Efficiency of the *Galleria* (wax moth) baiting technique for recovering infective stages of entomopathogenic rhabditids (Steinernematidae and Heterorhabditidae) from sand and soil

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SUMMARY

The efficiency of using Galleria larvae to quantify the numbers of entomopathogenic nematodes in naturally infested soil or in sand was assessed. Most tests were with an undescribed *Steinernema* sp. isolated from British soil and designated the Nashes isolate. Bioassays consisted of replacing all insects, whether dead or alive, with fresh living ones until infections ceased and counting the numbers of nematodes which established in the larvae. Whether using naturally infested soil or sterile sand, consecutive exposures of 36 days or more were required before infections ceased. However, two or three consecutive bioassays over twelve days recovered 75 % or more of the nematodes. A number of tests with 10-500 nematodes per *Galleria* larva showed a highly significant linear relationship between the dose of nematodes applied and the mean number which established in the insects. This applied for steinernematids and heterorhabditids, in sand and soil, for exposures lasting 72-144 h and at 15 or 20 °C. The proportion of the nematodes which could penetrate and establish in the hosts (the regression coefficient) varied and may provide a useful parameter for comparing the efficacy of different nematodes under specified conditions. Up to six consecutive 72 h exposures failed to recover more than 40 % of the nematodes applied. Comparison of two strains of *S. feltiae* (Filipjev) [= *S. bibionis* (Bovien)] with the Nashes isolate showed no significant differences in the percent that established in *Galleria* larvae after 72 h. The optimal temperature for infection and establishment of the Nashes isolate was 15-20 °C. There was no effect on efficiency of recovery if single insects were used with 25 ml of sand or five larvae with 300 ml of sand.

RÉSUMÉ

Efficacité de la technique utilisant Galleria *comme appât pour extraire du sable ou du sol les stades infestants de Rhabditides entomopathogènes (Steinernematidae et Heterorhabditidae)*

L'efficacité de l'utilisation de larves de *Galleria* pour dénombrer les nématodes entomopathogènes dans du sol ou du sable infesté a été testée. La plupart des tests ont été réalisés avec une espèce de *Steinernema* isolée du sol en Angleterre et désignée comme l'isolat Nashes. Les bioessais consistent à remplacer toutes les larves, vivantes ou mortes, par de nouvelles larves jusqu'à ce que l'infestation cesse et à compter les nématodes qui se sont établis dans les larves. Que l'on utilise du sol naturellement infesté ou du sable stérile, une durée d'exposition continue de 36 jours ou plus est nécessaire avant que l'infestation ne cesse. Cependant, deux ou trois bioessais consécutifs de 12 jours permettent la récupération d'au moins 75 % des nématodes. Des séries de tests comportant de 10 à 500 nématodes par larve de *Galleria* montrent une relation linéaire hautement significative entre la dose de nématodes utilisée et le nombre moyen de nématodes qui se sont établis dans les insectes. Ceci s'applique au Steinernématides et aux Hétérorhabditides, dans le sol ou le sable, pour des expositions s'étageant entre 72 et 144 h, à 15 ou 20 °C. La proportion de nématodes qui peuvent pénétrer dans l'hôte et s'y établir (le coefficient de régression) est variable et pourrait fournir un paramètre utile pour comparer l'efficacité de différents nématodes dans des conditions déterminées. Des expositions consécutives de 72 h, au nombre de six ou plus, ne permettent pas de récupérer plus de 40 % des nématodes mis en place. La comparaison de deux souches de *S. feltiae* (Filipjev) [= *S. bibionis* (Bovien)] avec l'isolat Nashes ne montre pas de différences significatives dans le pourcentage de nématodes qui se sont établis dans les larves de *Galleria* après 72 h. La température optimale pour l'infestation et l'établissement de l'isolat Nashes et de 15 à 20 °C. Il n'y a pas de différence dans l'efficacité de la récupération si un seul insecte est placé dans 25 ml de sable ou si cinq insectes sont placés dans 300 ml de sable.

Rhabditid nematodes belonging to the families Steinernematidae and Heterorhabditidae are obligate and lethal parasites of insects and have low host specificity. They act as vectors for specific bacteria, *Xenorhabdus* spp., which are harboured in the anterior intestine of third stage dauer larvae, which is the non-feeding, infective stage in the life cycle. Once inside the host haemocoele, the nematodes release the bacteria which cause a septicaemia and the host dies within hours or days, depending on the temperature. The bacteria provide optimal conditions for reproduction of the nematodes and usually within several weeks of infection,

thousands of new infective juvenile nematodes emerge from the cadaver and enter the soil. These nematodes can be reared on a commercial scale and numerous field trials against many target pests in soil, on vegetation and in cryptic habitats have been attempted with varying degrees of success. Bedding and Akhurst (1975) described a simple baiting technique using *Galleria mellonella* larvae for detecting these nematodes and since then there have been a number of studies which show that they are ubiquitous (Hominick & Briscoe, 1990*a*). Moreover, they can persist at some sites under natural conditions for two years or more (Hominick & Briscoe, 1990*b*). However, knowledge of the population biology of entomopathogenic nematodes is scanty because an understanding of population dynamics requires documentation of numbers of nematodes in the environment and such information is rarely available (Hominick & Reid, 1990). Most workers assume that percent mortality of hosts in bioassays is related to numbers of nematodes in the soil, but this assumption has serious limitations (Hominick & Reid, 1990). Therefore, we assessed the efficiency of the *Galleria* baiting technique for quantifying the infective stages of entomopathogenic rhabditids in sand or soil.

Materials and methods

In all experiments, late instar *Galleria mellonella* larvae were used and the infective nematodes had been reared in *Galleria* larvae and stored in water at 10 °C for no more than 14 days. The method to infect single larvae was based on that of Molyneux (1985). Sand was washed, autoclaved, oven-dried and passed through a 1.18 mm aperture sieve. It was moistened with 1 ml of tap water for every 25 ml of sand and then 25 ml of sand were placed in a 30 ml plastic universal tube (2.2 cm diameter, 8 cm high). Nematodes in 1 ml of tap water were introduced into a centrally-made hole 4 cm deep which was then obliterated by shaking the tube. The water content was thus 8 % (V/V). A *Galleria* larva was placed on the sand surface, the lid was screwed on and the tube inverted. Doses greater than ten nematodes were estimated by a dilution technique.

In all bioassays, depending on the temperature and period of exposure, *Galleria* larvae were recovered, washed with three rinses of water and either dissected immediately or maintained on moistened filter paper at 20 °C for a period of time before dissecting. The intention was to allow the nematodes sufficient time to kill the host and develop to adults, which facilitated counting.

Recovery from naturally-infested soil

Soil samples were randomly taken in cores of 100 cm^3, each core to a depth of 5 cm. Four cores sampled soil to a depth of 20 cm. Soil was bulked to provide 200-250 cm^3 of soil to be tested from each depth. The sampling site was Nashes Field, Silwood Park (Ordnance Survey Ref SU 945 692) and the nematodes recovered have been designated *Steinernema* sp. (Nashes isolate). DNA analysis and cross-breeding experiments (Reid & Fan, unpubl.) have shown that the species is a sibling of *S. feltiae* (Filipjev) [= *S. bibionis* (Bovien), see Poinar, 1989]. Each sample of 200-250 cm^3 was placed in a plastic dish (300 cm^3 in volume), five late instar *Galleria mellonella* larvae were placed on the soil surface and the dish was sealed with a tight-fitting lid. The dishes were incubated at 20 °C and the larvae replaced, whether living or dead, every 4 or 6 days until no further deaths occurred. All larvae were dissected in saline and the numbers of first generation adults were recorded. The percentage recovery on any particular occasion was calculated by taking the total accumulated number of nematodes recovered up to that time, divided by the number of nematodes accumulated by the end of the assay, which represented the infective nematode population in the samples.

Recovery from " Spiked " sand

Nematodes of the Nashes isolate were used at doses of 10, 50, 100, 150 and 300 per tube, replicated fifteen times. The tubes were incubated at 15 °C and the insects were changed weekly for 6 weeks. The dead ones were dissected and the numbers of nematodes counted.

Influence of time of exposure

One *Galleria* larva was added to each of 150 sand tubes containing 50 Nashes juveniles. After incubation at 15 °C for 24, 48 or 72 h, 50 tubes were removed, the *Galleria* larvae were recovered and washed in tap water and placed on moistened filter paper for a further 1 or 2 days at the same temperature. They were then dissected and the number of nematodes counted.

Influence of dose ani time of bioassay

Doses of 10, 50, 100, 150 and 300 infective Nashes juveniles were added to sand-filled tubes, a *Galleria* larva was added to each, and the tubes were incubated at 15 °C for 72 h. Then the insects, both dead and alive, were recovered and replaced with fresh ones. The larvae were rinsed in tap water, and placed on moistened filter paper for a further 1 or 2 days at the same temperature. They were then dissected and the number of nematodes counted. This was repeated until no more *Galleria* larvae were infected. There were twenty replicates at each dose.

Effect of nematode species/strain and temperature

Infective juveniles of the *S. feltiae* (= *S. bibionis*) T319 strain (Akhurst 1983), an *S. feltiae* (= *S. bibionis*)

strain recovered from England (Site 42, a hay field near Frensham Great Pond, Surrey) and Nashes isolate were tested at 15 °C. To test the effect of temperature, the Nashes isolate was used at 5, 10, 15, 20, 25 and 30 °C. There were 40 juveniles per tube, 20 replicates per strain or temperature, and tubes were incubated for 48 h. The insects were then recovered, washed, placed on moist filter paper at 20 °C and dissected 2 days later.

EFFECT OF GROUP AND INDIVIDUAL INFECTIONS

This experiment was to quantify the effect of using large containers with several insects, similar to those used in the *Galleria* trap. Thus, 300 ml plastic dishes were filled with sand and doses of 400, 600, 800 or 1 000 infective Nashes nematodes were added. Five *Galleria* larvae were placed on the surface in each dish and there were five replicates per dose. The containers were incubated at 15 °C and all *Galleria* larvae were replaced with fresh ones after 144 h. This was repeated until infections ceased. The procedure for determining numbers of nematodes in each host was then followed as documented above.

Results

It is possible to estimate the efficiency of recovery of infective nematodes from a soil sample by replacing *Galleria* larvae, whether dead or alive, with living ones until nematodes cease to infest. Then, the numbers of nematodes recovered from the larvae at any one time can be expressed as a percent of the total eventually recovered. Figure 1 presents the individual and cumulative percentages (Arcsin transformed) for nematodes recovered from soil taken from plots in Nashes Field in October, 1986 and 1987.

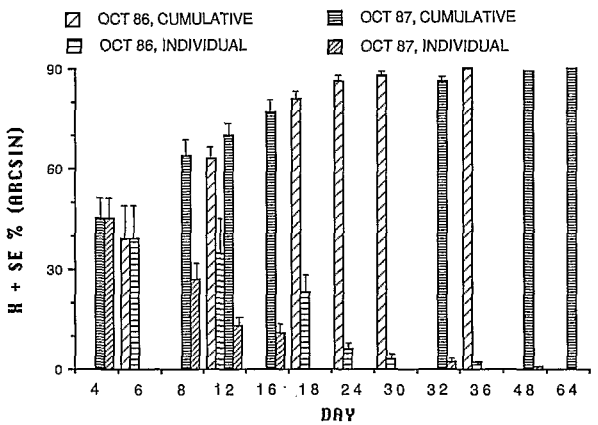

Fig. 1. Individual and cumulative means and standard errors, expressed as a percent of the total number of *Steinernema* sp. (Nashes isolate) which infected *Galleria* larvae during two prolonged bioassays at 20 °C of soil samples taken from Nashes Field. October 1986 : Six 1 m^2 plots, each providing four 250 cm^3 soil samples, collected from 0-5, 5-10, 10-15 and 15-20 cm depths; 5 *Galleria* larvae per sample, replaced at 6-day intervals until infections ceased; mean of 456 + 376 (SE) nematodes recovered per plot. October 1987 : Twenty-four 4 m^2 plots, each providing four 200 cm^3 soil samples, collected from 0-5, 5-10, 10-15 and 15-20 cm depths; 5 *Galleria* larvae per sample, replaced at 4-day intervals until infections ceased; mean of 286 + 129 (se) nematodes recovered per plot.

Table 1

Number (mean ± SE) and percent (calculated on the basis of the total eventually recovered) of *Steinernema* sp. (Nashes isolate) which established in *Galleria* larvae over a 36-day period (n = 15 tubes containing 25 ml sterile sand per dose; 15 °C; one *Galleria* larva per tube, larva changed at 6-day intervals).

Nematode juveniles/ 25 ml sand		Day of extraction						Total recovered	Total recovered as % of dose applied
		6	12	18	24	30	36		
10	N	3.4 ± 0.5	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0	0	3.9 ± 0.6	39 %
	%	87.2	7.7	2.6	2.6	0	0	100	
50	N	25.2 ± 1.9	2.3 ± 0.8	0.2 ± 0.1	0	0	0	27.7 ± 1.7	55.4 %
	%	91	8.3	0.7	0	0	0	100	
100	N	40.1 ± 2.6	1.2 ± 0.6	0.3 ± 0.1	0	0	0	41.6 ± 2.6	41.6 %
	%	96.4	2.9	0.7	0	0	0	100	
150	N	56.5 ± 2.4	11.9 ± 1.3	1.5 ± 0.3	0	0	0	69.9 ± 2.6	46.6 %
	%	80.8	17.0	2.1	0	0	0	100	
300	N	108.1 ± 7.6	18.3 ± 1.8	5.5 ± 1.2	4.1 ± 2.7	0.1 ± 0.1	0.1 ± 0.1	136.2 ± 8.0	45.4 %
	%	79.4	13.4	4.0	3.0	0.1	0.1	100	

In the October, 1986 samples, six consecutive 6-day exposures were required before nematodes ceased to infest fresh larvae. The largest proportion was obtained in the first exposure and the proportion decreased thereafter. In the first exposure, a mean of 39.8 % of the nematodes was recovered (CV = 24.5 %) and the standard error bars show that large variations existed between the six plots for the first two exposures (compare " individual " percentages). However, when the accumulated mean percent recovery was calculated, the variability between plots was much reduced. Thus, after 12 days the accumulated percent recovery was at a fairly consistent level approaching 75 % (untransformed), regardless of the nematode density in each plot.

The second assay in October, 1987 was carried out on samples from 24 plots to test whether this relationship was consistent. This time, sixteen consecutive 4-day exposures were required to recover all the nematodes. Nevertheless, the mean accumulated percent recovered again reached a high and fairly consistent level (mean 81.4 %, CV = 6.9 %, untransformed data) on day 12. This compares favourably with the results from the October, 1986 samples. Thus, a large number of consecutive bioassays is required to recover all the nematodes from a soil sample. However, two consecutive 6 day or three consecutive 4 day assays will extract about 80 % of the infective nematode population.

To simulate the conditions of naturally-infested soil samples, where the densities of nematodes are unknown and variable, 25 ml samples of sand were " spiked " with 10, 25, 50, 150 or 300 nematodes. Table 1 shows the numbers of nematodes recovered at 6-day intervals at each density while Figure 2 amalgamates the data from all five densities so that it is comparable to Figure 1.

Like the nematodes in soil from the field, those applied to sand could not be recovered by one exposure to a *Galleria* larva. Unlike the nematodes in field soil, those in sand were recovered more efficiently. Regardless of nematode density, a mean of 96.6 % (untransformed data) (CV = 3 %) of the nematodes which could be recovered were recovered with two consecutive 6-day bioassays. Note also that only 39-55 % of the dose applied eventually established (Table 1).

The mean (SE) numbers of Nashes isolate of *Steinernema* sp. which established in each *Galleria* larva after 24, 48 or 72 h exposures at 15 °C (50 replicates per time) were 7.8 (0.7), 18.5 (0.9) and 20.4 (1.0) respectively. Thus, there was a significant increase between 24 and 48 h but only a slight increase thereafter. A range of 36-40 % of the 50 infectives applied per *Galleria* had penetrated and established during the 72 h exposure.

The mean number of nematodes recovered after a 72 h exposure increased linearly as the dose of infectives increased from 10 to 300 per larva (Fig. 3). This implies that the proportion of the nematodes which could penetrate and establish in the hosts (the slope of the line : 36 %) was independent of the dose over the range tested. Such a dose-response test has been repeated a number of times by different workers in our research group, using different nematodes and conditions (Table 2). A high correlation between dose and the mean number of nematodes that establish in the replicates at each dose was always obtained, though protocols varied greatly. Thus, the test appears robust and the different slopes of the lines, which indicate different establishment rates of

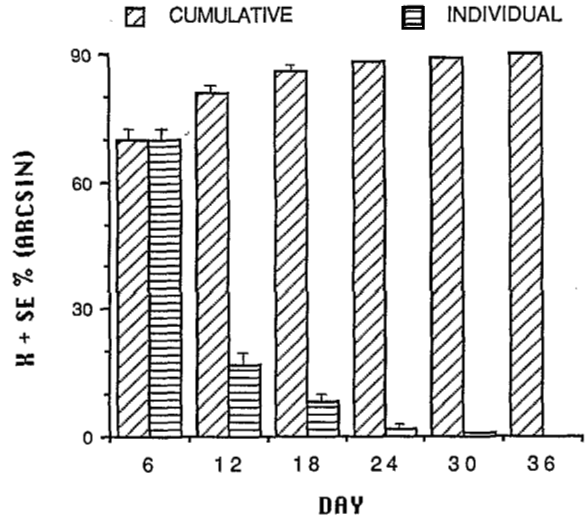

Fig. 2. Individual and cumulative means and standard errors, expressed as a percent of the total number of *Steinernema* sp. (Nashes isolate) which infected *Galleria* larvae during a 36-day bioassay. Universal screw-capped tubes contained 25 ml of sterile sand to which 10, 25, 50, 150 or 300 infective nematodes had been added (n = 15 tubes per dose; 15 °C; one *Galleria* larva per tube, larva replaced at 6-day intervals).

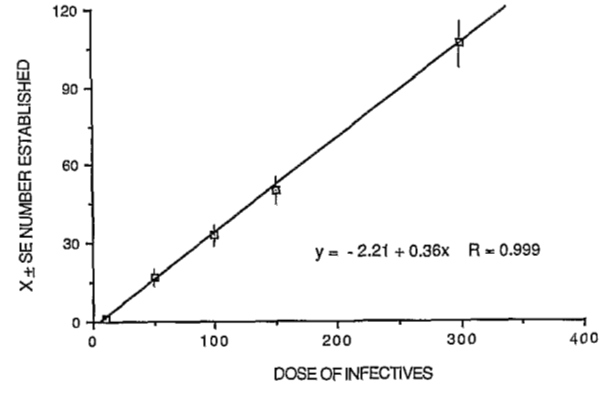

Fig. 3. Relationship between number of infective stages in 25 ml sterile sand and number (mean and standard error) of *Steinernema* sp. (Nashes isolate) which established in a *Galleria* larva after 72 h (twenty replicates per dose, 15 °C).

Table 2

Regression equations for mean number of nematodes per *Galleria* larva (y), exposed to increasing doses of infective stages (x) with n replicates/dose, water 8 % (vol/vol), using different nematode species/isolates and different protocols and obtained independently by different workers (Compare with Fig. 3).

Nematode	Protocol	Regression	R^2
Steiner nema sp. (Nashes)[1]	25 cm^3 sand, n = 15, 144 h exposure, x = 10, 50, 100, 150, 300/tube, 15 °C	$y = 3.94 + 0.35x$	0.995
Heterorhabditis sp. (UK)[2]	25 cm^3 sand, n = 15, 120 h exposure, x = 10, 50, 100, 150/tube, 15 °C	$y = 0.86 + 0.30x$	0.949
Heterorhabditis sp. (Dutch)[2]	25 cm^3 sand, n = 15, 120 h exposure, x = 10, 50, 100, 150, 200, 300, 500/tube, 15 °C	$y = 7.82 + 0.23x$	0.948
Heterorhabditis sp. (New Zealand)[2]	as for *Heterorhabditis* sp. (Dutch), but temperature = 20 °C	$y = -6.53 + 0.43x$	0.984
Heterorhabditis sp. (Trinidad)[2]	as for *Heterorhabditis* sp. (Dutch), but temperature = 20 °C	$y = -13.64 + 0.71x$	0.977
S. feltiae (= *S. bibionis*) (UK Site 76)[3]	300 cm^3 Corris Forest soil/dish, 5 *Galleria* per dish n = 10, 72 h exposure then fresh *Galleria* for another 72 h. Regression on cumulated mean numbers/dose x = 50, 250, 500, 750, 1 500/dish, 15 °C	$y = 1.99 + 0.15x$	0.985
S. feltiae (UK Site 76)[3]	identical protocol to the one above, but soil from Keilder Forest	$y = -4.40 + 0.13x$	0.972

[1,2,3] Unpublished data from : [1] Fan, X.; [2] Mason, J.; [3] Collins, S.

the nematodes, may provide a useful parameter for comparing the efficacy of different nematodes under specified conditions. For the trials documented in Table 2, *Steinernema* sp. (Nashes) in sand at 15 °C appears more effective than either *Heterorhabditis* isolate and all the nematodes in sand were better than *S. feltiae* (= *S. bibionis*) in forest soil. The most effective nematodes were *Heterorhabditis* sp. at 20 °C in sand, and the Trinidad isolate was the best.

Because a single exposure never recovers all infective nematodes, a fresh insect was added to each tube every 72 h until no further insects died. At all doses tested, most of the nematodes infected during the first two exposures and an additional four exposures resulted in little change. Thus, the overall mean percent of the infectives which established after two exposures to *Galleria* larvae for all the doses was 36.9 % compared to 37.6 % after six consecutive exposures.

There was no significant difference in the percent establishment of a dose of 40 infectives from the three strains tested after 72 h at 15 °C (ANOVA, $F = 1.25$, $P > 0.05$). The mean (SE) numbers per host were 14.7 (1.7) for T319, 17.9 (1.5) for Site 42 nematodes and 15.4 (1.3) for Nashes isolate. Again, for all three, the percent establishment was around 40 % of the dose applied.

The optimal temperature for infection and development of the Nashes isolate was 15-20 °C (Fig. 4), where the maximum percent establishment was 30-40 % of the dose applied. At temperatures outside this range, percent establishment never exceeded 20 %.

The results for group infections were similar to those for single infections. There were no significant differences between the percent establishment at the different doses tested and most nematodes infected after the first two exposures. Thus, after the first 144 h exposure, the overall mean percent establishment was 28.7 %.

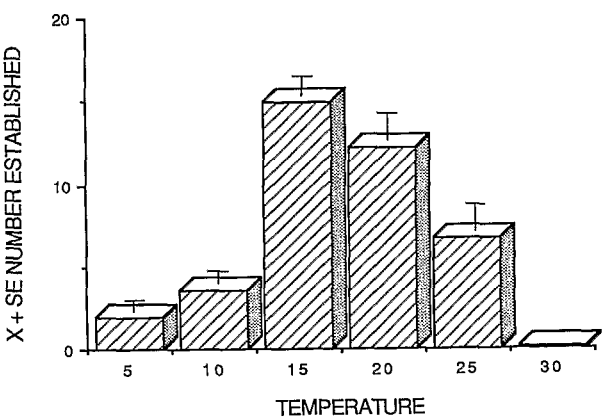

Fig. 4. Mean (+ se) number of *Steinernema* sp. (Nashes isolate) which established in each *Galleria* larva after 48 h at different temperatures (°C); 40 infectives per *Galleria* larva. (Replicated 20 times, 25 ml sterile sand).

After a second exposure of 144 h to fresh hosts, the overall mean percent establishment was 35.1 % and a further three exposures of 144 h each resulted in a final mean percent establishment of 37.9 %.

Discussion

Galleria traps have now become the standard method for recovering entomopathogenic rhabditids from soil. However, the results are usually treated qualitatively and the efficiency of the method has never been systematically examined. Hominick and Briscoe (1990*b*) showed that nematodes could sometimes be recovered from naturally infested soil in four consecutive tests. Because a first assay did not always recover nematodes, they used two consecutive bioassays during their survey of Britain (Hominick & Briscoe 1990*a*). This resulted in an estimate of the prevalence of the nematodes increasing from 39 to 49 % of the sites sampled. The present results confirm that a number of successive extractions is required to recover all the infective nematodes from a sample, even though conditions appeared optimal for infection. For population dynamics studies, it is essential to quantify the numbers of infective nematodes, but if all samples had to be successively bioassayed to exhaustion, the process would be tedious and labour intensive. Our results show that two or three consecutive assays over twelve days were sufficient for providing an estimate with an acceptable variance of the proportion of infective nematodes that would be recovered from a sample. Thus the procedure required to bioassay quantitatively a large number of samples would be to bioassay five to ten samples to exhaustion to calibrate the assay for soil type and nematode species. Then, the mean cumulative percent recovery after the second or third bioassay could be used as a recovery constant to estimate the number of infective nematodes in the other samples which need only be assayed two or three times.

Another way to assess the numbers of nematodes in a soil sample is to extract the nematodes using standard methods employed for phytophagous or free-living species. Saunders and All (1982) showed that the Baermann funnel technique recovered 71 % of *Neoaplectana carpocapsae* Weiser dauer larvae from spiked sand compared to 46 % recovered by a centrifugation-flotation method and 8 % by a flotation-sieving method. However, in field soils a community of nematodes is extracted and it is difficult and time-consuming to identify entomopathogenic species. Moreover, it is still necessary to assess the infectivity of the extracted nematodes with a bioassay because it is the number of infective juveniles present and not the total number which is important for population dynamics studies (Hominick & Reid, 1990). Hence this procedure adds an extra laborious step to assessing entomopathogenic nematode numbers and has not been adopted by workers.

Mraček (1982) estimated the numbers of nematodes in soil samples by yet another technique. He calibrated his *Galleria* bioassay by adding different concentrations of nematodes and relating them to the percent mortality and the time taken to attain different mortalities. However, this assumes that all larvae added to the sample are infective, an assumption that is incorrect. It is still necessary to count the numbers of nematodes per host to obtain an acceptable estimate of the numbers of infective nematodes in the soil sample.

Regardless of nematode strain, dose or exposure to single or several larvae, only about 30-40 % of the nematodes became established. These results confirm and extend observations of others. Bedding and Akhurst (1975) stated, without providing details, that 20-40 % of infective nematodes of various *Steinernema* species were recovered from *Galleria* larvae. Similarly, Mraček (1982) estimated that 10-20 % of a dose of *N. carpocapsae* applied to 0.3 dm^3 of soil was recovered from twenty *Galleria* larvae. Presumably, these estimates are based on a single test rather than on consecutive bioassays of the same sample. In more artificial conditions, Bednarek and Nowicki (1986) exposed groups of twenty *Galleria* larvae to 1000 *S. feltiae* nematodes on filter paper in Petri dishes. They even provided fresh insects every 5 h but the total numbers of nematodes recovered from the cadavers never exceeded 42 % of the dose applied.

It is not known why the majority of nematodes fail to establish when conditions are optimal. Estimates for infectivity based on *Galleria* larvae will be high since they are especially susceptible to entomopathogenic nematodes (Bedding, Molyneux & Akhurst, 1983). Kondo and Ishibashi (1986*a*) conducted filter paper tests with 500 infectives per *Spodoptera litura* larva (the common cutworm) and found that after 48 h exposures at 25 °C, 69 % of *S. feltiae* (DD-136), 4 % of *S. bibionis* and 2 % of *S. glaseri* had penetrated and developed to adults. Similar tests with DD-136 but with soil present showed that only 9 % of a dose of 1000 established after 24 h (Kondo & Ishibashi 1986*c*). They also found that replacing the insects with healthy ones every 24 h resulted in fewer nematodes establishing in successive exposures. Our results show a similar trend, though over weeks rather than days (Fig. 2). Also, different times of exposure to one host showed that most nematodes penetrated between 24 and 48 h after exposure began. Bednarek and Nowicki (1986) felt that nematodes in contact with *Galleria* feces become stimulated and rapidly deplete their energy reserves, hence losing their infectivity. Kondo and Ishibashi (1986*b*) also implicated host feces in affecting infectivity, but felt that differential attraction of host body and feces may explain differential infectivity. That is, some nematodes may be more attracted to host feces than to the host body, and so are less infective than others. Clearly, this is a fruitful area for further research. Infectivity is a complicated process, influenced by the host, environment and intrin-

sic properties of the parasite. Understanding the limitations could allow manipulations that would increase the efficacy of the parasites.

The relationship between dose and mean numbers of nematodes per host was remarkably strong. This is in contrast to the relationship between dose and % mortality for *Galleria* which is poor because it is so susceptible to the nematodes (see Bedding, Molyneux & Akhurst, 1983). A convenient bioassay for assessing efficacy and quality control has been sought by many workers (see Hominick & Reid, 1990) and one based on numbers of nematodes per *Galleria* may provide a useful first test to assess how different species/strains would perform under specific conditions. For example, the results for the effect of temperature clearly showed that the Nashes isolate establishes best over the temperature range 15-20 °C. This reflects soil temperatures commonly encountered in Britain (Hominick & Briscoe, 1990*a*) and the prediction would be that the species would be useful in control programmes where cooler temperatures prevail. To compare this species with other species/isolates, a dose-response test at the required operational temperature in the appropriate soil would be conducted and regression equations calculated. The nematode populations producing the largest regression coefficients would then be selected for further tests against the target pest. Alternatively, the target pest could replace *Galleria* if it is easily available. Then, regressions of the mean number of nematodes per host against dose should still provide a more sensitive comparison than the usual LD50 determinations. Also, fewer replicates would be required and the assumption that every nematode is equally infective, which the present results clearly show is incorrect, need not be made.

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