Survival of infective juveniles of *Heterorhabditis* spp., and *Steinernema* spp. (Nematoda : Rhabditida) at various temperatures and their subsequent infectivity for insects

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SUMMARY

In a comparison between two *Heterorhabditis* spp., (*Heterorhabditis* sp. D 1 and *H. heliothidis* T 327 strain) and two *Steinernema* spp. (*S. glaseri* KG strain and *S. feltiae* Agriotos strain), the steinernematids survived for longer periods of time than did the heterorhabditids in sand at various temperatures in the absence of insect hosts. Both heterorhabditids and *S. feltiae* showed an inverse relationship between survival time and temperature whereas *S. glaseri* survived for many months at each of the temperatures tested. Infective juveniles of *S. glaseri* became quiescent when insect hosts were not available; adopting a characteristic coiled-like posture. Exsheathment of infective juveniles had no noticeable effect on their survival in sand and nematode reproduction usually followed parasitization of the insect host by infective juveniles aged between one and 32 weeks. Nematode vigour in relation to age of infective juveniles was more accurately assessed by measuring their infectivity for insects rather than their motility through a Baermann sieve.

RÉSUMÉ

La survie des juvéniles infestants de Heterorhabditis *spp. et de* Steinernema *spp. (Nematoda : Rhabditida) en fonction de la température et leur pouvoir ultérieur de contamination à l'égard des insectes*

Une comparaison entre deux espèces de *Heterorhabditis* (*Heterorhabditis* sp. D 1 et *H. heliothidis* type T 327) et deux espèces de *Steinernema* (*S. glaseri*, type KG et *S. feltiae*, type Agriotos) a montré que les Steinernematides survivent pendant des périodes plus longues que les Heterorhabditides lorsqu'ils sont soumis à des températures variées en sol sableux et en l'absence d'insectes hôtes. Le temps de survie et la température varient en sens inverse tant pour les Heterorhabditides que pour *S. feltiae*, tandis que *S. glaseri* a survécu plusieurs mois à chacune des températures testées. Les juvéniles infestants de *S. glaseri* privés d'insectes hôtes entrent en quiescence, s'enroulant en posture caractéristique. Chez les juvéniles infestants la mue n'a aucun effet notable sur le pouvoir de survie dans un sol sableux et la reproduction des nématodes suit généralement la contamination de l'insecte hôte par des juvéniles infestants âgés d'une à trente-deux semaines. La vigueur des nématodes en fonction de l'âge des juvéniles infestants a pu être évaluée avec une meilleure précision en mesurant leur pouvoir de contamination des insectes plutôt que leur mobilité à travers le tamis d'un appareil de Baermann.

Entomopathogenic nematodes of the genera *Heterorhabditis* Poinar, 1975 and *Steinernema* Travassos, 1927 (syn. *Neoaplectana* Steiner, 1929; see Wouts *et al.*, 1982), along with their associated bacteria (*Xenorhabdus* spp.) are obligate pathogens of insects in nature (Poinar, 1979). Like most rhabditid nematodes, they have a free-living, non-feeding, infective-stage which is found usually in the soil. Within this environment, the infective juveniles locates the insect host (Bedding & Akhurst, 1975) and parasitizes via the insects' natural openings (Poinar, 1979); *Heterorhabditis* spp. may also enter via the intersegmental membranes (Bedding & Molyneux, 1983). On reaching the hosts' haemocoel the nematode releases its symbiotic bacteria which results in septicaemia and subsequent death of the insect. In addition, the bacteria produce wide spectrum antibiotics which inhibit the growth of other micro-organisms and thereby establish suitable conditions for nematode reproduction (Poinar, 1979; Akhurst, 1982). In due course, a new generation of infective juvenile nematodes is produced

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that leave the cadaver and are ready to parasitize another insect host. Once an infective juvenile has left the cadaver and entered the soil environment, changes in soil texture, moisture and temperature influence its behaviour and survival during this preparasitic stage of its life cycle.

Recently, Molyneux (1984) and Molyneux and Bedding (in press) described the effects of soil moisture and temperature on the infectivity of some of these nematodes for insects but little is known about their long-term survival in soil in the absence of insects. Most previous studies on the survival of infective juveniles have involv-

Jackson, 1973), with field studies involving mainly *Steinernema feltiae* Filipjev (syn. *Neoaplectana carpocapsae* Weiser) (Georgis & Hague, 1981; Saunders & All, 1982).

This paper provides information on the long-term survival of two *Heterorhabditis* spp., and two *Steinernema* spp. in sand at various temperatures and their subsequent infectivity for post-feeding, third instar larvae of the sheep blowfly, *Lucilia cuprina* (Wiedemann).

Materials and methods

Post-feeding, third instar larvae of *L. cuprina* reared on sheep liver at 23° and 76 % RH were used in all experiments. The nematodes were initially obtained from various sources (Table 1) and infective juvenile nematodes were obtained from *in vivo* cultures of the wax moth *Galleria mellonella* (L.) at 23° to ensure a uniform age of 4 ± 3 days.

Infective juveniles of *Heterorhabditis* sp. D 1, *H. heliothidis* strain T 327, *Steinernema glaseri* strain KG and *S. feltiae* Agriotos strain were kept in sand-filled, screwcap, specimen jars (4.2 cm diameter, 6 cm height) at 10°, 15°, 23° and 28° for one, two, four, eight, sixteen and 32 weeks. 1 000 infective juveniles were introduced in 1 ml of water into a centrally placed hole (0.5 cm diameter, 2 cm deep) which was then filled with sand for each nematode species/strain. The moisture content of the sand was kept at approximately 7 % (0.03 bars) by enclosing the sand-filled specimen jars in heavy duty plastic bags with water saturated tissue paper. There were 60 replications for each nematode/temperature/time combination.

At the required intervals, 56 replicates of each nematode/temperature combination were placed at 23° and left for 16 h to allow equilibration at that temperature. A single *L. cuprina* larva was then placed on the sand surface of each of 50 jars, the lids secured and the jars left at 23°. Final instar *G. mellonella* larvae were placed individually into the other six jars and left at 23° in order to test for nematode reproduction. *G. mellonella*

to reproduce in *L. cuprina* larvae (Molyneux, Bedding & Akhurst, 1983). After ten days, the jars were emptied and the sand sieved in water. The *L. cuprina* larvae and/or puparia and *G. mellonella* larvae were dissected in insect Ringers' solution and examined microscopically for nematode parasitization. If there was no parasitization of *L. cuprina* larvae then *G. mellonella* larvae were used to measure nematode parasitization at the later sampling times.

Concurrently, nematode survival in sand was measured by emptying the sand from the remaining four jars at each of the treatments onto modified Baermann sieves (Whitehead & Hemming, 1965) at 23°. Thus, there were four replicates for each nematode/temperature/time combination. At 24 h intervals the water plus the nematodes recovered from the sand were poured into 250 ml beakers and the nematodes counted after decanting the excess water. This procedure was repeated until nematodes ceased to appear.

Results

In the absence of their insect hosts and at all temperatures tested *S. glaseri* (Fig. 1 c) survived for longer periods of time than *S. feltiae* (Fig. 1 d) and both *Heterorhabditis* species (Fig. 1 a and 1 b). *S. feltiae* resembled *H. heliothidis* in ability to survive and *Heterorhabditis* sp. D 1 appeared to be the least well adapted of the four species for survival. Within two weeks at 23° and 28°, infective juveniles of both heterorhabditids and *S. feltiae* appeared transparent and were lethargic. Similarly, in-

Table 1

Sources of nematodes

Nematode	*Strain*	*Source*
Heterorhabditis sp.	Dl	Soil*, Darwin, Northern Territory, Australia.
Heterorhabditis heliothidis	T 327	Soil*, Dysart, Tasmania, Australia.
Steinernema glaseri	KG	H.K. Kaya, University of California, Davis, CA, U.S.A.
Steinernema feltiae	Agriotos	G.O. Poinar, University of California, Berkeley, CA, U.S.A.

* Isolated by the method of Bedding and Akhurst (1975).

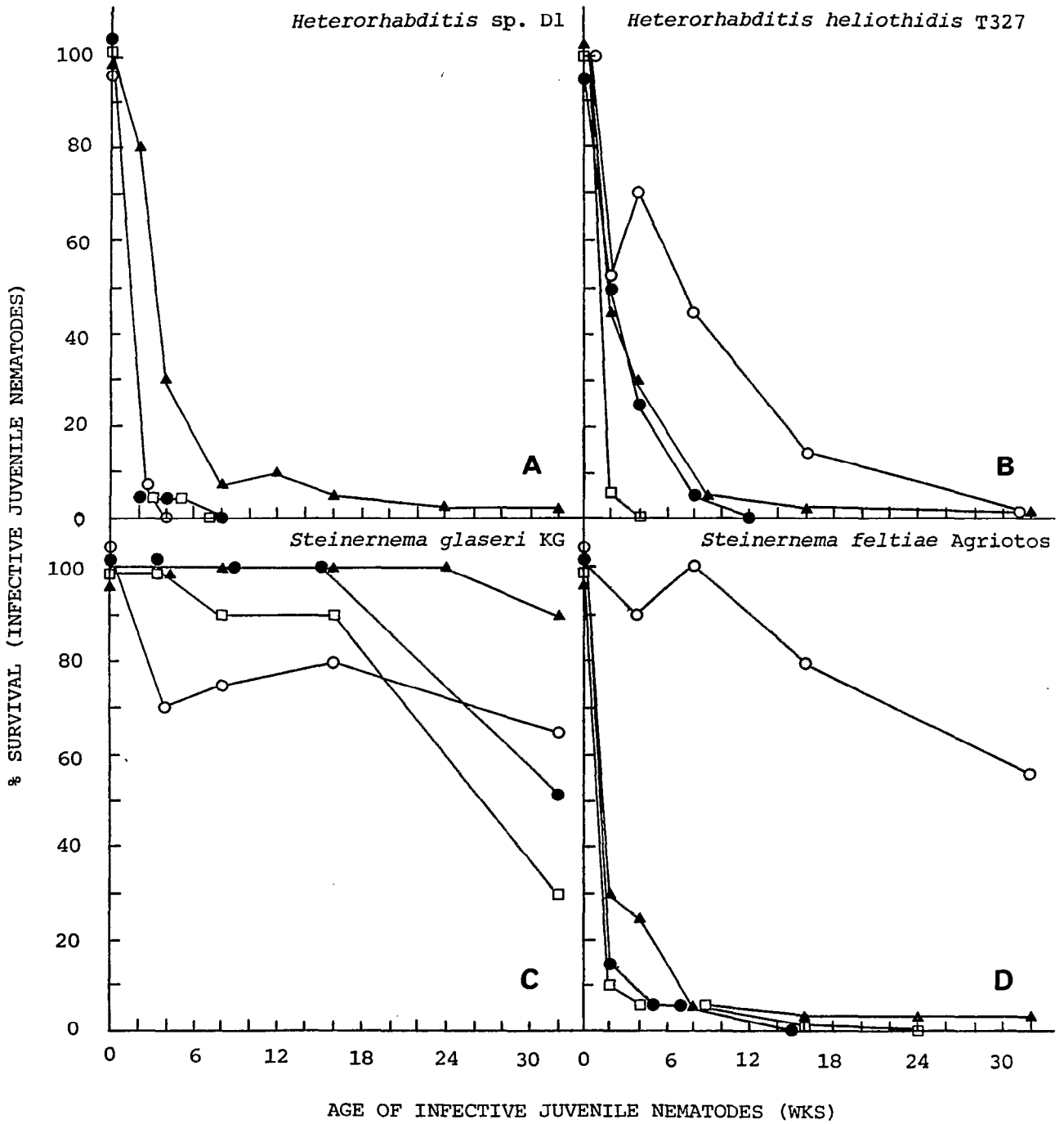

Fig. 1 : Survival of infective juveniles of *Heterorhabditis* spp., and *Steinernema* spp. after storage in sand (moisture content 7 % = 0.03 bars) at 10° (white circles), 15° (black triangles), 23° (white squares) and 28° (black circles) for one to 32 weeks.

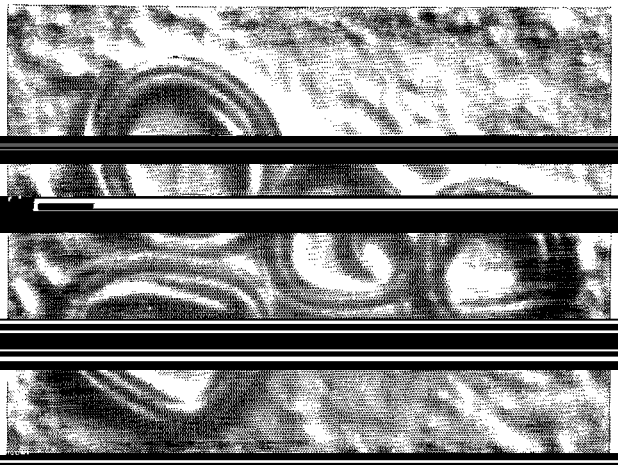

Fig. 2 : Infective juveniles of *Steinernema glaseri* strain KG in an immobile coiled position (× 70).

only after eight weeks at 28°. Unlike the heterorhabditids and *S. feltiae*, infective juveniles of *S. glaseri* were observed frequently in an immobile, coiled position when washed from the sides of sand-filled specimen jars (Fig. 2).

Percent parasitization (infectivity) by nematodes (Fig. 3) was correlated closely with nematode survival (Fig. 1). Although *Heterorhabditis* sp. D 1 and *H. heliothidis* infective juveniles failed to parasitize *L. cuprina* larvae after two weeks at 28° and 23°, *G. mellonella* larvae were parasitized by infective juveniles that had been kept in sand for sixteen weeks. After eight weeks at 15°, *H. heliothidis* did not parasitize *L. cuprina* larvae but continued to parasitize *G. mellonella* larvae after infective juveniles had been in sand for 32 weeks. Similarly, *L. cuprina* larvae were not parasitized by *S. feltiae* aged four weeks at 23° and 28° but continued to parasitize *G. mellonella* at sixteen and eight weeks of age respectively. In contrast, *S. glaseri* parasitized *L. cuprina* larvae throughout the 32 weeks at each temperature tested.

Nematode reproduction occurred generally in all *G. mellonella* larvae parasitized by infective juveniles aged between one and 32 weeks at each temperature. The one exception was when *G. mellonella* larvae were parasitized by infective juveniles of *heterorhabditis* sp. D 1 that had been in sand for one week at 10°. After the cadavers had been left for two weeks at 23°, they were found to contain dead first generation adult nematodes.

Nematode " vigour " was also measured in terms of the time taken for infective juveniles to move through a Bearmann sieve. Although there was no difference in the motility of *S. glaseri* throughout 32 weeks at each temperature tested, the motility of both heterorhabditids and *S. feltiae* declined with time; often three to five days being required to recover " active " *S. feltiae* infective juveniles.

Discussion

many phytoparasitic and zooparasitic nematodes must rely on their stored food reserves for the energy required to locate and infect their definitive hosts. Temperature is a major factor influencing nematode motility and the survival of the infective juvenile nematode.

Wallace (1966) stated that once a phytoparasitic nematode enters the soil its sources of energy are used at temperatures greater than 15° is likely to be related to their degree of motility, consumption of food reserves and an increase in respiration rates (Burman & Pye, 1980). The long-term survival of *Steinernema bibionis* (Molyneux, unpubl.) and some phytoparasitic nematodes (Croll, 1970), is probably responsible for its long-term survival. The increased longevity of the heterorhabditids and *S. feltiae* at lower temperatures is most likely due to their decreased motility although Danilov (1978) reported that *S. feltiae* became quiescent after two to four days in the field.

The difference in survival of these nematode species/strains at various temperatures may reflect, in part, their original climatic habitats. The warm and humid tropical origins of *Heterorhabditis* sp. D 1 probably account for its very short longevity at 10° whereas the cool and warm temperate climates from which *H. heliothidis* strain T 327 and *S. glaseri* strain KG were isolated and strain, favour their survival at low temperatures. These rapid declines in survival of *Heterorhabditis* spp. are not peculiar to sandy substrates. Similar mortalities were observed after infective juveniles had been kept in peat, vermiculite, loam and heat sterilized sand at similar moisture potentials. Furthermore, within a pH range of 4.5-6.5 (a pH range normally encountered in soils under agricultural or pastoral conditions), their survival did not appear to be affected by pH (Molyneux, unpubl.).

The Baermann sieve has been used previously to measure nematode motility (Whitehead & Hemming, 1965; Saunders & All, 1982) but Thomason, Van Gundy and Kirkpatrick (1964) hypothesised that infectivity (parasitization) is a more specific property than motility for measuring nematode " vigour ". Because *L. cuprina* larvae are invulnerable to parasitization after pupariation, the infective juvenile has only between four to five days to locate and enter the host at 23° (Molyneux, 1984). The [illegible] infectivity [illegible]

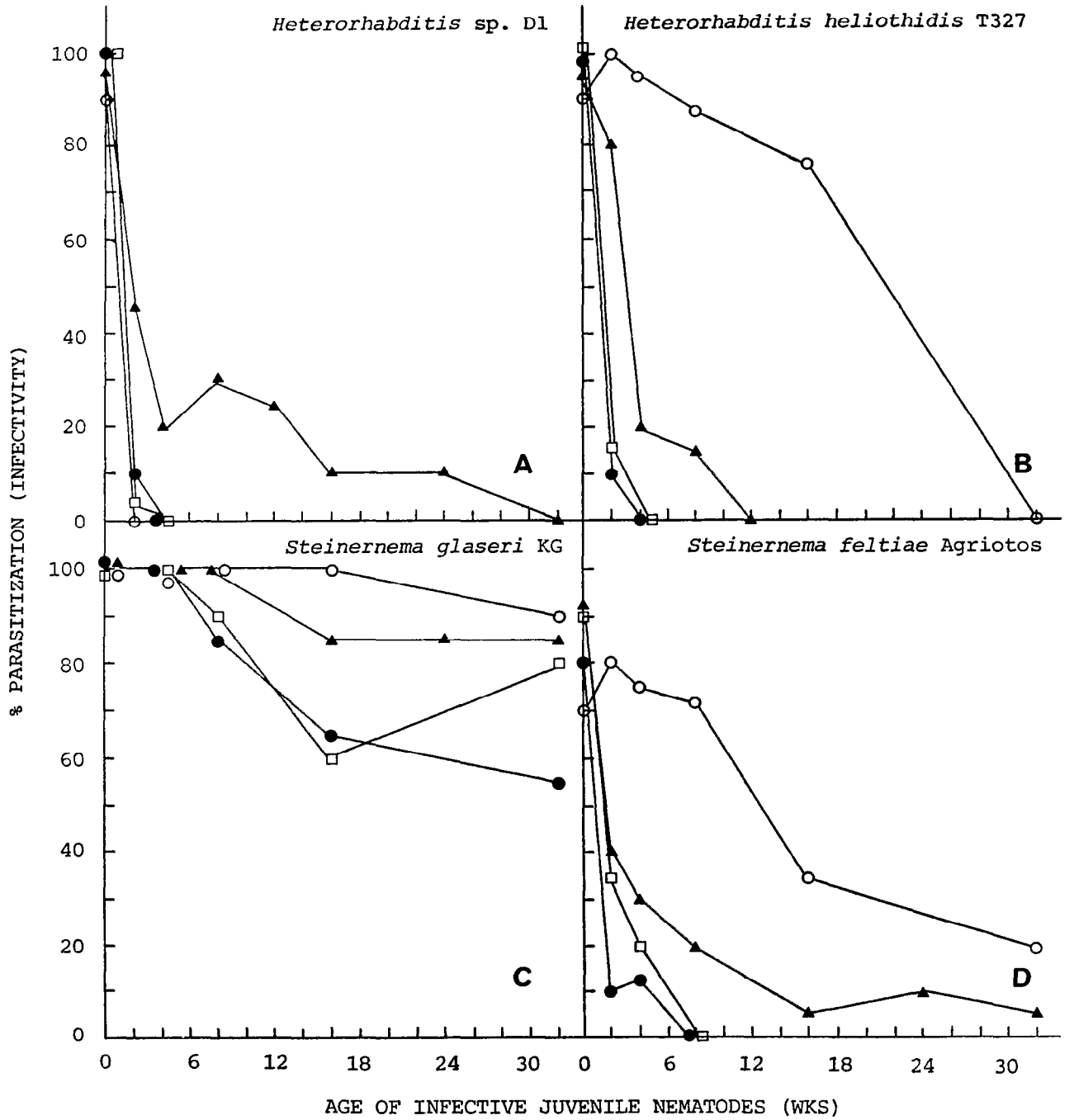

Fig. 3 : Parasitization of post-feeding, third instar larvae of *Lucilia cuprina* at 23 °C by infective juveniles of *Heterorhabditis* spp. and *Steinernema* spp. after storage of inefective juveniles in sans (moisture content 7 % = 0.03 bars) at 10° (white circles), 15° (black triangles), 23° (white squares) and 28° (black circles) for one to 32 weeks.

post-feeding, *L. cuprina* larvae rather than their motility *per se* is a more accurate means of assessing nematode " vigour " in relation to age.

Some infective juvenile nematodes survive appreciably longer when they remain ensheathed (Ellenby, 1969). However, the majority of *S. glaseri* readily exsheathed without any noticeable effect on their survival and infectivity. Similarly, dead ensheathed as well as exsheathed nematodes were recovered in the other species examined.

For permanent establishment of these nematodes in nematode parasitization and reproduction. In contrast, the steinernematids seem better able to survive extended periods of adverse climatic conditions and a shortage of insect hosts, with specific survival mechanisms being employed by some species.

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