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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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 docu-

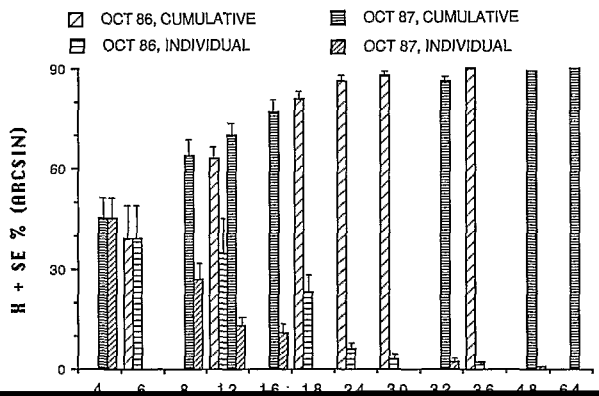
to a depth of 20 cm. Soil was bulked to provide 200-250 cm^3 of soil to be tested from each depth. The sampling site was Nashes Field, Silwood Park (Ordnance Survey Ref SU 945 692) and the nematodes recovered have been designated *Steinernema* sp. (Nashes isolate). DNA analysis and cross-breeding experiments (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

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

recovered from soil taken from plots in Nashes Field in October, 1986 and 1987.



In the October, 1986 samples, six consecutive 6-day

and variable, 25 ml samples of sand were " spiked " with

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
[redacted]	[redacted]	[redacted]	[redacted]

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

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

sic properties of the parasite. Understanding the limi-

BEDDING, R. A. & AKHURST, R. J. (1975). A simple technique