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Effect of *Steinernema glaseri*-infected host exudates on movement of conspecific infective juveniles

Brian A. Kunkel [a], David I. Shapiro-Ilan [a,*], James F. Campbell [b], Edwin E. Lewis [c]

[a] *USDA-ARS, Southeast Fruit and Tree Nut Research Lab, Byron, GA 31008, USA*
[b] *USDA-ARS, GMPRC, Manhattan, KS 66502, USA*
[c] *UC Davis, Department of Nematology, CA 95616, USA*

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Abstract

The entomopathogenic nematode's decision to infect a host is paramount because once the decision is made it is irrevocable; nematodes that invade a host either develop and achieve reproductive success, or they die. Entomopathogenic nematodes that have a cruiser foraging behavior, such as *Steinernema glaseri*, follow host-associated cues to locate insects to infect. Most of the host finding and infection dynamics research has focused on the infective juvenile nematodes' responses to cues from live insects such as host-associated volatiles and host contact cues. Few studies focus on how previously infected hosts influence infective juvenile infection behaviors. We investigated how exudates from nematode-infected hosts affect the behavior of *S. glaseri* infective juveniles. We hypothesized that the infective juvenile's behavioral response to cadavers would change as the state of a nematode-infected host changes during pathogenesis. We examined the effect of exudates collected from infected hosts on infective juvenile locomotory behavior. We detected no effects on nematode repulsion or attraction from exudates produced within the first 48 h post-infection. We observed repulsion from accumulated exudates during the 3–48, 3–72, 3–120, and 3–144 h intervals. Repulsion from exudates was observed during the 48–66, 72–90, and 120–138 h intervals in experiments evaluating daily exudate emissions. The repellent effect of infected host exudates may result in an infective juvenile discriminating between suitable and unsuitable hosts.
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1. Introduction

Entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae are used for controlling a broad range of insect pests (Gaugler, 2002; Grewal et al., 2005). Typically, once a nematode locates a host, it enters through body openings (i.e., mouth, anus, or spiracles), and releases symbiotic bacteria. Infected hosts die within 48–72 h, and the nematodes feed on the symbiotic bacteria and insect tissues, reproducing for 1–3 generations (Grewal and Georgis, 1999). Generally, the second generation of *Steinernema* spp. begins between 3 and 4 days after the initial infection and the third generation starts around day 6 (Lewis, 2002). As food resources dwindle, infective juveniles (IJs) are produced that emerge and search out new hosts.

The IJ stage is the only non-feeding, free-living stage in the entomopathogenic nematode life cycle. Their foraging strategies can be predicted from the behaviors they exhibit during host search (Grewal et al., 1994). Infective juveniles either sit-and-wait (ambushers), actively search (cruisers), or exhibit a combination of these to locate hosts (Lewis, 2002). Infective juveniles that ambush potential hosts forage near the soil surface and are generally best adapted to attacking mobile insects. Ambusher IJs raise most of their body off of the substrate (nictation) and jump towards host-associated volatile cues as a host passes nearby (Campbell and Kaya, 1999a,b). Cruisers are typically found deeper in the soil profile, and are more effective at

* Corresponding author. Fax: +1 478 956 2929.
E-mail address: dshapiro@saa.ars.usda.gov (D.I. Shapiro-Ilan).

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attacking sedentary hosts because they move towards host-associated cues (Lewis et al., 1993; Grewal et al., 1994).

Natural selection should favor IJs that are able to assess the condition of a potential host before invasion. Infective juveniles that encounter healthy hosts must overcome the insect's defensive behaviors and immune system to establish an infection (Gaugler et al., 1994; Wang et al., 1994; Wang et al., 1995). As an infection progresses, the host tissues are consumed by the nematodes and their symbionts. Thus, when considering a host that is already infected, the age of the infection can affect host suitability. An IJ that arrives soon after the initial infection encounters greater amounts of resources, whereas an IJ that invades after a host has died has fewer resources available. Other factors that can affect host suitability include plant compounds consumed by the host (Epsky and Capinera, 1994; Barbercheck et al., 1995; Barbercheck and Wang, 1996) and con- or heterospecific competition (Koppenhöfer et al., 1995; Kaya and Koppenhöfer, 1996).

Most literature pertaining to host finding and infection dynamics focuses on the IJ nematodes' responses to cues from uninfected insects such as host-associated volatiles (Lewis et al., 1993; Grewal et al., 1994) and host contact cues (Lewis et al., 1992). Few studies have focused on the effect an infected host has on IJ infection behaviors (Lewis, 2002). The attractiveness of previously infected hosts appears to be influenced by the duration of infection. *Steinernema carpocapsae* IJs were attracted to volatiles associated with hosts infected with conspecific nematodes 4 h after infection, but *S. carpocapsae* IJs were repelled from volatiles of conspecific-infected hosts 24 h after infection (Grewal et al. 1997). Chemical cues emitted from the infected host can affect the movement and infection behavior of surrounding IJs (Glazer, 1997; Shapiro et al., 2000).

The suite of cues emitted from an infected host may be gaseous (volatiles) or secretions (exudates) that are released into the environment. Previous research has examined cues emitted from infected hosts as volatiles or a combination of volatiles and exudates (Grewal et al., 1997; Glazer, 1997). In order to further understand effects of host cues on nematode behavior, the cues must be broken down into their components. Prior to our study, no research had been conducted to investigate the role of exudates (separate from volatile cues) as they change during the course of the infection process. In this study, we examined the effect of exudates secreted into the environment on entomopathogenic nematode movement behaviors. Our goal was to investigate the following questions: (1) are IJs attracted or repelled from host-associated exudates and (2) does the response change over time after host infection and if so, when? Research into these behaviors may provide insight into nematode dispersal, distribution, and infection dynamics in field situations.

Specifically, we examined the effects of exudates from infected hosts on the attraction or repulsion of *Steinernema glaseri* (Steiner). We chose *S. glaseri* because this cruiser nematode species is known to use chemical cues to locate its host (Lewis et al., 1992, 1993), thus we hypothesized that it would be sensitive to materials associated with infected insects.

2. Materials and methods

2.1. Insects and nematodes

Steinernema glaseri (NJ43 strain) were reared in the last instar of *Galleria mellonella* (Webster's Waxie Ranch, Webster, WI) following the methods described by Kaya and Stock (1997). Infective juveniles from the first 2 days of emergence and IJs older than 2 weeks were not used in these experiments.

2.2. Infections

We filled each well of 12-well cell culture plates (Corning Inc., Corning, NY) with approximately 3.0 g of sand and added 3000 IJs in 200 μl of water. We used this high rate of inoculation (3000 IJs) because our goal was to obtain 100% host infection in order to investigate nematode response to exudates close to the start of pathogenesis. One *G. mellonella* larva was placed in each well; the well plate was wrapped with parafilm and kept in an incubator at 25 °C for 3 h. After 3 h, the larvae were transferred to new wells and stored at 25 °C until they were used in a bioassay.

To determine the average number of founding IJs in our larvae, we exposed them to IJs as above, transferred the exposed insects without rinsing them to IJ-free wells after 3 h, and kept the insects in well plates at 25 °C for 72 h. After 3 days, the infected insects were frozen until the number of nematodes inside was determined using the pepsin digestion method (Mauléon et al., 1993).

2.3. Bioassay arenas

We modified arenas first described by Grewal and Wright (1992) in our exudate assays. All of our assays were conducted in 9.0 cm diameter Petri dishes (Fisher Scientific, Suwannee, GA) filled with 2% plain agar. The agar (Bacto Agar, Difco Laboratories, Sparks, MD) was poured 1.0 cm deep. Agar-filled dishes (assay arenas) were either stored at 4 °C until 24 h before use, or were used within 24 h. Lids were removed from assay arenas and air dried for 1 h immediately before use.

2.4. Accumulated exudates

In the first set of experiments, we determined how exudates that accumulate over progressively longer intervals of infection affect IJ movement. We collected infected host exudates on 2% agar disks. Agar disks were made by pipetting 1.0 ml of hot plain agar into each well of the 12-well cell culture plates and air drying them for 1 h before storage. Storage of the disks was similar to

storage for assay arenas. We set nematode-exposed insects on the agar disks to accumulate exudates over 3–24, 3–48, 3–72, 3–96, 3–120, and 3–144 h intervals. Thus, this experiment examined exudates that had accumulated from initial exposure (3 h) to the test-point interval. For comparison, we also exposed disks to uninfected hosts. Infected insects were placed on agar disks immediately after the 3 h exposure to IJs, and insects not exposed to nematodes (live hosts) were placed on agar disks 24 h before the set-up of an assay. During the set-up of an assay, live hosts, and IJ-infected hosts were taken off the agar disks, and the disks carefully removed from the well plates. Treated disks were inverted and placed within 2 mm of the rim of an agar-filled Petri dish, and a blank agar disk (not exposed to infected or live hosts) was placed directly opposite the treated disk. Thus, each assay arena had one treated and one blank 2% agar disk across from each other separated by 5.0 cm. Our control assay arena for this experiment consisted of two inverted blank (no host) agar disks placed opposite each other. To allow time for exudates to soak into the agar and for gradients to form, all Petri dishes were kept in an incubator at 25 °C for 18 h before use in an assay.

For the infected-host treatment, each trial used IJs from the same population of nematodes, but due to time constraints the initial exposure occurred in two groups. Insects exposed to IJs in the first infection-group were used to test accumulated exudates from 3 to 24, 3 to 72, and 3 to 120 h intervals and the second infection-group tested accumulated exudates from 3 to 48, 3 to 96, and 3 to 144 h. Insects exposed to IJs used during the first two intervals (3–24 and 3–48 h) were observed to ensure they eventually died from nematode infection; if insects did not die then the replicate was removed from the analysis (because the host was not infected).

In an assay arena similar to ours, Lewis et al. (1993) found that *S. glaseri* responded strongly to host volatiles. Thus, to confirm the validity of our assay and determine if our nematodes were responding normally, we included a live-host volatile treatment as a positive control. One *G. mellonella* larva was placed into a pipette tip and the wide end covered with parafilm. The insect-filled pipette tip and a corresponding empty pipette tip were placed into small holes made in a Petri dish lid opposite to each other so a volatile gradient could form on the agar. The pipette tips were about 2 mm above the surface of the agar. The volatile gradient formed over 2 h.

The number of IJs prepared for each assay arena was about 400. Infective juveniles were centrifuged for 1 min at 5000 rpm, removed with a micropipette and vacuum filtered to remove excess water. The clump of IJs was lifted off of the filter paper with a micro-spatula and placed onto the center of an assay arena through a 1.0 cm inoculation port in the lid of the dish. This inoculation port was covered with tape and assay arenas were kept at 23 °C under a large black plastic bag until the time of observation to prevent light from interfering with the assay. The IJs that were placed in the arena were from same batch used to infect insects in the infected-host treatment.

Measurement of IJ movement within the assay arenas was based on slightly modified procedures of those described by Grewal and Wright (1992). Briefly, concentric rings 0.5, 1, and 2 cm from the center of the lid were drawn, then the lids were quartered. The outermost ring (2 cm from center) contained either the treatment (treated disk) or controls (blank agar disk). We counted all IJs that moved from the inoculation point of the Petri dish including those located on the rim, after IJs had 1–1.5 h to disperse. Every nematode did not move from the center of the dish every time, thus there were occasions when more nematodes moved in one dish versus another. The main reason nematodes would not move from center is because occasionally IJs would stick to each other during the vacuum filtration step. To compare movement of IJs among treatments, we use an attraction index (Grewal and Wright, 1992). In this approach, variation in total number of IJs moved among dishes is not a factor because the attraction index measures relative nematode movement within each dish. We calculated the attraction index for each arena using the following Eq. (1):

$$A = [(5T_1 + 10T_2 + 20T_3) - (5C_1 + 10C_2 + 20C_3)] * N^{-1}. \quad (1)$$

N equals the total number of IJs that moved out of the center of the dish. The nematodes found in rings T_1, T_2, and T_3 correspond to the IJs found progressively closer to the treatment side, and nematodes in C_1, C_2, and C_3 were progressively closer to blank disks (control side). The subscripts on the variables refer to the relative distance from the center of the dish in centimeters. The experiment was set-up as a randomized complete block with five replicates per treatment. The entire experiment was repeated resulting in two trials for each infection-group and a total of 10 replicates for each treatment and time interval. If there were too few IJs moving (<50) on the agar plates after 1.5 h or if nematodes began to emerge from infected hosts (e.g., at 5 or 6 days post-infection) then the entire replicate was discarded.

2.5. Effects of exudates at daily intervals

We conducted this experiment to examine the effects of exudates during discrete intervals during the infection process. Our objective was to observe the intensity and direction of the movement in relation to exudates and to determine intervals where exudate emission probably occurs. We examined exudates from insects over the following intervals: 3–18, 24–42, 48–66, 72–90, 96–114, 120–138, and 144–162 h after initial exposure to IJs. The assays were conducted similar to the accumulated exudates experiment described above with the following exceptions: rather than using agar disks, the insects

exposed to IJs were confined on the agar plates with a wire mesh cage (8 gauge). Cages were used to prevent unrestricted crawling over the agar plate, and to concentrate exudates into one particular area (T_3). Empty (control) cages were placed directly opposite cages confining insects similar to the set-up above. The cages were used throughout all intervals and trials to maintain uniformity. Insects used in the first two intervals that did not die from nematode infection after observation had their replicate removed from the analysis. Our treatments were IJ-exposed insects (infected hosts) and a positive volatile control similar to above. An unexposed insect control was not included in this experiment because the results from all the prior (cumulative) assays established that no attraction or repulsion was detected from the live-host control. Infected hosts were confined on assay arenas for 18 h at 25 °C before use in an assay. Infected hosts were removed from the arena before the IJs were added to the dish through the inoculation port. The experimental design was a randomized complete block experiment with five replicates per trial and we conducted 2–3 trials for each time interval. If insects escaped from cages (3–18, 24–42, 48–66 h intervals), unacceptably low IJ movement (<50 IJs) was observed, or nematodes emerged onto the agar from the infected host (120–138 and 144–162 h intervals) then the replicate was discarded. When a replicate was discarded, we conducted an additional trial to increase the number of total successfully tested replicates. Thus, all time intervals had 8–13 replicates for each treatment.

2.6. Analysis

We analyzed the attraction indices to compare the overall directional movement rates of IJs among treatments, and used the number of IJs in treated (T_3) versus control (C_3) rings to determine IJ attraction within each treatment. Attraction indices were calculated to compare IJ movement in one treatment (e.g., infected host) to IJ movement to the other treatments (i.e., an evaluation of effects among treatments). Each time interval was analyzed separately and no statistical comparisons were made between intervals. To determine if there were differences between infection-groups, we examined the interactions between infection-group and treatment effects. The attraction indices were compared among treatments by ANOVA (analysis of variance) with $\alpha = 0.05$. Following a significant overall analysis of variance, the LSD means separation procedure was performed. We used *t*-tests to determine whether attraction or repulsion occurred within the treatments (i.e., a within treatment evaluation). The number of IJs found in T_3 and C_3 (treated side versus blank side) were square-root transformed before paired *t*-tests were conducted with $\alpha = 0.05$ (Sokal and Rohlf, 1981). All tests were performed using SAS 9.0. The means ± standard errors of untransformed numbers are presented.

3. Results

3.1. Accumulated exudates

The insects exposed to IJs for 3 h had 7.33 ± 1.24 IJs successfully invade and develop into adults. The degrees of freedom, *F*- and *p*-values for the accumulated exudate assay attraction indices are presented for each interval in Table 1. The positive volatile control had a higher attraction index than the infected host exudate treatment, live-host and no-host controls in every interval (Fig. 1A). No difference in attraction index was detected between the infected host exudate treatment and the live-host or no-host control for the 3–24, 3–48, and 3–96 h intervals (Fig. 1A). However, the attraction indices for IJs exposed to infected host exudates accumulated over 3–72, 3–120, and 3–144 h were lower than all other treatments (Fig. 1A). Infected host-associated exudates that accumulated over the 3–120 h interval (5 days) apparently caused the greatest repulsion of IJs relative to other treatments. No differences in attraction indices were detected between live-host and the no-host control for any interval of the experiment. We found no significant interaction between the infection-group and the treatment effects ($F = 1.44$, df = 3, 207, $p = 0.2311$).

The *t*-statistic, *p*-value and degrees of freedom for analyses within individual treatments are presented in Table 2. Exudates accumulated over 3–24 h from infected hosts did not attract or repel subsequent IJs (Fig. 2). Within the infected host treatment, exudates accumulated over 3–48 h on agar disks repelled IJs to the untreated side (C_3) of the Petri dish (Fig. 2). Infected host-associated exudates also repelled IJs to the untreated side at all other intervals thereafter except for the 3–96 h interval (Fig. 2). The agar disks treated with live hosts did not attract or repel IJs over any interval (Fig. 2). Additionally, the blank agar disks in the control assay arenas did not attract or

Table 1
F-test statistical values related to *S. glaseri* movement (measured as attraction indices) in response to *S. glaseri*-infected host exudates accumulated through the course of the experiment or from daily intervals

Experiment	Source	Interval	*F*-value	df	*p*-value
Accumulated					
	Trt	3–24	10.93	3, 24	0.0001
	Trt	3–48	13.30	3, 20	<0.0001
	Trt	3–72	15.80	3, 24	<0.0001
	Trt	3–96	24.96	3, 28	<0.0001
	Trt	3–120	24.35	3, 24	<0.0001
	Trt	3–144	16.01	3, 24	<0.0001
Daily intervals					
	Trt	3–18	2.70	1, 10	0.1312
	Trt	24–42	3.34	1, 8	0.1050
	Trt	48–66	99.88	1, 14	<0.0001
	Trt	72–90	33.47	1, 12	<0.0001
	Trt	96–114	35.01	1, 12	<0.0001
	Trt	120–138	62.38	1, 16	<0.0001
	Trt	144–162	16.62	1, 10	0.0022

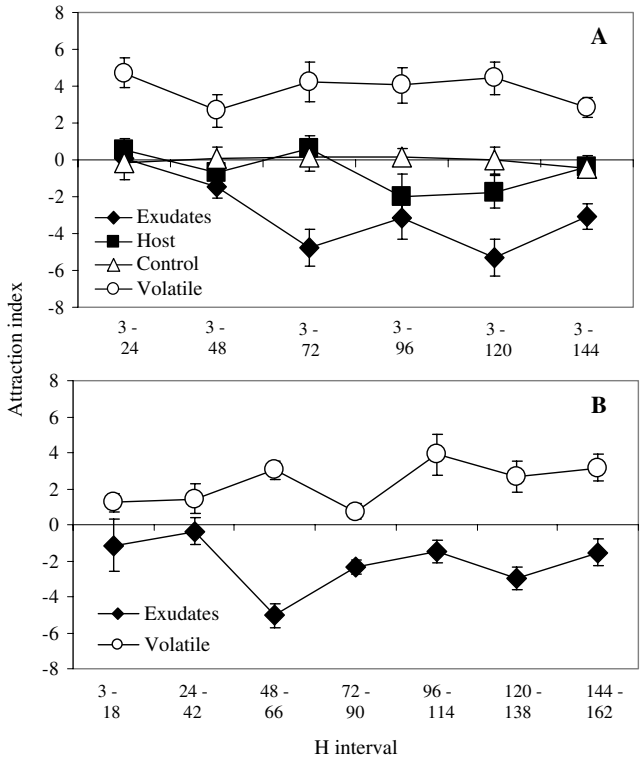

Fig. 1. Attraction index of *S. glaseri* IJs in response to accumulated exudates from hosts infected with *S. glaseri* (exudates), live hosts (host), untreated controls (control), or host-associated volatile cues (volatile) (A). Attraction index of *S. glaseri* in response to exudates at daily intervals from host infected with *S. glaseri* (exudates) or from host-associated volatile cues (volatile) (B). The mean attraction index displays the average distance moved per nematode from or towards the treated area. Averages are presented ± SEM.

repel IJs over any intervals (data not shown). Finally, the positive volatile control attracted more IJs to the treatment side (T_3) over every interval (Fig. 2).

3.2. Effects of exudates at daily intervals

The degrees of freedom, *F*- and *p*-values for the daily interval assay attraction indices are presented for each interval in Table 1. The attraction indices for the positive volatile control were positive for the entire series of experiments, and were higher than the indices of the infected host exudate treatment on all occasions after the 24–42 h interval (Fig. 1B). The *t*-statistic, *p*-value, and degrees of freedom for analyses within individual treatments are presented in Table 2. The infected host-associated exudates caused the repulsion of IJs as early as the 48–66 h interval (Fig. 3). We also detected IJ repulsion to the untreated side (C_3) of the Petri dishes at 72–90 and 120–138 h intervals (Fig. 3). No significant attraction or repulsion was observed for the 3–18, 24–42, 96–114, or 144–162 h intervals in the infected host treatment. The positive volatile treatment showed attraction of IJs to the treated side (T_3) of the Petri dishes for all intervals except 72–90 h (Fig. 3; Table 2). We found no significant interaction between infection-group and treatment effects during this experiment ($F = 1.15$, df = 1, 138, $p = 0.2862$).

Table 2
t-test statistical values related to *S. glaseri* movement in response to *S. glaseri*-infected host exudates accumulated through the course of the experiment or from daily intervals

Experiment	Source	Interval	*t*-statistic	df	*p*-value
Accumulated					
	Exudate	3–24	−0.58	8	0.5753
	Host	3–24	0.93	8	0.3783
	Control	3–24	−0.14	8	0.8891
	Positive volatile	3–24	6.01	8	0.0003
	Exudate	3–48	−2.38	7	0.0488
	Host	3–48	−1.68	7	0.1377
	Control	3–48	0.15	7	0.8861
	Positive volatile	3–48	3.35	7	0.0123
	Exudate	3–72	−6.00	8	0.0003
	Host	3–72	−0.36	8	0.7265
	Control	3–72	−0.05	8	0.9597
	Positive volatile	3–72	3.47	8	0.0085
	Exudate	3–96	−2.07	9	0.0684
	Host	3–96	−1.96	9	0.0814
	Control	3–96	0.86	9	0.4132
	Positive volatile	3–96	4.38	9	0.0018
	Exudate	3–120	−3.80	8	0.0052
	Host	3–120	−1.44	8	0.1884
	Control	3–120	−0.23	8	0.8230
	Positive volatile	3–120	6.05	8	0.0003
	Exudate	3–144	−3.81	8	0.0052
	Host	3–144	−0.20	8	0.8444
	Control	3–144	−1.77	8	0.1154
	Positive volatile	3–144	5.06	8	0.0010
Daily intervals					
	Exudate	3–18	−0.98	9	0.3538
	Positive volatile	3–18	2.99	9	0.0152
	Exudate	24–42	0.49	7	0.6390
	Positive volatile	24–42	3.06	7	0.0184
	Exudate	48–66	−7.40	11	<0.0001
	Positive volatile	48–66	5.69	11	0.0001
	Exudate	72–90	−4.58	9	0.0013
	Positive volatile	72–90	2.20	9	0.0550
	Exudate	96–114	−1.52	9	0.1622
	Positive volatile	96–114	3.53	9	0.0064
	Exudate	120–138	−4.16	12	0.0013
	Positive volatile	120–138	3.22	12	0.0074
	Exudate	144–162	−1.94	8	0.0886
	Positive volatile	144–162	4.61	8	0.0017

4. Discussion

The results of our experiments demonstrate that *S. glaseri* is repelled from exudates produced by conspecific-infected hosts after 48 h from the initial exposure to founding IJs. The repulsion from infected host-associated exudates began with the 3–48 and the 48–66 h intervals in the accumulated and daily exudates experiments, respectively. During both experiments we did not observe repulsion from exudates during the 3–96 and 96–114 h intervals. The decreased response may result from reduced production of the repellent compounds, and might signify a transition from actively feeding and developing

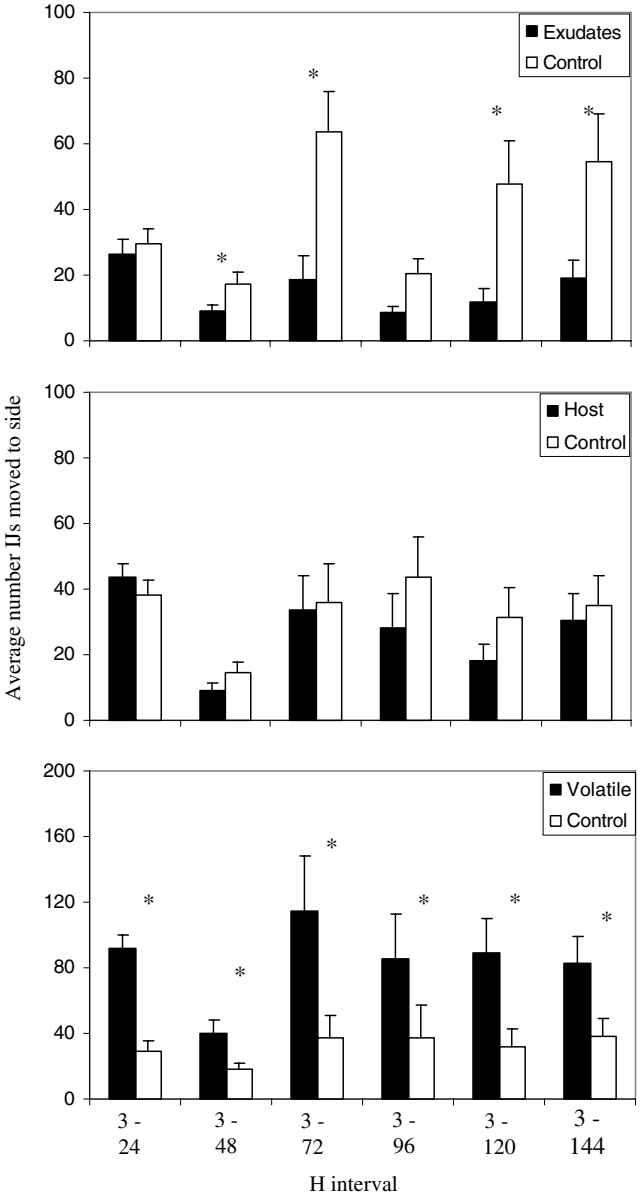

Fig. 2. Effects of exudates accumulated over time on subsequent *S. glaseri* IJ orientation in relation to *S. glaseri*-infected hosts (exudates), live hosts (host), or host-associated volatiles (volatile) within Petri dish arenas. Asterisks designate significant differences in the number of IJs that moved towards the treated side or the control side ($\alpha = 0.05$). Means (±SEM) for both sides of the dish are presented.

nematodes to the reproduction of the next generation of IJs. Additionally, the resumed repulsion effect observed at the 3–120 and 3–144 h intervals may be due to increasingly leaky-infected hosts.

The specific chemical constituents of the exudates we studied remain unknown. Although we did not observe visible signs of microbial growth on the agar plate, it is possible that microbial growth on the plate or on substances exuded by infected hosts could have contributed to the observed results. Conceivably, some of the active agents in the exudates were nitrogenous compounds. *S. glaseri* IJs respond to host feces by altering their behavior to increase the likelihood of locating a host

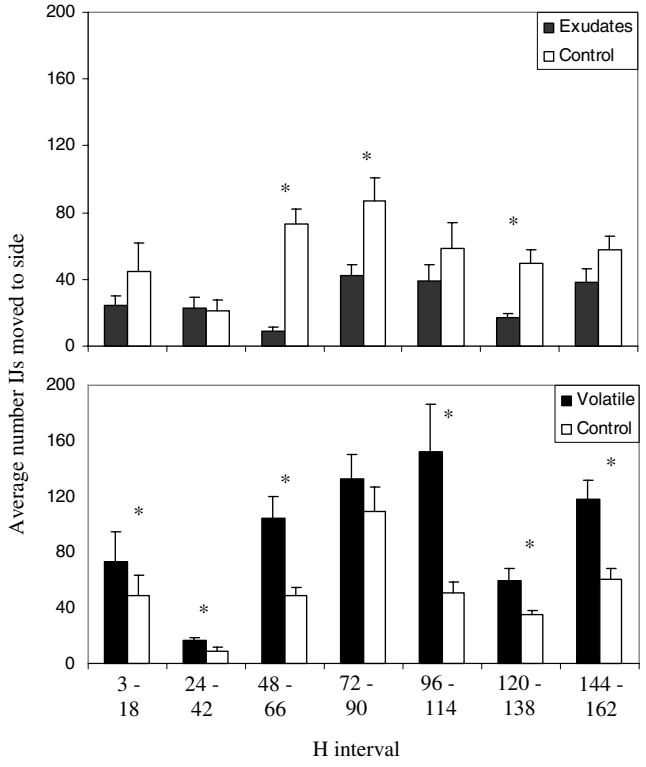

Fig. 3. Effects of exudates accumulated over time on subsequent *S. glaseri* IJ orientation in relation to *S. glaseri*-infected hosts (exudates), or host-associated volatiles (volatile) within Petri dish arenas. Asterisks designate significant differences in the number of IJs that moved towards the treated side or the control side ($\alpha = 0.05$). Means (±SEM) for both sides of the dish are presented.

(Lewis et al., 1992). In contrast, *S. glaseri* also exhibits avoidance behavior when they encounter cockroach feces that contain ammonia (0.9 μl/mg) (Grewal et al., 1993a). Shapiro et al. (2000) suggested *Heterorhabditis bacteriophora* Poinar infected hosts are attractive or repulsive to *H. bacteriophora* IJs depending upon the concentration of nitrogenous substances surrounding the host. We suggest that exudate composition probably changes as an infection progresses, and nitrogenous substances could be a dynamic component of the exudates.

The symbiotic bacteria may produce compounds that repel secondary nematode invaders. During the first 24–48 h of an infection, bacteria cell density increases and the bacteria produce a variety of molecules including some antimicrobial compounds (Forst and Clarke, 2002). Shapiro et al. (2000) observed peak nitrogen production within this time interval and hypothesized nitrogen losses were related to the activities of *Photorhabdus luminescens*, the symbiotic bacteria of *H. bacteriophora*. Although the peak repulsion in the experiments coincides with this stage of bacterial activity, previous research suggests that the symbiotic bacteria are not solely responsible for repulsion of secondary infective juveniles (Glazer, 1997; Grewal et al., 1997). Future research that investigates the composition of infected host exudates should elucidate factors responsible for repulsion of secondary invaders.

Our results support other studies that document steinernematid infected hosts can repel conspecific IJs. Three *Steinernema* spp. were not likely to invade conspecific-infected hosts after 6–9 h of initial injection of IJs into the host (Glazer, 1997). Similarly, *S. carpocapsae* injected into hosts did not decrease secondary-conspecific or *S. glaseri* IJ invasion until 6–9 h post-injection, and hosts injected with *S. glaseri* IJs were unlikely to be invaded by conspecific or heterospecific IJs (Wang and Ishibashi, 1998). Both *S. carpocapsae* and *S. glaseri* IJs were less attracted to conspecific-infected hosts when 24 h old compared to hosts infected for 4 h (Grewal et al., 1997). Although our findings were consistent with previous work in terms of observed repulsion, we did not observe repulsion from exudates until 48 h. The number of IJs injected into hosts in these previous studies was 30 and 50 IJs (Glazer, 1997; Wang and Ishibashi, 1998, respectively), and Grewal et al. (1997) exposed their hosts to 200 infective juveniles. The average number of IJs we had invade *G. mellonella* from our 3 h exposure was 7. Lewis (2002) suggests the amount of time required for an infection to progress to different stages is influenced by the number of founding IJs, and this may explain why we saw repulsion from the exudates after a greater duration of time. Additionally, the experimental arena used by Grewal et al. (1997) was similar to ours, however, they tested effects of cadaver-associated volatiles and our study focused on cadaver-associated exudates.

Nematode-infected hosts are both food resources and mating sites for steinernematid IJs. The ability to interpret these cues and determine host suitability prior to invasion is of great adaptive value to IJs. Infective juveniles that locate, invade, and overcome host immune defenses or arrive shortly thereafter are more likely to successfully acquire mates and other benefits of early (but not first) invasion (Grewal et al., 1993b; Stuart et al., 1998). Infective juveniles that arrive at hosts too long after the initial infection may encounter cues that repel them from the infected host. Grewal et al. (1997) proposed IJs repelled from conspecific-infected hosts might detect nematodes within the infected host that are at an incompatible stage of development. Alternatively, the repulsion of *S. glaseri* IJs from older nematode-infected hosts could be an evolved mechanism to avoid overcrowding. Selvan et al. (1993) found that there is an optimal number of infecting IJs for a host to contain for maximum progeny production. Additionally, overcrowded conditions reduce nutritional resources available to developing IJs and this can reduce nematode infectivity of the IJs produced from the overcrowded infection, produce shorter and less fecund females, and decreases lipid quality (Selvan et al., 1993; Nguyen and Smart, 1995; Abu Hatab et al., 1998; Ryder and Griffin, 2002).

Given our results indicating repulsion from host exudates one might expect that additional invasion would cease 48 h after an initial infection is established. Yet, *Steinernema* spp. IJs continue to invade nematode-infected hosts after 48 h (Fan and Hominick, 1991; Epsky and Capinera, 1993). The number of secondary invaders, however, decreases over time indicating that host exudates and repulsion may indeed play a role, but are not solely responsible for observed infection dynamics. A number of other cues are known to affect nematode foraging behavior [e.g., CO_2, contact with host cuticle (Gaugler et al., 1980; Gaugler and Campbell, 1991; Lewis et al., 1993)]; thus, it is likely the repelling effect of exudates interacts with these other cues to determine nematode infection.

In conclusion, the compounds exuded from an infected host may play a significant role in nematode infection dynamics, but interactions with volatiles from infected hosts remain to be explored. The composition of, and interactions with, exudates may vary depending upon the nematode species or strain, number of IJs initiating infection, bacteria species, and developmental stages of the nematodes within the host. In addition to conspecific interactions, prior research has demonstrated that infected hosts or compounds associated with them can affect interactions with other entomopathogenic nematode species as well as other soil fauna (Alatorre-Rosas and Kaya, 1991; Maxwell et al., 1994; Koppenhöfer et al., 1995; Kaya and Koppenhöfer, 1996; Baur et al., 1998; Zhou et al., 2002). Further research into infection dynamics is needed to clarify the roles that volatiles, exudates, nematodes, and bacteria play in overall nematode infection behavior. The concept of repulsion from infected hosts may also provide insight into nematode pest control efficacy and population dynamics under field conditions.

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