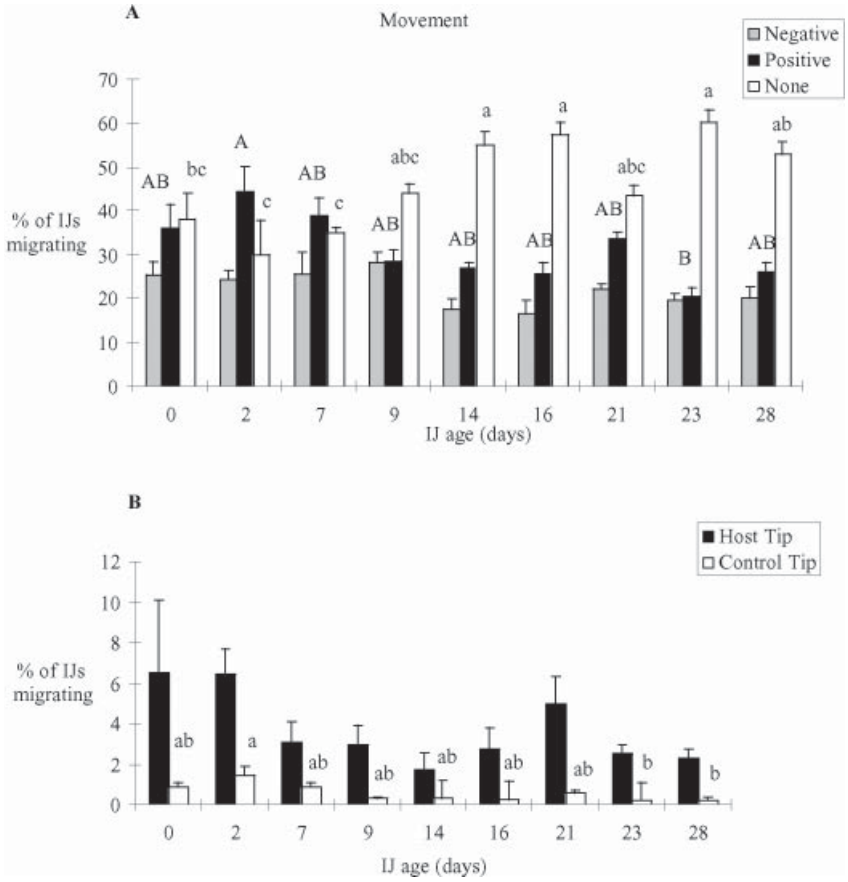

Fig. 4. (A) Mean percentage of IJs of *Heterorhabditis megidis* of different ages recovered from the host (positive), no host (negative) and no movement areas of an agar plate. The same letters indicate no significant differences. Each bar represents the mean of 5 replicates ± S.E.M. (B) The mean percentage of IJs of *H. megidis* of different ages recovered from the area under the host and control tips in the host finding migration assay. The same or no letters indicate no significant differences. Each bar represents the mean of 5 replicates ± S.E.M.

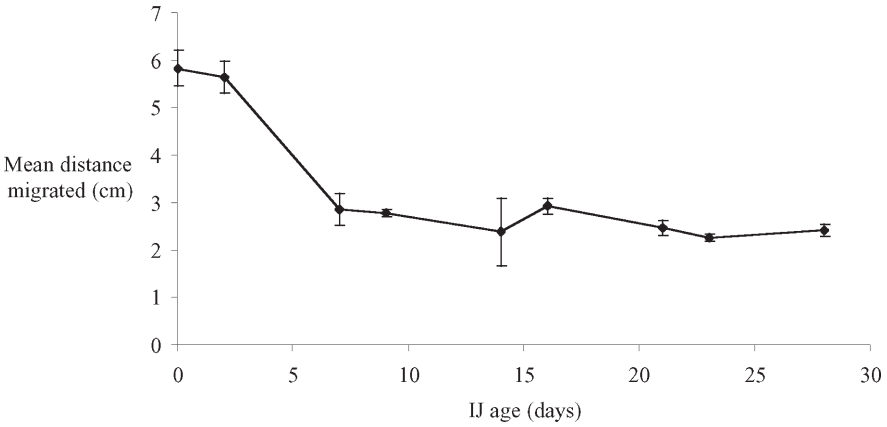

Fig. 5. The mean migration rate in sand of IJs of *Heterorhabditis megidis* of different ages. There was a significant difference ($P < 0{\cdot}05$) between test dates (Kruskal-Wallis). Each point represents the mean of 2 experiments ± S.E.M.

point of application dropped ($P < 0{\cdot}05$) from a maximum of 69 % on day 2 to 40 % on day 23. The decrease was due largely to a decline in the numbers moving to the host side of the arena, i.e. there was a decline in responsiveness to the host (Fig. 4A). The lowest percentage of IJs in the host side of the plate, 20 %, was recorded on day 23. This differed significantly from day 2 ($P < 0{\cdot}05$). No difference ($P > 0{\cdot}05$) was found in the percentage of nematodes found in the negative area of the plate over time (Fig. 4A). IJs accumulated preferentially under the host tip when compared to the percentage found under the control tip (Student's *t*-test, $P < 0{\cdot}05$) (Fig. 4B). However, the percentage of IJs found in these areas remained low, averaging 3·7 % and 0·6 % under the host tip and control tip respectively. There was a

http://dx.doi.org/10.1017/S0031182002001609

drop ($P < 0{\cdot}05$) in the number of IJs found underneath the control tip, from 1·5 % on day 2 to 0·2 % on days 23 and 28. There was no change ($P > 0{\cdot}05$) in the number of IJs found under the host tip.

Nematode migration in sand

The average distance IJs travelled in sand columns fell significantly ($P < 0{\cdot}05$) the longer they were stored (Fig. 5). The maximum distance migrated by IJs during the 4-h trial was 5·8 cm on day 0 and the minimum 2 cm on day 23.

DISCUSSION

The behaviour of *H. megidis* appears to be phased. Activity levels of the IJs were greatest within the first week of emergence from the insect host. This initial period was characterized by high levels of activity. Nearly all IJs were active in water and they spent a high proportion of time waving – the normal means of forward movement. There was also a high migration rate in sand and on agar, and greater migration to the host side than the non-host side of the agar arena. However, infectivity of the IJ population during this first week was low. This suggests that, on emergence from the host, the majority of the population of IJs are in a dispersive mode with only a small proportion infecting, at least under our time- and host-restricted assay conditions. This 'dispersive' phase was gradually replaced by a less-active but increasingly infective phase. The infective phase was characterized by a reduction in activity and migration behaviours. Coupled with this was an increase in infectivity. Eventually, both infectivity and activity declined somewhat towards the end of the 4-week test period, representing a third phase. As the IJs were stored in water a build up of excretory products may have affected their behaviour. However, infectivity did not differ between *H. megidis* IJs stored in the same manner as those used in these experiments and IJs stored in water which was frequently changed (Dempsey, 2000). Longer-term experiments (56 days) also showed no effect of changing the water on survival of *H. megidis* IJs (P. Dagg, unpublished data).

The increase in infectivity of *H. megidis* IJs following emergence from the host cadaver confirms the finding of Griffin (1996). Furthermore, the parallel records made here on ageing IJs give an indication of the specific behavioural changes contributing to the increase in infectivity. As both activity and specific (towards a host) and non-specific migration had already begun to decline as infectivity was increasing, neither a general increase in locomotory activity nor increased responsiveness to volatile host cues is likely to be the cause of the observed increase in infectivity with increasing storage time. There was, however, a tendency for the level of 'head thrusting' behaviour to follow the same pattern as infectivity as the IJs aged. In head thrusting, IJs push their anterior end against the bottom or the sides of the vessel containing them, and subjectively appear to be trying to penetrate it. Bedding & Molyneux (1982) described the ability of heterorhabditids to penetrate an insect cuticle using their dorsal tooth. They postulated that the high internal hydrostatic pressure coupled with the minute diameter of the head (*ca.* 8·15 μm) enables them to push through the thin inter-segmental membranes of the insect cuticle.

In our experiments, head thrusting behaviour occurred in the absence of any host stimuli. Penetrative behaviour has been previously observed in the presence of host cues. In the presence of host faeces and gut contents *H. bacteriophora* and *Steinernema* spp. increased the duration of time spent head thrusting or head thrusting into the substrate (Grewal, Gaugler & Lewis, 1993*a*; Grewal, Gaugler & Selvan, 1993*b*). The largest increase in the amount of time spent head thrusting in response to host cues was found in *H. bacteriophora* (Grewal *et al.* 1993*a*, *b*). Head thrusting described as 'repeated thrusting of head against the substrate' was presumed to be a host penetration behaviour (Grewal *et al.* 1993*a*, *b*) and is probably analogous to the behaviour observed here in *H. megidis*. Similar pre-infection behaviours have been described in other parasites in response to host stimuli, including stylet thrusting and head-end bending in *Heterodera schachtii* (Grundler, Schnibe & Wyss, 1991). Although head thrusting behaviour in *H. megidis* may be related to physical penetration of the insect host, in the present study it occurred in the absence of specific host cues, and may have been initiated by mechanical stimulation provided by the bottom or walls of the container. Observed changes in head thrusting behaviour might either reflect a change in touch sensitivity of the IJs or a change in the stimulus threshold for this behaviour. In soil, nematodes are not normally submerged in water. However, head thrusting behaviour was not exclusively observed in water but was also seen in IJs on 2 % agar prior to penetrating into it (Dempsey, 2000). The available evidence suggests that the observed increase in infectivity of *H. megidis* may be due to an increased tendency to attempt penetration rather than to an increase in either general levels of motor activity or responsiveness to volatile host cues. An increased responsiveness to contact chemical cues associated with the host, accompanying the generalized tendency to penetrate an object, cannot be excluded.

Age-dependent changes in foraging strategy have been described for many parasite groups (Evans & Perry, 1976; Croll & Matthews, 1977; Schad, 1977; Gautret & Motard, 1999). In entomopathogenic nematodes, a switch in behavioural tactics has been

proposed in *S. carpocapsae* (Lewis *et al.* 1995; Lewis, Campbell & Gaugler, 1997). When IJs of this species first emerge from the host they display a characteristically ambush type foraging strategy. Following storage at 25 °C they decreased the amount of time spent nictating, increased locomotory rate across agar and migrated more successfully towards insect host cues, indicating a change to an increasingly cruiser type foraging strategy. However, those authors concluded that the change in behaviour of *S. carpocapsae* IJs was not a true switch in foraging strategy as it did not result in an increase in the number of IJs invading subterranean hosts. *H. megidis* has been described as a cruiser type forager (Grewal *et al.* 1994). Activity of cruiser-type species of entomopathogenic nematodes, including *H. bacteriophora*, typically declines with age (Lewis *et al.* 1995). However, we have shown here that this is not the only change occurring in *H. megidis*. The behaviour of the *H. megidis* IJ population during the first 4 weeks after emergence from the host cadaver seems to be phased, with an initial dispersive phase gradually replaced by a less active but increasingly infective phase. This strategy would result in dispersal of many of the IJs away from the areas of high density around the source cadaver, reducing the risk of overcrowding in new hosts. The final phase recognized here, in which both dispersal and infectivity are declining, may be energy limited or some IJs may have entered quiescence to conserve energy. This phasing can be seen, not as a switch in foraging strategy, but as representing the foraging strategy itself, a strategy which can be summarized as 'disperse, then infect, and if a host has not been encountered within a certain distance then adopt energy conserving measures'. As the parameters were measured at a population level, we cannot say that all IJs follow the same strategy. There may be cohorts with different behavioural strategies such as 'infect early; don't disperse'. We have described here the behaviour of IJs that emerged from the host cadaver over a single 24-h period. Time of emergence affects the behaviour and stress tolerance of *H. megidis* IJs (O'Leary, Burnell & Kusel, 1999), and also the way in which infectivity changes during storage (Ryder & Griffin, unpublished observations).

Thanks to Micheal Keating of Trinity College Dublin (Ireland) for writing the programme for recording nematode behaviour and to Dr W. M. Hominick, CABI Biosciences UK who originally supplied *H. megidis* isolate UK211.

REFERENCES

BEDDING, R. A. & MOLYNEUX, A. S. (1982). Penetration of insect cuticle by infective juveniles of *Heterorhabditis* sp. (Heterorhabditidae: Nematoda). *Nematologica* **28**, 354–359.

BOFF, M. I. C., WIEGERS, G. L. & SMITS, P. H. (2000). Effect of storage time and temperature on infectivity, reproduction and development of *Heterorhabditis megidis* in *Galleria mellonella*. *Nematology* **2**, 635–644.

BOHAN, D. & HOMINICK, W. (1994). Infectivity of insect parasitic nematodes is inherently heterogeneous. *Proceedings of the VIth International Colloquium on Invertebrate Pathology and Microbial Control, Montpellier, France, 28 August–2 September, pp. 137–141.*

BOHAN, D. & HOMINICK, W. (1995*a*). Examination of the *Steinernema feltiae* (Site 76 strain) infection interaction with the *Galleria mellonella* host, using an infection model. *Parasitology* **111**, 617–625.

BOHAN, D. & HOMINICK, W. (1995*b*). Intra-population infectious structure and temporal variation in *Steinernema feltiae*. In *Ecology and Transmission Strategies of Entomopathogenic Nematodes* (ed. Griffin, C. T., Gwynn, R. L & Masson, J. P.), pp. 83–94. European Commission, Brussels.

BOHAN, D. & HOMINICK, W. (1996). Investigations on the presence of an infectious proportion amongst populations of *Steinernema feltiae* (Site 76 strain) infective stage. *Parasitology* **112**, 113–118.

BOHAN, D. & HOMINICK, W. (1997). Long-term dynamics of infectiousness within the infective-stage pool of the entomopathogenic nematode *Steinernema feltiae* (Site 76 strain) Filipjev. *Parasitology* **114**, 301–308.

CAMPBELL, J. F., KOPPENHÖFER, A. M., KAYA, H. K. & CHINNASRI, B. (1999). Are there temporarily non-infectious dauer stages in entomopathogenic nematode populations: a test of the phased infectivity hypothesis. *Parasitology* **118**, 499–508.

CASSADA, R. C. & RUSSELL, R. L. (1975). The dauer larva, a post embryonic developmental variant of the nematode *Caenorhabditis elegans*. *Developmental Biology* **46**, 326–342.

CROLL, N. A. (1970). *The Behaviour of Nematodes*. Academic Press, London.

CROLL, N. A. & MATTHEWS, B. E. (1973). Activity, ageing and penetration of hookworm larvae. *Parasitology* **66**, 279–289.

CROLL, N. A. & MATTHEWS, B. E. (1977). *Biology of Nematodes*. Halsted Press, New York.

CURRAN, J. (1993). Post-application biology of entomopathogenic nematodes in soil. In *Nematodes for the Biological Control of Insects* (ed. Bedding, R. A., Akhurst, R. J. & Kaya, H. K.), pp. 67–78. CSIRO, East Melbourne, Australia.

DEMPSEY, C. M. (2000). Temporal phasing of behaviour in the infective stages of the insect parasitic nematode: *Heterorhabditis megidis*. Ph.D. thesis, National University of Ireland, Maynooth, Ireland.

EVANS, A. A. F. & PERRY, R. N. (1976). Survival strategies in nematodes. In *The Organisation of Nematodes* (ed. Croll, N. A.), pp. 383–424. Academic Press, London.

FAN, X. & HOMINICK, W. M. (1991). Effects of low storage temperature on survival and infectivity of two *Steinernema* species (Nematoda: Steinernematidae). *Revue de Nématologie* **14**, 407–412.

GAUGLER, R., CAMPBELL, J. F. & MCGUIRE, T. R. (1989). Selection for host finding in *Steinernema feltiae*. *Journal of Invertebrate Pathology* **54**, 363–372.

http://dx.doi.org/10.1017/S0031182002001609

GAUTRET, P. & MOTARD, A. (1999). Periodic infectivity of *Plasmodium* gametocytes to the vector. A review. *Parasite* **6**, 103–111.

GREWAL, P. S., GAUGLER, R. & LEWIS, E. E. (1993*a*). Host recognition behaviour by entomopathogenic nematodes during contact with insect gut contents. *Journal of Parasitology* **79**, 495–503.

GREWAL, P. S., GAUGLER, R. & SELVAN, S. (1993*b*). Host recognition by entomopathogenic nematodes: behavioural response to contact with host faeces. *Journal of Chemical Ecology* **19**, 1219–1231.

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.

GRIFFIN, C. T. (1995). Failure of entomopathogenic nematodes to infect: a speculative analysis. *Abstracts from the 2nd International Symposium on Entomopathogenic Nematodes and their Symbiotic Bacteria, Honolulu, Hawaii, USA, 15–17 October, 1995*, pp. 33–37.

GRIFFIN, C. T. (1996). Effects of prior storage conditions on the infectivity of *Heterorhabditis* sp. (Nematoda: Heterorhabditidae). *Fundamental and Applied Nematology* **19**, 95–102.

GRUNDLER, F., SCHNIBE, L. & WYSS, U. (1991). *In vitro* studies on the behaviour of second-stage juveniles of *Heterodera schactii* (Nematoda: Heterorderidae) in response to host plant root exudates. *Parasitology* **103**, 149–155.

HOMINICK, W. M. & REID, A. P. (1990). Perspectives in entomopathogenic nematology. In *Entomopathogenic Nematodes in Biological Control* (ed. Gaugler, R. & Kaya, H. R.), pp. 327–345. CRC Press, Boca Raton, Florida.

ISHIBASHI, N. & KONDO, E. (1990). Behaviour of infective juveniles. In *Entomopathogenic Nematodes in Biological Control* (ed. Gaugler, R. & Kaya, H. R.), pp. 139–150. CRC Press, Boca Raton, Florida.

ISHIBASHI, N., WANG, X.-D. & KONDO, E. (1994). *Steinernema carpocapsae*: post-storage infectivity and sex ratio of invading infective juveniles. *Japanese Journal of Nematology* **24**, 60–68.

LEWIS, E. E., SELVAN, S., CAMPBELL, J. F. & GAUGLER, R. (1995). Changes in foraging behaviour during the infective stage of entomopathogenic nematodes. *Parasitology* **110**, 583–590.

LEWIS, E. E., CAMPBELL, J. F. & GAUGLER, R. (1997). The effects of ageing on the foraging behaviour of *Steinernema carpocapsae* (Rhabditida: Steinernematidae). *Nematologica* **43**, 355–362.

O'LEARY, S. A., BURNELL, A. M. & KUSEL, J. R. (1998). Biophysical properties of the surface of desiccation-tolerant mutants and parental strain of the entomopathogenic nematode *Heterorhabditis megidis* (strain UK211). *Parasitology* **117**, 337–345.

PATEL, M. N., STOLINSKI, M. & WRIGHT, D. J. (1997). Neutral lipids and the assessment of infectivity in entomopathogenic nematodes: observations on four *Steinernema* species. *Parasitology* **114**, 489–496.

SCHAD, G. A. (1977). The role of arrested development in the regulation of nematode populations. In *Regulation of Parasite Populations* (ed. Esch, G. W.), pp. 111–165. Academic Press, London.

STOREY, R. M. J. (1984). The relationship between neutral lipid reserves and infectivity for hatched and dormant juveniles of *Globodera* sp. *Annals of Applied Biology* **104**, 511–520.

VAN GUNDY, S. D. (1965). Factors in the survival of nematodes. *Annual Review of Phytopathology* **3**, 43–68.

WESTERMAN, P. (1992). The influence of time of storage on performance of the insect parasitic nematode, *Heterorhabditis* sp. *Fundamental and Applied Nematology* **15**, 407–412.

WOODRING, J. L. & KAYA, H. K. (1988). *Steinernematid and Heterorhabditid Nematodes: a Handbook of Techniques*. Southern Co-operative Series Bulletin 331. Arkansas Agricultural Research Station, Fayetteville, AK.