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# Effect of propagation temperatures on temperature tolerances of entomopathogenic nematodes

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**Summary** – Four isolates (= three species) of steinernematids were propagated at 25, 20 and when possible, 15 and 10 °C for 2 years, then their capacities for tolerating high temperatures and freezing determined. In all isolates, the temperature at which 50 % of the infective juveniles died ($LT_{50}$) increased with an increase in maintenance temperature. *Steinernema riobravis* had the highest $LT_{50}$ values, *Steinernema feltiae* strains the lowest, while *Steinernema carpocapsae* All strain displayed an intermediate degree of tolerance to high temperatures. Tolerance to freezing, as measured by times at which 50 % of the infective juveniles were killed by a –5 °C regime, was diminished at higher maintenance temperatures. The infectivity of nematodes that survived freezing was at least 90 % that of unfrozen nematodes (controls). Two strains of *S. feltiae* were the most cold tolerant of the four isolates, but their capacities to withstand freezing were diminished by propagation at warmer temperatures. © Elsevier - ORSTOM

**Résumé** – ***Effet de la température d'élevage sur la tolérance à la température de nématodes entomopathogènes*** – La capacité de quatre isolats (représentant trois espèces) de Steinernematides à tolérer des températures élevées, ou en dessous de 0 °C, a été déterminée après élevage à 25 et 20 °C et, quand cela a été possible, à 15 et 10 °C, pendant deux ans. La température à laquelle 50 % des juvéniles infestants de tous les isolats meurent ($LT_{50}$) augmente lorsque la température d'élevage s'élève. *Steirnernema riobravis* montre la $LT_{50}$ la plus élevée et les souches de *Steirnernema feltiae* les $LT_{50}$ les plus faibles, tandis que *Steinernema carpocapsae* isolat All montre un degré de tolérance intermédiaire aux températures élevées. La tolérance au gel, mesurée par le temps nécessaire pour tuer 50 % des juvéniles infestants à –5 °C, diminue lorsque les températures d'élevage sont hautes. Le pouvoir infestant des nématodes ayant survécu au gel est égal ou d'au moins 90 %, comparé à celui des nématodes n'ayant pas été congelés (témoins). Parmi les quatre isolats testés, deux isolats de *S. feltiae* sont les plus tolérants au froid, mais leur capacité à tolérer le gel diminue lorsque leur température d'élevage est élevée. © Elsevier - ORSTOM

**Keywords:** cold, entomopathogenic nematodes, freezing, heat, infectivity, *Steinernema carpocapsae*, *Steinernema feltiae*, *Steinernema riobravis*, tolerance, upper lethal temperature.

Temperature affects the dispersal, infectivity, reproduction and development of entomopathogenic nematodes (families Steinernematidae and Heterorhabditidae) (Molyneux, 1985; Kaya, 1990; Griffin, 1993; Grewal *et al.*, 1994; Mason & Hominick, 1995; Steiner, 1996). Infective juveniles of entomopathogenic nematodes have been recovered from the soils of a wide variety of climatic regions (Griffin *et al.* 1991; Mráček & Webster, 1993; Amarasinghe *et al.*, 1994; Cabanillas *et al.*, 1994; Jagdale *et al.*, 1996). Such nematodes are able to survive at habitat temperatures that undergo daily and/or seasonal cycles of fluctuation. To compensate for potentially lethal temperatures, infective juveniles may move deep into the soil (Kaya, 1990) or extend their thermal tolerance limits through acclimation.

The thermal tolerances of nematodes such as steinernematids may be adjusted through laboratory acclimation involving prolonged storage or propagation at defined temperatures. Dunphy and Webster (1986) concluded that *Heterorhabditis heliothidis* and two strains of *Steinernema feltiae* had no such capacity for temperature adaptation resulting from propagating the nematodes at various temperatures. However, after more prolonged propagation at temperatures different from the stock cultures, the temperature limits for virulence and establishment of *Heterorhabditis bacteriophora* and *Steinernema anomali,* as well as for reproduction of the former species, were extended (Grewal *et al.*, 1996). Heat tolerance, infectivity and reproduction of a strain of *H. bacteriophora* isolated from the Negev desert in Israel were enhanced by rearing the nematodes at a warmer temperature, whereas no such effects were noted for the commercially available HP88 strain of the same species (Shapiro *et al.*, 1996).

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Commercialization of entomopathogenic nematodes involves repetitive mass cultivation of infective juveniles *in vitro*. Thus, to maximize field efficacy it is important to obtain empirical data regarding the degree to which the temperature of the mass cultivation system may modify the capacities of such nematodes to withstand temperature extremes. The present *in vivo* study was carried out to investigate the extent to which the heat and cold tolerances of three species of *Steinernema,* isolated from varying climatic zones, were affected by the temperature regimes at which they were propagated.

## Materials and methods

### SOURCE OF NEMATODES

*Steinernema carpocapsae* All strain was provided by Plant Products Ltd., Brampton, Ontario, Canada; *S. riobravis* TX strain by Dr H. E. Cabanillas, USDA ARS, Crop Insects Research Unit, Weslaco, TX, USA. *Steinernema feltiae* Umeå strain was provided by Dr R. West, Canadian Forest Service (CFS), St. John's, NF, Canada from a stock colony that had been obtained initially from Biologic Biocontrol Products, Willow Hill, PA, USA. *Steinernema feltiae* NF strain is a new strain (Jagdale *et al.*, 1996) that was isolated, using *Galleria* bait traps (Woodring & Kaya, 1988), in summer 1994 from soil in an organic garden close to St. John's, NF, Canada.

### MAINTENANCE TEMPERATURE REGIMES

All nematode strains were maintained for two years (May, 1994-May, 1996) by propagation through *Galleria mellonella* larvae (Woodring & Kaya, 1988). The NF and Umeå strains of *S. feltiae* were propagated at 10, 15, 20 and 25 °C; *S. carpocapsae* All strain at 15, 20 and 25 °C; and *S. riobravis* TX strain at 20 and 25 °C. The numbers of generations perpetuated at the various temperatures were estimated to be 15-18 (10 °C), 21-25 (15 °C), 30-35 (20 °C) and 48-60 (25 °C).

### HEAT AND COLD TOLERANCES

*Heat tolerance ($LT_{50}$)*

Infective juvenile (IJ) suspensions of each of the four isolates propagated at the specified temperatures were prepared, with some modifications, as described by Gordon *et al.* (1996). The first IJ's (0-48 h-old) to emerge from insect cadavers into dilute formaldehyde solution inside White traps (Woodring & Kaya, 1988) were rinsed twice with distilled water and re-suspended in distilled water to a concentration of 100-400 IJ's/ml. One ml of this suspension was transferred into seven separate 50 ml beakers containing 9 ml distilled water. The beakers were then transferred into an incubator (Conviron, Controlled Environments Ltd., Winnipeg, Manitoba, Canada) at 26 °C, programmed to increase its temperature automatically (1 °C/h). Every hour, a 1 ml suspension of IJ's was removed from each of the seven beakers and the ratio of dead/total number of nematodes rapidly determined (<5 min) by probing each non motile nematode in the sample with a lachrymal needle under a stereomicroscope to determine whether it was alive or dead (Gordon *et al.*, 1996). After counting, each 1 ml sample was poured back into the beaker from which it had derived. Each beaker was considered as a separate replicate (n= 7).

A basal temperature of 26 °C was selected because we had previously determined that regardless of prior propagation temperature, all IJ's that were transferred to 26 °C were still active after 24 h. Nevertheless, controls were provided for each experiment. As a control, one 50 ml beaker containing 10 ml of the IJ's suspension of each isolate from each propagation temperature was transferred to an incubator at 26 °C until the end of each experiment and then examined for mortality of IJ's.

*Cold tolerance ($Lt_{50}$)*

Since we had previously determined that the IJ's of all isolates were tolerant of cold temperatures down to at least 5 °C, the time required to kill 50 % ($Lt_{50}$) of the nematodes at –5 °C was used to compare low temperature (subzero) tolerance. Two hundred pre-rinsed IJ's of each isolate were transferred into a set of twenty four 50 ml-beakers containing 10 ml of distilled water. In order to avoid thermal shock, all 24 beakers were transferred to an incubator at 5 °C for 12 h, 0 °C for another 12 h, then transferred to –5 °C. At 1, 2, 4, 6 and 8 h after transferring them to –5 °C, three beakers were removed from the incubator and allowed to thaw for 8 h at room temperature (25 °C). The ratio of "dead/total number of nematodes" in each of the beakers was determined as in the heat tolerance experiments. Each beaker was considered as a separate replicate (n= 3).

*Infectivity of* Steinernema *isolates subsequent to freezing*

The bioassay used to compare infectivities of IJ's subjected to subzero temperatures with unfrozen controls was similar to the *Tenebrio* Petri dish assay system used by Griffin and Downes (1994). IJ's of each of the four isolates (2500) that had been propagated at different temperatures were rinsed, transferred into beakers containing 10 ml distilled water, then transferred gradually to –5 °C as described above. After 5 h of freezing, the nematodes were removed from the incubator and allowed to thaw and recover for 8 h. For each isolate and propagation temperature, controls consisted of 2500 IJ's in a single 50 ml beaker containing 10 ml distilled water; control IJ's were pre-conditioned to 5 °C as experimental ones, but were then held at 0 °C in an unfrozen condi-

tion for 5 h. Two hundred active IJ's of each of the four isolates that recovered from freezing were transferred to a filter paper (Whatman no. 4) circle lining the base of each of five separate Petri dishes (5 cm diam.) and the filter paper evenly moistened by adding ≤ 0.2 ml distilled water (Gordon *et al.*, 1996). Ten last-instar larvae of *G. mellonella* were added to each Petri dish, all of which were then transferred to an incubator at 25 °C. Each Petri dish was considered as a separate replicate (n= 5 for controls and frozen nematodes of each isolate at the specified propagation temperatures).

Larval mortality was assessed 72 h after infection; all dead insects were transferred to White traps (Woodring & Kaya, 1988) for the emergence of IJ's to verify that these insects were infected by nematodes.

STATISTICAL ANALYSIS

Probit analysis (Norusis, 1990) was used to calculate the temperatures which killed 50 % of the IJ's ($LT_{50}$ = Upper Lethal Temperature), and the times at –5 °C necessary to kill 50 % of the IJ's ($Lt_{50}$ = Lower Lethal Temperature Times). The dependent variables (temperature, time) were subjected to $\log_{10}$ transformation in such analyses (Sokal & Rohlf, 1995). In both heat tolerance and cold tolerance studies, significant differences between pairs of $LT_{50}$ and $Lt_{50}$ values were based on the criterion of non overlap of 95 % confidence limits. Following arcsine transformation (Zar, 1996), the data on infectivity of *Steinernema* isolates subsequent to freezing was analysed by Kruskal-Wallis one way ANOVA (Jandel Corporation®, Sigma Stat, 1992).

## Results

HEAT TOLERANCE

There was no mortality of infective juveniles in any of the controls held at 26 °C. This is consistent with observations made over the 2 year-time frame in which nematodes were propagated, *i.e.*, negligible (<1 %) mortality occurred at ≤ 25 °C during the initial 48 h after removing the IJ's from the White traps. For all strains tested, the $LT_{50}$ increased progressively with increase in propagation temperature (Table 1). However, $LT_{50}$ values for each propagation temperature regime differed among strains. For propagation temperatures of 20 and 25 °C, the Umeå and NF strains of *S. feltiae* had the lowest $LT_{50}$ values of the four isolates. The TX strain of *S. riobravis* had the greatest $LT_{50}$ values. $LT_{50}$ values for the two strains (NF; Umeå) of *S. feltiae* were not significantly different. The All strain of *S. carpocapsae* appeared to display a level of high temperature tolerance intermediate between the two *S. feltiae* strains and *S. riobravis* TX strain. At propagation temperatures of 15, 20 and 25 °C, the All strain of *S. carpocapsae* had $LT_{50}$ values significantly higher than the values for *S. feltiae* Umeå strain. At the 25 °C propagation temperature, *S. carpocapsae* All strain had an $LT_{50}$ value significantly lower than that of *S. riobravis* TX strain.

COLD TOLERANCE

There was no mortality of infective juveniles in any of the unfrozen controls held at 0 °C. This accords with our observations that freshly harvested nematodes may be stored at 0-5 °C for up to 48 h with negligible (<1 %) mortality. For all isolates, survival times at –5 °C increased as propagation temperatures decreased (Table 1). Cold tolerance of the *S. feltiae* NF and Umeå strains propagated at 10 °C were not different. At a propagation temperature of 15 °C, IJ's of *S. carpocapsae* All strain survived freezing at –5 °C as well as *S. feltiae* IJ's (Table 1). At propagation temperatures of 20 and 25 °C, *S. riobravis* TX and *S. carpocapsae* All strain IJ's had $Lt_{50}$ values that were significantly greater than those of the *S. feltiae* strains. The freezing survival time for *S. riobravis* TX that had been propagated at 20 and 25 °C was the same as for *S. carpocapsae* All.

INFECTIVITY OF STEINERNEMATIDS SUBSEQUENT TO FREEZING (–5 °C)

All (100 %) of the insects were killed by control nematodes, a finding consistent with what we have routinely observed to occur during propagation of our stock colonies, in which the same host/parasite ratios were used. For all isolates, the propagation temperature did not significantly affect the infectivity of the IJ's that survived freezing (Table 2). Regardless of strain or propagation condition, nematodes that survived freezing for 5 h at –5 °C retained over 90 % of their infectivity compared to controls. It was also observed that the IJ's of each isolate emerged normally from the insect cadaver within 12-15 days after infection.

## Discussion

This study has shown that the heat and cold tolerances of entomopathogenic nematodes may be influenced by the temperature at which they are propagated. However, such manipulation of temperature tolerance is restricted by genetic differences among isolates.

Rearing at warmer temperatures increased the upper lethal temperatures and decreased survival times at –5 °C in all three species of *Steinernema*. Conversely, when nematodes were reared at colder temperatures, their upper lethal temperatures were decreased, while their freezing survival times were lengthened. In a related study, we have shown that the infectivity of these nematodes at cold temperatures (5-15 °C) was improved by propagation at cold temperatures, while infectivity at warm temperatures (0-

***Table 1.*** *Probit analysis of temperature tolerances of three species of* Steinernema *propagated at various temperature regimes.*

| Species and strains of *Steinernema* | Heat tolerance | | | Cold tolerance | |
|---|---|---|---|---|---|
| | PT[1] (°C) | Slope ± SE | $LT^1_{50}$ (°C) | Slope ± SE | $Lt^1_{50}$ (h) |
| *S. feltiae* NF strain | 10 | 38.3 ± 0.7 | 36.4*a* (36.0 - 36.7)[2] | 4.7 ± 0.2 | 7.3*b* (5.1 - 12.9) |
| | 15 | 56. 8 ± 1.2 | 38.0*b* (37.5 - 38.4) | 6.1 ± 0.3 | 6.7*b* (6.1 - 10.1) |
| | 20 | 49.1 ± 1.0 | 40.2*c* (39.8 - 40.7) | 3.3 ± 0.1 | 3.9*ab* (1.4 - 9.7) |
| | 25 | 51.2 ± 0.8 | 40.7*c* (40.3 - 41.1) | 4.1 ± 0.1 | 3.2*a* (2.1 - 4.4) |
| *S. feltiae* Umeå strain | 10 | 45.9 ± 1.8 | 35.0*d* (34.8 - 35.2) | 3.5 ± 0.1 | 6.6*c* (4.9 - 8.1) |
| | 15 | 36.4 ± 1.1 | 36.2*e* (35.9 - 36.5) | 3.4 ± 0.1 | 6.3*c* (4.9 - 9.0) |
| | 20 | 48.2 ± 1.9 | 37.7*f* (37.4 - 38.0) | 3.9 ± 0.1 | 3.0*d* (3.1 - 4.5) |
| | 25 | 35.5 ± 1.31 | 40.4*g* (40.2 - 40.6) | 8.7 ± 0.3 | 2.8*d* (2.5 - 3.0) |
| *S. carpocapsae* All strain | 15 | 72.3 ± 1.8 | 40.2*h* (39.9 - 40.5) | 3.8 ± 0.2 | 6.3*e* (4.8 - 10.5) |
| | 20 | 50.8 ± 0.7 | 42.4*i* (41.8 - 43.2) | 3.5 ± 0.2 | 6.3*e* (4.8 - 10.4) |
| | 25 | 44.1 ± 1.4 | 43.4*j* (43.2 - 43.7) | 6.4 ± 0.2 | 3.8*f* (3.1 - 4.1) |
| *S. riobravis* TX strain | 20 | 93.4 ± 1.7 | 44.4*k* (44.5 - 45.2) | 3.7 ± 0.2 | 6.2*g* (4.9 - 11.2) |
| | 25 | 61.6 ± 1.1 | 46.2*l* (45.9 - 46.5) | 5.7 ± 0.2 | 4.5*h* (3.9 - 5.0) |

[1]PT= Propagation temperature; $LT_{50}$= Upper lethal temperature; $Lt_{50}$ (h)= Lower lethal temperature time.

[2]Values in parentheses are the upper and lower 95 % confidence intervals.
Based on confidence intervals, the values for a given isolate in the same vertical column that are followed by the same letter are not significantly different from one another.

25 °C) was enhanced by propagation at warm temperatures (Jagdale & Gordon, 1997). Thus, it would appear that the efficacy of the nematodes, as displayed by their survival and infectivity, was adaptively modified by the temperatures at which they were propagated. The nematodes were propagated at specified temperature regimes for more than two years. Therefore, the observed shifts in heat and cold tolerances may be due to changes in their genetic constitution resulting from artificial selection and/or to environmentally-induced processes of thermal acclimation.

Acclimation to ambient temperatures has been demonstrated for several species of free-living nematodes (Mabbett & Wharton, 1986; Pickup, 1990; Wharton & Brown, 1991), as well as for the transmission stages of animal and plant parasitic ones (Ash & Atkinson, 1986; Forge & MacGuidwin, 1992). Survival at subzero temperatures was enhanced in *Heterorhabditis* sp. following 2-4 month-storage (Griffin, 1996) or in *H. bacteriophora*, *S. anomali* and *S. feltiae* following short-term (12-14 days) incubation (Brown & Gaugler, 1996) at temperatures lower than those at which propagation had been carried out. In this study, the possibility that temperature-induced acclimation may, at least in part, be responsible for the increased thermotolerance of strains that had been propagated at warmer temperatures is supported by Selvan *et al.* (1996), who showed that the infectivity and heat tolerance of *H. bacteriophora* to temperature regimes above the propagation temperature were enhanced by pre-conditioning the infective juveniles to 35 °C for a short time (1-3 h). On the basis of long term experiments, Shapiro *et al.* (1996) concluded that the heat tolerance trait of a desert dwelling strain

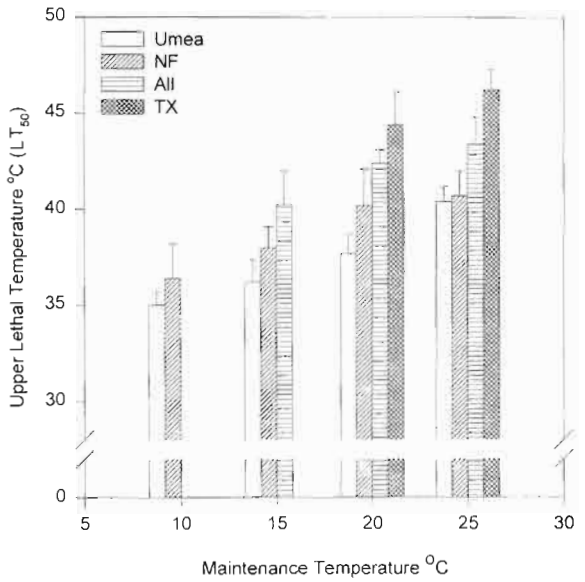


***Fig. 1.*** *Effect of maintenance temperature on the upper lethal temperature ($LT_{50}$) of four isolates (= three species) of* Steinernema *maintained at four different temperature regimes. NF = Newfoundland strain of* Steinernema feltiae*; Umeå = Umeå strain of* S. feltiae*; All = All strain of* Steinernema carpocapsae *and TX = TX strain of* Steinernema riobravis*. Vertical lines are standard error bars.*

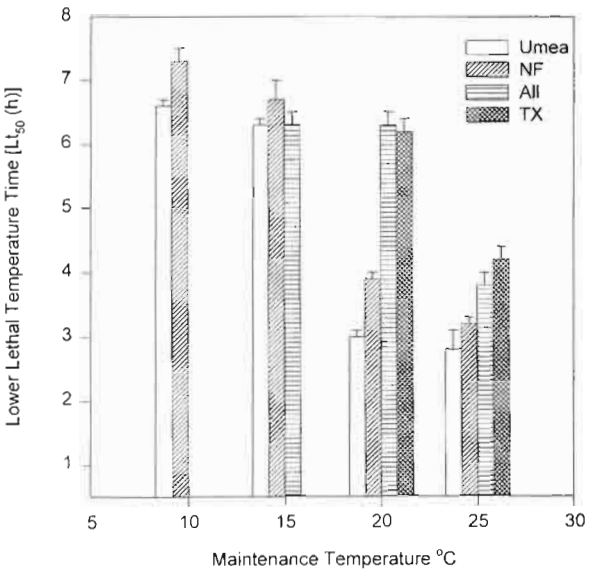


***Fig. 2.*** *Effect of four different maintenance temperature regimes on the lower lethal temperature time ($Lt_{50}$ [h]) required to kill 50 % steinernematids (three species = four isolates) following 5-h exposure to –5°C. NF = Newfoundland strain of* Steinernema feltiae*; Umeå = Umeå strain of* S. feltiae*; All = All strain of* Steinernema carpocapsae *and TX = TX strain of* Steinernema riobravis*. Vertical lines are standard error bars.*

***Table 2.*** *Effect of freezing on the infectivity of* Steinernema *species and strains propagated at various temperatures.*

| PT (°C) | Mortality (%) | | | |
|---|---|---|---|---|
| | *S. feltiae* NF strain ± SE | *S. feltiae* Umeå strain ± SE | *S. carpocapsae* All strain ± SE | *S. riobravis* TX strain ± SE |
| 10 | 82.7 ± 4.5*a* | 82.7 ± 4.7*b* | - | - |
| 15 | 81.0 ± 5.6*a* | 81.0 ± 5.6*b* | 82.6 ± 4.5*c* | - |
| 20 | 75.4 ± 3.6*a* | 81.0 ± 5.6*b* | 71.6 ± 0.0*c* | 75.6 ± 6.0*d* |
| 25 | 77.4 ± 5.4*a* | 77.4 ± 5.4*b* | 71.6 ± 0.0*c* | 75.6 ± 6.0*d* |

PT= Propagation Temperature. Dashes indicate absence of observations because *S. carpocapsae* and *S. riobravis* did not recycle at these temperatures.

Values (Arcsine transformed) followed by the same letter are not significantly different ($P \leq 0.05$), as determined by the Kruskal-Wallis one way ANOVA (n=5).

of *H. bacteriophora* was genetically based, but that the property could be influenced by the propagation temperature.

In this study, exposure to the subzero regime resulted in internal freezing of the IJ's. During the thawing process, we observed the presence of opaque ice crystals, followed by their disappearance, within the nematodes. Thus, our bioassay measured freezing tolerance. Given the seemingly adaptive manner in which propagation at cold temperatures enhanced freezing tolerance, it seems reasonable to infer that boreal nematodes such as the two strains of *S. feltiae* examined overwinter by tolerating freezing. However, more detailed studies are needed to validate this point. Brown and Gaugler (1996) also showed that *S. feltiae*, *S. anomali* and *H. bacteriophora* were freezing tolerant when the freezing event was experimentally induced by exogenous ice nucleation. In *Heterorhabditis zealandica*, however, the sheath is important in allowing the nematodes to avoid internal freezing as supercooling occurs (Wharton & Surrey, 1994). Steinernematids lose their sheaths easily (Campbell & Gaugler, 1991), so it seems probable that *S. feltiae* NF

strain, which overwinters in frozen soil (Walsh & Gordon, unpubl.), normally freezes.

In the present laboratory assays, 50 % of the nematodes died within 7 h subsequent to freezing at –5 °C. This survival percentage appears consistent with those reported for *H. bacteriophora*, *S. anomali*, and *S. feltiae*, *viz.*, 30-40 % and 10 % after 12 h and 1.3-2.7 day, freezing, respectively (Brown & Gaugler, 1996). However, such experiments, have not been designed to mimic the seasonal changes in soil temperatures that occur in cold climates. As in boreal insects (Sømme, 1982), a gradual sequence of environmental changes may be needed to induce cryoprotectant mechanisms for overwintering. Consequently, while such assays are useful for evaluating comparative tolerances to cold, the actual values yielded cannot be directly extrapolated to the field situation.

Our results do not accord with those of Dunphy and Webster (1986), who observed no temperature adaptation. In *H. heliothidis* and two strains of *S. feltiae*, times and dosages of IJ's required to kill *G. mellonella* larvae were unaffected by subculturing at various temperatures. These authors employed a shorter (9 months) maintenance period, so it is possible that adaptive mechanisms may not have had sufficient time to develop. Thermal adaptation was reported by Grewal *et al.* (1996) and Shapiro *et al.* (1996) who, like ourselves, used a more prolonged propagation period (twelve repeated passages through waxmoth larvae). Both upper and lower limits for infection of *H. bacteriophora* and *S. anomali* were extended when maintained at the lower (15 °C) and upper (30 °C) thermal limits of their reproduction (Grewal *et al.*, 1996). The adaptive responses that we have described, involving a shift, rather than an expansion, of the tolerance ranges accords more closely with what has been reported to occur in a wide range of other poikilotherms (Fry, 1967; Howling *et al.*, 1994; Quinn *et al.*, 1994).

The fact that genetic differences among the isolates limited the degree to which thermal adaptation occured is to be anticipated. Species and even strains of entomopathogenic nematodes were found to have distinct temperature limits for development and reproduction (Grewal *et al.*, 1994, 1996; Mason & Hominick, 1995). It has been suggested that the lower and upper temperature limits for the activity of entomopathogenic nematodes are correlated with the temperatures of their original habitats (Molyneux, 1986) and our studies appear to validate this proposition. With respect to the isolates used in the present study, the two strains of *S. feltiae*, NF and Umeå, originally isolated from Northern Sweden (Pye & Pye, 1985) and Newfoundland, Canada (Jagdale *et al.*, 1996) respectively, were the only ones able to propagate at 10 °C. *Steinernema riobravis* TX strain could not be propagated at any of the temperatures tested below 20 °C, undoubtedly a reflection of its subtropical habitat (Cabanillas *et al.*, 1994). The All strain of *S. carpocapsae*, an isolate that has been subjected to extensive laboratory and commercial subculturing displayed an intermediate minimum temperature for recycling (15 °C). The degree to which this nematode resembles natural field populations in Georgia, USA., from which it was originally derived (Poinar, 1979), is unknown.

All of the isolates, regardless of propagation temperature, had $LT_{50}$ values (35-45 °C) that were sufficiently high to indicate that they would survive soil temperatures in all except the most extreme situations. Provided that steps are taken to shield IJ from extreme heat during their application to the soil, tolerance to high temperatures should not limit their usefulness in pest management. With respect to the use of nematodes in cold climates, however, our data suggest that the boreally adapted strains of *S. feltiae* would need to be maintained at 10-15 °C in order to retain a high degree of cold tolerance. The subtropical *S. riobravis* and the commercially produced *S. carpocapsae* All strain recycle well and have a good capacity for tolerating high temperatures, when propagated at temperatures (20-25 °C) at which commercialization seems practical. Such nematodes appear suited for both inundative and inoculative releases in subtropical/tropical climates in which tolerance to freezing would not be an issue. Inundative short term applications of these nematodes in temperate or even boreal situations accords with their present usage and with the fact that ability to withstand freezing was compromised in all of the isolates when recycled at 25 °C.

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