THE EFFECT OF SELECTIVE CULTURE OF *STEINERNEMA FELTIAE* AT LOW TEMPERATURE ON ESTABLISHMENT, PATHOGENICITY, REPRODUCTION AND SIZE OF INFECTIVE JUVENILES

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*Steinernema feltiae* was selectively cultured in *Galleria mellonella* as follows: 13 infective cycles at 22°C, near to the optimum temperature for development of *S. feltiae*, four infective cycles at 10°C close to the lowest temperature at which reproduction occurs, or four infective cycles at 10°C followed by one cycle at 22°C. After four cycles at 10°C the LT$_{50}$ (Galleria mortality) was shorter, the establishment higher and the size of infective juveniles longer and wider than those produced after 13 infective cycles at 22°C. After treatment at four cycles at 10°C followed by one cycle at 22°C the decrease in LT$_{50}$ observed after four cycles at 10°C was lost when tested at 7°C and 10°C and partially lost when tested at 12°C and 15°C. Reproduction was not affected by previous culture temperatures.

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Entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae are effective biological control agents against a variety of soil living pests. The infective juveniles (IJ$s$) are the free living stage found in the soil which seek out a potential insect host (Poinar, 1972) penetrating through natural openings or through the cuticle. Once inside the hemocoel they release symbiotic bacteria which start to multiply and kill the insect converting the insect tissue to a suitable environment for the development and reproduction of the nematode: when the nutrients are depleted, IJ$s$ of a new generation leave the cadaver to seek a new host.

Under field conditions, low temperatures restrict the efficacy of these nematodes (Dolmans, 1983): a minimum temperature of 10 to 15°C being required for nematode penetration, establishment and reproduction (Grewal *et al.*, 1994; Schirocki & Hague, 1994). Nematodes active at low temperatures have been found in surveys (Gwynn, 1993; Mrácek & Webster, 1993) but none of these isolates has so far proved to be very effective under field conditions. Another approach to obtain nematodes effective at low temperatures is by selection, particularly as entomopathogenic nematodes combine many attributes which favour successful
selection programmes, such as ease of mass production and rapid generation time (Gaugler, 1987). The temperature at which 50% of exposed Tenebrio molitor L. larvae were killed by a strain of Heterorhabditis decreased after selection for improved performance at low temperatures (Griffin & Downes, 1994) and nematode establishment, pathogenicity and reproduction can be extended by selective culture at their temperature limits for reproduction (Grewal et al., 1996b). In this paper we describe the responses of a UK isolate of Steinernema feltiae Filipjev, selected by continuous culture for several generations at low temperature, which is then cultured for one generation at its optimum temperature for establishment.

MATERIALS AND METHODS

The UK isolate of Steinernema feltiae used in these experiments was recovered from soil in Berkshire (Hominick, personal communication) and commercialised by AGC Ltd (Agricultural Genetics Company, Cambridge, UK) as NEMASYS. The isolate was cultured in the laboratory in late instar larvae of Galleria mellonella, the greater wax moth, at 22°C and nematodes were harvested from dead Galleria larvae using a modified White trap (White, 1927). The IJs were used for experimental purposes within one week of harvesting.

The nematodes were repeatedly cultured through last instar Galleria larvae at 10°C, which is the lower temperature limit for reproduction of S. feltiae (Gwynn, 1993) and at 22°C, the optimum temperature for invasion and establishment of S. feltiae (Otto, 1996). For each infective cycle 800 IJs were applied in 1 ml of distilled water to two layers of filter paper in a 9 cm Petri-dish. Eight late instar G. mellonella larvae were placed in each dish and the Petri-dishes were sealed. After 7 days at 22°C and 15 to 20 days at 10°C, cadavers were transferred to modified White traps and returned to their respective temperature. After emergence, IJs were used to re-infect Galleria larvae. IJs from the following three selection treatments were used for experiments:

1. thirteen infective cycles at 22°C;
2. four infective cycles at 10°C;
3. four infective cycles at 10°C followed by one further cycle at 22°C.

Measurement of nematodes

Single IJs were transferred to a droplet of water on a microscope slide. Nematodes were heat killed and a cover slip was placed over the droplet. The edges of the cover slip were sealed with nail varnish and the length and greatest width of 20 IJs was determined within 2 hours of extraction.