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

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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.

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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 Nealon, 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-
TABLE 1

Sources of Steinernema feltiae Isolates Used in This Study

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

* Nematodes recovered with soil baiting technique.

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 and from the group of 24 that were exposed to S. feltiae (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 distilled 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...
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 \((F = 14.6; \text{df} = 4,211; P < 0.001)\). 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 \((F = 6.1; \text{df} = 2,121;\)
showed the lowest number of emerged infective juveniles among the Monterey, Rafaela, and Sinop isolates. Differences also occurred between the Sinop isolate and the other isolates. At 25°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).

Table 2: Mean ± Standard Error of Body Length of Five Isolates of Steinernema Feltiae Infective Juveniles Reared in Galleria mellonella Larvae at Different Temperatures

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>SN</th>
<th>Rafaela</th>
<th>Monterey</th>
<th>MG-14</th>
<th>Sinop</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>1033 ± 14A,a</td>
<td>990 ± 15A,a</td>
<td>1008 ± 11A,a</td>
<td>1002 ± 14A,B,a</td>
<td>939 ± 108A,a</td>
</tr>
<tr>
<td>15</td>
<td>916 ± 20A,b</td>
<td>935 ± 15A,a</td>
<td>964 ± 13A,b</td>
<td>915 ± 17A,a</td>
<td>928 ± 19A,b</td>
</tr>
<tr>
<td>23</td>
<td>843 ± 23A,c</td>
<td>850 ± 13A,b</td>
<td>870 ± 14B,c</td>
<td>788 ± 12A,a</td>
<td>903 ± 13A,B,c</td>
</tr>
</tbody>
</table>

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).

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).

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

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

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).

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 *Heterorhabditis* 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.

**DISCUSSION**

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, 1993), 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.

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