LOW TEMPERATURE ACTIVITY IN *HETERORHABDITIS* SP.  
(NEMATODA: HETERORHABDITIDAE)

BY

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Four isolates of *Heterorhabditis* sp. (H181, Hf85 and Hnhl 86 from the Netherlands and K122 from Ireland) were compared in laboratory bioassays. *Galleria mellonella* larvae were exposed to infective juveniles in sand for 2-5 days. There were significant differences between isolates in the number of infective juveniles that found and entered the larvae at 9° and 12°C. At 9°C, Hf85 was significantly better than all the other isolates. Success of Hf85 was considerably reduced at 7°C, and there was no parasitisation at 5°C. The infectivity for late instar *Tenebrio molitor* larvae was tested on filter paper at 5°, 7°, 9°, 12°, and 20°C. There was no mortality at 5°C. At 7°C, the LD50 of Hf85 was 23 IJs/insect; this was significantly lower than for the other three isolates. The LD50s of isolates differed significantly at 9° and 12° but not at 20°C. Differences between LT50s were significant at 20°C. The rank order of efficacy of the three Dutch isolates in the bioassays was the same as that established by other authors in pot trials at low temperature.

**Keywords:** *Heterorhabditis* spp., entomopathogenic nematodes, temperature, infectivity, bioassay, biological control.

Nematodes of the genera *Heterorhabditis* and *Steinernema* are obligate parasites of insects, mutualistically associated with entomopathogenic bacteria of the genus *Xenorhabdus* which play an important role in killing the host (Poinar & Thomas, 1966). *X. luminescens* is the symbiont of *Heterorhabditis* spp. The nematodes are capable of parasitising a wide range of insect species (Poinar, 1979) and are potentially useful for the biological control of many insect pests.

*Heterorhabditis* spp. are effective in controlling larvae of the black vine weevil (*Otiorhynchus sulcatus*) in glasshouses (Bedding & Miller, 1981; Simons, 1981; Dolmans, 1983; Georgis & Poinar, 1984). In the field, the larvae of *O. sulcatus* develop during winter and spring, and control measures may need to be applied at low soil temperatures. Outdoor trials with *Heterorhabditis* have given variable results; a soil temperature of 12-14°C is reported to be critical for control (Dolmans, 1983; Klingler, 1986; Simons & van der Schaaf, 1986; Rutherford et al., 1987; Scherer, 1987). The importance of low temperature in limiting the efficacy of the nematode-bacterial complex has also been shown under controlled conditions (Molyneux, 1986; Simons & van der Schaaf, 1986; Blackshaw & Newell, 1987).

A heterorhhabditid that gave effective control of vine weevil at low temperatures would be useful for outdoor application against the pest which
is otherwise difficult to control. European isolates of *Heterorhabditis* sp. have been tested against vine weevil in potted plants at controlled and in natural temperatures (Simons & van der Schaaf, 1986; Westerman & van Zeeland, 1989). A laboratory bioassay would help in screening more isolates to eliminate those nematodes that are least likely to perform well in pot trials, but the value of the bioassays in predicting control potential should be demonstrated.

Three Dutch isolates, Hf85, Hnh 86 and the commercially produced H181, have been compared at low temperatures in pot trials against vine weevil larvae (Westerman & van Zeeland, 1989). The results of laboratory bioassays using the same three isolates are presented here. A fourth nematode, Irish isolate K122 was included in the comparisons.

**MATERIALS AND METHODS**

Nematodes were cultured in larvae of the wax moth (*Galleria mellonella*). Infective juveniles (IJs) were stored in tapwater at 8°C and used between 2 and 8 weeks after harvesting. Three of the isolates originated in the Netherlands: H181 in Limburg, Hf85 in Friesland and Hnh1 86 in Noord Holland. K122 was isolated from soil in County Wexford, Ireland. The three Dutch isolates were obtained from Ir Paula Westerman, Friesland College of Agriculture.

The ability of infective juveniles to find and enter insects was tested in sand. Heat sterilised sand (particle size 0.2-0.4 mm) was mixed with 8% w/v tapwater. A cylindrical plastic container (diam. 36 mm, height 56 mm) with a last instar wax moth larva at the bottom was filled with the moist sand, capped and equilibrated overnight to the incubation temperature. A dose of 100, 500 or 1000 IJs in 100 µl tapwater was added to the sand surface and the containers returned to the incubator for 2-5 days. The insects were then removed from the sand, washed, patted dry with absorbent paper, and incubated on dry filter paper at 20°C. Mortality and the presence of the characteristic coloration (usually red) due to growth of the bacterial symbiont were assessed after 3 days. Dead insects were dissected and the number of first generation parasites counted. This was assumed to be the same as the number of IJs which had entered.

Infectivity of the various isolates for late instar *Tenebrio molitor* larvae was tested at 5°, 7°, 9°, 12° and 20°C. Exposures were performed in petri dishes (9 cm diam.) lined with three thicknesses of filter paper. IJs suspended in 3 ml tapwater were spread over the paper. Doses ranged from 1-1000 nematodes/insect and were prepared by serial twofold dilutions of a concentrated nematode suspension. Counts were made of nematodes in aliquots of the series to ensure that the levels obtained were as expected. For the lower doses (1-4 IJs/insect) suspensions were prepared by direct counts. Eight nematode doses were chosen for each temperature to give a suitable range of mortalities. Control dishes received 3 ml tapwater only. For each dose there