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Effect of salt and temperature stresses on survival and infectivity of *Heterorhabditis* spp. IJs

Michelle M. FINNEGAN [1], Martin J. DOWNES [1], Myra O'REGAN [2] and Christine T. GRIFFIN [1]

[1] *Department of Biology, NUI Maynooth, Maynooth, Co. Kildare;* [2] *Department of Statistics, Trinity College, Dublin, Ireland*

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Summary – *Heterorhabditis* is frequently found in coastal sandy soils where it may experience both high salinity and high temperatures. We tested the ability of infective juveniles (IJs) of three taxonomic groups of *Heterorhabditis* to infect insects in saline sand. We also tested whether salinity (sea water) affected the IJs' ability to tolerate elevated temperatures in aqueous suspension and in sand. IJs of all three taxonomic groups killed *Galleria mellonella* in saline sand (25.6% insects killed), but at a lower level than in non-saline sand (96.5% insects killed). Exposure of IJs in sand to high temperature reduced their ability to kill *G. mellonella* at 20° C; heating IJs in saline sand reduced *G. mellonella* mortality to a lesser extent (25.6% at 20° C, 18.3% at 39° C) than heating in non-saline sand (96.5% at 20° C, 17.5% at 39° C). In aqueous suspension, IJs of the North-West European and Irish types of *Heterorhabditis* tolerated high temperature better in sea water (at least 95% survived 1 h at 39° C) than in distilled water (none survived 1 h at 38° C). *H. bacteriophora* was more temperature tolerant: survival and subsequent infectivity of IJs was unaffected by temperature up to 39° C in either medium. It was concluded that high salinity (sea water) reduces the ability of *Heterorhabditis* IJs to infect, but improves their tolerance of high temperature.

Résumé – ***Effets des chocs dus à la salinité et à la température sur la survie et l'infestivité des juvéniles infestants d'* Heterorhabditis *spp.* –** Les *Heterorhabditis* sont fréquemment rencontrés dans les sols sableux côtiers où ils peuvent être soumis à des salinités et des températures élevées. Nous avons testé la capacité des juvéniles infestants (IJs) de trois groupes taxinomiques d' *Heterorhabditis* à infester des insectes dans du sable salé. Nous avons aussi cherché à savoir si la salinité (eau de mer) affecte la capacité des IJs à tolérer des températures élevées, soit en suspension aqueuse, soit dans du sable. Les IJs des trois troupes taxinomiques tuent les *Galleria mellonella* dans le sable salé, mais à un taux plus faible que dans le sable non salé (25,6 contre 96,5% des insectes tués). L'exposition des IJs dans du sable à des températures élevées réduit leur capacité à tuer *G. mellonella* à 20° C; le chauffage des IJs dans du sable salé réduit la mortalité des *G. mellonella* dans un moindre mesure (25,6 à 20° C contre 18,3% à 39° C) que dans du sable non salé (96,5% à 20° C contre 17,5% à 39° C). En suspension aqueuse, les IJs des types Europe du nord-ouest et d'Irlande tolèrent mieux les températures élevées dans l'eau de mer (au moins 95% survivent 1 h à 39° C) que dans l'eau distillée (aucune survie après 1 h à 38° C). *H. bacteriophora* montre la meilleure tolérance à la température: la survie et l'infestivité ultérieures des IJs ne sont pas affectées par la température jusqu'à 39° C dans l'un et l'autre milieux. Il en est conclu que les salinités élevées (eau de mer) réduisent la capacité d'infestation des IJs d' *Heterorhabditis*, mais améliorent leur tolérance aux températures élevées.

Keywords: entomopathogenic nematode, environmental stress, heat tolerance, *Heterorhabditis*, salinity, salt tolerance, temperature.

Entomopathogenic nematodes (*Heterorhabditis* spp. and *Steinernema* spp.) have considerable potential for the biocontrol of insect pests (Kaya & Gaugler, 1993). The transmission stage is a specialised third stage juvenile, the infective juvenile (IJ) which carries cells of a symbiotic insect-killing bacterium (*Photorhabdus luminescens* in *Heterorhabditis* spp.) in its intestine. On entry into the host's haemocoel, the nematode releases the bacteria which proliferate, killing the host by septicaemia. The IJs, like the dauer juvenile of *Caenorhabditis elegans*, does not feed and is more resistant than other stages to environmental stresses.

Griffin *et al.* (1994b) showed that *Heterorhabditis* was halotolerant. IJs survived prolonged storage in sea water as well as or better than in distilled water, and remained infective for up to nineteen weeks. Griffin *et al.* (1994b) suggested that *Heterorhabditis* IJs might be transported between coastal sites by sea currents. In that study, infectivity was tested in a non-saline medium. Thurston *et al.* (1994) reported that the infectivity of *H. bacteriophora* was not reduced in saline sand with an electroconductiv-

ity of 16-32 dS/m when the salt used was $CaCl_2$ or KCl, but NaCl did reduce infectivity.

Temperature is one of the most important factors affecting entomopathogenic nematodes (*e.g.*, Kaya, 1990; Griffin, 1993). The effects of temperature on infectivity and on long-term persistence are well documented (*e.g.*, Molyneux, 1985, 1986; Griffin & Downes, 1991; Kung *et al.*, 1991), but lethal temperature limits have received less attention. IJs may need to survive exposure to temperatures higher than those at which infection can occur, at least for short periods of the day, especially in warmer climatic zones or following foliar application. IJs of HP88, a Utah strain of *Heterorhabditis bacteriophora*, were all killed by short (1-2 h) exposure to 40°C, and their viability was reduced at 37°C (Glazer *et al.*, 1996; Shapiro *et al.*, 1996). Some Israeli isolates *of H. bacteriophora* and of *H. megidis* were considerably more heat tolerant than HP88 at both 37 and 40°C (Glazer *et al.*, 1993, 1996; Shapiro *et al.*, 1996).

Heterorhabditids are frequently found in sandy coastal soils (Hara *et al.*, 1991; Amarasinghe *et al.*, 1994; Griffin *et al.*, 1994a). Poinar (1993) cited this amongst the evidence for the origin of the genus in an arenicolous marine environment. The reason for this association with coastal sands is not clear, but one consequence is that in soils close to the sea, nematodes may encounter elevated levels of salinity and also of temperature. Sandy soils are more prone to heating than soils with better water holding capacity. The sites at which we have recovered *Heterorhabditis* in northern and central Europe typically have short vegetation, without much shading tree or shrub cover (Griffin *et al.*, 1994a, 1999), increasing the vulnerability of such soils to heating. In their natural environment, organisms are exposed to a variety of stresses simultaneously, and their viability is determined by the interaction of these factors. Here we test the effect of salinity on the ability of IJs of each of the three European types of *Heterorhabditis* to tolerate elevated temperatures, both in aqueous suspension and in sand. We also test their ability to infect insects in sand moistened with undiluted sea water.

Materials and methods

Source and maintenance of nematodes

Six *Heterorhabditis* isolates, two of each of three taxonomic groups, were used (Table 1). Nematodes were cultured at 20°C in late instar larvae of the wax-moth *Galleria mellonella*. IJs were harvested in modified White traps, washed by sedimentation in three changes of tap water and used within two weeks of harvesting.

Table 1. *Origin and taxonomic position of the* Heterorhabditis *isolates used in the study*

Code	Species/Type	Origin	Source
EU222	*H. bacteriophora*	Debrecen, Hungary	Maynooth
EU185	*H. bacteriophora*	Kesckemet, Hungary	Maynooth
UK211	North-west European	South coast of England	Hominick*
HF85	North-west European	Flevopolder, Netherlands	Westerman**
W48	Irish	South coast of Wales	Maynooth
M170	Irish	North-west coast of Ireland	Maynooth

* Dr W.M Hominick, International Institute for Parasitology.
** Ir Paula Westerman, Friesland College of Agriculture, The Netherlands.

Effect of high temperatures on IJs in aqueous suspension

Suspensions of 400 IJs/ml were prepared in sea water (from Sandycove, Co. Dublin; electroconductivity 54 dS/m) or distilled water. Suspensions were prepared 2 days before exposure to high temperatures took place. Eppendorf tubes containing aliquots of 1 ml suspension were suspended in water baths at 20, 32, 33, 34, 35, 36, 37, 38 and 39°C for 1 h. Temperature was monitored using a thermistor probe connected to a Grant Squirrel data logger immersed in an Eppendorf containing 1 ml distilled water; it reached the bath temperature within 5 min. Dissolved oxygen levels were recorded in saline and distilled water, with and without nematodes, at the start and end of a 1 h incubation at 20, 37 and 39°C, using a micro oxygen sensor (UMS, Kaltenordheim, Germany). Oxygen declined by up to 28% (sea water at 39°C) of starting level (saturation). After 1 h, tubes were removed from the water baths and allowed to return to room temperature (*c.* 23°C). There were two tubes for each treatment (isolate/temperature/medium). One set of tubes was examined immediately: IJs in the other set of tubes were washed by sedimentation three times in distilled water and left to recover in distilled water for 2 days at 20°C prior to examination. The numbers of living and dead IJs were counted.

Nematodes which failed to respond to probing with a fine glass rod were counted as dead.

For five of the test temperatures (20, 33, 35, 37 and 39°C) the infectivity of the surviving IJs, washed and unwashed, was assessed. Infectivity was assessed as the ability to kill *Galleria mellonella* larvae and as the proportion of nematodes that established in the killed insects. A late instar *G. mellonella* larva was placed in a 2.5 cm diam. Petri dish which was then filled with moist sand (heat sterilized silver sand moistened with 8% tap water (w/w)). Ten IJs were picked out by reference to preselected locations in a gridded dish and added to the sand in a minimum of water (*c.* 40 ml). Controls received water without nematodes. The assay dishes (five per treatment) were incubated at 20°C for 4 days. The insects were removed from the sand, washed in tapwater and patted dry with paper towelling to remove IJs adhering to the cuticle. They were returned to 20°C and dissected after a further 2-3 days. The number of first generation female nematodes was taken as representative of the number of IJs that had entered.

The experiment was repeated on three successive days using the same batches of IJs.

Effect of high temperature on IJs in sand

Flat-bottomed glass tubes (50 mm high × 11 mm int. diam.) were filled with *c.* 3.5 g moistened sand. The sand (washed, sterilised sea sand of particle diameter 170-250 μm) was moistened (8% w/w) with either sea water or distilled water. To each vial of this saline sand or non-saline sand was added 100 ml of IJs suspension containing 100 IJs in, respectively, either sea water or distilled water. The tubes were capped and suspended in water baths at 20, 37 or 39°C for 1, 2, 4, 6 or 24 h. Temperature within the sand was monitored by means of a thermistor probe inserted through the lid of a vial containing sand without IJs and attached to a Grant Squirrel data logger. The timing of the incubation period started when the test temperature was reached (which was within 5 min of immersion in the water bath).

Following incubation, the vials were allowed to return to room temperature (*c.* 23°C). The infectivity of the nematodes in the sand was tested by baiting for 2 days at 20°C with *G. mellonella* larvae. Ten vials per treatment were baited immediately, while another ten vials were left for 2 days at 20°C before baiting. One late instar *G. mellonella* larva was placed on the sand surface in each vial. It was then covered with saline or non-saline sand, as appropriate, to the top of the tube. After 2 days at 20°C, the insects were removed from the sand, washed in tap water and patted dry with paper towelling to remove IJs adhering to the cuticle. They were then incubated at 20°C for a further 3-4 days before dissection. The number of first generation females was taken as representative of the number of IJs that had entered. Note was taken of the appearance of the cadaver. Each of the *Photorhabdus luminescens* strains carried by the isolates tested here imparts a characteristic colour (yellow, purple or red, depending on the isolate) to the cadaver, which also develops a gummy consistency. Control tubes containing saline or non-saline sand without nematodes were incubated at 20, 37 or 39°C for 24 h and baited with *G. mellonella* as above.

The six isolates of the study were divided into two isolate sets (A and B). Each isolate set contained one isolate of each of the three taxonomic groups. The sand experiment was conducted twice, using a different isolate set on each occasion. The isolates of *H. bacteriophora* and the NWE and Irish types of *Heterorhabditis* were, respectively: EU222, HF85 and M170 in Set A, and EU185, UK211 and W48 in Set B.

Statistical analysis

An analysis of variance was carried out on the data. For the analysis of *G. mellonella* mortality, the dependent variable was the logit function = $\log(\frac{p}{1-p})$ where p was the proportion of insects that died. A suitable small constant was added to the numerator and denominator in the cases where $p = 0$ or 1.

Results

Exposure to high temperatures in aqueous suspension

Nematode survival

IJs of both of the *H. bacteriophora* isolates (EU185 and EU222) survived one hour at all temperatures up to and including 39°C without significant mortality, both in distilled water and in sea water (Table 2). At least 90% of the IJs of the NWE and Irish isolates survived an hour at 37°C in distilled water but none survived at 38°C. However, in sea water, at least 95% of the IJs survived at 39°C (Table 2).

Mortality of bait insects

The infectivity of IJs surviving the temperature treatments was tested against *G. mellonella.* Ability to kill

Table 2. *Percentage of infective juveniles of* Heterorhabditis *spp. alive following exposure to various temperatures for 1 h in aqueous suspension (sea water or distilled water)*

Temperature (°C)	*H. bacteriophora*				NWE type *Heterorhabditis*				Irish type *Heterorhabditis*			
	EU222		EU185		UK211		HF85		M170		W48	
	Dist	Sea	Dist	Sea	Dist	Sea	Dist	Sea	Dist	Sea	Dist	Sea
20	99.2	98.8	99.3	99.7	99.3	99.8	98.9	97.7	98.5	98.5	99.2	99.4
32	99.5	99.3	98.9	99.6	99.1	99.8	98.8	98.1	98.4	97.5	99.3	99.3
33	99.3	99.3	99.8	99.3	99.3	99.8	98.1	97.0	97.9	98.8	98.7	99.3
34	99.3	98.9	99.5	99.8	99.3	99.4	97.6	97.2	98.3	98.3	98.8	99.2
35	99.3	98.6	99.2	99.5	99.0	99.4	97.5	96.9	97.8	97.9	92.9	99.3
36	98.8	98.7	99.0	99.3	99.0	99.3	97.3	96.3	97.5	96.9	94.4	98.6
37	99.3	98.9	99.0	99.4	98.5	99.6	98.3	97.2	95.5	97.8	92.1	97.9
38	97.9	99.3	98.4	99.3	0	99.3	0	96.7	0	97.7	0	99.0
39	96.7	98.7	99.1	99.1	0	96.1	0	94.9	0	95.8	0	95.3

Data are mean of three replicates.

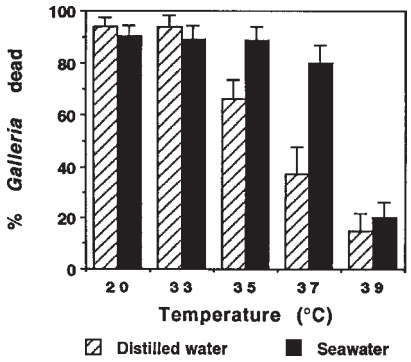

Fig. 1. *Mean percentage mortality of* Galleria mellonella *larvae exposed in sand at 20° C to* Heterorhabditis *IJs that had previously been heated for 1 h at temperatures ranging from 20 to 39° C. (Means are of six nematode isolates, both washed and unwashed treatments. Bars = SEM).*

G. mellonella was reduced by prior exposure to high temperatures (35°C and above), particularly when the exposure was in distilled water (Fig. 1). Mortality of control insects (no nematodes) was in all cases zero. High levels of *G. mellonella* mortality occurred in many of the nematode treatments, especially at lower temperatures (20-35°C inclusive) where 100% mortality was frequently recorded. This was true for all isolates except EU185, where the maximum mortality in any treatment was 93% (detailed results not shown). Due to the frequent occurrence of 100% mortality, these data were not as useful for discriminating between treatments as was the proportion of nematodes that established in the bait insects.

Establishment of nematodes in bait insects

Up to 60% of IJs established in the bait insects, but this proportion was considerably lower in several instances, especially for the Irish and NWE isolates following exposure to high temperatures (Fig. 2). An analysis of variance was carried out on these data. The independent variables were: temperature, salinity (distilled or sea water), washing (IJs washed or not washed after heating) and isolate (six isolates). The most important effects ($P < 0.0001$) identified in this analysis were the interaction terms temperature × salinity and temperature × isolate and the main effect washing. Each of these significant effects will now be discussed.

Temperature by salinity. At higher temperatures (35, 37 and 39°C), high salinity (sea water) tended to improve nematode viability (as assessed by subsequent ability to infect and establish in *G. mellonella* larvae), while at lower temperatures (20 and 33°C) the trend was reversed (Fig. 2). The largest effect of salinity occurred at 37°C: IJs that had been exposed to 37°C in sea water were almost three times more infective to *G. mellonella* than those that had been exposed in distilled water: 25% as against 9% nematodes established in the insects.

Temperature by isolate. The isolates differed in their response to temperature. For each of the NWE and Irish group isolates there was a marked effect of temperature: high numbers of nematodes established following exposure to the two lower temperatures (20 and 33°C); lower numbers established following exposure to 35°C and above; in the 39°C treatment, numbers of nematodes

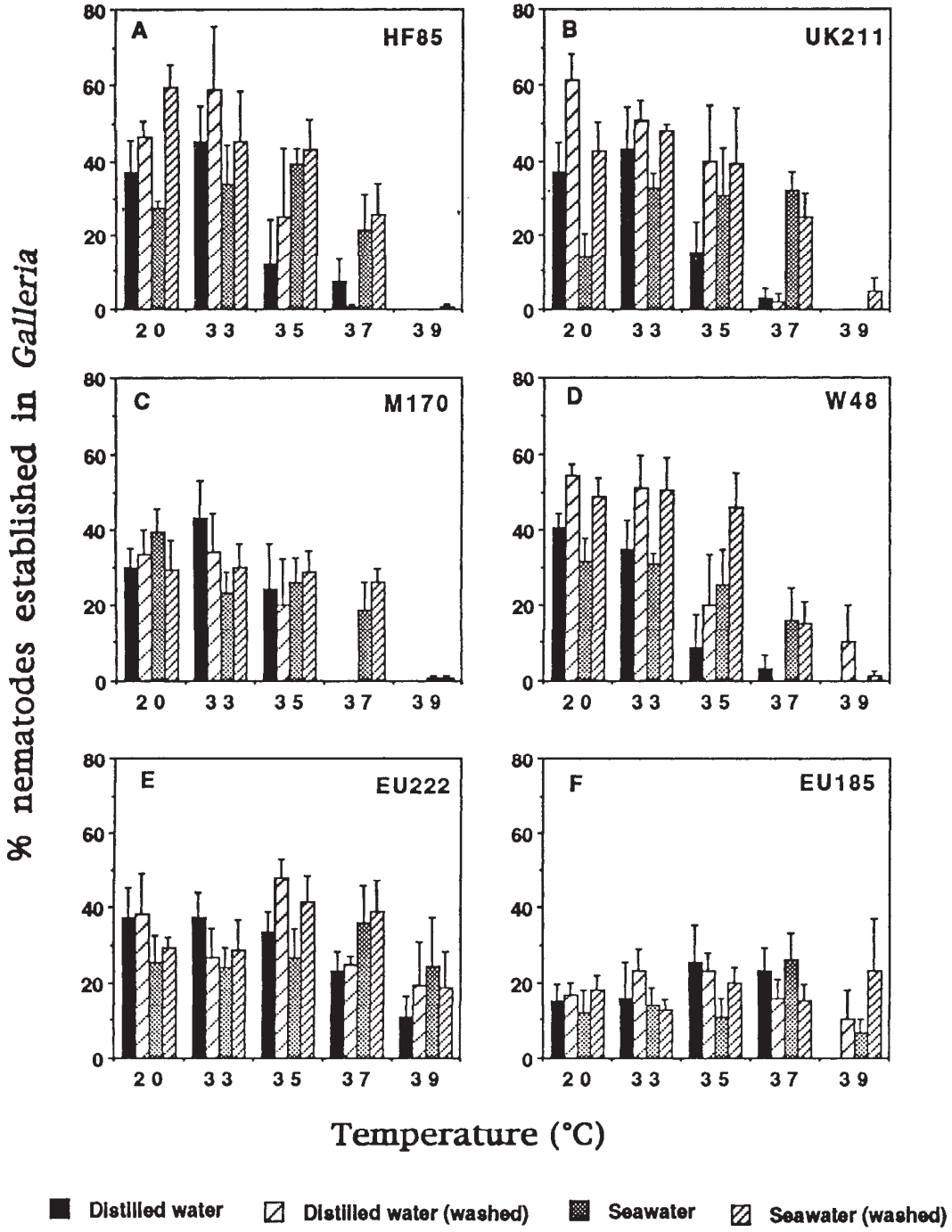

Fig. 2. *The percentage of IJs of six isolates of* Heterorhabditis *that established in* Galleria mellonella *larvae during two days in sand at 20° C. IJs in sea water or in distilled water had previously been exposed to five temperatures for 1 h. Infectivity was tested either immediately or after washing in distilled water. A: NWE type HF85; B: NWE type UK211; C: Irish type M170; D: Irish type W48; E:* H. bacteriophora *EU222; F:* H. bacteriophora *EU185. Bars = SEM.*

established were very low (Fig. 2 A-D). Temperature had much less of an effect on the two *H. bacteriophora* isolates (EU222 and EU185) (Fig. 2 E, F).

Washing. Washing the IJs following temperature treatment resulted in a higher proportion of the nematodes becoming established in the test insects: 27% of the washed nematodes established, compared to 21% of the unwashed nematodes (Fig. 2).

Exposure to high salinity and high temperatures in moist sand

In this experiment, IJs remained in the sand throughout the high temperature treatment and subsequent baiting with *G. mellonella*, and thus there is no direct measure of IJs survival. Results of the baiting are given both as *G. mellonella* mortality and as the proportion of nematodes that established.

Mortality of bait insects

Following 20°C exposure, IJs in non-saline sand (sand moistened with distilled water) killed nearly four times as many *G. mellonella* as IJs in saline sand (moistened with sea water) (Fig. 3A). Heating IJs to 37 or 39°C in non-saline sand greatly reduced their ability to kill *G. mellonella* while the killing power of IJs in saline sand was only slightly affected by these temperatures (Fig. 3A). This trend (high bait insect mortality in non-saline, 20°C-exposed sand compared to other salinity-temperature combinations) was found for every combination of the other factors tested (*i.e.*, for each of the six nematode isolates, at each exposure time, and in sand baited immediately after the temperature treatment or after a 2-day delay). There was no mortality of control insects (without nematodes).

Preliminary analysis revealed a large effect due to temperature × salinity, therefore the results for moderate (20°C) and high (37 and 39°C) temperature treatments were analysed separately in order to detect more subtle effects of the other factors. Separate analysis of variance was performed for each of the two isolate sets, which were tested on different occasions. Independent variables were salinity, isolate, exposure time (1-24 h), baiting (immediate or delayed) and, for the 37-39°C data sets, temperature.

For the 20°C treatment, salinity had a significant effect on *G. mellonella* mortality ($P < 0.001$ for each isolate set), with more *G. mellonella* killed by non-saline than by saline sand. This was the only significant effect detected in the analysis; exposure time, isolate and baiting time had

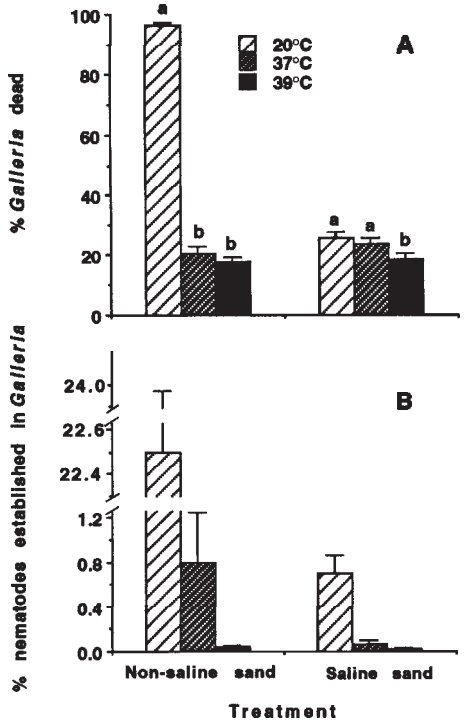

Fig. 3. *Infectivity of* Heterorhabditis *IJs in saline or non-saline sand for* Galleria mellonella *larvae during a two-day baiting at 20° C. The* Heterorhabditis *infested sand had previously been exposed to various temperatures for up to 24 h. A: Percentage of* G. mellonella *larvae dead. Within sand type, columns accompanied by the same letter are not significantly different (χ^2 test, $P < 0.05$; overall chi-square: non-saline sand: $\chi^2 = 970$, 2 d.f., $P < 0.001$; saline sand: $\chi^2 = 9.87$, 2 d.f., $P < 0.01$); B: Percentage of nematodes established in the* G. mellonella *larvae (Bars = SEM).*

no significant effect at this temperature in either isolate set.

For sand that had been exposed to 37 and 39°C, salinity was a less important factor, and was significant for only one of the two isolate sets (Set A, $P < 0.05$). In this case, more *G. mellonella* were killed in saline sand than in non-saline sand (21.2 compared to 16.5%), a reverse of the trend in the 20°C-treated sand. Temperature also had a significant ($P < 0.01$) effect for Set A: more *G. mellonella* larvae were killed by IJs-infested sand which had previously been exposed to 37°C than in sand that had been exposed to 39°C (22 and 15.5%, respectively). Exposure time had a significant effect for both isolate sets. IJs that had been exposed to high temperatures for longer periods resulted in lower *G. mellonella* mortality: mortality was 27% following 1 h heat-exposure and 16% following 24 h. Baiting time had a significant effect in both isolate

sets (A: $P < 0.01$; B: $P < 0.001$), but the nature of the effect was inconsistent. For Set A, immediate baiting resulted in higher bait insect mortality than delayed baiting, while the reverse was true for Set B.

Establishment of nematodes in bait insects

Dissecting the bait insects confirmed the large effect of salinity and temperature on *Heterorhabditis* infectivity (Fig. 3B). While 22.5% of the nematodes established following 20°C exposure in non-saline sand, fewer than 1% established in all other treatments. In non-saline sand, establishment was reduced nearly 30-fold by exposure to 37°C (from 22.5% at 20°C to 0.8% at 37°C), while in saline sand, it was reduced only 10-fold (from 0.7% at 20°C to 0.07% at 37°C). In both saline and non-saline sand, establishment was further reduced in the 39°C treatment (Fig. 3B).

In the case of the high temperature treatments, the majority of the bait *G. mellonella* that died (80 and 95% in the 37 and 39°C treatments, respectively) contained no nematodes when dissected, although they displayed the characteristic appearance of infection with *Photorhabdus luminescens*. Similarly, many (69%) of the dead *G. mellonella* in the 20°C saline sand treatment contained no nematodes. In the 20°C non-saline sand, only 7% of the dead bait *G. mellonella* did not contain any nematodes.

Discussion

Heterorhabditis IJs of each of the three taxonomic groups tested here were capable of parasitising insects in very saline sand (sand moistened with undiluted sea water). However, their infective ability was greatly reduced compared to those in non-saline sand. IJs were observed to be less active in sea water than in distilled water, and it is likely that reduced mobility due to osmotic stress (Wharton *et al.*, 1983) is the cause of the lowered infectivity. However, interference with host finding ability by constituents of the medium (Thurston *et al.*, 1994) could also contribute to the reduced invasion rate. The ability of IJs to invade insects in sea water-moistened sand is consistent with Poinar's (1993) hypothesis that the genus *Heterorhabditis* evolved from a marine ancestor, with ancestral types parasitizing arthropods in the littoral zone. However, comparison of six species of entomopathogenic nematodes showed that steinernematids were also capable of infecting insects in sea-water moistened sand, and their infectivity was no more severely affected by salinity than that of the heterorhabditids (Cribbin, unpubl.; Miller, unpubl.).

Sea water clearly protected *Heterorhabditis* IJs from the damaging effects of high temperature both in aqueous suspension and in sand. In aqueous suspension, the lethal limit for Irish and NWE type nematodes was at least 2°C higher in sea water than in distilled water and, in the sand experiment, the infectivity of all isolates was less affected by heating in the saline than in the non-saline medium. Several aquatic invertebrates tolerate supernormal temperatures best at the upper end of the salinity range, and an increase in salinity beyond normal habitat conditions tends to increase heat resistance (Kinne, 1970). In our experiments it is not clear whether IJs were protected from heat damage by the presence of the saline medium during heat exposure, or if protection was conferred before the heating, when the nematodes were transferred from tap water to sea water. In many organisms, prior exposure to one stress confers resistance to another stress by inducing common resistance mechanisms such as synthesis of heat shock proteins (Hoffman & Parsons, 1991).

In distilled water the two *H. bacteriophora* isolates tolerated higher (39°C) temperatures than isolates of the NWE and Irish groups (37°C). This correlates with the original source of the isolates used in the study: The *H. bacteriophora* isolates were from Hungary, which has a continental climate, while the isolates of the NWE and Irish groups were from Ireland, England and The Netherlands, where there is a greater maritime influence. The isolates used here are from locations representative of the distribution of these taxonomic groups in Europe: *H. bacteriophora* primarily has a continental and mediterranean distribution (Deseo & Miller, 1985; Smits *et al.*, 1991; Griffin *et al.*, 1999), while the NWE and Irish groups mainly occur along the cooler north-west coast of the continent and in Ireland and Britain (Smits *et al.*, 1991; Griffin *et al.*, 1994*a*; Hominick *et al.*, 1995). Here, however, they frequently occur in sand dunes where temperatures can exceed 40°C (Huiskes, 1979), and so the ability to tolerate elevated temperatures may be adaptive for these strains also. The greater similarity between the responses of the NWE and Irish types to each other than to the *H. bacteriophora* isolates corresponds with other findings regarding the biology of the IJs (Griffin *et al.*, 1994b) and the molecular characterization (Smits *et al.*, 1991) of these three taxonomic groups.

We have not determined the maximum temperature tolerated by the Hungarian *H. bacteriophora* isolates; however, they experienced very little mortality following

1 h in aqueous suspension at 39°C. Moreover, infectivity was unaffected by this treatment, suggesting that sublethal effects were also not important. Despite differences in methodology between our study and others, it would appear that the performance of the Hungarian isolates more closely resembles that of the heat tolerant Israeli IS-5 strain than that of the HP88 strain of *H. bacteriophora* (Glazer *et al.*, 1996; Shapiro *et al.*, 1996).

The maximum temperature tolerated by the Irish and NWE isolates was higher in sand (at least 39°C) than in aqueous suspension (37°C). Similarly, Gray and Johnson (1983) found that *S. carpocapsae* survived for up to two weeks at 40°C in soil, while the same species was reported to enter heat coma after 1 h at 35°C in tapwater (Schmiege, 1963). The inferior temperature tolerance in water in our experiments may be explained by the faster rate of heating or lower oxygen availability in that medium. Nematodes in aqueous suspension quickly settle; in contact with the bottom of the vial they would experience a more rapid increase in temperature than IJs in a comparatively large volume of sand. However, the difference in rate of heating was not great, as both media reached the test temperature within 5 min. Although measurements indicated that the water still contained abundant oxygen at the end of the incubation period, the conditions experienced by the IJs at the bottom of the vials may have been locally poorer. While the determination of lethal temperatures for nematodes in aqueous suspension is useful for comparative purposes, it may give an inaccurate indication of a species' tolerance in its natural habitat.

Most of the damage associated with elevated temperatures in sand occurred within 1 h of exposure; the killing power of IJs dropped from close to 100% mortality of *G. mellonella* (20°C non-saline treatment) to 27% after 1 h of 37 or 39°C, but a further 23 h of elevated temperature only reduced killing to 16%. Gray and Johnson (1983) found that the duration of incubation (1 or 2 weeks) did not have a significant effect on the survival of *S. carpocapsae* IJs in soil at temperatures from 20 to 40°C. Similarly, survival of *H. bacteriophora* in aqueous suspension dropped from 100 to 26% in the first 2 h at 37°C, but there was little further mortality during the next 6 h at that temperature (Glazer *et al.*, 1996). These findings suggest that once an IJs survives the initial shock of the temperature change, it has a relatively high chance of continued survival, and that the temperature change *per se* is more detrimental than the continuing exposure.

In general, "washing" increased the infectivity of IJs. For IJs incubated in sea water this could be explained by the restoration of an osmotically more favourable medium (sea water replaced by distilled water). The washing treatment included a 2-day delay between the time that the IJs were washed and the time that their infectivity was tested; this should favour the recovery of IJs that had been subjected to high temperature and/or osmotic shock. However, washing also resulted in increased infectivity following a relatively non-stressful treatment, incubation in distilled water at 20°C. The reason for this is not clear, but it is unlikely that differences between bait insects or other conditions of the infectivity assays for unwashed and washed nematodes was the cause, as the effect was detected each of the three times the experiment was run.

Following exposure of IJs to high temperatures in sand (both saline and non-saline), most of the dead bait insects contained no living nematodes, even though they were infected with *Photorhabdus luminescens*. This suggests that the IJs, although capable of invading, had been affected by the heat treatment and failed to develop, as has been reported for *Steinernema* (Grewal *et al.*, 1994; Henneberry *et al.*, 1996). The proportion of nematodes which can penetrate and establish is a useful parameter for comparing the efficacy of different nematodes under most conditions (Fan & Hominick, 1991), and was the most suitable parameter in the "aqueous medium" experiment reported here. However, bait insect mortality may be a better measure of infectivity in those rare circumstances when a large proportion of the invading nematodes fails to establish, such as in the "sand" experiment, where up to 95% of *Photorhabdus*-killed insects contained no nematodes.

The level of salinity used in the present experiments (54 dS/m) is above the tolerance level of all crop plants, and therefore would not be encountered by entomopathogenic nematodes applied inundatively for biocontrol purposes. However, *Heterorhabditis* has been isolated from sandy beaches devoid of vegetation but with crustaceans present (Amarasinghe *et al.*, 1994); the evidence presented here indicates that, not only would the high salinity of such a shoreline location not preclude infection of hosts there, but it would also protect IJs against possible high temperatures in this unshaded habitat.

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