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Persistence of *Heterorhabditis* Infective Juveniles in Soil: Comparison of Extraction and Infectivity Measurements[1]

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Abstract: The persistence of *Heterorhabditis megidis* in soil was studied over a 4-week period. On days 0, 2, 14, and 28, infective juveniles (IJ) were extracted by centrifugal flotation, Baermann funnel, and baiting of soil with *Tenebrio molitor* larvae, which were then dissected. Extraction efficiencies on day 0 were 82% by centrifugal flotation, 56% by Baermann funnel, and 19.8% by bait insect. The relative efficiency of the three methods changed over time. The relationship between the density of nematodes in the soil and the proportion recovered by dissection was non-linear. Up to a dose of approximately 60 IJ/insect, less than 12% became established, while at higher doses (up to 200 IJ/insect) the invasion efficiency was 23%. Mortality of bait insects increased from day 0 to day 2, but decreased to day 28. A novel method of assessing soil pathogenicity by preparing a soil density series and calculating the dose of soil or IJ that kills 50% of the bait insects gave a similar pattern. This method is recommended as a means of tracking changes in pathogenicity over time when bait insect mortality in undiluted soil is at or near 100%. Two methods of preparing a series of *Heterorhabditis* IJ densities in soil, either by diluting the soil itself with IJ-free soil or by adding diluted suspensions of IJ to the soil, resulted in the same bait insect mortalities.

Key words: Baermann funnel, centrifugal flotation, dissection, entomopathogenic nematode, *Heterorhabditis bacteriophora, Heterorhabditis megidis,* infectivity, LD50, nematode, persistence, soil baiting, soil dilution, soil ecology, soil extraction.

Entomopathogenic nematodes of the genera *Heterorhabditis* and *Steinermema* have been successfully used for pest control in the United States, Europe, and elsewhere (Kaya and Gaugler, 1990). These nematodes are parasites of a wide range of insects. Together with their symbiotically associated bacteria of the genera *Photorhabdus* and *Xenorhabdus,* respectively (Boemare et al., 1993), they kill insect hosts within days of infection. The nematodes develop and reproduce inside the insect cadaver from which they emerge into the soil as non-feeding dauer juveniles or infective juveniles (IJ) (Poinar, 1975). In soil, IJ may need to persist until an appropriate host is available.

There are two principal approaches to assessing entomopathogenic nematode survival in soil or other media. The first is to extract the IJ directly with standard techniques such as Baermann funnel (BF) and centrifugal flotation (CF) (Jenkins, 1964). The Baermann funnel technique depends on the mobility of the IJ, i.e. they extract themselves. The CF method, based on the separation of the nematodes from debris by gravity, recovers both mobile and immobile nematodes. Extraction may be followed by assessing the infectivity of extracted nematodes (e.g., Nguyen and Smart, 1990). The second approach is to assess the infectivity or pathogenicity of the soil without extracting the IJ. A baiting method (Bedding and Akhurst, 1975) was introduced as a simple method for the detection of entomopathogenic nematodes in soil samples. The percent mortality of bait insects can be used for monitoring changes in nematode pathogenicity over time (Ishibashi and Kondo, *Tech Ed:* 1986a; Kung et al., 1990a, 1990b, 1991; Molyneux, 1985). Bait insect mortality gives the best indication of residual pathogenicity of the nematodes, and hence the potential for continued control of insect populations. However, for a more detailed insight into nematode population dynamics it has been recommended that the infected insect hosts should be dissected in order to count the first-generation adults (Fan and Hominick, 1991a). This dissection method has been used in studies of persistence by Curran and Heng (1992) and Selvan et al. (1993a). The

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mpn (most probable number) method can also be used for estimating entomopathogenic nematode populations in soil (Hass et al., 1997). A series of IJ densities in soil is prepared by diluting nematode-infested soil with uninfested soil and baiting the mixture with insects. The bait insect mortality allows the calculation of the most probable number of nematode ''infectious units'' in the undiluted sample.

Several studies present comparative data for the extraction of steinernematids from soil by two or more methods. For example, Saunders and All (1982) and Ishibashi and Kondo (1986b) presented data for the extraction of steinernematids by CF and BF, while Curran and Heng (1992) compared CF, BF, and live-bait methods for extracting steinernematids from soil. Fan and Hominick (1991b) employed methods similar to each of these three in their study of steinernematid survival. However, such comparative data are not available for any species of *Heterorhabditis*.

The aims of this study were to compare the various methods for determining numbers of *Heterorhabditis* IJ in soil and their pathogenicity, and also to gain more detailed information about the relationship between survival, mobility, and infectivity of *Heterorhabditis* IJ by the concurrent application of several methods. Extraction methods chosen were CF, BF, and bait insect dissection. Pathogenicity of infested soil was measured by the percent mortality of bait insects at a single density of IJ in soil and by LD50, using mortality data from a baited soil density series.

Materials and Methods

Nematodes: Heterorhabditis bacteriophora (Poinar) isolate HI (an Italian isolate) and *Heterorhabditis megidis* (Poinar, Jackson & Klein) isolate HF85 (isolated in The Netherlands) were obtained from P. Westerman and W. R. Simons. The nematodes were reared in *Tenebrio molitor* larvae (mealworms) at 20 °C, and IJ were harvested from modified White traps (White, 1927). Harvested IJ were washed by sedimentation and stored in tap water at 9 °C for 6 (Experiment 1), 14 (Experiment 2), or 7 (Experiment 3) days before application to soil.

Soil: The soil used in these experiments came from a garden on Maynooth College campus and had the following composition by weight: 33% sand, 48% silt, and 19% clay; with 22.5% organic matter. The soil was air-dried on the laboratory bench to a moisture content of 23% (Experiments 1 and 2) and 27% (Experiment 3) by weight (pF 2.8 and 2.3, respectively). The soil used was unsterilized, but Maynooth soil is naturally free of *Heterorhabditis* (unpubl.). All experiments were conducted in 250-ml plastic beakers with snap-on lids (Wilscanco Plastics, Dungannon, Ireland). Beakers each contained 120 g of soil.

Survival of nematodes in soil: Nematodes were extracted from soil with Baermann funnel and centrifugal flotation methods. Soil was extracted in Baermann funnels lined with milk filters for two consecutive 48-hour periods at 20 °C. The catches from each of the two 48-hour periods were counted separately. The centrifugation flotation method (Jenkins, 1964) was modified slightly. Soil was washed twice through a 430-μm-pore sieve to remove large particles. The resulting aqueous soil suspension was poured through a 40-μm-pore sieve, and the residue in the sieve was rinsed into centrifuge tubes. The tubes were then centrifuged at 500*g* for 5 minutes. Pellets were resuspended in sucrose solution (500 g/liter) and centrifuged at 500*g* for 2 minutes. In contrast to the procedure described by Jenkins (1964), nematodes remaining in the water supernatant as well as those in the sucrose supernatant were counted. For each sample, the number of nematodes was estimated from the mean of 6 aliquots.

Infectivity and pathogenicity: Five (Experiment 1) or three (Experiment 2) replicate, two-fold, soil density series were baited with 40 *T. molitor* larvae/beaker and held for 4 days at 20 °C (Hass et al., 1997). A soil density series was prepared by diluting nematode-infested soil (120 g) with an equal volume of uninfested soil. Following thorough mixing the diluted soil was divided equally

and one half was diluted with uninfested soil; the process was repeated until the desired range of densities was prepared. Insect mortality data for the soil density series were used to calculate LD50 values with probit analysis. The dead bait insects were dissected in tap water between day 7 and day 9 after being placed in the soil, and first-generation nematodes were counted. This number was assumed to be the same as the number of IJ that had entered the insect.

Experiment 1, comparison of dilution of nematode inoculum in water with dilution in soil: This experiment was done to confirm the validity of the soil dilution method as a means of preparing a series of nematode densities in soil. Each of five beakers of soil was infested with 2,000 IJ of *H. bacteriophora* in 2 ml of tap water and then mixed with an equal volume of uninfested soil; this was repeated to give five independent 2-fold dilution series, with densities of 1,000, 500, and 250 IJ/beaker. The dilution was performed 30 minutes after the nematodes were added. At the same time a separate, aqueous 2-fold dilution series was prepared from the nematode suspension itself. From the diluted nematode suspension, 1-ml aliquots containing 1,000, 500, or 250 IJ each were pipetted into 5 replicate beakers of soil for each nematode density. The soil density series produced by diluting IJ in soil or in water should therefore have contained similar numbers of IJ. The soils from both methods, including all densities, were then baited as described above. Five additional uninfested beakers of soil were baited as controls.

Experiment 2, comparison of extraction and infectivity measurements: Sixty beakers of soil were each infested with 16,000 IJ (*H. megidis*) applied in 2 ml of tap water. An equal number of control beakers received tap water alone. The beakers were stored in a randomized block design in stacked plywood boxes (111 × 63 × 12 cm) in a controlled temperature room at 20 °C. Each block contained three infested and three uninfested beakers. In each box, a row of beakers containing uninfested soil was placed around the experimental beakers as a buffer. Nematode survival and infectivity were assessed immediately (day 0) and after 2, 14, and 28 days. At each assessment date 5 of 20 blocks, randomly chosen, were removed and replaced by buffer blocks. Of the three infested soil beakers in each block, one was extracted with BF, one was extracted with CF, and the third was used to prepare a soil density series, which was then baited with mealworms for assessment of infectivity and pathogenicity. The density series was prepared by serial 2-fold dilution with uninfested soil to give final densities of 8,000, 4,000, 2,000, 1,000, 500, and 250 IJ/beaker (based on day 0 levels of IJ). The three uninfested soil beakers were similarly treated. Changes in soil pathogenicity over time were measured as the percent mortality of bait insects in the least dilute (8,000 IJ/beaker) baited soil density, representing a dose of 200 IJ/insect. In addition, insect mortality at different soil density levels was used to calculate LD50s using two methods. For LD50A, dose was based on original inoculum level at day 0 and measured in beakers of soil (1 beaker = 8,000 IJ). For LD50B, dose was based on IJ remaining in soil (as assessed by CF) at the time of testing.

Experiment 3, relationship between T. molitor mortality and IJ dose: This experiment was conducted to confirm the results of experiment 2. Five beakers of soil were each infested with 20,000 IJ (*H. megidis*) applied in 2 ml of tap water. Five additional beakers received tap water only (controls). Soil pathogenicity and infectivity were assessed directly after inoculation, by baiting, and by dissection of hosts following soil dilution. Dissected hosts were from beakers containing 5,000, 2,500, 1,250, 625, and 313 IJ/beaker, representing a dose range of 8–125 IJ/insect.

Statistical analysis: Student's *t*-test was used to evaluate differences in mean values of two samples. In cases where the *t*-test could not be applied, the Mann Whitney *U*-test was used as a non-parametric alternative. ANOVA was used for comparison of more than two samples. Percentages were arcsin-transformed prior to analysis. LD50s were calculated by probit analysis (PROC

PROBIT, SAS Institute, Cary, NC), with a correction for natural mortality.

Results

Comparison of dilution through water with dilution through soil: The two methods of preparing a series of *Heterorhabditis* IJ densities in soil, either by diluting the soil with soil or by adding a diluted suspension of IJ to the soil (Experiment 1), showed no significant difference between bait insect mortalities at any density level (Fig. 1). In the control beakers natural mortality of *T. molitor* was 1.5% (0.6 dead insects/beaker).

Recovery of nematodes from soil by BF, CF, and dissection of bait insects: Immediately after inoculation (Experiment 2), 82.3% of the IJ were recovered from the soil with the CF method, and represented the total number of extractable IJ. A lower proportion of the applied nematodes was recovered by each of the other methods: 56% by BF (mobile nematodes) and 19.8% by dissection (infective nematodes) (Fig. 2). Recovery of IJ by all three methods declined over the 28 days of the experiment, with the exception that the percentage of IJ recovered by insect dissection was higher on day 2 than on day 0 ($P < 0.005$) (Fig. 2). In contrast, the percentage extracted by CF declined over the same period ($P < 0.01$), while the percentage recovered by BF showed an insignificant decline over this period ($P = 0.410$) (Fig. 2). For each date, numbers of nematodes recovered by BF and by dissection were expressed as a percentage of CF (Fig. 3A). The percentages did not remain constant over time. On day 0, 67.7% of the nematodes extracted by CF could also be extracted by BF, but on day 28, the number of IJ extracted by BF was only 48.9% that of CF. Numbers of IJ found by dissection as a percentage of the number from CF declined from 24.1% to 9.6% over the same period (Fig. 3A), indicating that the proportion of nematodes present that were infective was lower after a month than immediately after application. When expressed as a proportion of BF, the numbers recovered by dissection declined from 35.5% on day 0 to 19.6% on day 28 (Fig. 3B).

When IJ were extracted from soil by two-step CF, the relative proportion of IJ found in the two supernatants (water and sucrose) changed with the length of time that the IJ had been stored in soil (Fig. 4). The proportion of IJ remaining in the water supernatant after centrifugation more than doubled between day 0 and day 14. The increase began with a rapid rise within the first 2 days of storage, followed by a more gradual increase over the remaining period. After 28 days the proportion of IJ in the water phase was more than three times as high as on day 0.

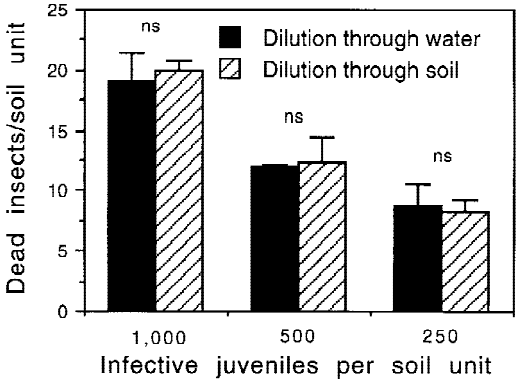

Fig. 1. Number of dead *Tenebrio molitor* larvae out of 40/beaker in soil infested with three levels of *Heterorhabditis bacteriophora* HI infective juveniles (IJ). Infestations were prepared by dilution through water or through soil. Bars are the means (± SEM) of five replicates. Differences between dilution methods were not significant at any infestation level (Student's *t*-test, $P < 0.05$).

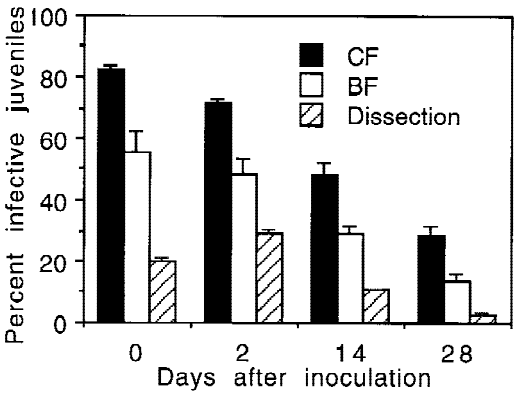

Fig. 2. Effect of extraction method on recovery of applied *Heterorhabditis megidis* HF85 infective juveniles (IJ) added to soil in beakers. Extraction methods: centrifugation flotation (CF); Baermann funnel (BF); bait dissection. Soil was inoculated with 16,000 IJ/beaker on day 0. Bars are the means (± SEM) of three replicates.

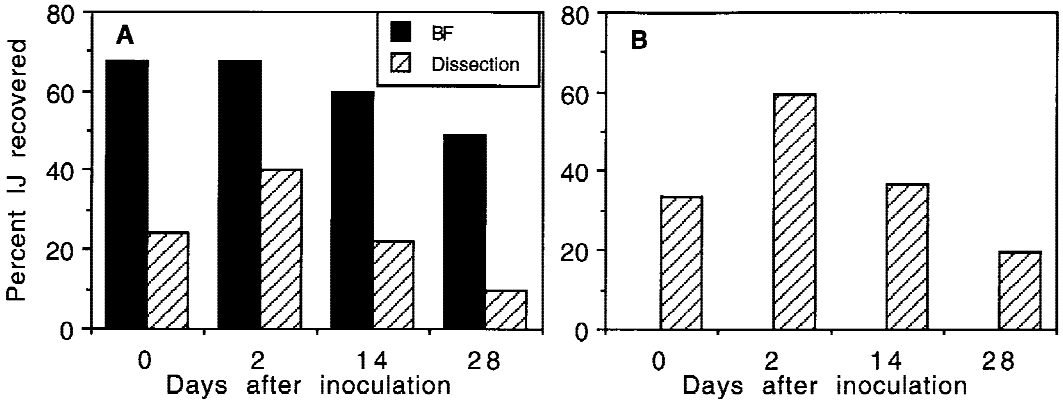

FIG. 3. Relative efficiency of extraction methods for recovery of *Heterorhabditis megidis* HF85 infective juveniles (IJ). A) Efficiency of Baermann funnel (BF) extraction of IJ and dissection of insect hosts, as a percentage of IJ extracted with centrifugation flotation (CF). B) Efficiency of IJ recovery by dissection of insect hosts as a percentage of IJ recovered with BF.

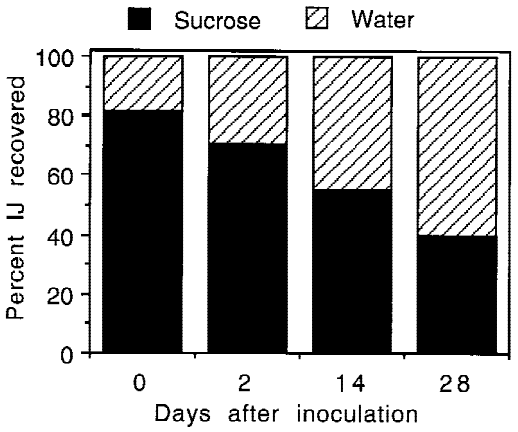

FIG. 4. Proportion of *Heterorhabditis megidis* HF85 infective juveniles (IJ) extracted in the two supernatants of a two-step centrifugation flotation procedure, as a percentage of the total number of IJ extracted. In the first extraction phase, nematodes were centrifuged in water; in the second phase, water was replaced with a sucrose solution (500 g/liter water).

A single Baermann funnel extraction period of 48 hours was enough to recover at least 95% of the total of IJ extracted by BF in 96 hours. This percentage did not vary significantly over the 4 weeks of the experiment ($P > 0.05$).

Measures of changes in soil pathogenicity over time: The proportion of bait insects killed in the soil sample with the highest IJ density dropped from 92% on day 0 to 68% 1 month after inoculation (Table 1). Mortality remained above 90% for the first three assessment dates, and there was no evidence of a decline between day 0 and day 14 ($P = 0.155$). There was, however, an initial increase between day 0 and day 2 ($P < 0.01$). A similar pattern was observed when LD50 was used as a measure of pathogenicity (Table 1). An increase in soil pathogenicity from

TABLE 1. Pathogenicity of *Heterorhabditis megidis* HF85 infective juveniles (IJ) in infested soil as indicated by percent mortality of *Tenebrio molitor* larvae (for the least dilute soil density level of 8,000 IJ/beaker on day 0) and by LD50 values (with 95% fiducial limits) based on beakers of soil (LD50A) and numbers of IJ per insect present in the soil (LD50B).

Day	Percent dead[a]	LD50A[b]	LD50B[c]
0	92.0 (0.94)	0.12 (0.081–0.167)	776.9 (NE-1444.6)
2	98.5 (0.61)	0.080 (0.050–0.113)	309.2 (NE-803.9)
14	93.5 (2.92)	0.137 (0.121–0.155)	732.7 (284.0–090.8)
28	68.0 (5.72)	0.567 (0.385–1.023)	1,487.0 (1,188.7–1,958.2)

[a] Numbers are means with standard error of the mean in parentheses.
[b] LD50A = dose expressed as number of soil units required to kill 50% of *Tenebrio molitor* larvae. A dose of 1.0 soil unit is equivalent to 8,000 IJ/beaker, or 200 IJ/insect, on day 0.
[b] LD50B = dose of IJ per insect (expressed in terms of IJ recovered from the soil by centrifugal flotation) required to kill 50% of *Tenebrio molitor* larvae. NE = not estimated.

day 0 to day 2 was indicated by the decrease in both LD50A and LD50B values, but day 0 and day 2 did not differ significantly in either case (95% fiducial limits overlap). Neither LD50 value showed much difference between days 0 and 14, but there was a decline in pathogenicity between day 14 and day 28. The difference between days 14 and 28 was significant in each case, based on non-overlap of the 95% fiducial limits. Nearly twice as many of the remaining IJ (as indicated by CF) were required to kill 50% of the insects on day 28 as on day 0 (i.e., the surviving IJ were less infective). By day 28, nearly five times as much soil was required to achieve 50% insect mortality as on day 0, representing both a decline in numbers of IJ and a decline in their infectivity. Pathogenicity as assessed by each of the LD50 parameters (LD50A, LD50B) was highly correlated with simple *T. molitor* mortality at an inoculum of 8,000 IJ per beaker (for LD50A, $y = 1.69 - 0.042x$, $R^2 = 0.984$; for LD50B, $y = 44.2 - 0.011x$, $R^2 = 0.941$).

Dose response relationships of Experiments 2, 3: The relationship between the dose of IJ in the soil and the numbers of nematodes established in the insects was non-linear in Experiment 2, and this relationship was confirmed for day 0 in Experiment 3 (Fig. 5).

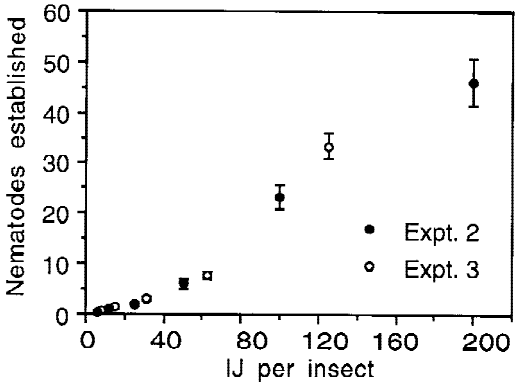

FIG. 5. Relationship between applied dose of *Heterorhabditis megidis* HF85 infective juveniles (IJ) and numbers of nematodes established in *Tenebrio molitor* as estimated by insect dissections. The soil was baited on the same day as nematode infestation. The nematode doses were prepared from soil infested with 16,000 (Experiment 2) or 20,000 (Experiment 3) IJ by a serial 2-fold dilution using uninfested soil. Points represent means (±SEM) with 5 replicates/point.

The regression line for data of both experiments combined was $y = -4.282 + 0.280x$, $R^2 = 0.938$. Up to a dose of about 60 IJ/insect the relationship approached linearity ($y = -0.762 + 0.133x$, $R^2 = 0.975$), with less than 13% of the IJ invading an insect; at higher doses the invasion efficiency was 23% and the slope of the regression line was steeper than at lower doses ($y = 4.146 + 0.213x$, $R^2 = 0.955$). Similar results (not shown) were found for each of the other assessment dates of Experiment 2.

DISCUSSION

The soil dilution method introduced by Hass et al. (1997) for estimating most probable numbers (mpn) of entomopathogenic nematodes in soil was a valid alternative to the application of an aqueous dilution series for obtaining soil infested with a range of IJ densities. Density series prepared by both methods had equal pathogenicity. Aqueous dilution series have been used in measuring nematode infective potential (Peters and Ehlers, 1994), but diluting infested soil with uninfested soil has not. The use of LD50s calculated from the baited soil density series was shown to be a viable method for studying nematode persistence; LD50 values showed changes in soil pathogenicity comparable to those for simple *T. molitor* mortality at a single IJ density, although they did not have the same discriminatory power due to large fiducial limits. Soil dilution may be particularly useful for the study of soil pathogenicity above levels that kill 100% of the bait insects. Ishibashi and Kondo (1986a) found that bait insect mortality for some treatments was 100% throughout a 6-week experimental period. A single low IJ application rate (avoiding 100% kill) may not suit all treatments under investigation (for example, when comparing species of high and low pathogenicity). An alternative would be to infest soil samples with a range of IJ densities at day 0; however, this might be impracticable, and might permit unpredictable density-dependent interactions of soil organisms (e.g., fungi, other nematodes) with the IJ, complicating experiments

run over long periods. The soil dilution technique permits long-term experimentation with soils containing very high densities of entomopathogenic nematodes, which are adjusted only at the end of the experimental period. There was no evidence that the soil dilution procedure affected the performance of IJ, as shown by similar insect mortalities in soil prepared by the soil dilution and the water suspension dilution methods.

A 48-hour BF extraction recovered at least 94% of the total number of *H. megidis* IJ extracted by two 48-hour periods of extraction. The time required for efficient nematode recovery is species-specific and related to soil texture (Robinson and Heald, 1989) and nematode age (Molyneux, 1985). Molyneux (1985) found that the time necessary for IJ of two heterorhabditids and *S. feltiae* to move through a Baermann funnel increased when they had been stored in sand for longer periods; often 3 to 5 days were required to recover active *S. feltiae* IJ. There was no evidence in the present experiments that the time needed to recover *H. megidis* increased with time of storage in soil, as the proportion of IJ extracted in the first 48-hour period was the same after 28 days in soil as on day 0.

The CF extraction efficiency (82%) for *H. megidis* IJ immediately after inoculation was high compared to reported efficiencies for *Steinernema* spp. extracted with similar methods, in which typically less than 65% of applied nematodes are extracted (Kung et al., 1990a; Saunders and All, 1982; Ishibashi and Kondo, 1986a). One factor contributing to the high extraction rate reported here may be that the water supernatant in the first extraction step was examined for nematodes instead of being discarded, as is usual in this procedure. Had the water supernatant been discarded, the extraction efficiency on day 0 would have been only 67% instead of 82%. Since the proportion of IJ remaining in the water supernatant increased with time, an even greater proportion of IJ would have been lost at the later extraction dates, had it been discarded. Differences in recovery rates of various nematode species have led to the use of individually adjusted solutions for extraction of given species or groups (Kung et al., 1990a), presumably minimizing losses of IJ in the water supernatant.

The fact that the proportion of nematodes recovered in the water supernatant of the CF extraction increased with time suggests that *H. megidis* HF85 IJ undergo a reduction in their specific gravity during storage in soil. The cause of this change is unknown, but it could be due to changes in the biochemical composition of the nematodes due to metabolic activity or to age-related changes in the permeability of the cuticle (Searcy et al., 1976; Selvan et al., 1993b). The increase in the proportion of IJ in the water phase began between day 0 and day 2, suggesting rapid acclimatization of some kind. This change probably is adaptive rather than pathological since soil infectivity and bait insect mortality increased over this period. Fan and Hominick (1991b) recorded increased infectivity in steinernematid IJ 1 day after application to sand and recorded it as an "unexplained abnormality." Maturation of IJ (Griffin, 1996) in soil or sand is a possible alternative to acclimatization.

For *H. megidis* HF85, the most efficient method of extracting IJ from soil was CF, followed by BF and dissection. This result was not unexpected, as CF in principle extracts all nematodes, while BF relies on their mobility, and dissection requires that IJ are capable of infecting bait insects. However, the relative efficiency of these methods for extraction of entomopathogenic nematodes can be variable. Saunders and All (1982) found that BF was considerably more efficient at extracting *Steinernema carpocapsae* from soil than was CF (71% vs. 46%). Fan and Hominick (1991b) also found that the Whitehead extraction method (Whitehead and Hemming, 1965), which is similar to BF, was slightly more efficient than a sucrose flotation method for extracting steinernematids from sand. Curran and Heng (1992) reported that a flotation method using salt was more efficient than BF, while recovery of nematodes by dissection of bait insects was intermediate.

After a storage period of 14 days, the num-

ber of IJ extracted by BF declined more rapidly than the number extracted by CF, and there was a decline in the number of IJ extracted by BF as a percentage of CF. This decline suggests a tendency for IJ to become less mobile before dying, since the BF extraction relies on nematode mobility while CF does not. Numbers of IJ found by dissection declined even more quickly with time than numbers of IJ extracted with Baermann funnels. These results may indicate that heterorhabditid IJ lose the ability to find and penetrate an insect faster than they lose their ability to move through soil. However, entomopathogenic nematodes may become quiescent in soil (Ishibashi and Kondo, 1986b); the possibility that the loss of mobility in *H. megidis* was temporary (a form of quiescence or diapause) rather than permanent cannot be discounted.

If the proportion of IJ establishing in bait insects is the same at all nematode densities, a linear relationship with strong correlation between dose and established numbers should result (Fan and Hominick, 1991a). However, in the present experiments the proportion of IJ that established depended on the dose of nematodes applied, with a lower proportion of nematodes established at the lower doses. Data from two similar experiments (2, 3), performed on two different occasions, showed a similar relationship between dose and establishment, with similar points of inflection at a dose between 60 and 100 IJ/insect. Differences between the experiments in soil moisture content (23% and 27% in experiments 2 and 3, respectively) and in the length of time IJ were stored at 9 °C before use (14 and 7 days, respectively) did not change this pattern. The finding of a dose-dependent establishment rate is in contrast to certain other studies (Bohan and Hominick, 1996; Fan and Hominick, 1991a), where the proportion of heterorhabditid or steinernematid nematodes establishing in *G. mellonella* larvae was independent of the dose of IJ in sand or soil. However, those studies used a broader range of doses and had fewer dose levels below 100 IJ/insect than the present experiments. Hay and Fenlon (1995), focusing on the lower dose range, also found that the proportion of establishing *S. feltiae* increased with dose. They suggested that the population consists of subpopulations: one that initiates infection (pioneers) and another that colonizes only already-infected hosts (followers). This concept, or the concept that colonized hosts simply become more attractive to conspecifics (Grewal et al., 1997), could explain the density-dependent recovery rates from insects for *H. megidis* in the current study.

If "pioneers" and "followers" exist in species of entomopathogenic nematodes, nematode establishment data as a means of assessing the infective potential of IJ should be adjusted, perhaps by the use of appropriate statistical treatments (Hay and Fenlon, 1995), or by manipulating experimental conditions. However, a departure from linearity may be a consequence of restricted baiting time (4 days in the present study and 12 to 48 hours in Hay and Fenlon's [1995] experiments). Fan and Hominick (1991a) concluded from their experiments that long periods of time are required to estimate the proportion of invasive IJ in a population of nematodes, and recommended a minimum of 12 days of repeated baiting. If pioneers differ from followers in speed of response rather than in absolute terms, then repeated baitings should yield a more linear relationship.

Literature Cited

Bedding, R. A., and R. J. Akhurst. 1975. A simple technique for the detection of insect parasitic nematodes in soil. Nematologica 21:109–110.

Boemare, N. E., R. J. Akhurst, and R. G. Mourant. 1993. DNA relatedness between *Xenorhabdus* spp. (Enterobacteriaceae) symbiotic bacteria of entomopathogenic nematodes, and a proposal to transfer *Xenorhabdus luminescens* to a new genus, *Photorhabdus* gen. nov. International Journal of Systematic Bacteriology 43: 249–255.

Bohan, D. A., and W. M. Hominick. 1996. Investigations on the presence of an infectious proportion amongst populations of *Steinernema feltiae* (Site 76 strain) infective stages. Parasitology 112:113–118.

Curran, J., and J. Heng. 1992. Comparison of three methods for estimating the number of entomopathogenic nematodes present in soil samples. Journal of Nematology 24:170–176.

Fan, X., and W. M. Hominick. 1991a. Efficiency of

the *Galleria* (wax moth) baiting technique for recovering infective stages of entomopathogenic rhabditids (Steinernematidae and Heterorhabditidae) from sand and soil. Revue de Nématologie 14:381–387.

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

Grewal, P. S., E. E. Lewis, and R. Gaugler. 1997. Response of infective stage parasites (Nematoda: Steinernematidae) to volatile cues from infected hosts. Journal of Chemical Ecology 23:503–515.

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

Hass, B., C. T. Griffin, J. G. Keating, and M. J. Downes. 1997. Application of the most probable number method to estimations of entomopathogenic nematode populations in soil. Annals of Applied Biology 131: 471–480.

Hay, D. B., and J. S. Fenlon. 1995. A modified binomial model that describes the infection dynamics of the entomopathogenic nematode *Steinernema feltiae* (Steinernematidae; Nematoda). Parasitology 111:627–633.

Ishibashi, N., and E. Kondo. 1986a. *Steinernema feltiae* (DD-136) and *Steinernema glaseri*: Persistence in soil and bark compost and their influence on native nematodes. Journal of Nematology 18:310–316.

Ishibashi, N., and E. Kondo. 1986b. A possible quiescence of the applied entomogenous nematode, *Steinernema feltiae*, in soil. Japanese Journal of Nematology 16:66–67.

Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter 48:692.

Kaya, H. K., and R. Gaugler, eds. 1990. Entomopathogenic nematodes in biological control. Boca Raton, FL: CRC Press.

Kung, S.-P., R. Gaugler, and H. K. Kaya. 1990a. Soil type and entomopathogenic nematode persistence. Journal of Invertebrate Pathology 55:401–406.

Kung, S.-P., R. Gaugler, and H. K. Kaya. 1990b. Influence of soil pH and oxygen on persistence of *Steinernema* spp. Journal of Nematology 22:440–445.

Kung, S.-P., R. Gaugler, and H. K. Kaya. 1991. Effects of soil temperature, moisture, and relative humidity on entomopathogenic nematode persistence. Journal of Invertebrate Pathology 57:242–249.

Molyneux, A. S. 1985. Survival of infective juveniles of *Heterorhabditis* spp., and *Steinernema* spp. (Nematoda: Rhabditida) at various temperatures and their subsequent infectivity for insects. Revue de Nématologie 8: 165–170.

Nguyen, K. B., and G. C. Smart. 1990. Preliminary studies on survival of *Steinernema scapterisci* in soil. Proceedings of the Soil and Crop Science Society, Florida 49:230–233.

Peters, A., and R.-U. Ehlers. 1994. Susceptibility of leatherjackets (*Tipula paludosa* and *Tipula oleracea,* Tipulidae; Nematocera) to the entomopathogenic nematode *Steinernema feltiae*. Journal of Invertebrate Pathology 63:163–171.

Poinar, G. O., Jr. 1975. Description and biology of a new insect parasitic rhabditoid, *Heterorhabditis bacteriophora* n. gen., n. sp. (Rhabditida; Heterorhabditidae n. fam.). Nematologica 21:463–470.

Robinson, A. F., and C. M. Heald. 1989. Accelerated movement of nematodes from soil in Baermann funnels with temperature gradients. Journal of Nematology 21:370–378.

Saunders, M. C., and J. N. All. 1982. Laboratory extraction methods and field detection of entomophilic rhabditoid nematodes from soil. Environmental Entomology 11:1116–1165.

Searcy, D. G., M. Kisiel, and B. M. Zuckerman. 1976. Age-related increase of cuticle permeability in the nematode *Caenorhabditis briggsae*. Experimental Aging Research 2:293–301.

Selvan, S., R. Gaugler, and J. F. Campbell. 1993a. Efficacy of entomopathogenic nematode strains against *Popillia japonica* (Coleoptera: Scarabaeidae) larvae. Journal of Economic Entomology 86:353–359.

Selvan, S., R. Gaugler, and P. S. Grewal. 1993b. Water content and fatty acid composition of infective juvenile entomopathogenic nematodes. Journal of Parasitology 79:510–516.

White, G. F. 1927. A method for obtaining infective nematode larvae from cultures. Science 66:302–303.

Whitehead, A. C., and Y. R. Hemming. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Annals of Applied Biology 55:25–38.