Short Communication

Directional movement of steinernematid nematodes in response to electrical current

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A B S T R A C T

Steinernematid nematodes are parasites that are important natural regulating agents of insect populations. The infective juvenile nematodes respond to a variety of stimuli that aid in survival and host finding. Host finding strategies among steinernematids differ along a continuum from ambush (sit & wait) to cruiser (search & destroy). In this paper we describe directional movement in response to an electrical current, which was generated on agar plates. Specifically, Steinernema glaseri (a cruiser) moved to a higher electric potential, whereas Steinernema carpocapsae, an ambusher, moved to a lower electric potential. Thus, we hypothesize that steinernematids may detect electrical currents or electromagnetic fields in nature, and these stimuli may be used differentially among species for host finding or enhancing other fitness characters.

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1. Introduction

Nematodes in the genus Steinernema are parasites of insects. These entomopathogenic nematodes can act as important natural regulators of insect populations and are also commonly applied as biological control agents of pest populations (Grewal et al., 2005). The developmentally arrested infective juvenile nematodes (IJs), the only free-living stage, typically occupy soil habitats until they infect an insect, which they must do in order to resume development and reproduce. Once the nematodes enter the host (generally through natural openings) the nematodes release symbiotic bacteria (Xenorhabdus spp.), which are the primary agents in killing the host (Kaya and Gaugler, 1993). The infected hosts die within 24–72 h, and the nematodes feed on the symbiotic bacteria and insect tissues, reproducing for one to three generations (Kaya and Gaugler, 1993). As food resources dwindle, IJs are produced that emerge and search out new hosts.

To persist in the soil environment and successfully find and recognize suitable hosts, steinernematids respond to various cues and display an array of host-seeking strategies. In terms of search strategies, IJs either sit-and-wait (ambushers), actively search (cruisers), or exhibit a combination of these behaviors to locate hosts (Campbell and Kaya, 1999; Lewis, 2002). Additionally, IJs respond to a variety of stimuli such as CO₂ (Lewis et al., 1993; Lewis, 2002), vibration (Torr et al., 2004), temperature (Burman and Pye, 1980; Byers and Poinar, 1982) and chemical compounds (Pye and Burman, 1981; Shapiro et al., 2000), presumably to increase their chances of finding a host. Response to stimuli may also be useful in other aspects of fitness such as sensing and avoiding adverse environmental conditions, e.g., temperature or moisture extremes (Ishibashi and Kondo, 1990).

Certain plant parasitic, mammal parasitic, and free-living nematodes have been observed to respond electrical fields (Sukul et al., 1975; Croll and Matthews, 1977; Viglierchio and Yu, 1983; Riga, 2004). In the case of plant parasitic nematodes, it has been suggested that such responses may assist in host finding (Bird, 1959; Riga, 2004). Directional response to electric fields has not been previously investigated in insect parasitic nematodes such as steinernematids. Conceivably, a directional response to electric fields in insect parasitic nematodes could play a role in the organisms’ foraging behavior. Our objective was to determine directional response of an ambusher-type steinernematid, Steinernema carpocapsae (Weiser) and a cruiser-type Steinernema glaseri (Steiner) in the presence of electric fields.

2. Materials and methods

Experiments designed to test directional movement of nematodes were adapted from previous studies testing response to other cues (e.g., Ramos-Rodriguez et al., 2007). The nematodes, S. carpocapsae (All strain) and S. glaseri (NJ43 strain), were cultured...
in larvae of the greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) according to *Kaya and Stock* (1997). After harvest, IJs were stored in 13 °C for less than 2 weeks before being used in experiments. Experiments were conducted in plastic Petri dishes (90 mm diam.) with 2% agar at approximately 0.5 mm depth. Sodium Chloride (0.01%) was incorporated into the agar to facilitate conductance of electricity (*Sukul et al.*, 1975); the low salt concentration we used was not adverse to steinernematids (*Thorston et al.*, 1994). An electric field was generated across the agar plate using an electrophoresis power supply (model EC 105, E-C Apparatus Corporation, Holbrook, NY). An electrode from the cathode (negative on the power supply) with copper wire (0.5 mm diam.) was inserted vertically on one side of dish (approximately 1 mm from edge), and the other electrode from the anode was placed on the opposite side.

Two sets of experiments were conducted. In the first set of experiments, approximately 2000 *S. carpocapsae* IJs or 1000 *S. glaseri* IJs were concentrated by vacuum and placed in the middle of the agar dish. A higher number of *S. carpocapsae* was used relative to *S. glaseri* because the latter are known to be naturally more dispersive on various media (*Lewis*, 2002). The power supply was set at 36 V (3.0 mA) and current was applied for 60 min, at which time the number of IJs within a 2.5 cm semicircle around the cathode or anode was counted. Controls consisted of plates treated identically but with no current.

The protocol for the second set of experiments was the same as the first except that the dispersal time was shortened. Three treatments were included: (1) nematodes placed on agar plates with an electric current (from voltage of 36 V) running concurrently with nematode migration and the number of IJs at the cathode or anode determined after 20 min, (2) electrical current (from voltage of 36 V) applied to the agar for 20 min and then removed (electrodes removed from the agar); nematodes were then placed on the agar and movement to the opposite sides determined after an additional 20 min without current, (3) electrical current (from voltage of 36 V) applied to the agar for 40 min and then removed; nematodes were then placed on the agar and movement to the opposite sides determined after an additional 20 min without current. Given that the dispersal time was shorter than the first set of experiments, the number of *S. carpocapsae* IJs used was increased to 4000 to allow sufficient numbers to move during the assay interval (the number of *S. glaseri* remained the same).

For each treatment and control, preferential movement of nematodes to one side of the plate was determined through paired *t*-tests comparing the number of IJs at the anode side versus cathode side (*SAS, 2001, \( \alpha = 0.05 \)). Additionally, in each experiment the percentages of nematodes that moved to the cathode side (relative to the total that moved to either side) were calculated for each treatment and control and compared through analysis of variance (ANOVA, \( \alpha = 0.05 \)); when three treatments were evaluated (i.e., the 20 min assays) the Student–Newman–Keuls’-test was used to further elucidate treatment effects when a significant *F* value was detected in the ANOVA (*SAS, 2001*). Percentage data were arc sine transformed prior to analysis (*Steel and Torrie, 1980*). Each treatment or control was replicated in time a minimum of five times (i.e., 5–16 replicate plates per treatment).

### 3. Results

The steinernematids exhibited directional movement in an electrical field when migration was determined 60 min after the current was initiated. Specifically, a higher number of *S. carpocapsae* moved to the cathode relative to the anode (*t* = 4.04, *df* = 15, *P* = 0.001), whereas *S. glaseri* moved in the opposite direction (*t* = −5.29, *df* = 14, *P* = 0.0001) (Fig. 1). For both species, no significant difference in movement to the cathode versus anode was detected on the control plates (for which no electrical current was applied) (*t* = 1.03, *df* = 4, *P* = 0.3610 for *S. carpocapsae*, and *t* = −2.27, *df* = 4, *P* = 0.086 for *S. glaseri*) (Fig. 1). Additionally, for each nematode species, the percentage of nematodes that moved to the cathode was different between treatment and control plates (*F* = 18.14, *df* = 1,18, *P* = 0.005 for *S. carpocapsae*, and *F* = 5.6, *df* = 1,17, *P* = 0.03 for *S. glaseri*) (Fig. 1).

Directional movement in the presence of electric fields was also detected in assays that measured nematode movement over a 20 min period. Similar to the 60 min assays, when the nematodes were continually exposed to an electrical field for 20 min a higher number of *S. carpocapsae* moved to the cathode relative to the anode, and *S. glaseri* moved in the opposite direction (*t* = 7.70, *df* = 7, *P* = 0.0001 for *S. carpocapsae*, and *t* = −5.21, *df* = 7, *P* = 0.002 for *S. glaseri*) (Fig. 2). When nematodes were applied to plates that had electrical current removed after 20 min (and nematode movement was then determined after an additional 20 min without current) no directional movement was detected (*t* = −0.2, *df* = 7, *P* = 0.85 for *S. carpocapsae*, and *t* = −1.69, *df* = 14, *P* = 0.11 for *S. glaseri*) (Fig. 2). Similarly, when the amount of time electrical current was applied prior to removal of the current and addition of nematodes was increased to 40 min, no directional movement was detected in *S. glaseri* (*t* = −0.33, *df* = 8, *P* = 0.75). In contrast, when *S. carpocapsae* was added to plates that had electric current running...
for 40 min, a higher number of IJs moved to the anode (after an additional 20 min without current) relative to the cathode (t = -3.7, df = 13, P = 0.003) (Fig. 2). For both nematode species, the percentage of nematodes that moved to the cathode was different between treatment and control plates, and control plates (40 or 20 min of current applied before nematode addition) were not different from each other (F = 48.53, df = 2,27, P = 0.0001 for S. carpocapsae, and F = 9.28, df = 2,29, P = 0.0008 for S. glaseri) (Fig. 2).

4. Discussion

Our results demonstrate directional movement in two steiner-nematids in response to electrical fields. When current was applied to agar plates S. carpocapsae consistently moved to a lower electric potential, whereas S. glaseri moved to a higher electric potential. When current was not applied to plates no directional response was detected. Additionally, when current was applied and then removed no directional response was detected with exception of S. carpocapsae on plates that had current removed after 40 min (and IJ migration subsequently determined after an additional 20 min). We suggest that the 40 min of current created a sufficient gradient of ions on the plate so that removal of the current from the power supply resulted in a weak electrical field in the opposite direction (which S. carpocapsae responded to); this hypothesis explains the reversed directional movement of S. carpocapsae observed on these control plates relative to the plates with continuous current applied. Additionally, we suggest that removal of applied current in the other 20 min assays did not create a sufficient gradient or resulting current to affect nematode movement. It should be noted that we did not detect any temperature differences between the anode and cathode sides of the agar plates (data not shown) thus indicating the directional movement was not due to temperature gradients.

The ability to sense and use electrical fields for prey or host location has been suggested or demonstrated in a number of vertebrate and invertebrate animals (Proskie and Gregory, 2003; Kim, 2007; Steullet et al., 2007). The ability of plant parasitic nematodes to sense electrical fields has also been suggested to aid in host finding, as the nematodes may be attracted to electric potentials in roots (Bird, 1959; Riga, 2004). Generally, plant parasitic nematodes among various genera move toward the cathode (Croll and Matthews, 1977), yet exceptions exist such as Meloidogyne javanica (Treub), which has been observed to move toward the anode (Viglierchio and Yu, 1983). We observed differential movement in response to electrical fields between two insect parasitic species within a single genus.

The different responses to electrical fields observed in S. carpocapsae and S. glaseri (an ambusher and cruiser, respectively) may be related to their different foraging behaviors. Cruiser and ambusher nematodes are adapted to target and infect different host species, e.g., cruisers tend to be most efficient at targeting sedentary hosts, whereas ambushers most efficiently infect mobile hosts (Lewis, 2002). Insects are associated with electrical charges and potentials, which can vary among different species and circumstances (e.g., movement through different substrates) (Scheie and Smyth, 1967; McGonigle and Jackson, 2002); possibly S. carpocapsae and S. glaseri orient to electrical fields differentially in order to find suitable hosts or optimum areas to forage.
Similar to what has been suggested for plant parasitic nematodes, insect parasitic nematodes may also orient to plant roots based on attraction to electrical fields. Steinernematids as well as other entomopathogenic nematodes (i.e., heterorhabditids) have been observed to migrate toward plant roots, which is a behavior that can enhance hosts finding (Kanagy and Kaya, 1996; Wang and Gaugler, 1998). The attraction of certain entomopathogenic nematodes to roots for the purpose of finding phytophagous hosts may be facilitated, at least in part, by the release of volatile chemicals from the plant that is being attacked, a kind of distress call (Wang and Gaugler 1998; Rasmann et al., 2005). Given that roots also react to stress such as wounds with changes in electric potential (Filek and Kościelniak, 1997), it is conceivable that insect parasitic nematode’s orientation to electrical signals contributes to their migration toward damaged roots as well. Additional research is needed to determine the biological significance of steinernematid nematode movement in response to electrical fields.

Acknowledgments

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References