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Temperature Effects on *Heterorhabditis megidis* and *Steinernema carpocapsae* Infectivity to *Galleria mellonella*

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Abstract: The effect of temperature on the infection of larvae of the greater wax moth, *Galleria mellonella,* by *Heterorhabditis megidis* H90 and *Steinernema carpocapsae* strain All, was determined. For both species, infection, reproduction, and development were fastest at 20 to 24 °C. Infection by both *H. megidis* and *S. carpocapsae* occurred between 8 and 16 °C; however, neither species reproduced at 8 °C. Among the nematodes used in experiments at 8 °C, no *H. megidis* and very few *S. carpocapsae* developed beyond the infective juvenile stage. Compared with *H. megidis, S. carpocapsae* invaded and killed *G. mellonella* larvae faster at 8 to 16 °C. By comparing invasion rates, differences in infectivity between the two nematode species were detected that could not be detected in conventional petri dish bioassays where mortality was measured after a specified period. Invasion of *G. mellonella* larvae by *H. megidis* was faster at 24 than at 16 °C.

Key words: entomopathogenic nematode, *Heterorhabditis megidis,* infectivity, invasion rate, nematode, *Photorhabdus luminescens, Steinernema carpocapsae,* temperature, *Xenorhabdus nematophilus.*

Nematode infectivity as measured by insect mortality varies with the species and strain of both the insect and the nematode and is affected by abiotic factors, especially temperature (Mason and Hominick, 1995; Molyneux, 1983). Temperature influences nematode mobility, reproduction, and development (Mason and Hominick, 1995; Molyneux, 1983; Simons and van der Schaaf, 1986; Zervos et al., 1991). Infectivity is commonly used to evaluate the efficacy of entomopathogenic nematodes as biocontrol agents. Heterorhabditids are sometimes regarded as more virulent than steinernematids (Bedding et al., 1983; Dunphy and Webster, 1986) and, generally, have a greater host range (Molyneux, 1983), but they have not been as extensively studied as the steinernematids. To evaluate heterorhabditids as biological control agents, detailed comparisons of their infectivity relative to steinernematids are required. The purpose of this research was to compare the effects of temperature on the entry of *Heterorhabditis megidis* strain H90 and *Steinernema carpocapsae* strain All into a host and the subsequent development of the nematodes and their respective bacteria, *Photorhabdus luminescens* and *Xenorhabdus nematophilus.*

Materials and Methods

Sources of insects and nematodes: Greater wax moth (*Galleria mellonella*) larvae were reared according to the method of Dutky et al. (1962). The wax moth larvae were obtained from the insectary at the Great Lakes Forestry Centre (Forestry Canada) in Sault Sainte Marie, Ontario. All larvae used in the experiments were future generations from that cohort. *Heterorhabditidis megidis* H90, isolated from the Okanagan Valley and the Thompson Canyon areas of British Columbia (Mráček and Webster, 1993), was cultured at ca. 20 °C in late instar *G. mellonella* larvae, using a modification of the method of Dutky et al. (1964). Each larva was challenged with ca. 20 infective juveniles (IJ) in 9-cm-diam. petri dishes (10 larvae and 200 IJ/dish) lined with one piece of filter paper. After 2 days, the larval cadavers were transferred to White traps (White, 1927), and IJ, which emerged in 7 to 10 days, were maintained in water at ca. 20 °C until used within 7 days. *Steinernema carpocapsae* strain All, obtained as Biosafe (Biosys, Columbia, MD), was similarly cultured.

Experimental protocol: For all experiments, 2 ml water containing ca. 300 IJ was added

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to 9-cm-diam. petri dishes lined with one piece of filter paper. Untreated petri dishes received 2 ml sterile-distilled water. Petri dishes were sealed with Parafilm (American National Can., Greenwich, CT) and placed in an incubator at the required temperature for 6 hours to allow the IJ to thermoequilibrate. Subsequently, all petri dishes received one late instar *G. mellonella* larva and then were resealed and reincubated in darkness at temperatures and durations specific to each experiment.

Mortality of G. mellonella by H. megidis and S. carpocapsae: Groups of 100 petri dishes (50 containing *H. megidis,* 50 untreated) were incubated at 8, 12, 16, 20, or 24 °C. For the first 4 days post treatment, *G. mellonella* larvae were checked four times a day at 4-hour intervals and twice a day at 8-hour intervals for the following 27 days or until larval death, whichever occurred first. Dead larvae and larvae that were alive at the end of the experiment were dissected and examined for IJ. The experiment was repeated with *S. carpocapsae.*

Invasion by H. megidis and S. carpocapsae: Galleria mellonella larvae were placed singly into 200 petri dishes, of which 100 contained *H. megidis,* and the remaining 100 were untreated. All 200 petri dishes were placed at 24 °C and after 1, 2, 4, 6, and 8 hours the *G. mellonella* larvae were removed from 20 treated and 20 untreated petri dishes; rinsed for 30 seconds under running, distilled water to remove IJ adhering to the larval surface; and placed in sterile petri dishes lined with one moist filter paper. After 7 days at 24 °C, the larvae were dissected and the number infected with nematodes recorded. The experiment was repeated with *S. carpocapsae.*

Infection by H. megidis and development of P. luminescens: Two hundred petri dishes (100 containing IJ, 100 untreated) were incubated at 16 °C. At 4-hour intervals, larvae were removed from 20 petri dishes (10 containing IJ, 10 untreated). By removing a proleg from each larva, hemolymph was obtained that was then examined with a hemocytometer under phase-contrast microscopy to determine the number of bacteria. When bacterial counts were high, hemolymph was diluted with phosphate-buffered saline (initial pH 6.2–6.5) to facilitate counting. Subsequently, each insect was dissected and the IJ in the host were counted. The number of bacteria in the hemolymph was estimated until collection of hemolymph was not possible due to the gummy consistency of the cadavers that is characteristic of advanced infection. To evaluate the effect of temperature on bacterial growth, 200 petri dishes (100 containing IJ, 100 controls) were incubated at 24 °C; at 4-hour intervals, the larvae were checked for presence of bacteria in the hemolymph at levels greater than 1,000/ml. Quantification was conducted as described previously.

Development of H. megidis IJ to hermaphrodites: Sixty petri dishes (30 containing IJ, 30 untreated) were incubated at 16 °C for 32 hours. *Galleria mellonella* larvae were subsequently rinsed with distilled water to remove any IJ adhering to the surface and placed into sterile petri dishes containing moistened filter paper. The petri dishes were held at 24 °C for 7 days, after which the larvae were dissected and nematode hermaphrodites were counted. The experiment was repeated at 24 °C.

Data analysis: Data are expressed as means ± standard errors and the significance of mean differences was determined with Studentized *t*-tests (SAS Institute, Cary, NC). LT50 values (length of time needed to kill 50% of the *G. mellonella* population) were determined by probit analysis with SAS. Comparison of invasion rates of *H. megidis* and *S. carpocapsae* were made with the X^2 test (Zar, 1974) and a contingency table.

Results

Mortality of G. mellonella by H. megidis and S. carpocapsae: The time required for *H. megidis* or *S. carpocapsae* to kill *G. mellonella* larvae decreased with increasing temperature (Fig. 1). The LT50s at 24 °C of 42 (*H. megidis*) and 40 (*S. carpocapsae*) hours were not significantly different. Similarly, the LT50s at 20 °C of 54 (*H. megidis*) and 52 (*S. carpocapsae*) hours were not significantly different. At

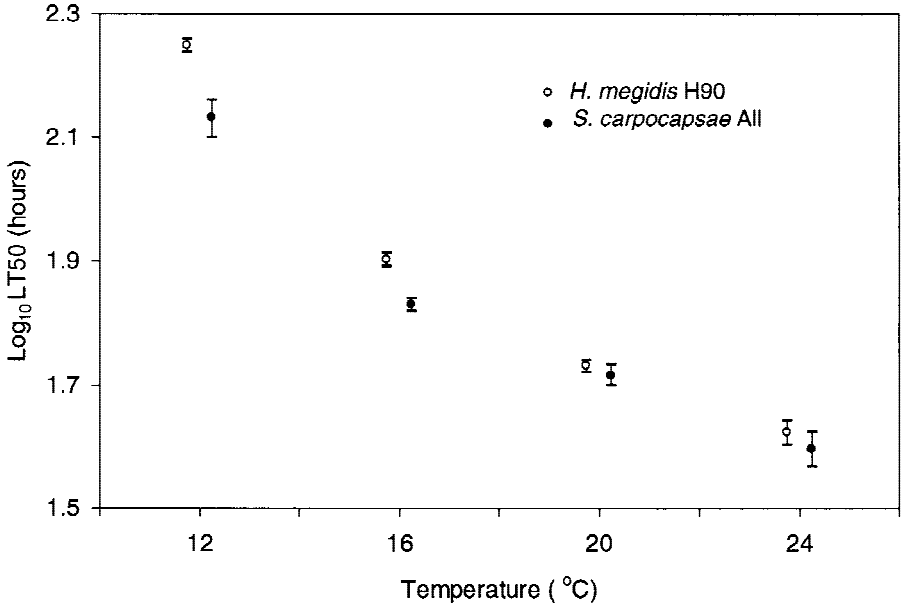

FIG. 1. LT50 (hours) of *Heterorhabditis megidis* H90 and *Steinernema carpocapsae* strain All in late instars of wax moth larvae at 12, 16, 20, and 24 °C. Bars are 95% fiducial limits; n = 50 for each nematode species at each temperature.

16 °C, the LT50 of 80 hours for *H. megidis* was significantly higher than the 68 hours recorded for *S. carpocapsae,* and, at 12 °C, the 179 hours recorded for *H. megidis* was significantly higher than the 136 hours reported for *S. carpocapsae.* At 8 °C, all larvae treated with either nematode species remained uninfected for the initial 10 days. By 33 days at 8 °C, 72% of larvae exposed to *H. megidis* were dead, of which 8% were red from *P. luminescens* and contained IJ, 30% were black from contaminating bacteria and contained dead IJ, and another 34% were dead and did not contain IJ. The latter group may have been killed by invading IJ that subsequently were not found because of contaminating bacteria. By 33 days at 8 °C, no developing hermaphrodites were found and all control larvae remained alive. By 33 days at 8 °C, 94% of *S. carpocapsae*-treated larvae died from IJ infections, nematode development beyond the IJ stage occurred in two larvae, and all control larvae survived.

Invasion by H. megidis and S. carpocapsae: None of the *G. mellonella* larvae exposed to *H. megidis* for 2 hours were killed or contained nematodes, and these larvae were pupating when examined 7 days after exposure. By contrast, 40% of the larvae exposed to *S. carpocapsae* for 2 hours became infected. However, after an 8-hour exposure to IJ at 24 °C, there was no significant difference in the percent larval mortality caused by the two nematode species (Fig. 2). There was no nematode infection in the controls.

Infection by H. megidis and development of P. luminescens: By 14 hours at 16 °C, IJ were

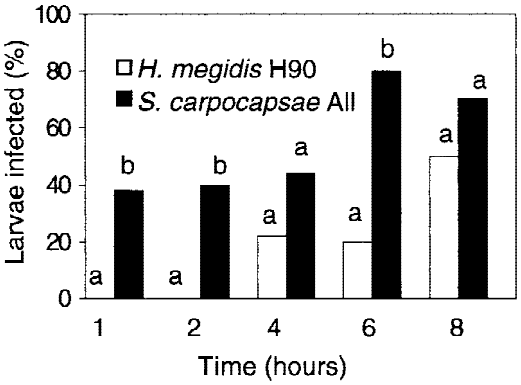

FIG. 2. Invasion rate of wax moth larvae challenged with either *Heterorhabditis megidis* H90 or *Steinernema carpocapsae* strain All at 24 °C for different periods; n = 20 larvae per duration. Bars in the same period with the same letter are not significantly different ($P > 0.05$).

present in all 10 larvae dissected (Table 1). The low recovery of IJ during the first 42 hours was partially due to the difficulty in locating them in freshly killed hosts. By 135 hours, nematodes were easier to enumerate since development to hermaphrodites had begun, and almost 8% of the initial inoculum was found in each host. *Photorhabdus luminescens* was first detected in the hemolymph 18 hours after exposure of the hosts to the IJ and multiplied rapidly after 32 hours. Detection of bacteria did not occur earlier at 24 °C than at 16 °C. However, at 24 °C, the count of 9,519,000 ± 8,529 rods/ml hemolymph exceeded 829,500 ± 181 rods/ml recorded at 16 °C 42 hours after exposure. All control larvae used in these experiments were alive and uninfected at the end of the experiment.

Development of H. megidis IJs to hermaphrodites: Significantly more IJs entered the larvae at 24 °C than at 16 °C ($t = 6.19$, d.f. = 29, $P < 0.001$). After 7 days, the 30 larvae at 24 °C and the 30 at 16 °C contained 275 ($X = 9.17 \pm 1.23$ per larva) and 37 ($X = 1.23 \pm 0.35$ per larva) hermaphrodites, respectively.

Discussion

Galleria mellonella larval mortality and nematode reproduction and development were faster at 20 and 24 °C than at lower temperatures for both *H. megidis* and *S. carpocapsae*. The timing of the presence of significant numbers of *P. luminescens* in the hemocoel is closely associated with the time of death of the insect larva. *Steinernema carpocapsae* invaded the larvae faster than did *H. megidis*. The high larval mortality at 20 to 24 °C, as expressed by LT50 values, is probably due to the combined effect of high insect larval activity and the high level of bacterial growth. At 24 °C, the LT50 values of 44 hours (*H. megidis*) and 42 hours (*S. carpocapsae*) were not significantly different and were similar to the values of 37 hours and 48 hours obtained at 24 °C for *H. heliothidis* NC 1 strain and *S. feltiae* DD136 strain, respectively, by Dunphy and Webster (1986). The observation that at lower temperatures (12 to 16 °C) nematode reproduction and development occurred at a slower rate, compared with that at 20 to 24 °C despite the fact that nematodes had entered the larvae, demonstrates the need to continue observations of nematode-treated insects for an extended period so as to avoid mistaking delayed infection for non-infection.

Fujiie et al. (1995) investigated the effect of temperature on the infectivity of *S. kushidai* against *Anomala cuprea* (Coleoptera: Scarabaeidae) and found that feeding activity of the insect was lower at 10 to 15 °C than

Table 1. Presence of *Heterorhabditis megidis* infective juveniles (IJ) and *Photorhabdus luminescens* in the hemolymph of *Galleria mellonella* larvae in petri dishes at 16°C.

Hours after exposure[a]	Percent larvae containing IJ	No. IJ/larva	Percent larvae containing bacteria	No. bacteria per larva ($X \pm SE \times 1{,}000$)
10	40	0.4 ± 0.5	0	0
14	100	2.4 ± 2.1	0	0
18	100	2.3 ± 1.6	30	—[d]
22	100	2.7 ± 1.2	50	—[d]
32	100	2.8 ± 2.6	30	1.5 ± 0.8
38	100	3.6 ± 1.7	70	113.5 ± 28.4
42	100	3.6 ± 1.7	90	829.5 ± 180.6
66	100	—[b]	100	3,046.0 ± 622.4
111	100	7.9 ± 5.6[c]	100	—[b]
135	100	22.7 ± 11.5[c]	100	—[b]
159	100	24.9 ± 14.4[c]	100	—[b]

[a] $n = 10$ for each sample time.
[b] Values not determined.
[c] IJ had already developed into hermaphrodites.
[d] <1,000 rods/ml.

at 20 to 30 °C. They suggested that opportunities for IJ infection may decrease with lower feeding activity. *Galleria mellonella* larvae are less active at 8 to 16 °C, and, although the site of infection of *A. kushidai* larvae may differ from *G. mellonella* larvae, it is possible that the lower level of *G. mellonella* activity was partly responsible for decreased IJ infectivity at these temperatures.

At 12 °C, the LT50 values of *H. megidis* (179 hours) and *S. carpocapsae* (136 hours) were significantly higher than their respective values at 20 or 24 °C. The observation that *H. megidis* is less infective than *S. carpocapsae* at lower temperatures is in agreement with earlier studies that investigated other strains of these genera (Dunphy and Webster, 1986; Molyneux, 1986; Wright, 1992). Infectivity of heterorhabditids between 13 and 22 °C has been reported to vary between isolates (Mráček and Webster, 1993). Although the lower temperature range (12 to 16 °C) over which the two species are infective overlap, *S. carpocapsae* killed *G. mellonella* larvae faster than did *H. megidis*.

Molyneux (1986) found that different strains of the same nematode species had different temperature limits for reproduction and development. At 33 days at 8 °C, larval mortality due to IJ infections reached 38% with *H. megidis* and 94% with *S. carpocapsae* but no reproduction was recorded for either nematode species. At 8 °C, IJs of *H. megidis* did not mature and maturation of *S. carpocapsae* IJs was very limited. Brown and Gaugler (1997) reported that although IJ can survive low temperatures and relative humidities for limited periods of time in the host cadaver, they fail to emerge and they eventually die. Generally, reproduction has been reported to cease at about 10 °C for heterorhabditids (Mason and Hominick, 1995; Wright, 1992; Zervos et al., 1991). Wright (1992) reported reproduction in steinernematids (*S. feltiae* strains CA and AKLD), and Mason and Hominick (1995) reported reproduction in a heterorhabditid (*H. megidis* isolate U.K.) to occur at 10 °C, but reproduction took greater than 60 days. Wright (1992) noted also that the reproduction rate at 10 °C for several nematode strains was related to the rate of growth of the bacterial symbiont. Mráček et al. (1997) reported on several strains of steinernematids isolated in Canada that were able to kill *G. mellonella* larvae in laboratory tests conducted at 4 °C. The temperature regime at which nematodes are propagated influences their capacity to infect *G. mellonella* larvae (Jagdale and Gordon, 1997; Schirocki and Hague, 1997), and this observation may be used to optimize the efficacy of a nematode strain.

The more rapid invasion rate of *G. mellonella* larvae by *S. carpocapsae* strain All than by *H. megidis* H90 supports earlier studies (Glazer, 1992) that reported LT50 values of 0.5 to 2.0 hours for *S. carpocapsae* strain All and 5.0 to 14.1 hours for *H. bacteriophora*. Our study showed that within 8 hours of exposure, differences in infectivity of the two nematode species could be ascertained, which is similar to the finding of Glazer (1992), who showed that differences in infectivity could be determined after 4 to 6 hours' exposure. Similarly, Mason and Hominick (1995) were able to discriminate between four heterorhabditid species by documenting the mean number of nematodes that became established in *G. mellonella* larvae following a 72-hour exposure of the larvae to IJ in a sand-filled tube. Epsky and Capinera (1994) advocated that a quantification of the IJ entering a host within a prescribed time period should be incorporated with assessments of mortality as an estimate of nematode efficacy. On the basis of these collective findings and the results described here, it is suggested that infectivity be measured by both the number of dead hosts and the number of IJ gaining entry into the host over a prescribed time period between 1 and 8 hours. Such tests are particularly useful in the evaluation of heterorhabditid infectivity because each IJ becomes a hermaphrodite that is easily counted due to its large size.

LD50 (lethal dose to kill 50% of a population) values may be used for comparison of nematode species and strains, but they were not considered in this paper because of the high susceptibility of *G. mellonella* larvae

to entomopathogenic nematodes, and the consequently low LD50 values. A more meaningful measure of nematode infectivity may be the use of a moderately resistant insect species in petri dishes or soil in comparative bioassays with *G. mellonella* larvae. For example, Griffin and Downes (1991) have described a laboratory test utilizing *G. mellonella* for the primary screening of *Heterorhabditis* isolates for low-temperature infectivity.

Temperature influences the number of IJ entering the host, subsequent bacterial growth, and nematode development. Collectively, these parameters influence the time it takes to kill *G. mellonella* larvae. Although the effect of temperature on insect feeding was not addressed in this work, it may be a crucial factor affecting *H. megidis* infectivity even though there is a report of heterorhabditids infecting insects directly through the cuticle (Molyneux, 1985). Further comparative studies between *Heterorhabditis* spp. and commercial products of *Steinernema* spp. are needed to ascertain the potential of using heterorhabditids in insect management programs.

Literature Cited

Bedding, R. A., A. S. Molyneux, and R. J. Akhurst. 1983. *Heterorhabditis* spp., *Neoaplectana* spp., and *Steinernema kraussei:* Interspecific and intraspecific differences in infectivity for insects. Experimental Parasitology 55:249–257.

Brown, J. M., and R. Gaugler. 1997. Temperature and humidity influence emergence and survival of entomopathogenic nematodes. Nematologica 43:363–375.

Dunphy, G. B., and J. M. Webster. 1986. Temperature effects on the growth and virulence of *Steinernema feltiae* strains and *Heterorhabditis heliothidis.* Journal of Nematology 18:270–272.

Dutky, S. R., J. V. Thompson, and G. E. Cantwell. 1962. A technique for mass rearing the greater wax moth (Lepidoptera: Galleridae). Proceedings of the Entomological Society of Washington 64:56–58.

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

Epsky, N. D., and J. L. Capinera. 1994. Invasion efficiency as a measure of efficacy of the entomogenous nematode *Steinernema carpocapsae* (Rhabditida: Steinernematidae). Journal of Economic Entomology 87: 366–370.

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

Glazer, I. 1992. Invasion rate as a measure of infectivity of steinernematid and heterorhabditid nematodes to insects. Journal of Invertebrate Pathology 59:90–94.

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

Jagdale, G. B., and R. Gordon. 1997. Effect of recycling temperature on the infectivity of entomopathogenic nematodes. Canadian Journal of Zoology 75: 2137–2141.

Mason, J. M., and W. M. Hominick. 1995. The effect of temperature on infection, development, and reproduction of heterorhabditids. Journal of Helminthology 69:337–345.

Molyneux, A. S. 1983. The biology and ecology of the entomopathogenic nematodes *Heterorhabditis* spp. (Heterorhabditidae) and *Steinernema* spp. (Steinernematidae). M. Ag. Sc. thesis. University of Tasmania, Hobart.

Molyneux, A. S. 1985. Survival of infective juveniles of *Heterorhabditis* spp. and *Steinernema* spp. (Nematoda: Rhabditida) at various temperatures and their subsequent infectivity for insects. Revue de Nématologie 8: 165–170.

Molyneux, A. S. 1986. *Heterorhabditis* spp. and *Steinernema* (= *Neoaplectana*) spp.: Temperature, and aspects of behavior and infectivity. Experimental Parasitology 62:169–180.

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

Mráček, Z., and J. M. Webster. 1993. Survey of Heterorhabditidae and Steinernematidae (Rhabditida, Nematoda) in western Canada. Journal of Nematology 25:710–717.

Schirocki, A. G., and N. G. M. Hague. 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.

Simons, W. R., and D. A. van der Schaaf. 1986. Infectivity of three *Heterorhabditis* isolates for *Otiorhynchus sulcatus* at different temperatures. Pp. 285–289 *in* R. A. Samson, J. M. Vlak, and D. Peters, eds. Fundamental and applied aspects of invertebrate pathology. Wageningen, The Netherlands.

White, G. F. 1927. A method for obtaining infective nematode larvae from cultures. Science 66:302–303.

Wright, P. W. 1992. Cool temperature reproduction of steinernematid and heterorhabditid nematodes. Journal of Invertebrate Pathology 60:148–151.

Zar, J. H. 1974. Biostatistical analysis. Englewood Cliffs, NJ: Prentice Hall.

Zervos, S., S. C. Johnson, and J. M. Webster. 1991. Effect of temperature and inoculum size on reproduction and development of *Heterorhabditis heliothidis* and *Steinernema glaseri* (Nematoda: Rhabditoidea) in *Galleria mellonella.* Canadian Journal of Zoology 69:1261–1264.