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Canadian Steinernematid (Nematoda) Isolates and Their Infectivity, under Cold Conditions, to Greater Wax Moth (*Galleria mellonella*) Larvae

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Nematode species, strains, or isolates of the entomopathogenic family Steinernematidae differ in their ability to infect insects at low temperatures. Some steinernematid isolates from British Columbia, probably a new species, killed *Galleria mellonella* larvae at 7°C. In laboratory experiments, these nematode isolates (isolates 69, 76, 99, 102, or D) were applied to petri dishes at concentrations of 50, 100, 200, 400, or 800 infective juvenile nematodes (IJs)/dish. Each petri dish contained 10 *Galleria* larvae and was kept at 4, 7, or 10°C for 250 to 255 h. More than 5600 *G. mellonella* larvae were examined to determine larval mortality and the number of adult nematodes developing in the insect. Excluding 69, isolate, all other isolates showed a significant level of infectivity at these temperatures. At 10°C the mortality reached 100% at 400 and 800 IJs/dish of the D isolate, and at 4°C the highest mortality was 81.7% at 800 IJs/dish of isolate 99. The penetration efficiency, as determined by the number of adult nematodes recovered when the larvae were dissected, decreased with temperature and IJ concentration and ranged from 28.5% (isolate D at 10°C and 800 IJs/dish) to 0% (isolates 102 and 69 at 4°C and 50 IJs/dish). *Steinernema cubana* with supposed warm temperature infectivity was used as a comparative control and was only slightly infective at 10°C and 800 IJs/dish. The rate of infection, evaluated by Anderson's model, proved to be a useful tool for comparing the infectivity of various isolates and species of entomopathogenic nematodes, as it is independent of experimental conditions. © 1997 Academic Press

KEY WORDS: *Steinernema* spp.; entomopathogenic nematode; cold temperature.

INTRODUCTION

Infectivity of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) under cold conditions is an important attribute in temperate regions of the world, where significant insect damage to crops may occur at relatively low temperatures. It is generally believed that those nematode isolates that are cold adapted should be most effective as biological control agents of pest insects in the cooler, temperate regions. Laboratory experiments with cold-adapted *Heterorhabditis* species showed significantly greater mortality of black wine weevil larvae at 9 and 12°C, but not at 20°C (Simons and van der Schaaf, 1986). Consequently, the low temperature strains of *Heterorhabditis* spp. in combination with their low temperature bacterial symbiont strain were recommended for effective field control at temperatures below 12°C. Griffin and Downes (1991) exposed larval *Galleria mellonella* (L.) to four heterorhabditid isolates at 5, 7, 9, 12, and 20°C and found significant differences among isolates in the number of infective juveniles (IJs) that entered the host larvae at these experimental temperatures. Hf85 was significantly better than all other isolates, but its success was considerably reduced at 7°C and there was no infectivity at 5°C.

Generally steinernematids are more effective at lower temperatures than are heterorhabditids. However, in outdoor experiments with steinernematids at soil temperatures of 12–14°C, the results were variable. This is reported to be the critical minimum temperature for effective pest control (Griffin *et al.,* 1989) when using a steinernematid strain adapted for use at 20°C. Commercial companies in the cooler agricultural regions of the world are seeking nematode isolates that are effective at temperatures below 10°C in order to control pest insects in the spring and late autumn when soil temperatures are low. Temperature influences nematode mobility (Molyneux, 1986), reproduction and development (Wright, 1992), and infectivity (Finney and Bennet, 1984), and all these factors influence the effectiveness of entomopathogenic nematodes as biological control agents against insects. Unlike heterorhabditids where the mobility threshold is about 10–14°C, the steinernematids remain mobile at temperatures as low as 4°C (Molyneux, 1986).

Five isolates of *Steinernema* spp., which according to morphological characters probably included a new spe-

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cies from British Columbia, infected *G. mellonella* larvae at 7°C (Mráček and Webster, 1993). Most of these isolates originated from high mountain elevations or subboreal habitats and did not reproduce at temperatures greater than 20°C. Our objective was to delineate the infectivity of such steinernematid nematode isolates from Canada under cold conditions and to compare their infectivity with that of *S. cubana,* a nematode species originating from the subtropics (Mráček *et al.,* 1994).

MATERIALS AND METHODS

Steinernematid isolates 69, 76, 99, 102, and D (for description of their origin see Mráček and Webster (1993)) were tested in the laboratory for their infectivity to *G. mellonella* (reared for many generations in the Simon Fraser University Insectary) larvae at 4, 7, and 10°C and at 50, 100, 200, 400, and 800 IJs/dish with six replications (for details of the petri dish bioassay procedure see Mráček and Webster (1993)). The *G. mellonella* larvae were exposed to IJs for 250 to 255 h (approximately 10 days) in an incubator.

Experimental arenas were made of petri dishes (9 cm diameter) filled with sterilized, wet sand (7% moisture) and inoculated with different sized inocula of each isolate in 1 ml cold water (1–3°C) on the sand in the center of each dish. Ten *G. mellonella* larvae were placed around the edge of each dish. Both IJs and *G. mellonella* were acclimatized at the experimental temperature for 2 h. The number of IJs was counted in a water drop for concentrations of 50 and 100 IJs, and higher concentrations were based on the average of five aliquots of nematode suspension. In part of this experiment for comparative purposes, *S. cubana* inocula treatments were used in the experimental arenas in the same way as described above.

After 250–255 h, the insect cadavers were washed in tap water and dried on an absorbent paper to remove IJs from the cadavers' surface. The cadavers were kept at room temperature for 1 to 2 days after completion of the experiment to enable nematodes to mature before dissection. The mortality caused by nematodes was determined by dissecting the *Galleria* larvae and counting the adult nematodes in the body cavity.

Statistical Analysis

Differences in the numbers of nematodes established among the experimental groups were analyzed by three-way ANOVA. Experimental data were fitted by a modification of Anderson's (1978) model of the dynamics of the infection process so as to evaluate the instantaneous rate of infection for each *Steinernema* sp. isolate and temperature. This model was used to simulate the infective process by entomopathogenic nematodes (Hudson and Norman, 1995). The instantaneous rate of infection is a useful tool for comparing the infectivity of different clones or species of parasites because it is consistent for any pair of host–parasite species at a particular temperature and is independent of experimental conditions such as initial host species or parasite numbers, size of the experimental arena, duration of the experiment, etc. (Řezáč *et al.,* 1993).

Anderson's Model

In using the Anderson (1978) model for any particular temperature, the number of IJs in the dish is denoted by $I = I(t)$, the total number of successful nematodes penetrating into all hosts at time t by $P = P(t)$, and the initial conditions (the numbers at time zero) were $I(0) = I_0$ and $P(0) = 0$, respectively. The rate of infection, α, can be assumed to be directly proportional to I and to the number of living hosts, $H = H(t)$, and indirectly proportional to the volume of the experimental arena, V,

$$dI/dt = -\alpha IH/V, \quad [1]$$

$$dP/dt = \alpha IH/V, \quad [2]$$

where d/dt is the first derivative. The number of susceptible hosts declines as they are being killed by the penetrating nematodes. Due to the possibility of superparasitism, however, the rate of decline in susceptible hosts is lower than the rate of infection, α; therefore, a different parameter, β, where $\beta < \alpha$, is used for the description of the dynamics of numbers of susceptible hosts:

$$dH/dt = -\beta IH/V, \qquad H(0) = H_0. \quad [3]$$

A typical behavior of the system [1]–[3] is easily derived by the separation of variables. With respect to time, both the number of IJs, I, and the number of living hosts, H, approach the constant values, $I_\omega = \beta H_0$ and $H_\omega = 0$, respectively, with an increase of α, I_0, and $1/V$ causing increases in the rate of approach to this equilibrium. Parameter α, called "the rate of infection," is therefore an important characteristic of the nematodes that describes their ability to penetrate the insect hosts. The total number of nematodes found in the hosts before equilibrium has been reached increases with the increase in the initial number of infective juveniles, but also approaches an equilibrium value.

RESULTS

More than 5600 *Galleria* cadavers were dissected during the experiments. All tested *Steinernema* isolates were capable of infecting *G. mellonella* larvae. At 10°C, the average *Galleria* mortality, from all isolates

except *S. cubana,* was over 90% and reached 100% at 800 and 400 IJs/dish using isolate D. Even at 50 IJs/dish the average mortality for all isolates ranged from 25 (isolate 69) to 56.7% (isolate D) (Table 1). At 7°C the mortality ranged between 96.7 and 100% at 800 IJs/dish (except for isolate 69) and between 20 and 63.3% at 50 IJs/dish (Table 1). At the lowest temperature tested (4°C) relatively few *Galleria* larvae were killed. At 800 IJs/dish, the average percentage mortality of *Galleria* ranged from 6.7 (isolate 69) to 81.7 (isolate 99), and at 50 IJs/dish and at 4°C the average percentage mortality ranged from zero (isolate 69) to 38.3 (isolate 76) (Table 1).

Isolate 69 consistently caused lower *Galleria* mortality at all temperatures and concentrations than did any other *Steinernema* isolate tested, except for *S. cubana.* The highest mortality obtained with isolate 69 was 73% at 10°C and 800 IJs/dish. In combinations of lower temperatures and concentrations, the mortality of *Galleria* larvae caused by this isolate decreased rapidly to very low levels and to zero at 4°C (Table 1). The control nematode, *S. cubana,* infected only a very small percentage of *Galleria* and that was at 10°C with 800 IJs/dish.

The average number of adult nematodes per *Galleria* cadaver at the end of the experiment is given in Table 2. A comparison of the percentage of adults that developed in the *Galleria* at different temperatures from the different IJ inocula showed that the highest average percentage of recovered adults varied with temperature. Thus, at 10°C the highest average percentage ranged from 17 (50 IJs/dish) to 28.5 (800 IJs/dish) for isolate D; at 7°C from 17 (50 IJs/dish) to 26.7 (800 IJs/dish) for isolate 102; and at 4°C from 3.6 (50 IJs/dish) to 4.5 (800 IJs/dish) for isolate 99. The number of recovered nematode adults of isolate 69 was the lowest among all isolates at all temperature treatments. If this number is expressed as a percentage of the size of IJ inocula, it reached a maximum of 7.4 at 10°C (200 IJs/dish) and no adults were recovered at 4°C (Table 2).

There was a high rate of infection (α in Table 2) for all tested isolates (excluding isolate 69 and *S. cubana*), when evaluated by Anderson's model. This value was highest at 10°C for isolate D and at 7°C for isolate 102, and at 4°C the differences were relatively very small (Table 2). Results of a three-way ANOVA on the mortality of *Galleria* larvae showed that with infectivity as a dependent variable, the F ratio was 236.2 and $df = 2$ for the effect of temperature, $F = 410.6$ and $df = 5$ for the effect of isolates and $F = 145.1$ and $df = 4$ for the effect of the IJ inoculum size. The influence of each of these factors was highly significant ($P < 0.001$). As well, the interactions of temperature × isolate, isolate × IJ inoculum, and temperature × IJ inoculum were highly significant ($P < 0.01$, $F = 23.8$, 2.8, and 8.1 and $df = 10$, 8, and 20, respectively). A three-way ANOVA on the number of adult steinernematid nematodes that developed in the cadavers of *G. mellonella* larvae showed $F = 119.8$, $df = 2$ for temperature, $F = 55.4$, $df = 5$ for isolate, $F = 155.4$, $df = 4$ for IJ inoculum, $F = 16.4$, $df = 10$ for the interactions temperature × isolate, $F = 26.3$, $df = 8$ for temperature × IJ inoculum, and $F = 13.8$, $df = 20$ for isolate × IJ inoculum.

DISCUSSION

All Canadian isolates (excluding isolate 69) were shown to have high infectivity at the relatively low

TABLE 1

Average Percentage Mortality of *Galleria mellonella* Larvae (% ± Standard Deviation) Caused by Five Different Sized Inocula of Infective Juveniles (IJs) of *Steinernema* sp. (Isolates 69, 76, 99, 102, and D) and *S. cubana* (cub.) at Three Temperatures (T)

		% Mortality by isolates of *Steinernema* sp. ± SD					
T (°C)	No. IJ/dish	69	76	99	102	D	cub.
10	50	25 ± 22.7	53.3 ± 13.3	33.3 ± 21.5	43.3 ± 24.7	56.7 ± 11.3	0.0 ± 0.0
	100	41.7 ± 23.4	66.7 ± 16.5	48.3 ± 14.6	60.0 ± 15.5	69.6 ± 21.7	0.0 ± 0.0
	200	66.7 ± 22.6	75.0 ± 9.2	90.0 ± 9.8	76.7 ± 13.3	95.0 ± 6.0	0.0 ± 0.0
	400	70.0 ± 22.4	93.3 ± 5.7	90.0 ± 17.0	86.7 ± 11.3	100.0 ± 0.0	0.0 ± 0.0
	800	73.3 ± 17.9	96.7 ± 8.9	98.3 ± 4.5	96.7 ± 5.7	100.0 ± 0.0	3.3 ± 5.7
7	50	20.0 ± 7.9	23.3 ± 23.7	63.3 ± 22.6	55.0 ± 18.0	42.0 ± 22.9	0.0 ± 0.0
	100	23.3 ± 13.3	53.3 ± 13.3	78.3 ± 16.1	65.0 ± 11.5	48.0 ± 25.5	0.0 ± 0.0
	200	33.3 ± 13.3	58.3 ± 30.5	93.3 ± 8.9	81.7 ± 21.3	86.7 ± 19.2	0.0 ± 0.0
	400	35.0 ± 16.6	85.0 ± 13.4	98.3 ± 4.5	95.0 ± 6.0	96.7 ± 5.7	0.0 ± 0.0
	800	43.3 ± 26.5	96.7 ± 8.9	100.0 ± 0.0	100.0 ± 0.0	98.3 ± 4.5	0.0 ± 0.0
4	50	0.0 ± 0.0	38.3 ± 14.6	13.3 ± 5.4	13.3 ± 5.7	35.0 ± 18.0	0.0 ± 0.0
	100	0.0 ± 0.0	43.3 ± 21.5	26.7 ± 25.6	23.3 ± 8.9	40.0 ± 0.0	0.0 ± 0.0
	200	0.0 ± 0.0	58.3 ± 4.5	60.0 ± 24.0	36.7 ± 11.3	48.3 ± 16.1	0.0 ± 0.0
	400	3.3 ± 5.7	65.0 ± 6.0	53.3 ± 24.7	35.0 ± 6.0	65.0 ± 18.0	0.0 ± 0.0
	800	6.7 ± 8.9	75.0 ± 11.5	81.7 ± 10.8	53.3 ± 8.9	80.0 ± 25.0	0.0 ± 0.0

TABLE 2

Average Number (±Standard Deviation) of Adult Steinernematid Nematodes in Cadavers of *Galleria mellonella* Larvae at Three Temperatures (*T*) and for Different Inoculum Levels of Infective Juveniles (IJs); Expressed Also for the Parameters α (the Rate of Infection) and β (See Text) of Anderson's (1978) Model

		No. of adult nematodes in *Galleria* cadavers ± SD					
T (°C)	No. IJ/dish	69	76	99	102	D	cub.
10	50	1.7 ± 1.3	8.3 ± 7.0	4.2 ± 4.2	5.5 ± 3.8	8.5 ± 5.2	0.0 ± 0.0
	100	6.3 ± 5.7	18.8 ± 7.6	6.8 ± 2.7	13.2 ± 5.3	11.8 ± 5.9	0.0 ± 0.0
	200	14.8 ± 8.3	28.0 ± 5.7	33.8 ± 20.9	33.7 ± 11.1	55.2 ± 14.4	0.0 ± 0.0
	400	20.4 ± 8.8	66.5 ± 20.6	53.2 ± 29.5	55.5 ± 13.3	85.7 ± 13.6	0.0 ± 0.0
	800	31.3 ± 18.4	114.7 ± 47.1	84.0 ± 37.6	129.8 ± 36.2	227.8 ± 91.9	0.3 ± 0.9
7	50	0.4 ± 0.6	1.8 ± 2.8	8.2 ± 5.5	8.5 ± 3.3	3.4 ± 1.7	0.0 ± 0.0
	100	3.0 ± 4.0	7.5 ± 3.4	15.2 ± 9.7	15.7 ± 3.1	5.8 ± 6.0	0.0 ± 0.0
	200	12.3 ± 15.0	9.0 ± 4.3	44.0 ± 22.0	38.2 ± 16.5	44.3 ± 25.4	0.0 ± 0.0
	400	18.8 ± 10.9	41.5 ± 28.6	76.2 ± 17.3	95.7 ± 15.8	80.7 ± 20.3	0.0 ± 0.0
	800	32.3 ± 25.2	84.5 ± 46.4	158.3 ± 73.8	213.7 ± 48.3	135.7 ± 83.5	0.0 ± 0.0
4	50	0.0 ± 0.0	1.7 ± 1.6	1.0 ± 0.7	0.0 ± 0.0	1.8 ± 2.4	0.0 ± 0.0
	100	0.0 ± 0.0	2.2 ± 2.3	2.3 ± 2.3	0.0 ± 0.0	2.7 ± 1.8	0.0 ± 0.0
	200	0.0 ± 0.0	4.8 ± 3.7	13.3 ± 11.4	1.2 ± 0.8	3.2 ± 2.4	0.0 ± 0.0
	400	0.0 ± 0.0	14.2 ± 11.0	7.7 ± 7.6	1.5 ± 1.8	5.5 ± 3.4	0.0 ± 0.0
	800	0.0 ± 0.0	18.5 ± 8.3	36.3 ± 16.2	2.7 ± 1.3	24.2 ± 29.0	0.0 ± 0.0
10	α	0.0806	0.1942	0.1782	0.1743	0.3193	0.0019
	β	0.0205	0.0057	0.0130	0.0000	0.0000	0.0000
	RSS	10	41	111	88	823	1
	log(β)	−1.69	−2.24	−1.89	−6.39	−8.65	−8.11
7	α	0.0573	0.1095	0.2230	0.3017	0.2383	0.0000
	β	0.0084	0.0000	0.0000	0.0000	0.0062	0.0000
	RSS	16	157	51	411	310	0
	log(β)	−2.07	−5.17	−5.37	−8.67	−2.21	
4	α	0.0000	0.0387	0.0427	0.0036	0.0273	0.0000
	β	0.0000	0.0127	0.0000	0.0000	0.0000	0.0000
	RSS	0	11	120	0	40	0
	log(β)		−1.90	−6.76	−7.83	−6.95	

Note. RSS is the corresponding residual sum of squares. As β is very small, log(β) is also given.

temperatures of 4, 7, and 10°C by penetrating and killing *Galleria* larvae. Therefore, these isolates may be suitable for the biological control of some insect pests at similar low temperatures. No large differences were found in infectivity at these cold temperatures among isolates 76, 99, 102, and D, but each of these isolates infected *Galleria* in significantly greater numbers than did *S. cubana,* the nematode species originating from Cuba in the subtropics. Surprisingly, isolate 69, which originated from the most northerly latitude, namely at the Liard Hot Spring settlement in northern British Columbia, infected most *Galleria* at higher temperatures. This may be due to the fact that the location of the sampling site may have been thermally influenced by an adjacent (100 m away) wetland with hot springs.

It is probable that the climatic character of the biotope where an isolate is found has an essential impact on the optimum temperature for infectivity of an isolate. Griffin and Downes (1994) reported that 29 of 151 of their tested isolates were infective at cold temperatures (i.e., 9°C). All these isolates originated from northern and western Europe. The Canterbury strain of *Steinernema feltiae* (Filipjev) (syn. *bibionis*) that was isolated at a higher altitude with a prevailing cooler climate (Wright and Jackson, 1988) killed 93% of porina (*Wiseana copularis*) larvae at 10°C in laboratory experiments.

The highest percentage *Galleria* mortality was caused by isolate D at 10°C and this corresponded with the highest value for the number of recovered nematode adults. At 7°C, the highest *Galleria* mortality was caused by isolate 99 and, as claimed by Griffin and Downes (1994), there is a strong correlation between the number of nematodes recovered from the host and host mortality. However, at 4°C, a relatively small number of IJs of isolate 99 penetrated into *Galleria* larvae, but the percentage of *Galleria* mortality was relatively high (excepting isolate 69) and varied from 13.3 to 81.7%.

These Canadian steinernematid isolates had a higher rate of infectivity of *Galleria* larvae than did cold infective *Heterorhabditis* isolates reported earlier (Griffin and Downes, 1994). Only *Heterorhabditis* Hf85 was infective at 9°C (Griffin and Downes, 1991) and its

infectivity was significantly reduced below this temperature. These Canadian steinernematid isolates readily killed their *Galleria* hosts at 7°C, and even at 4°C with a *Galleria* mortality 80% (isolate 99) at 800 IJs. However, the duration of exposure of *Galleria* larvae was longer in the present experiments than in those of Griffin and Downes (1991, 1994) and this could account for the difference in percentage mortality.

The Canterbury strain of *S. feltiae* appeared to have greater infectivity under cold conditions than did other steinernematid and heterorhabditid strains when tested at 10°C against grass grub (*Costelytra zealandica*) and porina larvae (Wright *et al.,* 1988). This strain was isolated from the wild at a higher altitude, which may reflect an associated colder climate. Molyneux (1986) considered the geographic origin and associated temperature to be important factors influencing nematode activity, host penetration and host mortality.

The duration of exposure to *Galleria* used in our experiments was somewhat longer (2–5 days) than that used by Griffin and Downes (1991, 1994) (3 days), which probably explains some of the differences in observations between their reports and our observations. We regard an exposure period of 3 days as too at low temperature to obtain optimum infection of the host and it does not simulate natural conditions.

The number of nematodes within the *Galleria* larvae increased with increasing nematode inoculum concentration which is consistent with the prediction of Anderson's model. The number of adult nematodes in the *Galleria* increased with increasing temperature, which is reflected in Anderson's model by the increased rate of penetration, α, with increasing temperature (Table 2). As the rate of penetration, α, is independent of inoculum size and/or host concentration, length of the experiment, etc., it can be used as a good measure of the differences between the infection capabilities of various nematode strains under constant temperature conditions.

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