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Effects of prior storage conditions on the infectivity of *Heterorhabditis* sp. (Nematoda : Heterorhabditidae)

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Summary – When dauer juveniles (DJs) of *Heterorhabditis* sp. (NWE Group) were stored in tapwater at 9, 20 or 25 °C, they became progressively more infective. Infectivity was measured at 9 and 20 °C, as the proportion of nematodes entering *Galleria mellonella* in sand. Cold-infectivity (the ability to infect insects at 9 °C) was particularly affected by storage, showing a 120-fold increase from time of harvest. At higher storage temperatures (20 or 25 °C), infectivity reached a peak after 2-3 weeks. When DJs were stored at 9 °C, maximum infectivity occurred later. The pattern that was observed during 9 °C storage varied with experimental conditions, and the age of DJs at the time when they were placed at 9 °C. The experimental results are consistent with the involvement of two storage phenomena : a progressive increase of infectivity, which occurs over a range of storage temperatures, and a temporary cold-induced state of reduced activity which masks or delays the appearance of the increase in infectivity in cold-stored DJs for a period of some weeks.

Résumé – ***Effets des conditions préalables de stockage sur le pouvoir infestant d'*Heterorhabditis *sp. (Nematoda : Heterorhabditidae)*** – Lorsque des dauer juvéniles (DJs) d'*Heterorhabditis* (groupe NWE) sont conservés dans l'eau du robinet à 9, 20 ou 25 °C, leur pouvoir infestant augmente progressivement. Le pouvoir infestant, mesuré à 9 et 20 °C, est la proportion de nématodes pénétrant *Galleria mellonella* dans du sable. Le pouvoir infestant à froid (la capacité à infester des insectes a 9 °C) est particulièrement dépendant des conditions de conservation et augmente 120 fois à partir de la date de récupération des DJs. A des températures de conservation plus élevées (20 ou 25 °C), le pouvoir infestant atteint un maximum après 2 à 3 semaines. Quand les DJs sont conservés à 9 °C, l'infestivité maximale apparaît plus tard. Pendant la conservation à 9 °C, on observe une variation de l'infestivité avec les conditions expérimentales et l'âge des DJs au moment où ils sont placés à 9 °C. Les résultats expérimentaux sont compatibles avec l'intrication de deux phénomènes liés à la conservation : une augmentation progressive du pouvoir infestant qui apparaît dans une gamme de températures de conservation, et une réduction temporaire d'activité induite par le froid qui masque ou retarde l'apparition de l'augmentation du pouvoir infestant pour les DJs conservés au froid pendant quelques semaines.

Keywords : biocontrol, cold activity, entomopathogenic nematode, *Heterorhabditis* sp. HF85 and UK211, storage temperature.

The entomopathogenic nematodes *Heterorhabditis* spp. and *Steinernema* spp. have considerable potential as biocontrol agents. The infective stage is a non-feeding third stage infective juvenile or dauer juvenile (DJ). The infectivity of these DJs declines during prolonged storage, and this is presumed to be largely due to the depletion of energy reserves (Vänninen, 1990; Westerman & Stapel, 1992). There is normally an inverse relationship between survival time and temperature, related to the greater mobility and faster utilisation of reserves at higher temperatures (Molyneux, 1985). However, there is increasing evidence, both for heterorhabditids and for the related steinernematids, that changes in infectivity occur prior to the eventual decline associated with starvation or ageing of stored DJs. At least two storage phenomena have been reported : First, an increase in infectivity, documented especially for various *Heterorhabditis* spp. at both high (Wojcik *et al.*, 1986; Griffin *et al.*, 1994 *a*) and low (Griffin, 1993) storage temperatures. Second, a temporary suppression of infectivity, or " U-shaped curve ", induced by cold (e.g., Fan & Hominick, 1991) and, up until now, documented only for steinernematids. The relationship between the two patterns is unclear, though results presented by Curran (1993) and by Ishibashi *et al.* (1995) show an overall increase in the infectivity of cold-stored steinernematids, interrupted by a U-shaped curve, leading to a bimodal pattern of infectivity over time which I shall call " peak-trough-peak ".

The North-West European (NWE) Group of *Heterorhabditis* shows good potential for pest control, particularly of black vine weevil, *Otiorhynchus sulcatus* (Simons, 1981; Van Tol, 1994), and strains of this species are commercially produced for the purpose. Among the factors hampering the further development of these organisms for biocontrol are poor activity at low temperatures, and the variability of bioassay and trial results. The exact storage history of the dauers is one possible source of variation. The objective of this study was to document the effect of storage conditions (time and

temperature) on the infectivity of the NWE group of *Heterorhabditis*. As dauers are normally stored at low temperatures in the laboratory, and as infectivity at low soil temperatures is an important limiting factor for them, particular emphasis was placed on both the effects of cold storage and on the impact of storage conditions on cold-infectivity.

Materials and methods

Source and maintenance of nematodes and insects

Heterorhabditis sp. HF85 and UK211 were originally obtained from Ir Paula Westerman (Van Hall Institute, Leeuwarden, The Netherlands) and from Dr W. Hominick (International Institute of Parasitology, St. Albans, England), respectively. These isolates, which originated in the Netherlands and England, respectively, both belong to the North-West European group of *Heterorhabditis* (Smits *et al.*, 1991; Griffin *et al.*, 1994 *b*). Nematodes were cultured at 20 °C in late instar larvae of the wax moth *Galleria mellonella* (twenty insects exposed on filter paper to 4000 dauer juveniles (DJs) in a 9 cm Petri dish; two to four dishes per culture batch of each isolate). After 2 days, cadavers displaying the characteristic red colouration were transferred to clean Petri dishes containing moist paper. After a further 12 days, cadavers were placed in modified White traps (14 cm Petri dishes, with ten insects per trap). DJs were harvested daily; those used in experiments had emerged from the cadavers either 18 (Experiments I and II) or 20 (Experiment III) days after infection. DJs were washed by sedimentation in three changes of tapwater on the day of harvest.

G. mellonella larvae used for culturing nematodes and for assays originated from a culture continuously maintained in the laboratory for 5 years. They were reared at 30 °C under uncrowded conditions on a diet of honey (500 g), beeswax (125 g), glycerol (425 g), wholemal flour (850 g), brewer's yeast (250 g), and wheat bran (approx 30 g).

Storage conditions

Suspensions of DJs (1000 DJs/ml tapwater) which had been harvested over a single 24-h period were stored in a shallow layer of tapwater at constant temperatures, in darkness. They were either in Petri dishes (5 cm diam., 8 ml/dish) sealed with Parafilm (Exp. I) or in loosely stoppered glass bottles (2 cm diam., 2 ml/bottle) (Exps II and III). At intervals, the infectivity of stored DJs was assessed using a *Galleria* sand bioassay. The storage and assay temperatures of the various experiments are summarised in Table 1. Counts were made of stored DJs prior to testing. Because dead DJs may decompose, counts were of living DJs/ml. Where necessary, the concentration of surviving DJs was adjusted to 1000/ml before testing, so that the number of live DJs added in the bioassay was always approximately 100. A storage treatment was terminated when the survival of DJs dropped below 80 %, to minimise confusing the effects of selective mortality with those of physiological change. Experiments 1 and 2 overlapped in time (assays for week 8 of Exp. 1 and for week 0 of Exp. 2 were conducted simultaneously), while Exp. 3 began immediately after the end of Exp. 2.

Table 1. *Prestorage, storage and assay temperatures used for* Heterorhabditis *sp. dauer juveniles in each of three experiments. Storage was in tapwater; infectivity assays were conducted against* Galleria mellonella *in sand.*

Experiment	Storage temperature °C	Pre-storage treatment	Assay temperatures °C
I	9	–	9
II a	9	–	9
II b	9	20 °C, 1 week	9
II c	9	20 °C, 2 weeks	9
III a	9	–	9, 20
III b	20	–	9, 20
III c	25	–	9, 20
III d	9	20 °C, 3 weeks	9, 20

Infectivity test

Infectivity was tested using the *Galleria* sand bioassay described by Griffin and Downes (1994 *a*). Last instar *G. melonella* larvae of equal size were selected by eye and were maintained non-feeding in Petri dishes at 15 °C for 6-8 days prior to use. One late instar *G. mellonella* was placed at the bottom of a plastic vial (40 mm height × 45 mm diam.) which was then packed with moist sand. The sand used was washed seasand (the fraction retained between 400 and 250 μm pore diam. sieves), heat-sterilized and moistened with 8 % w/w tapwater. Vials were capped and thermoequilibrated to the test temperature. Thermoequilibration of sand vials lasted either 24 h at 9 °C or 2 h at 20 °C. The DJs were assayed immediately on removal from the storage temperature, without prior equilibration to the test temperature. DJ suspension (100 dauers in 100 μl tapwater) was pipetted to an indentation in the surface of the sand. There were twelve replicate vials per treatment. The vials were incubated in sealed insulated boxes in constant temperature rooms. The temperature within the boxes was monitored at 30 min intervals by thermistor probes attached to a Grant data logger, and typically deviated by no more than about ± 0.2 °C across time or space. DJs were added to, and the insects removed from, the sand at the incubation temperature. Infectivity assays lasted either 3 days at 9 °C or 16 h at 20 °C. At

the end of the test period, the insects were removed from the sand, rinsed in tapwater to remove surface DJs, dried with paper towelling and incubated at 20 °C. Insects which died were placed on moist paper. They were dissected after 5 days at 20 °C and the number of first generation adult nematodes was recorded. Insects which did not die were not dissected, but were assumed to harbour no nematodes.

EXPERIMENT I

This experiment investigated the effect of storage at 9 °C on the infectivity of the NWE Group of *Heterorhabditis*. DJs of two isolates (UK211 and HF85) were stored at 9 °C for up to 22 weeks and, at intervals during storage, their infectivity was assayed at 9 °C.

EXPERIMENT II

Freshly harvested DJs (UK211 and HF85) were divided into three lots. One lot was stored at 9 °C as in Exp. 1. The other two lots were pre-stored at 20 °C for 1 or 2 weeks, respectively, before being stored at 9 °C for up to 5 months. At intervals during storage, infectivity was assayed at 9 °C.

EXPERIMENT III

Freshly harvested UK211 DJs were divided into four lots; three were stored at a single constant temperature (9, 20, and 25 °C) throughout. The fourth lot of DJs was pre-stored at 20 °C for 3 weeks before being transferred to 9 °C for further storage, for up to 13 weeks. The infectivity of these DJs was tested before transfer and subsequently at intervals during cold-storage. Infectivity of DJs from all storage regimes was tested at both 9 and 20 °C.

STATISTICAL ANALYSIS

The Mann Whitney U Test (n = 24) was used for pairwise comparisons.

Results

INFECTIVITY OF DJS STORED AT 9 °C FOR VARIOUS PERIODS FROM TIME OF EMERGENCE FROM THE HOST CADAVER

The infectivity of *Heterorhabditis* sp. DJs for *G. mellonella* at 9 °C increased during prolonged storage at constant 9 °C (Figs 1 A, B, E, F; 2 A). The infectivity of DJs in each experiment displayed a similar pattern over time. Immediately after harvesting (week 0), infectivity was less than two nematodes/insect, i.e., fewer than 2 % of applied DJs penetrated. There was a lag period before an increase in infectivity was detected. It first differed significantly from week 0 after 5 or 6 weeks of storage ($P \leq 0.05$). Thereafter, infectivity remained high for several weeks, especially in Expts I and II, where levels of ten or more nematodes/insect were recorded from

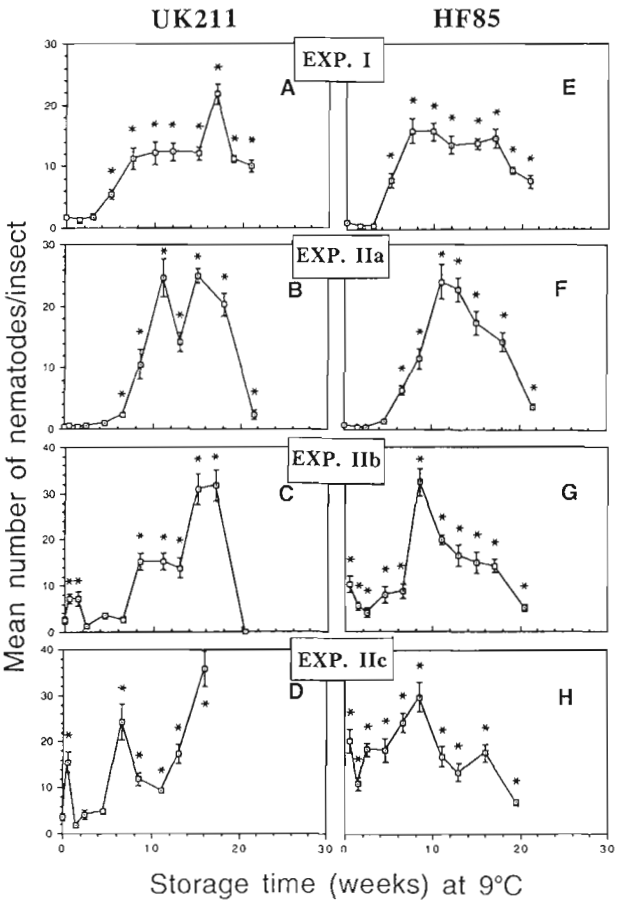

Fig. 1. *Infectivity (mean number of first generation nematodes established per insect, with standard error) of* Heterorhabditis *sp. DJs for* Galleria mellonella *larvae in sand at 9 °C following storage of the DJs in tapwater at 9 °C for various periods. (A-D : isolate UK211; E-H : isolate HF85. Culture batch I stored at 9 °C since harvest [A, E]; culture batch II stored at 9 °C since harvest [B, F] or following 1 week [C, G] or 2 weeks [D, H] pre-storage at 20 °C. An asterisk indicates that a value differs from that of time 0 [Mann Whitney U test, P ≤ 0.05]. For G and H, the comparison is with time 0 of F [i.e. the day the DJs were harvested].).*

week 8 to at least week 17 (Fig. 1 A, B, E, F). This same general pattern (which I shall call " delayed peak ") was shown by the three culture batches of UK211 DJs, each of which was tested in a separate experimental run (Figs 1 A, B; 2 A) and by the two culture batches of HF85 DJs (Fig. 1 E, F). Although the same general trends were recorded when the experiment was repeated with different culture batches, there were differences in the shape and height of the curves. For example, infectivity of UK211 DJs at 9 °C after 10-11 weeks cold storage was twice as high in Exp. II (25 nematodes/insect; Fig. 1 B) as in Exp. I, where it was twelve nematodes/insect (Fig. 1 A).

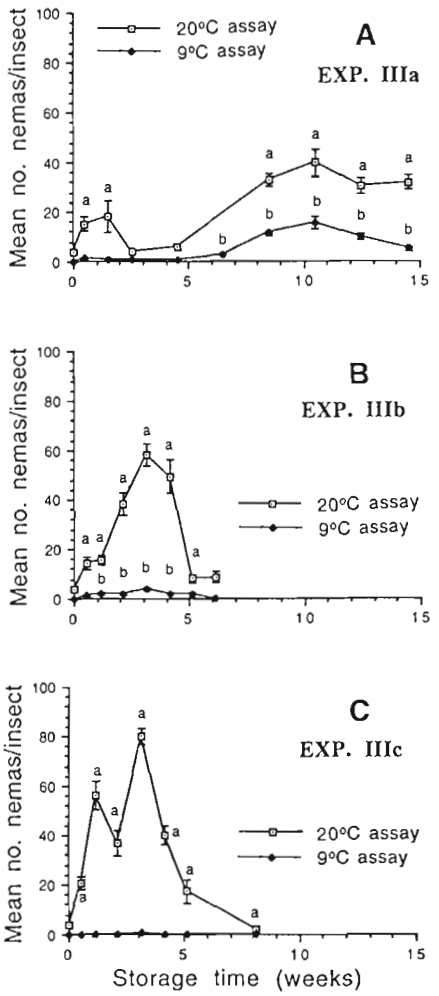

Fig. 2. *Infectivity (mean number of first generation nematodes established per insect, with standard error) of* Heterorhabditis *sp. UK211 DJs for* Galleria mellonella *larvae in sand at 9 °C and 20 °C following storage of the DJs in tapwater at 9 °C (A), 20 °C (B) or 25 °C (C) for various periods. (A point accompanied by a letter [*a *for 20 °C assay,* b *for 9 °C assay] differs from time 0 [Mann Whitney U test,* $P \leq 0.05$*]).*

In each experiment in which HF85 and UK211 DJs were cultured, stored, and tested in parallel, the two isolates displayed a similar pattern of infection at 9 °C (Exp. I : Fig. 1 A, E; Exp. II : Fig.1 B, F). Indeed, there is a more striking similarity between the two isolates within an experiment than there is between experiments for a given isolate. The most notable difference between isolates was seen in week 17 of Exp. I, when UK211 DJs performed exceptionally well, with 22 nematodes/insect recovered (Fig. 1 A), while HF85 DJs tested at the same time did not (Fig. 1 E).

When DJs stored at 9 °C were tested for infectivity at 20 °C (Exp. III only), there was evidence of two peaks, a minor one (maximum eighteen nematode/insect) within 2 weeks, and the main peak (maximum 40 nematodes/insect) after 10 weeks of cold-storage (Fig. 2 A) giving a " peak-trough-peak " pattern. In the trough between the two peaks, infectivity did not differ from that at week 0. The minor peak that occurred within 2 weeks was not seen in the 9 °C tests (when DJs stored at 9 °C were tested at 9 °C; Figs 1 A, B, E, F; 2 A). The later part of the curve for the 20 °C test mirrors very well that of the 9 °C test, with all values from week 8 onward differing ($P \leq 0.05$) from the value at week 0 (Fig. 2 A).

Infectivity of DJs stored at 20 and 25 °C for various periods following emergence from the host cadaver

Storage at 20 °C resulted in a progressive increase in the infectivity of UK211 DJs, reaching a peak (58 nematodes/insect in 20 °C tests, four nematodes/insect in 9 °C tests) after 3 weeks (Fig. 2 B). There was no evidence of a lag period before the increase in infectivity, such as was seen in 9 °C storage; after 4 days storage, infectivity was significantly (20 °C test, $P < 0.01$) or almost significantly (9 °C test, $P = 0.06$) higher than at day 0. This simple pattern I call " early peak ".

Comparison of Fig. 2 A, B shows that, in addition to differences in the timing of the peak, cold- and warm-storage were not otherwise equivalent in effect. Infectivity at 9 or 20 °C was higher in those DJs that had been stored at the assay temperature. For example, maximum cold-infectivity of cold-stored DJs was sixteen nematodes/insect (recorded after 10 weeks; Fig. 2 A) compared to a maximum cold infectivity of four nematodes/insect of DJs stored at 20 °C (at 3 weeks; Fig. 2 B).

DJs stored for up to 3 weeks at 25 °C performed even better at 20 °C than DJs stored at 20 °C for equivalent periods (Fig. 2 B, C); the difference was significant at 1 ($P < 0.001$) and 3 ($P < 0.01$) weeks. Peak infectivity of the 25 °C stored DJs was reached after 3 weeks, as for the 20 °C storage. DJs stored at 25 °C performed very poorly in the 9 °C test (Fig. 2 C); less than one (mean number) DJ established per insect on any test occasion.

Effect of cold-storage on the infectivity of DJs following pre-storage at 20 °C for 1-3 weeks

After DJs from isolates UK211 and HF85 had been stored for either 1 or 2 weeks at 20 °C, they were stored at 9 °C for 4 to 5 months, and assayed (also at 9 °C) at intervals during this time. The results (Fig. 1, C, D, G, H) can be compared with the performance of DJs of the same harvest which were stored at 9 °C from time of emergence from the host cadaver, i.e., without pre-storage at 20 °C (Fig. 1 B, F).

When UK211 DJs were tested immediately after harvest, an average of 0.3 nematodes/insect became

established in the 9 °C assay (Fig. 1 B). Prestorage at 20 °C for 1 or 2 weeks improved the cold infectivity of these DJs to 2.5 and 3.7 nematodes/insect, respectively (represented as 0-time of cold storage in Fig. 1 C and 1 D, respectively) even prior to cold storage. Cold-storage of these 20 °C-"matured" DJs at 9 °C for 3 days further improved their performance in the 9 °C assay, resulting in an "initial peak" in the plot of infectivity against time of cold storage (Fig. 1 C, D). With further cold storage for periods of less than 6 weeks, infectivity was lower and did not differ from that seen at time of transfer. Thereafter, infectivity again increased, but the 1-week and 2-week pre-stored UK211 DJs behaved differently. Infectivity of 1-week pre-stored DJs showed a plateau from week 8 to week 13, and rose again at weeks 15 to 17 (Fig. 1 C), while 2-week pre-stored nematodes showed a total of three infectivity peaks (after 3 days, 6 weeks and 16 weeks of cold storage), each of which being progressively higher (Fig. 1 D). The occurrence of a peak after 4 months cold-storage here (Fig. 1 C, D) recalls the finding of a similar peak in Fig. 1 A, derived from DJs of a different culture batch and tested at a different time.

Even after 16 weeks, no significant mortality of these stored DJs had occurred, although some of them were pale in appearance (having depleted their food reserves) and had exsheathed. The majority (not quantified) were still ensheathed.

DJs of HF85 which had been pre-stored for 1 or 2 weeks were not assayed before transfer to the cold, therefore it is not possible to describe a rise to an "initial peak". However, the combination of 1 or 2 weeks at 20 °C followed by 3 days at 9 °C dramatically increased cold-infectivity over that of freshly harvested worms, as can be seen by a comparison of the initial values of Fig. 1 F-H. HF85 DJs pre-stored for either 1 or 2 weeks at 20 °C showed peak infectivity after 8 weeks of cold-storage (32 and 29 nematodes/insect, respectively; Fig. 1 G, H). DJs from the same culture batch stored at 9 °C from day of harvest (i.e., not pre-stored), attained peak infectivity (of 24 nematodes/insect) after 11 weeks (Fig. 1 F). A later peak at 15-17 weeks, such as was seen in UK211 DJs (Fig. 1 C, D), is not seen in HF85 DJs, although there is a small rise at the appropriate time in the 2-week pre-stored treatment (Fig. 1 H) and an echo of the same in the 1-week pre-stored treatment (Fig. 1 G). Again, as for UK211, pre-stored HF85 DJs showed a higher base-line level of infectivity following transfer to 9 °C than those which had not been pre-stored.

UK211 DJs were pre-stored at 20 °C for 3 weeks before transfer to 9 °C, and the infectivity of the transferred DJs was assayed at both 9 and 20 °C. Infectivity of these DJs during pre-storage at 20 °C reached a maximum after 3 weeks (Fig. 2 B). The results (Fig. 3) can be compared with the results in Fig. 2 A for DJs from the same harvest stored at 9 °C throughout. Infectivity in the 20 °C assay followed a "U-shaped curve" (Fig. 3 B) similar to that described by Fan and Hominick (1991), while there was little change in infectivity in the 9 °C assay; the initial level was not significantly exceeded ($P \leq 0.05$) during the 13 weeks of cold-storage (Fig. 3 A).

Discussion

The experiments reported here show that the performance of *Heterorhabditis* DJs in bioassay is profoundly affected by prior storage conditions (temperature and time). In these experiments, infectivity is defined as the number of DJs that enter and establish in the test insect during a defined period; the DJs were not allowed unlimited time in which to enter. Thus, "infectivity" here is not an absolute measure of the "infective proportion" (Bohan & Hominick, 1994) of the population; rather, it reflects the proportion of the population that is capable of infecting within the specific constraints of the assay.

Probably the most significant of the findings are those relating to the impact of storage conditions on cold-infectivity. The use of entomopathogenic nematodes, especially of *Heterorhabditis* spp., is limited by low soil

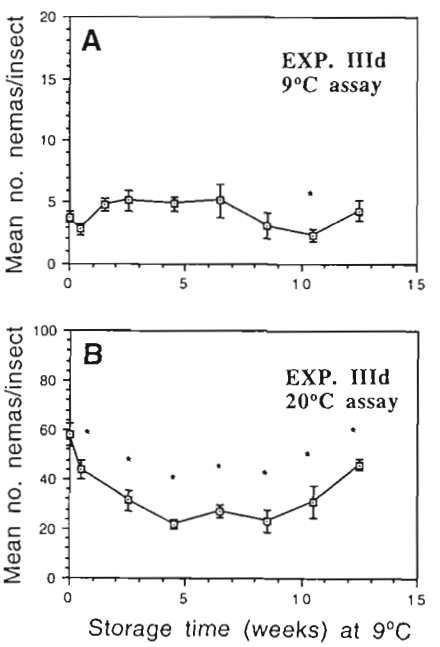

Fig. 3. *Infectivity (mean number of first generation nematodes established per insect, with standard error) of* Heterorhabditis *sp. UK211 DJs for* Galleria mellonella *larvae in sand at 9 °C (A) and 20 °C (B), following storage of the DJs in tapwater at 9 °C for various periods. (Week 0 : the time at which the DJs were transferred to 9 °C after 3 weeks prestorage at 20 °C. An asterisk indicates that a value differs from that of time 0 [Mann Whitney U test, P ≤ 0.05]).*

temperatures. Approaches to improving the performance of these nematodes at low temperatures have concentrated on genetic improvement, either by the isolation of cold-infective wild-types (e.g., Griffin & Downes, 1994 *a*) or by selective breeding for this trait (Griffin & Downes, 1994 *b*). The North-West European group of *Heterorhabditis* includes some of the most cold-active strains known (Griffin & Downes, 1994 *a*; Westerman & Van Zeeland, 1989). The experiments reported here demonstrate that the cold-infectivity of members of this group could be dramatically improved by appropriate storage conditions. They confirm the observation of Griffin (1993) that storage of freshly harvested DJs of *Heterorhabditis* UK211 at 9 °C for about 2 months results in a highly significant increase in their infectivity at 9 °C. As this was also found to be true for a geographically distinct isolate of the NWE Group, HF85 from mainland Europe, it may therefore be considered a more widespread characteristic of the group. There is also evidence for at least one of the isolates, UK211, that even more prolonged cold storage – approximately 4 months – leads to yet further improvement in cold-infectivity. The effect of cold-storage was not limited to DJs that were freshly harvested when placed in storage. Indeed, the highest cold-infectivity recorded in any of the experimental regimes was for DJs that had been pre-stored for 2 weeks at 20 °C, and then stored for 4 months at 9 °C. The value recorded was 36 nematodes/insect, i.e. 36 % of the applied DJs migrated through a 4 cm sand column, penetrated into, and established in, an insect larva at 9 °C; as only 0.3 % of unstored DJs from the same harvest established, this represents a 120 fold improvement in infectivity during storage. An improvement in cold-activity was also produced by pre-storing DJs at 20 °C and then storing them for a short period (3 days) at 9 °C; however, this did not produce an effect equal in magnitude to storage of either prestored or freshly harvested DJs for 2 months or longer at 9 °C. Progressive physiological adaptation to 9 °C may contribute to the improvement in cold infectivity during prolonged cold-storage. However, this is evidently not the only cause of the improvement, as evidenced by the fact that infectivity at 20 °C mirrors that seen at 9 °C, reaching a peak at the same time. Furthermore, storage of DJs for up to 3 weeks at 20 °C also results in an improvement in infectivity, and this is seen not just in assays at 20 °C but also in assays conducted at 9 °C, pointing to a general post-emergence improvement of infective capacity.

The simplest explanation for this improvement of infective capacity is that it represents a release of DJs from inhibition. Womersley (1993) proposed that DJs of entomopathogenic nematodes may be in diapause on leaving the cadaver, and that this diapause is terminated when they are subjected for specific periods to specific environmental stimuli- in the present case, various temperature/time combinations. An alternative hypothesis is that the progressive improvement in infective capacity represents a maturation process, which occurs at both high (20-25 °C) and low (9 °C) storage temperatures. However, at lower storage temperatures the improvement does not follow the same smooth course as at higher temperature. The rise may be either delayed (" delayed peak " pattern : Figs 1 A, B, E, F; 2 A) or interrupted, showing an initial small peak followed by a trough and subsequently a larger peak (" peak-trough-peak " pattern : Figs 1 C, D; 2 A). These patterns were seen in DJs which were either freshly harvested or had been stored at 20 °C for relatively short periods (1 or 2 weeks) prior to cold-storage, and in both delayed peak and peak-trough-peak, the cold-stored DJs eventually attain a higher level of infectivity during cold-storage than was shown before it. When more " mature " DJs (those which had been stored at 20 °C for 3 weeks) were transferred to the cold, there was a drop in infectivity which later returned to starting level (" trough " pattern, Fig. 3 B). Each of these three patterns is consistent with cold-storage inducing a state of temporary inhibition from which the DJs recover spontaneously, as was described for steinernematids (Fan & Hominick, 1991). According to this hypothesis, then, two processes are involved in generating changes in infectivity : maturation, which occurs over a range of temperatures, and a cold-induced inhibition of infectivity which is perhaps analogous to the U-shaped curve of infectivity shown by *Steinernema* spp. stored at 5 °C (Fan & Hominick, 1991). In immature (freshly-harvested) or maturing (pre-stored for 1-2 weeks at 20 °C) *Heterorhabditis* DJs, exposure to low temperatures interferes with the maturation process, delaying or interrupting it. When the DJs are already fully mature (e.g., following storage for 3 weeks at 20 °C) on transfer to 9 °C, infectivity returns following inhibition to that seen at the time of transfer.

Whatever the nature of the underlying mechanism, it is clear that the behaviour of DJs of the NWE Group of *Heterorhabditis* is labile. Indeed, it is becoming increasingly apparent that changes in infectivity other than the eventual decline may be an important feature of entomopathogenic nematode biology (Fan & Hominick, 1991; Curran, 1993; Griffin, 1993; Bohan & Hominick, 1994; Ishibashi *et al.*, 1995). The most consistent findings relate to DJs stored at a low temperature and assayed at a higher temperature; under such conditions, DJs either initially become less infective and subsequently regain high levels of infectivity after storage, i.e., display the U-shaped curve (Fan & Hominick, 1991), or show an initial peak of infectivity followed by a larger peak after some weeks. Interestingly, such a " peak-trough-peak " pattern which is seen here in NWE *Heterorhabditis* stored at 9 °C and tested at 20 °C (Fig. 2 A) is also apparent in the data shown for *S. feltiae* stored at 5 °C and tested at 15 °C (Curran, 1993) and in *S. carpocapsae* stored at 8 °C and tested at 25 °C (Ishibashi *et al.*, 1995). While this pattern is best documented

for low storage temperatures (5-9 °C), there is an indication of it in 15 °C-stored *Steinernema* sp (Nashes strain) when the data are presented as infectivity of surviving DJs (Fan & Hominick, 1991).

For heterorhabditids at least, an improvement of infectivity occurs during storage at 20-25 °C, as shown here for the NWE Group, and previously for *H. heliothidis* (Wojcik *et al.,* 1986) and for *H. bacteriophora* (Griffin *et al.,* 1994 *a*). For steinernematids, an improvement in infectivity of DJs stored at temperatures higher than 15 °C has not yet been reported.

Changes in the infectivity of populations of *S. feltiae* and of the closely related Nashes isolate involve individuals shifting between infective and non-infective states (Fan & Hominick, 1991; Bohan & Hominick, 1994). Thus, fewer *Steinernema* sp. DJs were infective during the trough period of the U-shaped curve, even with repeated exposure to insects (Fan & Hominick, 1991). On the other hand, although the infectivity of newly emerged NWE *Heterorhabditis* DJs at optimal temperatures (20 °C) was recorded as low when they were given a 16 h exposure in the present experiments, at least 60 % invaded a host when incubated with it for 3 days (unpublished data). I suggest that when low infectivity of populations of these nematodes is recorded, it is associated with a lower likelihood of each DJ infecting within the time of the assay, rather than an absolute inability to infect, and the increase in infectivity of NWE *Heterorhabditis* with storage represents an incremental improvement in infective capabilities – such as improved responsiveness to the host (Lei *et al.,* 1992) or an increase in general locomotor activity (Thomas & Ollevier, 1993) – rather than the switch from non-infective to infective states seen in the *S. feltiae* group. Selective mortality of less effective dauers can be ruled out as a factor in generating improved infectivity in my experiments, as treatments were discontinued when the survival of stored DJs dropped below about 80 %.

While the gradual increase in infectivity of DJs stored under constant conditions is suggestive of an endogenously timed process, we cannot rule out the possibility that this observed behaviour reflects a response of the nematodes to a progressive unplanned change in the storage medium (non-sterile, unbuffered, non-aerated tapwater). However, the fact that similar phenomena have been recorded for entomopathogenic nematodes stored in both sand (Fan & Hominick, 1991; Curran, 1993) and water (this work; Ishibashi *et al.,* 1995) suggest that they are not an artefact of the storage conditions. Whether the changes in infectivity in heterorhabditids and steinernematids are the same, the similarity of the timing suggests a common clock mechanism, possibly the same as that employed by other nematodes (Evans & Perry, 1976).

It is not surprising that the most clearcut results were those of experiments where the DJs were stored throughout at a single constant temperature and were also tested at the storage temperature. Transfer of organisms from one temperature to another introduces complexity associated with physiological adaptation and shock effects (Precht *et al.,* 1973). There are limits to the extent of change tolerated : while UK211 DJs were capable of infecting following a sudden drop in temperature from 20 to 9 °C, those transferred from 25 to 9 °C displayed very poor ability to infect by comparison.

Although the same general pattern of changes in infectivity during storage was observed in repeated runs of an experiment, there were some differences in the precise timing and levels of infectivity. Such between-batch variation in performance is a well-known feature of entomopathogenic nematode DJs, and may be attributed to physiological differences in the harvested DJs as a result of culture conditions (Gaugler & Georgis, 1991) or to differences in conditions during storage. Among factors that may have contributed to between-batch variation are the known differences between experiments, such as the nature of the container used for storage (plastic Petri dishes in Exp. I, glass vials in Exps II and III), and the time at which the DJs were harvested (18 days post infection in Exps I and II, 20 days in Exp. III). It is likely that a higher proportion of the DJs was produced by second generation females in the later-emerging DJs used in Exp. III. Furthermore, DJs used to initiate the cultures for the three experiments may themselves have differed in " quality ", with consequent unquantified effects on the within-cadaver culture conditions. There are obvious similarities between the two isolates in their response to storage conditions in each of the two experiments in which they were tested in parallel (Exps I and II), contrasting with the differences between batches for each isolate. Elucidation of the cause of between-batch variation should make it possible to further standardise the results of bioassays conducted on repeat occasions, and to predict more accurately the performance of DJs in bioassay. Because of the between batch variation, further repetition would be necessary to clarify to what extent the apparent differences between isolates (such as the continued improvement in infectivity of UK211 DJs, but not of HF85 DJs, from months 2 to 4 of cold storage) have a genetic basis.

I have documented reproducible changes in the infectivity of *Heterorhabditis* sp. DJs during storage, and suggested a conceptual framework for explaining these changes in terms of two phenomena, maturation and inhibition, complicated by the effects of thermal shock and acclimation. The proposed explanation is offered as a framework in which to investigate the underlying physiological and behavioural mechanisms, as well as the ecological significance, and is by no means complete; for example, it does not account for the three peaks in Fig. 1 D. The alternative approach to understanding the documented phenomena, as the termination of diapause by storage conditions (Womersley, 1993), cannot be discounted.

The phenomena described here are of great importance for bioassay, both for screening DJs of newly acquired strains for useful attributes, and for quality control of commercial product. In particular, assessments of " cold-infectivity " of species or strains should take into account the physiological lability of the DJs, including their capacity for thermal acclimation. Experiments are planned to test the field significance of the phenomena, particularly with a view to effecting an improvement in performance at low temperatures.

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