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Storage temperature and duration affect *Steinernema scarabaei* dispersal and attraction, virulence, and infectivity to a white grub host

Albrecht M. Koppenhöfer *, Lemma Ebssa, Eugene M. Fuzy

Department of Entomology, Rutgers University, New Brunswick, NJ 08901-8524, United States

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ABSTRACT

The entomopathogenic nematode *Steinernema scarabaei* has exceptional potential for the control of many white grub species. Initial studies suggested this species to have a widely ranging foraging strategy based on its attraction to hosts in soil columns, however, with a generally low but very variable dispersal rate even in the presence of hosts. The objective of this study was to develop a better understanding of the dispersal behavior of *S. scarabaei* IJs and the influence of storage conditions on its dispersal and infectivity. We found that storage temperature and duration had a strong effect on *S. scarabaei* IJ dispersal, virulence, and infectivity. But even under conditions conducive to movement only a small proportion of IJs moved towards a host. IJ dispersal declined with storage time by a factor of around 100 between 1 week and 12 weeks of storage whether the IJs were stored at room temperature or 8 °C; however, the decline was more than twice as fast after storage at 8 °C. Host attraction also diminished with storage duration. IJ virulence and infectivity declined with storage time for IJs stored at room temperature. In contrast, for IJs stored at 8 °C virulence remained high and infectivity increased over time. The decrease in dispersal and infectivity when stored at room temperature may reflect an adaptation to conserve energy in the absence of hosts since *S. scarabaei* IJs have to persist through extended periods in summer during which infections are unlikely to occur. The even faster decrease in dispersal rate when stored at 8 °C may suggest a cold-induced dormancy that may serve as an overwintering strategy. The parallel increase in infectivity, however, seems to contradict such a strategy. Future studies should examine whether and how *S. scarabaei* IJs 'inactivated' by the absence of hosts can be 'reactivated' and whether this behavior could be used to improve the efficacy of nematode applications against insect pests.

1. Introduction

Entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) are lethal parasites of soil-dwelling insects and are used for the biological control of insect pests in several ornamental and crop production systems (Grewal et al., 2005). The infective juvenile (IJ) is the only free-living, non-parasitic stage of these nematodes. The IJ's purpose is to locate and infect a suitable host. The success of nematode applications for insect control in soil and the survival of naturally occurring nematode populations depends on the IJ's ability to disperse and persist until it can locate a host. Numerous intrinsic factors (e.g., behavioral, physiological, and genetic characteristics) and extrinsic factors [e.g., temperatures, soil moisture, soil texture, UV radiation (Kaya, 1990; Smits, 1996)] have been shown to affect IJ dispersal and persistence.

The entomopathogenic nematode *Steinernema scarabaei*, isolated from epizootics in oriental beetle, *Anomala* (=*Exomala*) *orientalis*, and Japanese beetle, *Popillia japonica*, larvae in turfgrass areas in central New Jersey, USA (Stock and Koppenhöfer, 2003), has exceptional potential for the control of many species of white grubs, root-feeding larvae of scarab beetles (Coleoptera: Scarabaeidae). Its high virulence (e.g., Koppenhöfer et al., 2006) combined with longevity of the IJ stage under a wide range of soil conditions (Koppenhöfer and Fuzy, 2006, 2007) and its ability to effectively reproduce in white grubs (Koppenhöfer and Fuzy, 2003) enable *S. scarabaei* to suppress white grub populations and persist in the field for several years after application (Koppenhöfer and Fuzy, 2009).

Initial studies on *S. scarabaei*'s biology and ecology suggested this species to have a widely ranging foraging strategy ('cruiser' type) (Lewis et al., 2006) based on the IJs' ability to infect caged third-instar *A. orientalis* better at 2, 5, and 10 cm depth than at the surface of soil (sandy loam) columns within 4 days of inoculation onto the soil surface (Koppenhöfer and Fuzy, 2003). In support of the cruiser type foraging strategy, Koppenhöfer and Fuzy (2008) found 2–18-times higher numbers of *S. scarabaei* travelling to the bottom 1-cm section of 5.5-cm sand columns if the bottom

* Corresponding author. Address: Department of Entomology, Blake Hall, 93 Lipman Dr., Rutgers University, New Brunswick, NJ 08901-8524, United States. Fax: +1 (732) 932 7229.
E-mail address: koppenhofer@aesop.rutgers.edu (A.M. Koppenhöfer).

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contained a larva of several white grub species or of the greater wax moth, *Galleria mellonella* L., than if no larva was present. However, in most experiments even in the presence of a larva only around 5% of *S. scarabaei* were recovered from the bottom sections of the columns, a proportion generally several times lower than for other known cruiser-type nematode species tested [on average: *Heterorhabditis bacteriophora* (GPS11 and NC1 strains), 16%; *Heterorhabditis zealandica* (X1 strain), 42%; *Steinernema glaseri* (NC strain), 66%] (Koppenhöfer and Fuzy, 2008). In contrast, the infectivity (percentage of inoculum infecting) in laboratory experiments (30- to 80-ml containers with soil) to white grubs of *S. scarabaei* (averaged across four experiments: 14%) was at least as high to several times higher than that of the other above species, with some variation among white grub species (on average: *H. zealandica*, 7%; *H. bacteriophora*, 5%; *S. glaseri*, 3%) (Koppenhöfer and Fuzy, 2006, 2007; Koppenhöfer et al., 2007).

The high infectivity of *S. scarabaei* in laboratory trials despite its low dispersal rate can at least in part be explained by the fact that IJs do not have to move far in laboratory trials to reach a host. However, this is to a decreasing extent true in turfgrass greenhouse pot trials and field trials in which *S. scarabaei* has shown excellent efficacy (e.g., Cappaert and Koppenhöfer, 2003; Koppenhöfer and Fuzy, 2009). The greater size of arenas in those trials, even when considering the higher number of hosts per arena and that the white grubs typically are concentrated in the top 5 cm of soil, results in around 5–10 × lower host densities per soil volume. In addition, *S. scarabaei* has also performed very well against *A. orientalis* larvae in highbush blueberries, *Vaccinium corymbosum* L., in greenhouse trials and field trials where the larvae were found down to 30 cm depth (Polavarapu et al., 2007). Also, both dispersal rate in absence of hosts and especially attraction to hosts in sand or soil column experiments has been highly variable with over 20% of the *S. scarabaei* IJs dispersed to hosts at 5–10 cm depth in some experiments (Koppenhöfer and Fuzy, 2003, 2008).

The objective of this study was to develop a better understanding of the dispersal behavior of *S. scarabaei* IJs, knowledge crucial for optimizing its use in white grub management. To this end we examined IJ dispersal on a finer scale than in previous studies and studied the effect of storage conditions (temperature and length) and host exposure period on dispersal. For factors that had an effect on dispersal, any effects on IJ virulence and infectivity were also examined.

2. Materials and methods

2.1. Soils, insects, nematodes

Third-instar *A. orientalis* were used in all experiments as host. They had been collected in turf areas at the Rutgers University Plant Sciences Research Farm (Adelphia, NJ) and kept individually in the wells of 24-well tissue culture plates at 8 °C for 2–14 weeks in a mixture of organic compost and loamy sand. *S. scarabaei* (AMK001 strain) was maintained and cultured in *A. orientalis* and *P. japonica* larvae because its production in wax moth larvae is unreliable (Koppenhöfer, unpublished data). The emerging infective juveniles (IJs) were harvested from White traps and stored in tap water at room temperature (21–24 °C) or 8 °C (Kaya and Stock, 1997) for 1–12 weeks before use, depending on experiment (see below). IJs stored at 8 °C were acclimatized at room temperature for 6 h before inoculation. In experiments where IJ storage duration was a factor, all IJ batches were produced from the same original stock batch to avoid any genetic differences among the different storage duration batches. Since *S. scarabaei* IJs can be stored for very long periods (>1 year) at 8 °C without apparent loss in viability and virulence (Koppenhöfer, unpublished data) it was assumed that IJ batches originating from the same stock batch (at least 3 weeks old) and reared no more than 11 weeks apart (see below) would be very similar in quality.

In most experiments a fine sand was used as substrate that had been washed and air-dried before use. The sand was prepared at 8% (w/w) moisture (−5 kPa soil water potential). In two experiments a sandy loam (61% sand, 27% silt, 12% clay, 2.3% organic matter, pH 6.0) was used that had been pasteurized (3 h at 70 °C) and air-dried before use. After addition of the treatments, the sandy loam's final moisture content was 13% (w/w) (−10 kPa soil water potential).

2.2. Measurement of IJ migration in vertical sand columns

To determine the effect of various factors on *S. scarabaei* movement, we quantified IJ migration through vertical sand columns (Westermann, 1995; Koppenhöfer and Fuzy, 2003, 2008) at room temperature (21–24 °C). The columns consisted of polyvinyl pipe (40 mm inner diameter; 12.6 cm^2 cross section) sections held together with adhesive tape. The columns were filled from the bottom to 0.5 cm below the top with sand and closed on both ends with bottoms of 60 mm diameter Petri dishes. In Experiments 1, 2, and 4 the columns consisted of an upper (5 cm length) and lower (1 cm length) section of polyvinyl pipe; in Experiments 5–6 columns consisted of five 1-cm sections. To minimize moisture loss the columns were also kept in sweater boxes containing moist paper towel.

The bottom 1-cm section of the columns contained one third-instar *A. orientalis* restrained in a cage made of aluminum mesh (1 mm openings) stapled carefully around the larva. The cages were just large enough to allow limited movement of the larva such as turning and grooming. Larvae were allowed to warm up at room temperature for 24 h before they were placed in the cage and some sand was rubbed into the cage through the mesh. The caged larva was then placed in the bottom section and the columns filled with moist substrate. After 24 h (except for Experiment 1), to allow for a potential gradient of nematode attractants to build up, 500 IJs in 1 ml water were inoculated onto the substrate surface. Controls contained a cage filled with sand only. The arenas were incubated for different times depending on experiments (see below).

To evaluate IJ migration the IJs were extracted from the sand using a decant-and-sieve method. The sand from each segment was rinsed into a separate Petri dish. The larvae were also rinsed and the rinsate kept with the sand of the lower segment. The contents of each Petri dish were extracted separately. The Petri dish content was rinsed into a 1-L beaker, tap water added to about 750 ml, and the beaker contents vigorously stirred to suspend the sand. After allowing the sand to settle for 30 s, the water was decanted through a fine sieve (No. 635, 20 μm openings) to catch the IJs. The contents of the fine sieve were washed into a Petri dish. The whole process was repeated once. Then the number of IJs in the dish was counted under a dissecting microscope. The larvae were incubated for another 4 days in soil in the wells of 24-well tissue culture plates with germinated perennial ryegrass, *Lolium perenne* L., seeds provided as food. The plates were evaluated daily and larvae that died during the incubation period were dissected 2 days after death (allowing for development of penetrated nematodes to adults to facilitate counting) and digested in a 0.5% pepsin solution for 2 h at 37 °C (Mauléon et al., 1993) to count the nematodes established in them. Throughout the experiments, *S. scarabaei* extraction efficiency was very consistent (71–80%). Nematodes found in the larvae were added to the nematode count of the bottom section(s) of the column.

2.3. Measurement of nematode virulence and infectivity

Assays to determine the effect of various variables on *S. scarabaei* virulence and infectivity were conducted at room temperature