Journal of Invertebrate Pathology **73,** 243–247 (1999)
Article ID jipa.1998.4833, available online at http://www.idealibrary.com on IDEAL®

Factors Influencing the Infectivity of a Canadian Isolate of *Steinernema kraussei* (Nematoda: Steinernematidae) at Low Temperature

Zdeněk Mráček, Stanislav Bečvář, Pavel Kindlmann, and John M. Webster*

*Department of Insect Pathology, Institute of Entomology CAS, Branišovská 31, 370 05 České Budějovice, Czech Republic; and * Department of Biological Sciences, Simon Fraser University, Burnaby, Vancouver, B.C. V5A 1S6, Canada*

Received July 1, 1997; accepted October 7, 1998

The effectiveness of Canadian isolate 76 of *Steinernema kraussei,* at 10°C, in penetrating *Galleria mellonella* larvae (percentage parasitism and number of IJs developed to adult nematodes) was measured at different host densities (differing number of larvae and size of experimental arena) and for different durations of exposure. The greater the size of the inoculum of infective juvenile nematodes per unit area and the longer the duration of exposure, the greater the number of larvae that were killed and the larger the number of mature nematodes in the larval host. The infection rate (α) and the adjusted infection rate (β) were determined using the modified Anderson model. This model successfully described the behavior of the *S. kraussei–G. mellonella* interaction. © 1999 Academic Press

***Key Words: Steinernema kraussei;* cold infective isolate; *Galleria mellonella;* time exposure; size of arena; host density; penetration ability; instantaneous rate of infection.**

INTRODUCTION

Entomopathogenic nematodes from the families Heterorhabditidae and Steinernematidae are lethal parasites of many insect species. They have a ubiquitous distribution that includes virtually all major climatic zones and they occur in virtually all the main natural and modified terrestrial ecosystems (e.g., forests, agricultural fields, meadows), but have not been recorded from water (Weiser and Mráček, 1989). Due to the rapidity with which they kill the host they are applied as biological control agents against insect pests. The abiotic and biotic factors that influence the biology of these nematodes, and their efficacy as biological control agents of insect pests, are the subject of extensive research (Kaya, 1990).

An effect of temperature on nematode survival, pathogenicity, and reproduction have been thought to be due to the different geographic origins of the nematode (Kung *et al.,* 1991). Grewal *et al.* (1994) observed, however, that the breadth of the thermal niches of specific populations of *Steinernema carpocapsae, S. feltiae,* and *S. glaseri* isolated from different localities do not differ. Many reports support the importance of the geographic origin of strains of entomopathogenic nematodes in determining the temperature range at which they infect insects (Griffin and Downes, 1991, 1994; Fujiie *et al.,* 1995; Shapiro *et al.,* 1996; Mráček *et al.,* 1997). Other abiotic factors such as humidity (Molyneux and Bedding, 1984; Koppenhöfer *et al.,* 1995) and soil texture (Molyneux and Bedding, 1984; Jaworska, 1992) also influence nematode infection of insects. Consequently, laboratory experiments are usually performed under prescribed experimental conditions in which the temperature, substrate, and moisture level are controlled.

Cold activity can be considered as the ability of these entomopathogenic nematodes to seek out hosts at and below 10°C. This term must also include the ability of nematode isolates to penetrate and kill the host. This phenomenon of cold activity, which is composed of several factors, is a significant trait of each isolate, and it may be an important consideration when determining the most effective steinernematid or heterorhabditid to be used in a field application. The activity and associated infectivity of different species, strains, and isolates of *Steinernema* vary significantly with temperature. For example, Fujiie *et al.* (1995) showed that the percentage mortality of *Anomala cuprea* caused by *S. kushidai* increased from 10° to 25°C, decreased slightly at 30° and greatly at 35°C. Similarly, increasing exposure time increases the number of nematodes penetrating into the insect, causing a more rapid death. For example, Ricci *et al.* (1996) found that *Galleria* host mortality caused by three steinernematid and one heterorhabditid species increased with time of exposure.

The number of insects killed in field or laboratory experiments depends also on the quantity of infective juvenile (IJ) steinernematids to which they are exposed. In the experiments of Koppenhöfer and Kaya

0022-2011/99 $30.00
Copyright © 1999 by Academic Press
All rights of reproduction in any form reserved.

(1995), the nematode's penetration efficiency, measured as percentage of the initial inoculum established in the host, decreased significantly with increasing number of IJs present in the soil and entering the host.

Anderson's (1978) model has been successfully modified and used to simulate the infection process of entomopathogenic nematodes (Hudson and Norman, 1995; Mráček *et al.,* 1997). The instantaneous rate of infection (representing the number of nematodes penetrating the host) estimated by this model is a useful tool for comparing the infectivity of different clones or species of parasite 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 or parasite density, size of the experimental arena, duration of the experiment, etc. (Řezáč *et al.,* 1993). Consequently, experiments were designed to test the adequacy of the model's assumptions for entomopathogenic nematodes. The objectives of the research, therefore, were to determine the number of *Galleria mellonella* larvae parasitized by *S. kraussei* 76 and to measure the infection rate of IJs at 10°C at different host and parasite densities, sizes of experimental arenas, and times of exposure.

MATERIAL AND METHODS

S. kraussei isolate 76 was obtained from soil collected in the vicinity of Hudson Hope, British Columbia, Canada (Mráček and Webster, 1993). Since that time the nematodes have been passaged (Dutky *et al.,* 1964) frequently through *G. mellonella.* IJs from this culture were stored for a maximum 12 weeks at 4 to 8°C before use in the experiments. Last instar *G. mellonella* larvae, reared according to Haydak (1936), were used for the experiments.

Experiments

The experiments were done in petri dishes containing sterilized, moist sand (7% w/v) acclimatized at 10°C for 2 h before adding the nematodes. Two hundred IJs were added to 1 ml of cold water (1–3°C) to the center of each petri dish, last instar *G. mellonella* larvae were placed peripherally on the sand surface in each dish, and the dish was acclimatized at 10°C. There was fivefold treatment replication of each experiment.

In the first experiment, the time of exposure of *G. mellonella* to IJs varied from 28, 73, 120, 168, and 216 to 265 h. The petri dish diameter was 11.15 cm and the number of target *G. mellonella* larvae was 10 per dish. In the second experiment, the petri dish diameter (height, volume) varied from 7.3 (1.25, 52.3), 9.15 (1.5, 98.6), 11.15 (1.7, 166.0), and 14.05 (2.15, 333.0) to 18.7 (2.50, 686.0) cm; host number (10 *G. mellonella* larvae per dish) and time of exposure (250 h) were constant. In the third experiment, the number of *G. mellonella* varied from 2, 4, and 8 to 16 larvae per dish, and the constant factors were time of exposure (250 h) and size of petri dish (11.15 cm).

Living and dead *G. mellonella* larvae were removed from the experimental dishes after the required exposure time, rinsed immediately in tap water, and dried on absorbent paper to remove IJs adhering to the insect cuticle. The living (some of them were invaded with nematodes but still living) and dead larvae were held at laboratory temperature for 2 days to allow any nematodes within them to kill the living larvae, to mature, and to facilitate counting. Dead larvae were then dissected and the number of nematodes counted. For each set of replications the average and standard deviation (SD) were calculated.

Statistical Analysis

Data were processed using a modification of the Anderson model (Mráček *et al.,* 1997) in the following equations:

$$dI/dt = -\alpha IH/V, \qquad I(0) = I_0 \tag{1}$$

$$dP/dt = \alpha IH/V, \qquad P(0) = 0 \tag{2}$$

$$dH/dt = -\beta IH/V, \qquad H(0) = H_0 \tag{3}$$

where, d/dt is the first derivative, $I = I(t)$ denotes the number of IJs in the dish at time t, and $P = P(t)$ the total number of nematodes penetrating into all 10 hosts at time t. The parameter α is called "the rate of infection" and is assumed to be directly proportional to I and to the number of living hosts, H, and indirectly proportional to the size of the experimental arena, V. The number of susceptible hosts decline as they are killed by the penetrating nematodes. Due to the possibility of multiple parasitism, however, the rate of decline in susceptible hosts is lower than the rate of penetration, α. Therefore, a different parameter, β (adjusted penetration rate), where $\beta < \alpha$, is used to reflect the changing dynamics of the number of susceptible hosts in the arena. The model Eq. (1)–(3) were explicitly solved by means of separation of the variables. The data were fitted by using the least squares method and the module SOLVER in the EXCEL package.

RESULTS

All three factors tested, namely time of exposure, size of arena, and number of host larvae, significantly influenced *G. mellonella* larval mortality and parasitism and larval penetration by *S. kraussei.* The percentage of *G. mellonella* that were parasitized by *S. kraussei* increased with time of exposure to the IJs and

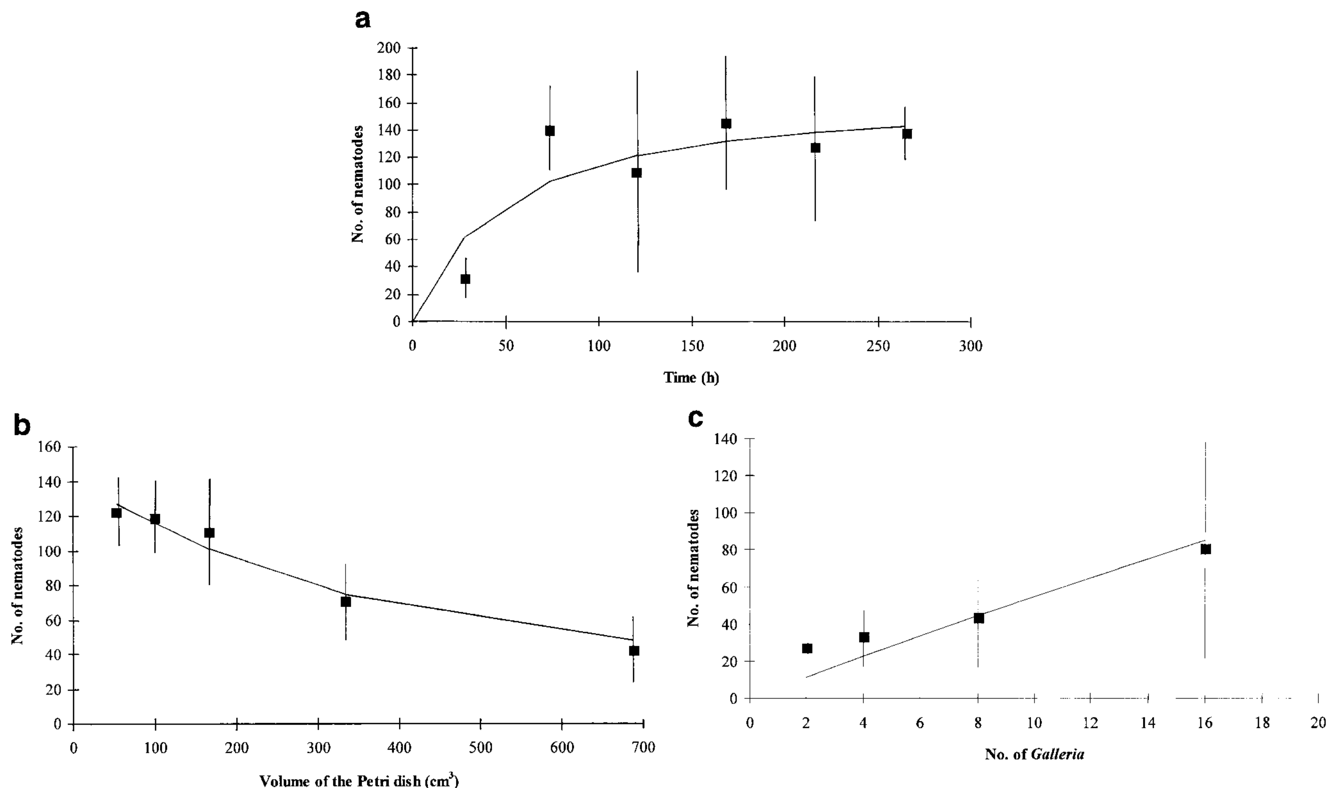

FIG. 1. The average number of *Steinernema kraussei* infective juveniles per dish that penetrated into *Galleria mellonella* larvae at different (a) lengths of exposure, (b) petri dish arena size, and (c) number of larval *Galleria* hosts. Points are empirical data, the lines are derived from Anderson's model.

ranged from 80 ± 14.1% at 28 h to 100% at 265 h. The average number of adult nematodes per dish recovered from the *G. mellonella* larvae increased with time from 31.4 ± 13.9 (15.7% of the IJ inoculum) at 28 h to 137.8 ± 22 (68.9% of the IJ inoculum) at 265 h but there was no increase after 73 h exposure (Fig. 1a).

The percentage of larvae that were parasitized with *S. kraussei* and the average number of adult nematodes recovered from them decreased with increasing size of the petri dish in the experiment (Fig. 1b). In a 7.3-cm-diameter petri dish 100% of the larvae were parasitized and an average of 122.2 ± 20.8 adults per dish were recovered from the cadavers (61.1% of the IJ inoculum), but in a 18.7-cm-diameter dish percentage decreased to only 76 ± 18.2% and the average number of adult nematodes per dish was 42.2 ± 19.6 (21.1% of the IJ inoculum), respectively.

In the third experiment, parasitism of *G. mellonella* attained a maximum of 95.0 ± 6.8% with eight larvae per dish and was lowest, 70 ± 44.7%, when only two larvae per dish were exposed to IJs. The average number of adult *S. kraussei* per dish recovered from the cadavers increased with the number of larvae per dish from 27.3 ± 14.5 (13.65% of the IJ inoculum) at two to 80.6 ± 55.2 (40.3% of the IJ inoculum) at 16 larvae per dish (Fig. 1c).

The estimated parameters α and β of the modified Anderson's model are given (Table 1) together with the corresponding residual sums of squares (RSS). In the modified Anderson's model (Eq. (1)–(3)) only simple linear assumptions about the dependence of the number of penetrating nematodes per unit time, dP/dt, on the number of infective juveniles, I, on the number of susceptible hosts, H, and on the inverse size of the arena (petri dish), 1/V, are used. Data shown in Fig. 1 and the residual sums of squares in Table 1 both confirm that these simple assumptions apply to the description of the infection process. The calculated curves based on Anderson's model are a good fit compared with the absolute number of nematodes penetrat-

TABLE 1

Summary of the Estimated Parameters α and β of the Modified Anderson's Model and the Residual Sums of Squares (RSS) for the Data from Each of the Three Experiments

	1	2	3
α	0.284	0.095	0.061
β	0.018	0.007	0.01
RSS	2818	193.3	382

Note. α, rate of infection. β, rate of host number decline.

ing into the hosts, P (Fig. 1). This is supported by the small residual sums of squares (Table 1): The "average deviation" of the model curve from empirical data calculated as $\sqrt{RSS}/n$, where n is the number of data points, makes only 15% or less of the average P. Larger RSS in experiment 1 is inevitable because of large scatter in empirical data for short exposure times.

DISCUSSION

All three factors that were investigated at the relatively low temperature of 10°C strongly influenced the number of (a) *Galleria* larvae killed and (b) adult nematodes in the *Galleria* cadavers when the larvae were exposed to the cold infective Canadian *S. kraussei* isolate 76 (Fig. 1). However, the instantaneous rate of infection (α) and the rate of decline in number of *Galleria* hosts that represents the changing number of susceptible hosts (i.e., adjusted infection rate) (β), were similar in these experiments (Table 1).

The total proportion of IJs becoming parasitic was assumed to remain constant (see also Bohan and Hominick, 1996). However, this presumption is probably valid for the experiments concerning time exposure and arena size but is slightly different for the different number of hosts. In this latter experiment *Galleria* parasitism never attained 100% even with only two hosts, and this may have been due to the age of the IJs (i.e., 12 weeks) used in this experiment. Storage time of IJs is known to affect the infection capability of entomopathogenic nematodes (Griffin, 1996; Fan and Hominick, 1991).

A very interesting relationship is apparent when the data on duration of exposure are compared with that from experiments by Ricci *et al.* (1996). They performed their experiments with *S. riobravis, S. feltiae, S. scapterisci,* and *H. bacteriophora* at 200 IJs per dish inoculum and at 25°C. The strains and species of nematodes they used can be considered to be representative of normal or even heat-tolerant species. In the experiments of Ricci *et al.* (1996), after 24 h, the mortality of *G. mellonella* larvae attained 100% by *S. riobravis,* approximately 70% by *S. feltiae,* and was negligible by the other nematode species. In our experiments, in which parasitized larvae were held for 2 days so as to ensure that this reflected the true level of mortality, *G. mellonella* parasitism by *S. kraussei* was 80% after 28 h and comparable with that of *S. feltiae.* Similarly, in their experiments the rate of infection, expressed as the percentage of recovered adult nematodes from the initial IJ inoculum, was 44% for *S. riobravis* and 30% for *S. feltiae* after 48 h, which is comparable with the approximately 40% value derived from our data (see Fig. 1a). To summarize, the cold active nematode isolate of *S. kraussei* had an infection rate and host parasitism at low temperature similar to that of the normal or heat active nematode isolates at significantly higher temperatures. Epsky and Capinera (1995) did similar experiments with two strains of *S. carpocapsae* against three insect species at 25°C. They obtained very similar results to these reported here when the invasion efficiency (infection rate) was positively related to increases in length of host exposure time.

Usually, the level of insect host mortality is significantly influenced by the different dose or inoculum size of nematodes (Selvan *et al.,* 1993). However, less is known about the level of mortality and infection rate caused by a constant dose of IJs within different sized arenas or with different number of hosts. Our results indicate that a greater arena size functions similarly to a diluted IJ inoculum and that the capability of IJs to find and penetrate the insect host decreases with their density per unit volume. Similarly, Epsky and Capinera (1995) recorded that the invasion efficiency is positively related to increases in number of hosts and negatively related to increases in substrate surface area (volume arena) per host. Together, these three factors reflect the parasitism of *G. mellonella* and the number of penetrating IJs per host per unit area. The results confirm the expected trait that with increasing number of hosts the number of recovered adult nematodes increases significantly (Fig. 1c). However, in our experiments the IJs failed to achieve a correspondingly high percentage of mortality and parasitism, probably due to lack of IJ vigor through their prolonged pre-experiment storage.

These results may suggest that applications of cold infective, steinernematid isolates at low temperatures (down to 10°C) may be used to control insect pests during that part of the season when or in those geographic regions where the soil temperature is so low as to minimize adaptability to insects and infectivity by the local, native isolates.

ACKNOWLEDGMENTS

We thank the Natural Sciences and Engineering Research Council of Canada (NSERC) for an International Exchange Award to Zdeněk Mráček, the Czech National Grant Agency (Grant 2373) to Zdeněk Mráček, and NSERC (Grant 4679) to John M. Webster for supporting the research.

REFERENCES

Anderson, R. M. 1978. Population dynamics of snail infection by miracidia. *Parasitology* **77,** 201–224.

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

Dutky, S. R., Thompson, J. V., and Cantwell, G. E. 1964. A technique for the mass propagation of the DD-136 nematode. *J. Insect. Pathol.* **6,** 417–422.

Epsky, D. N., and Capinera, J. L. 1993. Quantification of invasion of two strains of *Steinernema carpocapsae* (Weiser) into three lepidopteran larvae. *J. Nematol.* **25,** 173–180.

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

Fujiie, A., Tachibana, M., Takata, Z., Yokoyama, T., Suzuki, N., and Uechi, T. 1995. Effects of temperature on insecticidal activity of an entomopathogenic nematode, *Steinernema kushidai* (Nematoda: Steinernematidae), against *Anomala cuprea* (Coleoptera: Scarabaeidae) larvae. *Appl. Entomol. Zool.* **30,** 23–30.

Grewal, P. S., Selvan, S., and Gaugler, R. 1994. Thermal adaptation of entomopathogenic nematodes: Niche breadth for infection, establishment, and reproduction. *J. Therm. Biol.* **19,** 245–253.

Griffin, C. T. 1996. Effects of prior storage conditions on the infectivity of *Heterorhabditis sp.* (Nematoda: Heterorhabditidae). *Fundam. Appl. Nematol.* **19,** 95–102.

Griffin, C. T., and Downes, M. J. 1991. Low temperature activity in *Heterorhabditis* sp. (Nematoda: Heterorhabditidae). *Nematologica* **37,** 83–91.

Griffin, C. T., and Downes, M. J. 1994. Recognition of low-temperature infective isolates of the entomopathogenic nematode *Heterorhabditis* spp. *Nematologica* **40,** 106–115.

Haydak, M. H. 1936. A food for rearing laboratory insects. *J. Econom. Entomol.* **9,** 1026.

Hudson, P., and Norman, R. 1995. The role of entomopathogenic nematodes in regulating the abundance of pest species: A generalised model. *COST 819 workshop,* Biotechnology, Ecology and Transmission Strategies of Entomopathogenic Nematodes. pp. 52–57.

Jaworska, M. 1992. Effect of soil texture and temperature on the activity of *Steinernema feltiae* and *Heterorhabditis bacteriophora. Entomonematologia* **1,** 23–29.

Kaya, H. K. 1990. Soil ecology. *In* “Entomopathogenic Nematodes in Biological Control” (R. Gaugler and H. K. Kaya, Eds.), pp. 93–115. CRC Press, Boca Raton, FL.

Koppenhöfer, A. M., and Kaya, H. K. 1995. Density-dependent effects on *Steinernema glaseri* (Nematoda” Steinernematidae) within an insect host. *J. Parasitol.* **81,** 797–799.

Koppenhöfer, A. M., Kaya, H. K., and Taormino, S. P. 1995. Infectivity of entomopathogenic nematodes (Rhabditida: Steinernematidae) at different soil depths and moistures. *J. Invertebr. Pathol.* **65,** 193–199.

Kung, S. P., Gaugler, R., and Kaya, H. K. 1991. Effects of soil temperature, moisture, and relative humidity on entomopathogenic nematode persistence. *J. Invertebr. Pathol.* **57,** 242–249.

Molyneux, A. S., and Bedding, R. A. 1984. Influence of soil texture and moisture on the infectivity of *Heterorhabditis* sp. D1 and *Steinernema glaseri* for larvae of the sheep blowfly, *Lucilia cuprina. Nematologica* **30,** 358–363.

Mráček, Z., and Webster, J. M. 1993. Survey of Heterorhabditidae and Steinernematidae (Rhabditida, Nematoda) in Western Canada. *J. Nematol.* **25,** 710–717.

Mráček, Z., Bečvář, S., Řezáč, P., Kindlmann, P., and Webster, J. M. 1997. Canadian steinernematid (Nematoda) isolates and their cold infectivity to the greater wax moth (*Galleria mellonella*) larvae. *Biol. Control* **8,** 160–164.

Ricci, M., Glaser, I., Campbell, J. F., and Gaugler, R. 1996. Comparison of bioassays to measure virulence of different entomopathogenic nematodes. *Biocontrol Sci. Technol.* **6,** 235–245.

Řezáč, P., Kindlmann, P., Dostálková, I., and Holasová, E. 1993. Dynamics of gastropod infection by first-stage larvae of protostrongylid nematodes—a model. *Parasitology* **107,** 537–544.

Selvan, S., Campbell, J. F., and Gaugler, R. 1993. Density dependent effects on entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) within an insect host. *J. Invertebr. Pathol.* **62,** 278–284.

Shapiro, D. I., Glaser, I., and Segal, D. 1996. Trait stability and fitness of the heat tolerant entomopathogenic nematode *Heterorhabditis bacteriophora* IS5 strain. *Biol. Control* **6,** 238–244.

Weiser, J., and Mráček, Z. 1989. “Parasitic Nematodes of Insects.” (in Czech). Academia Press, Prague, 258 pp.