

Temperature Effects on the Growth and Virulence of *Steinernema feltiae* Strains and *Heterorhabditis heliothidis*¹

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Temperature influences the successful application of steinernematids and heterorhabditids as biological control agents against soil dwelling insects (1,3). It affects also the maturation and growth rates of *Steinernema feltiae* strains on meridic diets (10), nematode reproduction (12), growth in *Galleria mellonella* (4) and *Hylobius pales* (12), dauer juvenile infection potential and emergence time (11), migratory activity (3), sex ratios (4), and insect death rates (12). Studies of temperature effects on *Heterorhabditis* spp. are scarce. However, larvae of *Choristoneura fumiferana* parasitized by *H. heliothidis* died more slowly when incubated at 17 C than at 24 C (8).

Adapting *S. feltiae* strains and *H. heliothidis* to selected soil temperatures would likely improve control of soil dwelling insects. Burman and Pye (3) acclimated *S. feltiae* dauer juveniles to soil temperatures of 15-25 C by culturing the nematodes at those temperatures, but the acclimation was transient.

Our objectives were to test the potential of selecting for temperature adapted strains of *S. feltiae* and *H. heliothidis* by serial passaging the nematodes through *G. mellonella* larvae at specific temperatures and by prolonged culture of the nematodes on semi-defined diet in vitro at selected temperatures. Temperature adaptation was assessed by determining the yield of nematodes on artificial diet, monitoring changes in the concentration of dauer juveniles required to kill 50% (LC50) of the larvae,

and determining the time required for dauer juveniles to kill 50% (LT50) of the insects at designated temperatures.

The Mexican and DD136 strains of *S. feltiae* (supplied by G. O. Poinar, Jr., University of California, Berkeley) and *H. heliothidis* NC-1 (supplied by W. Brooks, North Carolina State University, Raleigh) were cultured monoxenically on fortified lipid agar (7). Larval *G. mellonella* were reared until they weighed 120 mg as the sixth instar (6).

The LC50s were determined by placing individual insect larvae in 9-cm-d petri dishes containing 1-100 dauer juveniles on moistened filter paper (Whatman no. 1). The larvae were restrained with a covering of moist filter paper. Ten larvae were used for each of five replicates and each concentration of nematodes. Larval mortality was assessed at 72 hours.

The LT50s were determined by exposing individual larvae to 10 dauer juveniles as described above. Five replicates containing 50 insect larvae per replicate were examined at 4-hour intervals for mortality until more than 75% of the larvae had died. Both the LC50s and LT50s were determined algebraically and analyzed for treatment effects by regression analysis (9).

Nematodes were passaged through *G. mellonella* larvae several times at a given temperature, and the LT50s were determined after either two or five passages. Dead larvae were incubated on White's water traps at the test temperature.

Only the DD136 strain of *S. feltiae* was subcultured monthly at 15, 20, and 25 C in vitro. LT50s were determined for dauer juveniles at their culture temperatures and at temperatures at which they had not been previously cultured; e.g., the LT50s of dauer juveniles from cultures at 15 C were determined at 15, 20, and 25 C. The total number of nematodes per dish was determined after prolonged culture at a particular temperature for a designated time and

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propagating the nematodes at that temperature and at two additional temperatures; e.g., nematodes from 15 C were cultured at 15, 20, and 25 C. Nematode yields were determined after 3 weeks incubation (7).

LC50s and LT50s of the nematodes tested declined as the incubation temperature increased when dauer juveniles from 25 C were used as inoculum (Table 1). Based upon decreasing LC50s and LT50s with increasing temperature, *H. heliothidis* was more sensitive to temperature than the Mexican strain of *S. feltiae*. In terms of LC50s at 25 C, *H. heliothidis* was the most virulent of the nematodes; next were the steinerneematids. In terms of LT50s, *H. heliothidis* and the Mexican strain of *S. feltiae* were equally virulent and more virulent than the DD136 strain of *S. feltiae*.

The DD136 strain was passed through the insect five times at both 15 and 20 C. At the final passage at a stated temperature (LT50 at 15 C on fifth passage = 143 ± 3 hours; LT50 at 20 C = 64 ± 2 hours, $P > 0.05$) the LT50s were not statistically different from the initial LT50s shown in Table 1. The Mexican strain failed to develop and emerge from dead larvae after the first passage at 15 C. Dead infective nematodes were located in the hemocoels of the larvae 30 days after larval death, which confirms the ability of the infective juveniles to penetrate the host. The Mexican strain was passed through the larvae five times at 20 C. There was no significant difference in the LT50 at 20 C and the initial LT50 ($P > 0.05$). *H. heliothidis* failed to emerge from the insects during an attempted third passage at 15 C, but they readily passed through the larvae five times at 20 C. Insects at 15 C died and turned ochre in color, confirming penetration of the host by the dauer larvae and release of *Xenorhabdus luminescens*. The LT50s for *H. heliothidis* at 15 C (178 ± 2 hours) and 20 C (68 ± 5 hours) did not differ from the initial values shown in Table 1.

Prolonged in vitro culturing of *S. feltiae* strains and *H. heliothidis* at 20 and 25 C for 9 months did not alter the LT50s from those of the initial values at the respective temperatures. Prolonged culture of the DD136 strain at 15 C did not alter the LT50.

The absence of changes in LT50s and

TABLE 1. Temperature effects on the LC50s and LT50s of selected nematodes reared at 25 C and exposed to sixth-instar *Galleria mellonella* at designated temperatures.

Nematode	Incubation temperature (C)	LC50* (dauer juveniles/insect)	LT50† (hours)
<i>Steinernema feltiae</i> , DD136 strain	15	5.2 ± 0.3	142 ± 5
	20	2.1 ± 0.4	64 ± 4
	25	1.5 ± 0.1	48 ± 3
<i>S. feltiae</i> , Mexican strain	15	12.2 ± 0.3	136 ± 8
	20	4.1 ± 0.2	57 ± 4
	25	3.5 ± 0.2	39 ± 2
<i>Heterorhabditis heliothidis</i> , NC-1 strain	15	8.4 ± 0.2	175 ± 5
	20	7.2 ± 0.3	60 ± 3
	25	1.0 ± 0.1	37 ± 3

* Concentration of dauer juveniles required to kill 50% of the insect larvae. Mean \pm standard error of the mean, $n =$ five replicates, mortality determined at 72 hours.

† Time required for dauer juveniles to kill 50% of the insects. Mean \pm standard error of the mean, $n =$ five replicates, nematode dosage of 10 dauer juveniles per larva.

LC50s and nematode yields during prolonged in vitro culture at 15 and 20 C demonstrates that the populations of steinerneematids and *H. heliothidis* in the present study do not adapt to different temperatures. Transient temperature adaptation in terms of behavioral changes has been observed for the Agriotos (= Leningrad) strain of *S. feltiae* (3). It is possible that over long periods cultural practices may have reduced the genetic variability of nematode stocks and (or) their bacteria and thus changed their ability to permanently adapt to different temperatures. The ecritic temperature of *S. feltiae* is believed to represent a balance between a constant driving force toward colder temperatures and a plastic drive toward warmer temperatures (3). Burman and Pye (3) suggested that permanent selection for a given temperature may be possible by mutagenesis or by isolation of nematodes from cool zones in the soil.

Our study establishes that the Mexican strain has a faster rate of kill of insect larvae than does the DD136 strain of *S. feltiae*. This finding may represent differences in host seeking and penetration activity, differences in toxin production by either the nematode-bacterium complex or the bacteria, differences in bacterial virulence, or

the number of bacteria per dauer juvenile. Preliminary data established similar LD50s and LT50s for *X. nematophilus* subsp. *nematophilus* (from the DD136 strain) and *X. nematophilus* subsp. *nematophilus* var. *mexicanus* (from the Mexican strain). The absence of toxin production by either *S. feltiae* strain or bacterial types in a culture medium similar to the host's hemolymph has been reported (5,6). Thus behavioral differences or differences in the number of bacteria per dauer juvenile may account for differences in virulence between the *S. feltiae* strains. In the present study, differences in virulence were influenced by temperature. Although the rasping denticle of *H. heliothidis*, which is not present in steinernematids, may account for the accelerated kill rate by the heterorhabditid (2), T. A. Rutherford (pers. comm.) has observed that steinernematid dauer juveniles kill *Tenebrio molitor* larvae faster than do the heterorhabditids.

LITERATURE CITED

1. Bedding, R. A., and L. A. Miller. 1982. Use of the nematode *Heterorhabditis heliothidis* to control blackvine weevils, *Otiorhynchus sulcatus*, on potted plants. *Annals of Applied Biology* 99:211-216.
2. Bedding, R. A., and A. S. Molyneux. 1982. Penetration of insect cuticle by infective juveniles of *Heterorhabditis* spp. (*Heterorhabditidae*: *Nematoda*). *Nematologica* 28:354-359.
3. Burman, M., and A. E. Pye. 1980. *Neoalectana carpocapsae*: Movements of nematode populations on a thermal gradient. *Experimental Parasitology* 49:258-265.
4. Danilov, L. G. 1976. Effect of the temperature on the infestation of caterpillars of the greater wax moth (*Galleria mellonella* L.) by entomopathogenic nematodes (*Neoalectana carpocapsae* Weiser, 1955, strain "agriotos") and on the development of the latter within the host. (In Russian; English summary.) *Byulleten Vesoyuznogo Nauchno-Issledovatel'skogo Instituta Zashchity Rastenii* 37:17-21.
5. Dunphy, G. B., and J. M. Webster. 1984. Interaction of *Xenorhabdus nematophilus* subsp. *nematophilus* with haemolymph of *Galleria mellonella*. *Journal of Insect Physiology* 30:883-889.
6. Dunphy, G. B., and J. M. Webster. 1985. Influence of *Steinernema feltiae* (Filipjev) Wouts, Mracek, Gerdin and Bedding DD136 strain on the humoral and haemocytic responses of *Galleria mellonella* (L.) larvae to selected bacteria. *Parasitology* 91:369-380.
7. Dunphy, G. B., T. A. Rutherford, and J. M. Webster. 1985. Growth and virulence of *Steinernema glaseri* influenced by different subspecies of *Xenorhabdus nematophilus*. *Journal of Nematology* 17:476-482.
8. Finney, J. R., and G. F. Bennett. 1984. Susceptibility of early instars of spruce budworm (*Lepidoptera*: *Tortricidae*) to *Heterorhabditis heliothidis* (*Nematoda*: *Rhabditidae*). *The Canadian Entomologist* 116:285-287.
9. Hewlett, P. S., and R. L. Plackett. 1979. An introduction to the interpretation of quantal responses in biology. New York: University Park Press.
10. Kaya, H. K. 1977. Development of the DD136 strain of *Neoalectana carpocapsae* at constant temperatures. *Journal of Nematology* 9:346-349.
11. Kaya, H. K., J. M. Marston, J. G. Lindegren, and Y. Peng. 1982. Low susceptibility of the honeybee, *Apis mellifera* L. (*Hymenoptera*: *Apidae*), to the entomogenous nematode *Neoalectana carpocapsae* Weiser. *Environmental Entomology* 11:920-924.
12. Pye, A. E., and M. Burman. 1978. *Neoalectana carpocapsae*: Infection and reproduction in large pine weevil larvae, *Hyllobius abietis*. *Experimental Parasitology* 46:1-11.