Journal of Invertebrate Pathology **77,** 243–250 (2001)
doi:10.1006/jipa.2001.5029, available online at http://www.idealibrary.com on IDEAL®

Developmental Temperature Effects on Five Geographic Isolates of the Entomopathogenic Nematode *Steinernema feltiae* (Nematoda: Steinernematidae)

Selçuk Hazir,*,† S. Patricia Stock,* Harry K. Kaya,* Albrecht M. Koppenhöfer,‡ and Nevin Keskin†

**Department of Nematology, University of California, One Shields Avenue, Davis, California 95616-8668; †Department of Biology, Faculty of Science, University of Hacettepe, 06532, Beytepe, Ankara, Turkey; and ‡Department of Entomology, Rutgers University, New Brunswick, New Jersey 08901*

E-mail: hkkaya@ucdavis.edu

Received September 11, 2000; accepted March 1, 2001; published online June 6, 2001

The development of five geographic isolates of *Steinernema feltiae* at 5, 8, 10, 15, 20, 25, and 28°C in wax moth, *Galleria mellonella*, larvae was examined. The isolates were from Mediterranean (Sinop from Turkey, SN from France, and Monterey from California), subtropical (Rafaela from Argentina), and tropical (MG-14 from Hawaii) regions. All isolates caused 100% mortality of wax moth larvae and developed and produced progeny between 8 and 25°C. At 28°C, mortality was 100%, but no progeny was observed. The highest infective juvenile production was observed at 15°C for all isolates. In general, the tropical isolate, MG-14, had the lowest production of infective juveniles. The time of emergence of the infective juveniles from the host cadaver showed some differences among isolates, with the Sinop isolate having the earliest emergence time from cadavers at 15°C (10 days) and 20°C (8 days). At 25°C, the infective juveniles of the Sinop, SN, and Rafaela isolates emerged from the cadavers from 5 to 7 days. Time of host death by all isolates showed no differences at 8, 10, 15, 20, and 28°C. At 25°C for all isolates (except the MG-14), shorter times to host death were observed. Host death occurred at 12 days at 8°C, 9 to 11 days at 10°C, 4 to 5 days at 15°C, 3 days at 20°C, and 2 days at 25 and 28°C. For penetration efficiency, the Sinop, SN, and Rafaela isolates penetrated their hosts at 5, 8, and 10°C. Penetration of the infective juveniles was consistently high for all isolates at 15, 20, 25, and 28°C, but it was significantly lower for the MG-14 isolate at 15, 25, and 28°C. No progeny production occurred at 28°C, but nematode penetration did occur with the MG-14 isolate having significantly lower penetration than the other isolates. When nematodes were produced at 8, 15, and 23°C in wax moth larvae, all isolates had infective juveniles with longer body lengths at 8°C followed by 15 and 23°C. To further verify body length at the different temperatures, beet armyworm, *Spodoptera exigua*, larvae and dog-food agar medium were used, respectively, for *in vivo* and *in vitro* culture of the Sinop isolate. Infective juvenile body length showed the same trends, with the longest being at 8°C and decreasing in length from 15 to 23°C. The data suggest that quality of food for the nematode and temperature (that is, developmental time) influence the body length of the infective juvenile. © 2001 Academic Press

Key Words: **Insect-pathogenic nematode; Steinernematidae; *Steinernema;* size variation.**

INTRODUCTION

Entomopathogenic nematodes in the family Steinernematidae, currently represented by 25 species in the genus *Steinernema* and 1 species in the genus *Neosteinernema*, are obligate, lethal parasites of insects (Burnell and Stock, 2000). Steinernematids are mutualistically associated with enteric bacterial species (*Xenorhabdus* spp.) that occur in the intestine of the infective juvenile nematode stage (Boemare *et al.*, 1996; Forst and Nealson, 1996), and each nematode species is associated with a specific bacterial species. The infective juvenile, which is the only free-living stage, searches for a susceptible insect host, enters it through natural openings, penetrates the hemocoel, and releases the bacterial cells that kill the host within 48 h (Kaya and Gaugler, 1993). The bacterial symbiont digests the host tissues, thereby providing suitable nutrient conditions for nematode growth and development.

Entomopathogenic nematodes can be very effective biological control agents against a number of insect pests and possess several advantages over chemical pesticides (Kaya and Gaugler, 1993). For example, they can actively find their hosts, can recycle in the soil environment (Kaya and Gaugler, 1993), and are envi-

0022-2011/01 $35.00
Copyright © 2001 by Academic Press
All rights of reproduction in any form reserved.

TABLE 1
Sources of *Steinernema feltiae* Isolates Used in This Study

Isolate	Geographic origin	Habitat	Climatic region	Insect host	Source
SN	Southern France	Unknown	Mediterranean	Unknown[a]	G. O. Poinar
Rafaela	Santa Fe, Argentina	Alfalfa field	Subtropical	*Graphognathus leucoloma*	S. P. Stock
Monterey	California, USA	Grassland	Mediterranean	Unknown[a]	S. P. Stock
MG-14	Hawaii, USA	Under tree	Tropical	Unknown[a]	A. Hara
Sinop	Sinop, Turkey	Grassland	Mediterranean	Unknown[a]	S. Hazir

[a] Nematodes recovered with soil baiting technique.

ronmentally safe (Akhurst, 1990). Moreover, they have a global distribution (reviewed by Hominick *et al.*, 1996), and many surveys have been conducted to isolate new strains and species for biological control programs or to determine their biodiversity (Akhurst and Bedding, 1986; Constant *et al.*, 1998; Yoshida *et al.*, 1998; Stock *et al.*, 1999).

A significant amount of research has been conducted to elucidate the biology of these nematodes, including the systematics, ecology, and biological control potential (Gaugler and Kaya, 1990; Kaya and Gaugler, 1993; Burnell and Stock, 2000). Much of the research has focused on individual species or comparisons between and among species. Researchers are interested in intraspecific variations that occur among different isolates of the same species for determining variation in morphology (Poinar, 1992; Stock *et al.*, 2000), genetics (e.g., Gaugler *et al.*, 1989), physiology (e.g., Jagdale and Gordon, 1997; Fitters *et al.*, 1999; Solomon *et al.*, 1999), infectivity (Griffin and Downes, 1991), and climatic adaptation (Solomon *et al.*, 1999). Temperature is one of the important factors affecting the infectivity, time of death, development, reproduction, and storage of entomopathogenic nematodes (Griffin, 1993; Grewal *et al.*, 1994; Finnegan *et al.*, 1999; Koppenhöfer and Kaya, 1999). In this paper, we report findings on the effect of temperature on four of these parameters (infectivity, time of death, development, and reproduction) in five isolates of *Steinernema feltiae* using the wax moth, *Galleria mellonella*, as a host. We also studied the effect of temperature on infective juvenile length in these isolates and the effect of temperature in relation to food source on infective juvenile length in one of these isolates.

MATERIALS AND METHODS

Nematode Source and Culture

Five isolates of *S. feltiae* from different geographical regions of the world were obtained and information about their source, geographic location, and host are detailed in Table 1. The isolates originated from America (Monterey and MG-14), Argentina (Rafaela), France (SN), and Turkey (Sinop). Each isolate was reared separately in wax moth larvae (Rainbow Mealworms, Compton, CA) (Kaya and Stock, 1997). After the wax moth larvae died, they were placed in White traps, and the infective juveniles were allowed to emerge and were collected every other day. The infective juveniles were stored at 15°C.

To verify that the isolates were the same species, we conducted a cross-mating study. We used 24-well tissue culture plates which were lined with a piece of filter paper (1.5 cm diameter) in each well. One infective juvenile from each of the two test isolates was placed in each well by use of a 10-μl microdispenser. Then, one wax moth larva (200–300 mg) was placed into each well. This protocol was repeated for each nematode isolate. As a control, two infective juveniles from the same population were placed together as described above. Each dead wax moth larva was placed on a White trap and the emergence of infective juvenile progeny was used as an indicator that breeding between isolates occurred.

Infectivity and Developmental Studies at Different Temperatures

Tissue culture plates with 24 wells were filled with 0.5 g of pasteurized air-dried sandy loam soil to which 60 μl of nematode inoculum containing 50 infective juveniles was added. Two plates per isolate per temperature were placed in incubators set at 5, 8, 10, 15, 20, 25, 28, and 30°C. After 1 h acclimation, one wax moth larva was added to each well. Whereas both sets of plates were used to determine the time of death of the wax moth larvae, one set of plates was used for the infectivity study based on penetration efficiency, and the other set was used for the developmental study. In addition, two control plates without nematodes (60 μl of distilled water) were included in the study to verify the health of the last instar wax moth larvae (Koppenhöfer and Kaya, 1999). The plates were placed into polyethylene bags to prevent desiccation. All five *S. feltiae* isolates were included in this study and the experiment was conducted twice.

To determine the time of death, each larva was probed daily, and if the larva did not respond to the probe, it was considered dead. At 5°C the living wax

moth larvae were so sluggish that time of larval death could not be determined adequately and death was assumed based on the characteristic color associated with a *S. feltiae* infection.

To determine the penetration efficiency of the infective juveniles, the wax moth larvae were checked daily for mortality. When a larva died, it was rinsed in distilled water to remove the external nematodes. Dead larvae from 5, 8, 10, or 15°C were placed at room temperature (23°C) for 2 additional days, whereas larvae recovered at 20, 25, and 28°C were held for 1 day (modified after Koppenhöfer and Kaya, 1999). No infection was observed at 30°C and this temperature was discontinued. After this time period, each cadaver was dissected and digested with pepsin solution (Mauleon *et al.*, 1993) and the number of penetrated (i.e., established) nematodes was counted.

For the developmental study, 8 dead wax moth larvae from the group of 24 that were exposed to *S. feltiae* were selected to determine the time and number of infective juvenile emergence. These cadavers were placed individually on an emergence White trap which consisted of a 35 × 10-mm petri dish lid lined with filter paper, situated in a 100 × 15-mm petri dish containing sterilized distilled water (Koppenhöfer and Kaya, 1999). The traps were kept at the original incubation temperatures and checked daily. We recorded the first day that infective juveniles were observed emerging from the host. Once they entered the water, they were collected at regular intervals until no more infective juveniles entered the water. The total number of infective juveniles emerged was estimated by the counting of subsamples at a magnification of 50×.

Temperature Effects on Infective Juvenile Length

A separate set of experiments with wax moth larvae was conducted to determine the effect of temperature on the body length of emerging infective juveniles of the five geographic isolates of *S. feltiae.* The infective juveniles of each isolate were allowed to infect wax moth larvae at 8, 15, and 23°C as described above. Six cadavers were used for progeny production and placed individually on White traps. Three days after the infective juveniles emerged, they were collected from the White trap; a pool of 40–50 infective juveniles/cadaver was heat-killed at 60°C and stored in triethanolamine formalin (TAF) solution (Kaya and Stock, 1997). From this pooled sample, 25 infective juveniles were measured with a Nikon Eclipse E600 microscope with Scion Image software (1.62a version).

To determine whether infective juvenile body length was also influenced by temperature (8, 15, or 23°C) if reared on a different host or *in vitro,* we conducted the following experiments with one *S. feltiae* isolate (Sinop). To determine the effect of a different host, infective juveniles were produced in last-instar *Spodoptera exigua* larvae (obtained from Agraquest, Davis, CA) with the protocol described above for the wax moth larvae. This experiment was done only once with 24 *S. exigua* larvae.

To determine the effect of *in vitro* production, the infective juveniles were grown on a dog-food medium. One-week-old infective juveniles were surface-sterilized in 0.1% Hyamine 1622 solution (benzethonium chloride) (Sigma, St. Louis, MO) (Kaya and Stock, 1997) for 30 min and rinsed three times in sterile water. About 250 infective juveniles in 100 μl were placed in a 35-ml test tube containing 5 ml of sterilized dog-food agar medium and held at 23°C. When infective juveniles were produced, they were harvested for the temperature studies. Three tubes were inoculated each with ca. 250 infective juveniles and were maintained at 8, 15, or 23°C and checked every other day for nematode development. When infective juveniles were observed on the side of the tubes, they were harvested by the adding of 15 ml of sterile water and the pouring of the contents into a beaker. The nematodes were allowed to settle, and the supernatant was discarded. More water was added and the process repeated until the nematode suspension appeared clear. Infective juveniles were obtained by the adding of 0.1% sodium hypochlorite (NaOCl) to the beaker for 15 min, which killed the developing nematodes, leaving behind the infective juveniles (Kaya and Stock, 1997). The infective juveniles from the tubes at each given temperature were pooled and approximately 250 to 300 of them were fixed in TAF, and 25 infective juveniles from each temperature were measured as described previously. This experiment was repeated twice.

Statistics

Data on time of death, nematode penetration, reproduction, and total body length of the infective juveniles were analyzed with analysis of variance (ANOVA), and significant differences among means were separated by Tukey's test (SAS Institute, 1996).

RESULTS

When each of the five geographic populations of *S. feltiae* was crossed with each other, viable progenies were produced (data not shown). No infectivity of *S. feltiae* was observed at 30°C. In general, the wax moth larvae used as controls in the developmental studies at various temperatures appeared healthy.

Infectivity and Developmental Studies at Different Temperatures

Time of death. Temperature had a significant effect on the time of host death by *S. feltiae* (Fig. 1A). All isolates elicited the fastest time of death at 25 and 28°C and the slowest time of death at 8°C. The time

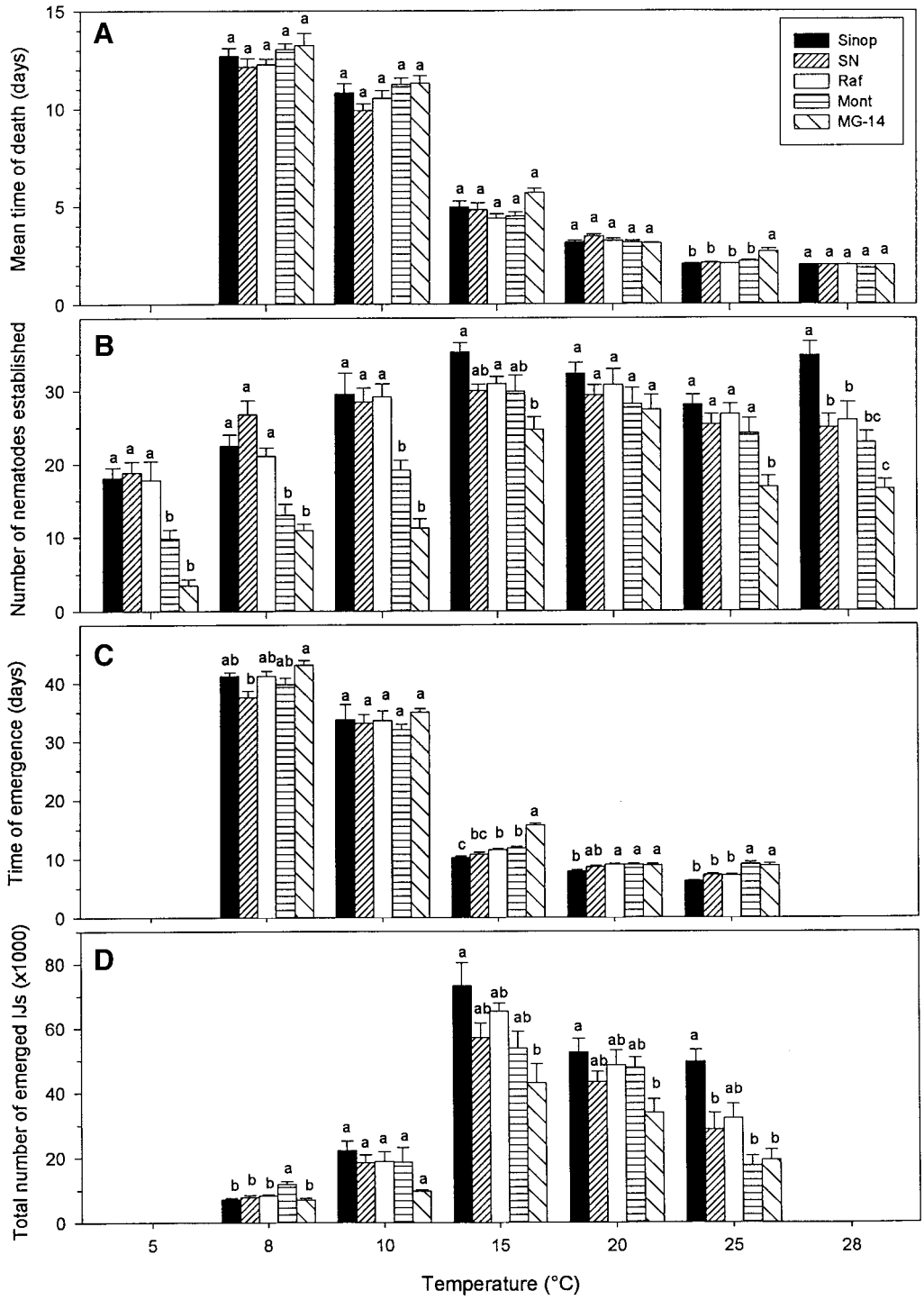

FIG. 1. The effect of temperature on four different parameters on five geographical isolates of *Steinernema feltiae*. (A) Time to death of wax moth host. (B) Number of nematodes established in wax moth larvae. (C) Time of emergence of first infective juvenile from host cadaver. (D) Total number of infective juveniles (IJs) emerged per wax moth larva.

of death was not significantly different among the isolates at 8, 10, 15, 20, and 28°C ($P > 0.05$). At 25°C, time of death was significantly longer for MG-14 than for the other isolates ($F = 14.64$; $df = 4,211$; $P < 0.001$). We were unable to determine the time of death at 5°C because the wax moth larvae did not respond to probing even though they appeared to be alive.

Penetration efficiency. At 15 and 20°C, all *S. feltiae* isolates penetrated in relatively high numbers (>20) in the wax moth larvae. At 15°C ($F = 6.1$; $df = 2,121$;

TABLE 2

Mean ± Standard Error of Body Length of Five Isolates of *Steinernema feltiae* Infective Juveniles Reared in *Galleria mellonella* Larvae at Different Temperatures

Temperature (°C)	SN	Rafaela	Monterey	MG-14	Sinop
8	1033 ± 14A,a (803–1098)	990 ± 15A,a (885–1100)	1008 ± 11A,a (795–1079)	1002 ± 14AB,a (853–1048)	939 ± 10B,a (901–1173)
15	916 ± 20A,b (716–1138)	935 ± 15A,a (774–1071)	964 ± 13A,b (795–1033)	915 ± 17A,a (764–1045)	928 ± 19A,b (689–1081)
23	843 ± 23A,c (738–1016)	850 ± 13A,b (749–1001)	870 ± 14B,c (694–926)	788 ± 12A,a (798–1013)	903 ± 13AB,c (601–1036)

Note. Measurements are in micrometers and the range is shown in parentheses. Means within the same row followed by the same capital letter are not significantly different ($P < 0.05$; Tukey's test). Means within the same column followed by the same lower case letter are not significantly different ($P > 0.05$; Tukey's test).

$P < 0.001$), however, the MG-14 isolate penetrated and established at significantly lower numbers than the Sinop and Rafaela isolates (Fig. 1B). At 5°C ($F = 10.2$; $df = 3,115$; $P < 0.001$), 8°C ($F = 17.94$; $df = 3,115$; $P < 0.001$), and 10°C ($F = 13.68$; $df = 3,100$; $P < 0.001$), the MG-14 and Monterey isolates penetrated and established at significantly lower numbers than the Sinop, SN, and Rafaela isolates. At 25°C ($F = 4.93$; $df = 4,136$; $P < 0.001$), the MG-14 isolate had significantly lower establishment than the other isolates. At 28°C ($F = 13.64$; $df = 4,159$; $P < 0.0001$), the MG-14 isolate had significantly lower penetration (16.5 infective juveniles/host) than the other isolates (range 22.9–34.8 infective juveniles/host). No significant differences in penetration efficiency were observed at 20°C ($P > 0.30$) (Fig. 1B).

Emergence time. All isolates showed the fastest emergence time (first emergence from the cadaver) at 20 and 25°C and the slowest emergence time at 8°C; no infective juveniles emerged at 5 and 28°C (Fig. 1C). No significant differences among isolates were observed at 10°C ($F = 0.58$; $df = 4,22$; $P > 0.68$). However, at 8°C ($F = 4.9$; $df = 1,24$; $P < 0.005$), there was a significant difference between the MG-14 and SN isolates. At 20°C ($F = 4.6$, $df = 3,75$; $P < 0.002$), significant differences were observed among the Sinop, Rafaela, Monterey, and MG-14 isolates. Additionally, significant differences were observed between one group (MG-14 and Monterey) and the other group (Sinop, Rafaela, and SN) at 25°C ($F = 16.1$; $df = 1,72$; $P < 0.001$). At 15°C ($F = 30.2$; $df = 4,60$; $P < 0.001$), MG-14 emergence started significantly later than the other isolates. Differences also occurred among the Monterey, Rafaela, and Sinop isolates.

Number of emerged infective juveniles. All isolates showed the lowest number of emerged infective juveniles at 8°C and the highest number of emerged infective juveniles at 15°C (Fig. 1D). Significantly more Monterey infective juveniles emerged at 8°C ($F = 12.3$; $df = 4,15$; $P < 0.001$) than the four other isolates. At 15°C ($F = 3.33$; $df = 1,82$; $P < 0.01$) and 20°C ($F = 1.80$; $df = 1,82$; $P > 0.136$), there were significant differences only between the Sinop and the MG-14 isolates, with the MG-14 isolate producing less infective juveniles than the Sinop. However, significant differences were observed between the Sinop and SN and the Monterey and MG-14 isolates at 25°C ($F = 8.43$; $df = 1,58$; $P < 0.001$). No significant differences were observed among any of the isolates at 10°C ($P > 0.10$).

Temperature Effects on the Length of Infective Juveniles

Our results indicated that the developmental temperatures affected the length of emerging infective juveniles (Table 2). For all isolates, the longest infective juveniles were recovered at the lowest temperature (8°C) and the shortest were at the highest temperature (23°C).

The Sinop ($F = 24.4$; $df = 2,72$; $P < 0.0001$), SN ($F = 24.2$; $df = 2,72$; $P < 0.0001$), and Monterey ($F = 54.8$; $df = 2,72$; $P < 0.0001$) isolates exhibited significant differences in the total body length of the infective juveniles among the three temperatures. The Rafaela isolate ($F = 30.04$; $df = 1,72$; $P < 0.0001$) exhibited significant differences only between 8 and 23°C and 15 and 23°C, respectively. In the MG-14, the longest infective juveniles were observed at 8°C and the shortest at 23°C, but the means did not differ significantly ($F = 1.72$; $df = 1,72$; $P = 0.185$) (Table 2).

In the *in vivo* method with *S. exigua,* maximum infective juvenile length was obtained from cadavers kept at 8°C and the minimum infective juvenile length was obtained from nematodes at 23°C. However, length of the Sinop infective juveniles differed significantly ($F = 9.72$; $df = 1,72$; $P < 0.0002$) between 8 and 15°C and 8 and 23°C, but not between 15 and 23°C (Table 3).

In the *in vitro* method with dog-food agar, infective juvenile length of the Sinop isolate differed signifi-

TABLE 3

Mean ± Standard Error of Body Length of the Sinop Infective Juveniles Reared from *Spodoptera exigua* Larvae and Dog-Food Agar at Different Temperatures

Temperature (°C)	*Spodoptera exigua*	Dog-food agar
8	903 ± 18A,a (755–1005)	923 ± 19A,a (781–1000)
15	851 ± 20A,b (651–999)	906 ± 16A,a (689–1003)
23	814 ± 6A,b (765–897)	815 ± 14A,b (701–941)

Note. Measurements are in micrometers and the range is shown in parentheses. Means within the same row followed by the same capital letter are not significantly different ($P > 0.05$; Tukey's test). Means within the same column followed by the same lower case letter are not significantly different ($P > 0.05$; Tukey's test).

cantly only between 8 and 23°C and 15 and 23°C ($F = 10.6$; $df = 2,72$; $P < 0.0001$). Although the infective juveniles were longer at 8 than at 15°C, no significant difference was observed between these two temperatures ($P > 0.05$). There was no significant difference in body length at each temperature ($P = 0.31$) between *S. exigua*-reared and *in vitro*-reared infective juveniles (Table 3).

DISCUSSION

Our study demonstrated that similarities and differences occur among isolates of the same entomopathogenic nematodes in their response to temperature. Temperature had a direct effect on the time of death, penetration rate, emergence time of infective juveniles, and number of emerging infective juveniles of the five isolates of *S. feltiae*. However, differences among the five isolates in some of the parameters indicated that geographic isolation resulted in adaptation of the isolate to the given region.

The Sinop, SN, and Monterey isolates are from Mediterranean areas, Rafaela is from a subtropical area, and the MG-14 isolate is from a tropical area. At 28°C, none of the isolates produced progeny, and the nematodes developed to the first generation adults but were unable to proceed to the next generation. At the other extreme, the MG-14 and the Monterey isolates had very low numbers of penetrated nematodes in the host at 5, 8, and 10°C compared with the other isolates. At 25°C, the MG-14 took significantly longer to kill its host than the other isolates. In general, the Monterey and MG-14 isolates were similar in many of the parameters that were measured, and the Sinop, SN, and Rafaela isolates were likewise similar in many parameters. All isolates had the most progeny production at 15°C. Clearly, *S. feltiae* is adapted to lower temperatures (Grewal *et al.*, 1994, 1996; Schirocki and Hague, 1997). Even the tropical isolate (MG-14) did comparatively well at low temperatures.

Other researchers have shown that different isolates of the same species of entomopathogenic nematodes have differential responses to various factors. Gaugler *et al.* (1989) screened 22 isolates of *S. carpocapsae* from four different continents for host-finding ability and ultraviolet light (UV) tolerances. They found differences among the 22 isolates and selected 10 of them for further analysis. Host-finding ability of the infective juveniles of 3 isolates was significantly better than that of the other isolates, but there was a clear gradation from very good to poor host-finding ability. However, the 10 isolates showed a narrow UV tolerance, indicating that genetic variability for this trait was low. Solomon *et al.* (1999) demonstrated that a *S. feltiae* isolate from Israel's Negev Desert was better adapted for desiccation tolerance than an isolate from Germany. In temperature studies, Wright (1992) showed that two isolates of *S. feltiae* from New Zealand had differences in the number of infective juveniles produced, and the time for reproduction and emergence was less for one isolate than another isolate. Griffin and Downes (1991), working with four different *Heterorhabditis* isolates, showed that some isolates were better than others in infecting their host at low temperatures. Our data confirm Wright's (1992) study that differences in progeny production, time of death of host, time of emergence, and number of penetrated infective juveniles in a host can vary among *S. feltiae* isolates.

Temperature can also affect the length of the infective juvenile progeny that are being produced. Schirocki and Hague (1997) demonstrated that infective juveniles of *S. feltiae* that were produced for four reproductive cycles at 10°C were longer (966 μm) than those that were produced continuously at 22°C (896 μm). When *S. feltiae* was reared at 10°C and then reared for one cycle at 22°C, the resulting infective juveniles measured an average of 892 μm. Our study confirmed that *S. feltiae* infective juveniles from five geographical isolates were longer when reared at lower temperature (8°C) than at higher temperature (23°C). Schirocki and Hague (1997) speculated that the longer development time at the lower temperatures results in more nutrient uptake and larger infective juveniles.

We also observed the same temperature effect on infective juvenile body length when the Sinop isolate was reared on another insect host (*S. exigua*) or on dog-food medium. Although we could not statistically compare the body length of the infective juveniles from *S. exigua* and dog-food agar medium with those reared on the wax moth larvae, our data strongly indicate that infective juveniles tend to be longer when reared in wax moth larvae than in *S. exigua* and on dog-food agar. It appears that the host species or artificial medium can influence the body length of the infective

juveniles. *S. exigua* larvae probably have a lower nutritional quality than wax moth larvae.

Nguyen and Smart (1995) observed that when steinernematids and heterorhabditids were reared in wax moth larvae at 25°C, there was a negative linear relationship between the body length of the infective juveniles and the time of harvest. With respect to an unidentified strain of *S. feltiae,* Nguyen and Smart (1995) showed that on the 1st day of harvest the infective juveniles averaged 914 μm but by the 15th day of harvest they averaged 794 μm. The shortest body length of the infective juveniles (632 μm) was obtained from artificial medium (brain–heart infusion, corn oil, cholesterol, agar, and water). In our study, the body length of the infective juveniles of the Sinop isolate reared on dog-food medium was longer than the body length of the infective juveniles of *S. feltiae* reared on artificial diet as reported by Nguyen and Smart (1995). The data suggest that nutritional quality affects body length. The infective juveniles that emerge first from the insect cadaver may have greater access to better nutrition in the cadaver than those that develop later in the same cadaver. Similarly, dog-food medium may be more nutritious for nematode development (i.e., longer infective juveniles) than the brain–heart infusion, corn oil, cholesterol diet used by Nguyen and Smart (1995). Other factors affecting body size of the infective juvenile include host size (Gouge and Hague, 1995) and number of nematodes established per host (Selvan *et al.*, 1993).

Our results show that there are variations among the five geographical isolates of *S. feltiae.* The Sinop, Rafaela, Monterey, and MG-14 isolates have been cultured under laboratory conditions for only a few generations, whereas the SN isolate has been under laboratory cultivation for a much longer time. We expected to see greater variation in the tropical isolate than in those that were from the Mediterranean climatic regions. The tropical isolate, MG-14, had some differences compared with those from the Mediterranean climatic region, with the Monterey isolate being intermediate. Our data suggest that these isolates have retained the cold tolerant characters.

ACKNOWLEDGMENTS

This study was supported in part through a Scientific and Technical Research Council of Turkey grant to S. Hazir. We thank S. Nadler for use of microscope and video image software for conducting the morphometric observations and the Genetics Resources Conservation Program for their continued support for maintenance of the entomopathogenic nematode collection.

REFERENCES

Akhurst, R. J. 1990. Safety to nontarget invertebrates of nematodes of economically important pests. *In* "Safety of Microbial Insecticides" (M. Laird, L. A. Lacey, and E. W. Davidson, Eds.), pp. 233–240. CRC Press, Boca Raton, FL.

Akhurst, R. J., and Bedding, R. A. 1986. Natural occurrence of insect pathogenic nematodes (Steinernematidae and Heterorhabditidae) in soil in Australia. *J. Aust. Entomol. Soc.* **25,** 241–244.

Boemare, N. E., Laumond, C., and Mauleon, H. 1996. The entomopathogenic nematode–bacterium complex: Biology, life cycle and vertebrate safety. *Biocontr. Sci. Technol.* **6,** 333–3435.

Burnell, A. M., and Stock, S. P. 2000. *Heterorhabditis, Steinernema* and their bacterial symbionts—Lethal pathogens of insects. *Nematology* **2,** 31–42.

Constant, P., Marchay, L., Fischer-LeSaux, M., Briand-Panoma, S., and Mauleon, H. 1998. Natural occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Guadeloupe islands. *Fundam. Appl. Nematol.* **21,** 667–672.

Finnegan, M. M., Downes, M. J., O'Regan, M., and Griffin, C. T. 1999. Effect of salt and temperature stresses on survival and infectivity of *Heterorhabditis* spp. IJs. *Nematology* **1,** 69–78.

Fitters, P. F. L., Patel, M. N., Griffin, C. T., and Wright, D. J. 1999. Fatty acid composition of *Heterorhabditis* sp. during storage. *Comp. Biochem. Mol. Biol.* **124,** 81–88.

Forst, S., and Nealson, K. 1996. Molecular biology of the symbiotic pathogenic bacteria *Xenorhabdus* and *Photorhabdus* spp. *Microbiol. Rev.* **60,** 21–43.

Gaugler, R., Campbell, J. F., and McGuire, T. R. 1989. Selection for host finding in *Steinernema feltiae. J. Invertebr. Pathol.* **54,** 363–372.

Gaugler, R., and Kaya, H. K. 1990. "Entomopathogenic Nematodes in Biological Control." CRC Press, Boca Raton, FL.

Gouge, D. H., and Hague, N. G. M. 1995. The development of *Steinernema feltiae* (Nematoda, Steinernematidae) in the sciarid fly *Bradisia paupera* (Diptera, Sciaridae). *Ann. Appl. Biol.* **126,** 395–401.

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.

Grewal, P. S., Gaugler, R., and Wang, Y. 1996. Enhanced cold tolerance of the entomopathogenic nematode *Steinernema feltiae* through genetic selection. *Ann. Appl. Biol.* **129,** 335–341.

Griffin, C. T. 1993. Temperature responses of entomopathogenic nematodes for the success of biological control programs. *In* "Nematodes and the Biological Control of Insect Pests" (R. Bedding, R. Akhurst, and H. Kaya, Eds.), pp. 101–111. CSIRO Publications, East Melbourne, Australia.

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

Hominick, W. M., Reid, A. P., Bohan, D. A., and Briscoe, B. R. 1996. Entomopathogenic nematodes: Biodiversity, geographical distribution and the convention on biological diversity. *Biocontr. Sci. Technol.* **6,** 317–331.

Jagdale, G. B., and Gordon, R. 1997. Effect of propagation temperatures on temperature tolerances of entomopathogenic nematodes. *Fundam. Appl. Nematol.* **211,** 177–183.

Kaya, H. K., and Gaugler, R. 1993. Entomopathogenic nematodes. *Annu. Rev. Entomol.* **38,** 181–206.

Kaya, H. K., and Stock, S. P. 1997. Techniques in insect nematology. *In* "Manual of Techniques in Insect Pathology" (L. Lacey, Ed.), pp. 281–324. Academic Press, San Diego.

Koppenhöfer, A. M., and Kaya, H. K. 1999. Ecological characterization of *Steinernema rarum. J. Invertebr. Pathol.* **73,** 120–128.

Mauleon, H., Briand, S., Laumond, C., and Bonifassi, E. 1993. Utilisation d'enzyme digestives pour l'etude du parasitisme des

Steinernematidae et Heterorhabditidae envers les larves d'insects. *Fundam. Appl. Nematol.* **16,** 185–186.

Nguyen, K. B., and Smart, G. C., Jr. 1995. Morphometrics of infective juveniles of *Steinernema* spp. and *Heterorhabditis bacteriophora* (Nemata: Rhabditida). *J. Nematol.* **27,** 206–212.

Poinar, G. O., Jr. 1992. *Steinernema feltiae* (Steinernematidae: Rhabditida) parasitizing adult fungus gnats (Mycetophilidae: Diptera) in California. *Fundam. Appl. Nematol.* **15,** 427–430.

SAS Institute. 1996. SAS 6.11 for windows. Cary, NC.

Schirocki, A. G., and Hague, N. G. M. 1997. The effect of selective culture of *Steinernema feltiae* at low temperature on establishment, pathogenicity, reproduction and size of infective juveniles. *Nematologica* **43,** 481–489.

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.

Solomon, A., Paperna, I., and Glazer, I. 1999. Desiccation survival of the entomopathogenic nematode *Steinernema feltiae:* Induction of anhydrobiosis. *Nematology* **1,** 61–68.

Stock, S. P., Pryor, B. M., and Kaya, H. K. 1999. Distribution of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in natural habitats in California, USA. *Biodiversity Conserv.* **8,** 535–549.

Stock, S. P., Mrácek, Z., and Webster, J. M. 2000. Morphological variation between allopatric populations of *Steinernema kraussei* (Steiner, 1923) (Rhabditida: Steinernematidae). *Nematology* **2,** 143–152.

Wright, P. J. 1992. Cool temperature reproduction of steinernematid and heterorhabditid nematodes. *J. Invertebr. Pathol.* **60,** 148–151.

Yoshida, M., Reid, A. P., Briscoe, B. R., and Hominick, W. M. 1998. Survey of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Japan. *Fundam. Appl. Nematol.* **21,** 185–198.