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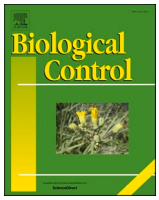

Responses of two entomopathogenic nematode species from the genus *Steinernema* to ethanol and 1-nonene

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HIGHLIGHTS

- Two novel behaviorally active compounds for EPN IJs were revealed.
- The two species tested (*Steinernema feltiae* and *S. carpocapsae*) responded differently to the behaviorally active compounds.
- Within the blend of volatiles emitted by EPN-infected insect cadavers, 1-nonene plays a behavior-active role.
- Ethanol is a novel attractant for *S. feltiae* IJs.
- The differences in the responses to the compounds support known data on scavenging trends of the EPNs tested.

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ABSTRACT

Entomopathogenic nematodes (EPNs) parasitize insects in the soil and are applied as environmentally friendly means for pest control in agriculture. Knowledge of how EPN infective juveniles (IJs) find their prey can be used to increase their effectiveness. Chemical signals in the soil are undoubtedly important but exactly which ones is little known. We hypothesized that volatile compounds emitted by EPN-infected larval cadavers could act as such signals. The objective of the study was to test the behavioral effects of 1-nonene which is known as a volatile compound emitted by several EPN-infected insect cadavers. Behavioral tests revealed that 1-nonene was attractive to IJs of both *Steinernema feltiae* and *S. carpocapsae*. High concentrations of the compound were repellent to *S. feltiae* and attractive to *S. carpocapsae* IJs. Low concentrations were attractive to *S. feltiae* (those from 10^4 to 10^6 times lower than the repellent concentrations) but did not affect the behavior of *S. carpocapsae*. Ethanol (solvent used for control tests) was attractive to *S. feltiae* IJs and not to those of *S. carpocapsae*. Both compounds are new agents involved in the behavior control of these EPN species. Different responses of IJs of two taxonomically closely related EPN species to chemical compounds could indicate interspecific difference in foraging. Behavioral reactions of *S. carpocapsae* IJs are more in line with the strategy of the scavenger.

1. Introduction

Entomopathogenic nematodes (EPNs) from families of Heterorhabditidae and Steinernematidae are obligate parasites of insects and are applied as biological control agents for economically important pests (Grewal et al., 2005). Nematodes form symbiotic associations with pathogenic bacteria: Steinernematidae EPNs with bacteria from the genus *Xenorhabdus* and Heterorhabditide – with those from *Photorhabdus* (Poinar, 1990). Infective juveniles (IJs) of EPNs are the only free-living non-feeding stage searching for insect hosts in the soil. Following contact with a suitable insect, IJs enter the host through natural openings (oral cavity, anus, spiracles, and in some cases through the cuticle) (Dowds and Peters, 2002) and release their symbiotic bacteria into the hemocoel, which in turn release a variety of secondary metabolites that kill the insect within 48–72 h (Poinar, 1990). Two to three generations of EPNs develop within the insect cadaver and after the resources are depleted the newly formed IJs exit the cadaver and search for new hosts (Grewal and Georgis, 1999).

Despite many abiotic and biotic factors that are important for IJs to locate suitable insect hosts perceived by thermosensation,

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mechanosensation, *etc.*, chemosensation is among the most important ones. Several volatile cues that are important for EPNs' behavior control during insect host searching are known. Among these is carbon dioxide which indicates the presence of certain biological activity within soil substrate; host-insect derived volatiles that help IJs accurately locate the host; herbivore-induced plant volatiles that help EPNs to detect herbivore insects from the distance, and volatiles released by EPN-infected cadavers that inform EPNs about the prey infection status (reviewed by Zhang et al., 2021). In general, both attractants and repellents are important for EPN IJs in searching for suitable insect hosts and avoiding unsuitable objects within the soil environment. However, not much is known about the particular chemical compounds involved in EPN behavior, especially if compared to plant parasitic nematodes where over 500 such compounds that induce behavioral responses are known (Čepulytė and Būda, 2022). Also, though EPNs already are commercially available and even broadly applied as biological control agents of insect pests in agriculture, behavior traits related to prey location remain insufficiently known.

It was demonstrated that volatile blends released by EPN-infected insect cadavers differ depending on the species (either EPN, symbiotic bacteria, or insect) participating in the infection and almost do not overlap (Grunseich et al., 2021; Fu et al., 2021; Zhang et al., 2019). However, recently it was revealed that larval cadavers of two insect species, namely cucumber beetle *Acalymma vittatum* (Fabricius 1775) and *Galleria mellonella* (Linnaeus 1758) (laboratory model insect for EPN studies) infected by three different EPN species (either *Heterorhabditis bacteriophora* Poinar, 1976, *Steinernema riobrave* Cabanillas et al., 1994 or *S. carpocapsae* (Weiser, 1955) Wouts et al., 1982) emit mixtures of volatiles that contain alkene 1-nonene as a common compound (Grunseich et al., 2021). Since the larvae of both above-mentioned insect species were suitable prey for a few EPN species, we hypothesized that the chemical compound common in the emissions could act as a chemical signal involved in the behavioral control of IJs. The objective of the study was to test the behavioral reactions of *S. feltiae* (Filipjev, 1934) Wouts et al. (1982) and *S. carpocapsae* IJs to 1-nonene.

2. Materials and methods

2.1. Nematodes

Initial stock of *S. feltiae* (strain RM-107, GenBank Accession number MW480131 (Blanco-Pérez et al., 2020)) was provided by Dr. Raquel Campos-Herrera, University of La Rioja, Spain, and *S. carpocapsae* was purchased commercially from Koppert, The Netherlands. The EPNs were propagated on *G. mellonella* larvae, the initial culture of which was provided by Dr. Raquel Campos-Herrera. Five last instar larvae were placed in a 5.5 cm Petri dish lined with filter paper and approximately 300 IJs in 500 μL deionized water were applied on top of each larva. Petri dishes were kept at room temperature (21–22 °C) for 7 days in the dark and emerged IJs were collected using White traps (White, 1927). Briefly, the lid of the 5.5 cm Petri dish was lined with filter paper dampened with deionized water and placed in a 9 cm Petri dish filled with 5–7 mL deionized water. Insect cadavers were transferred on filter paper and placed in a star-like pattern. After 4–7 days in White traps, IJs were collected from the water in the 9 cm Petri dish and rinsed three times with deionized water by sedimentation. IJs were stored in vented culture tissue flasks placed horizontally for 5 to 15 days at 12 °C. Before the behavioral test, *S. feltiae* were maintained at room temperature and *S. carpocapsae* at 24 °C for 24 h. EPN IJs up to three weeks old were tested.

2.2. Galleria mellonella

The culture of *G. mellonella* was maintained at 21 °C on natural honeybee combs in a 5 L glass vessel. Last instar larvae were collected and stored in aerated plastic boxes with sawdust (Flamingo, Belgium) at 12 °C until used for nematode rearing and in experiments.

2.3. Chemical compounds

As 1-nonene is not soluble in water but soluble in ethanol, this solvent was tested first on the behavior of *S. feltiae* and *S. carpocapsae* IJs. Ten times and 100 times dilutions of ethanol (96 %, Vilniaus Degtinė, Lithuania) were prepared in deionized water. 1-Nonene (96 %, Sigma Aldrich, USA) concentrations of 1 M, 500 mM, 200 mM, 20 mM, 2 mM, 200 μM, 20 μM, 2 μM, 0.2 μM, and 0.02 μM were prepared in undiluted ethanol. Both ethanol and 1-nonene solutions were stored at 4 °C in dark glass vials sealed with parafilm until used. Fresh ethanol and 1-nonene concentrations were prepared every two weeks.

2.4. Chemotaxis assay

EPN IJ behavioral condition in the assays was evaluated using two controls – positive and neutral. A supernatant of *G. mellonella* last instar larva crushed in 300 μL of deionized water served as a positive control as nematodes in the preliminary experiments chose *G. mellonella* over water *i.e.*, *G. mellonella* supernatant was highly attractive to nematodes. Also, this control indicated and reflected the nematode viability and activity. Water served as a neutral control, as in preliminary experiments it was not attractive nor repellent to IJs. Besides, this control indicated whether under uniform conditions IJs in the Petri dish spread evenly and if their movement was unaffected by other factors. If the IJs were attracted to *G. mellonella* supernatant and were equally distributed in the water *vs.* water assay, such a batch of IJs was used for further assays.

EPN IJs behavioral response to chemical compounds was tested using a two-choice chemotaxis assay. Petri dishes of 9 cm diam. were filled with approximately 15 mL of 1.5 % or 1.8 % agar for *S. feltiae* and *S. carpocapsae*, respectively, and stored at 4 °C. Concentration of agar was slightly increased for *S. carpocapsae* bioassay to prevent IJs entering the agar layer as such behavior was observed in preliminary assays. Before the assays, Petri dishes with agar were kept in a fume hood at room temperature for 15 min to dry the excess moisture. Two dots on the bottom of each Petri dish were marked using a nematode scoring template prepared in advance. One dot was marked on one side of the dish 1 cm away from the border and the other one – on the opposite side in the same manner. The dot in the center of a dish was marked as the nematode application point. The dots on the opposite sides of the Petri dish were in the center of a 1 cm diameter circle marked on the template for nematode counting. Four hundred IJs of *S. feltiae* or 600 IJs of *S. carpocapsae* in 10 μL of deionized water were applied to the center. The numbers were based on unequal IJs mobility of the species resulting in different participation in the assay (*e.g.* Baiocchi et al., 2019; Lewis et al., 1995). To balance participation, the preliminary assay was performed, and the proportion was established. When checking nematode response to ethanol, 10 μL of ethanol (undiluted or diluted in deionized water) was applied on one side of the Petri dish and 10 μL of deionized water was applied on the opposite side. When checking nematode response to 1-nonene, 10 μL of 1-nonene (diluted in ethanol) was applied on one side of the Petri dish, and 10 μL of ethanol (undiluted solvent) was applied on the opposite side. Ethanol *vs.* ethanol, *G. mellonella* larvae supernatant *vs.* water, and water *vs.* water controls were used for both assays and applied on agar in the same manner. The assay start was recorded immediately after the water of the EPN pellet had dried. The dishes containing *S. feltiae* were kept at room temperature (~22 °C) for 1 h and those containing *S. carpocapsae* at 24 °C for 2 h in the dark. The temperature conditions were close to optimal for the IJs of both species (*e.g.* Radová and Trnková 2010; Grewal et al., 1994), and duration balanced the difference in mobility of both species. Then the number of nematodes in scoring circles was counted using the nematode scoring template mentioned above. For each ethanol dilution and each 1-nonene concentration tests were performed in 3 Petri dishes simultaneously. Each assay was repeated 6 times.

2.5. Statistical analysis

EPN IJs choice between control (A) and stimulus (B) was calculated as a percentage, where 100 % was considered all the IJs falling into scoring circles A and B. IJs in the stimulus circle were calculated as a percentage of the number of nematodes divided by the sum of nematodes in the stimulus and control circles $\frac{B}{A+B} \times 100\,\%$, and IJs in the control circle – as a percentage of the number of nematodes divided by the sum of nematodes in the stimulus and control circles $\frac{A}{A+B} \times 100\,\%$. The percentage values were then used for the statistical analysis and the Wilcoxon signed-rank test was applied. The difference between data was considered statistically significant when $p < 0.05$. The data was processed, and graphs were plotted using Microsoft Excel. Statistical analysis was performed using PAST 4.03.

3. Results

3.1. Behavioral condition of EPN

The behavioral condition of EPN IJs was evaluated following two criteria: responses to insect larva (crushed *G. mellonella* larva supernatant) *vs.* water and water *vs.* water. *Steinernema feltiae* and *S. carpocapsae* were strongly attracted to *G. mellonella* – approximately 90 % of IJs for both species ($p < 0.01$) (Fig. 1; Fig. 2; Supplementary Material, Table S1; Table S2). In the water *vs.* water assay water was neither attractive nor repellent to *S. feltiae* and *S. carpocapsae* IJs *i.e.*, nematodes chose scoring circles equally and no statistically significant differences were observed (Fig. 1; Fig. 2; Supplementary Material, Table S1; Table S2). Nematode batches that met both experimental criteria were used for further experiments (66 % of *S. carpocapsae* and 100 % of *S. feltiae*).

Besides, in the two-choice behavioral assay when checking *S. feltiae* and *S. carpocapsae* response to different dilutions of ethanol and those of 1-nonene, control ethanol *vs.* ethanol was included. Distribution of IJs of both species in the scoring circles was equal and no statistically significant differences were recorded (Fig. 1; Fig. 2; Supplementary Material, Table S1; Table S2).

3.2. EPN response to ethanol: S. feltiae

In the two-choice behavioral test (ethanol *vs.* water), undiluted and 10 times diluted ethanol was attractive to IJs ($p < 0.01$ and $p < 0.05$ correspondingly), however, 100 times diluted lost this feature (Fig. 1; Supplementary Material, Table S1). The EPN response to undiluted ethanol was nearly identical to the response to *G. mellonella* larva: 93.5 % and 91.7 % of IJs chose the stimulus respectively (Fig. 1; Supplementary Material, Table S1). Ten times diluted ethanol was slightly less attractive to IJs than undiluted ethanol as 75.2 % of nematodes chose the stimulus. Attractivity of 100 times diluted ethanol disappeared and no statistically significant difference to that *vs.* water control was recorded. Thus, 100 times diluted ethanol was neither attractive nor repellent to *S. feltiae* IJs (Fig. 1; Supplementary Material, Table S1). In summary, the solvent of 1-nonene – ethanol was attractive to *S. feltiae* IJs at high concentrations.

3.3. EPN response to ethanol: S. carpocapsae

Undiluted, 10 times, and 100 times diluted ethanol was neither attractive nor repellent to *S. carpocapsae* IJs as the response to ethanol was not significantly different from the water control: a similar percentage of IJs chose the stimulus *i.e.*, 53.9 %, 58.4 %, and 52.4 % over control (Fig. 1; Supplementary Material, Table S1).

3.4. EPN response to 1-nonene: S. feltiae

The highest concentrations of 1-nonene tested (1 M, 500 mM, and 200 mM) were strongly repellent to *S. feltiae* IJs, as most of the EPNs avoided the scoring circle with the stimulus, and over 90 % of the nematodes chose control scoring circle (Fig. 2; Supplementary Material, Table S2). Differences between the response to these concentrations and ethanol as control were statistically significant ($p < 0.001$) (Fig. 2; Supplementary Material, Table S2). Twenty mM 1-nonene concentration was significantly repellent as well, with only 14.7 % of nematodes choosing the stimulus side ($p < 0.01$). Another 10 times and more reduced concentrations (200 μM, 20 μM, and 0.02 μM) of 1-nonene were neither repellent nor attractive to *S. feltiae* IJs – the repellency effect disappeared, and no statistical differences were observed in the EPN response to the stimulus over control. Furthermore, low concentrations of 1-nonene, such as 2 μM and 0.2 μM, became even attractive to *S. feltiae* IJs, as statistically significant differences were recorded compared to water control ($p < 0.01$) and the attractiveness was like that demonstrated towards *G. mellonella* larva. Finally, the lower concentration of 1-nonene (0.02 μM) tested was not attractive to *S. feltiae* IJs *i.e.*, the attractivity effect disappeared and no statistically significant differences were recorded in IJs response to the stimulus compared to the control. In summary, the highest concentrations of 1-nonene were repellent to *S. feltiae* IJs, and the lower – attractive.

3.5. EPN response to 1-nonene: S. carpocapsae

The highest concentrations of 1-nonene tested (1 M, 500 mM, and 200 mM) were attractive to *S. carpocapsae* IJs, statistically significant differences between these and control were recorded: approximately 80 % of IJs chose 1-nonene over control (ethanol) and attractiveness was similar to that of *G. mellonella* larva (Fig. 2; Supplementary Material,

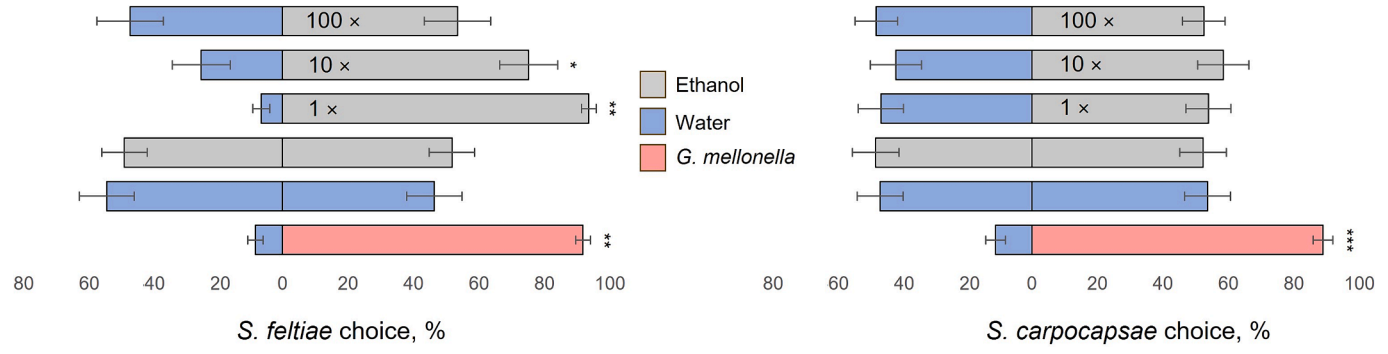

Fig. 1. Response of *Steinernema feltiae* and *S. carpocapsae* infective juveniles (IJs) to different dilutions of ethanol (solvent of 1-nonene). Percentages (mean ± SEM) represent the ratio of IJs choosing the control (water) and stimulus scoring circles. The supernatant of crushed *Galleria mellonella* larva *vs.* water, water *vs.* water, and ethanol *vs.* ethanol served as controls. Statistically significant differences were assessed using the Wilcoxon signed-rank test, *$p < 0.05$; **$p < 0.01$, ***$p < 0.001$, SEM – standard error of the mean.

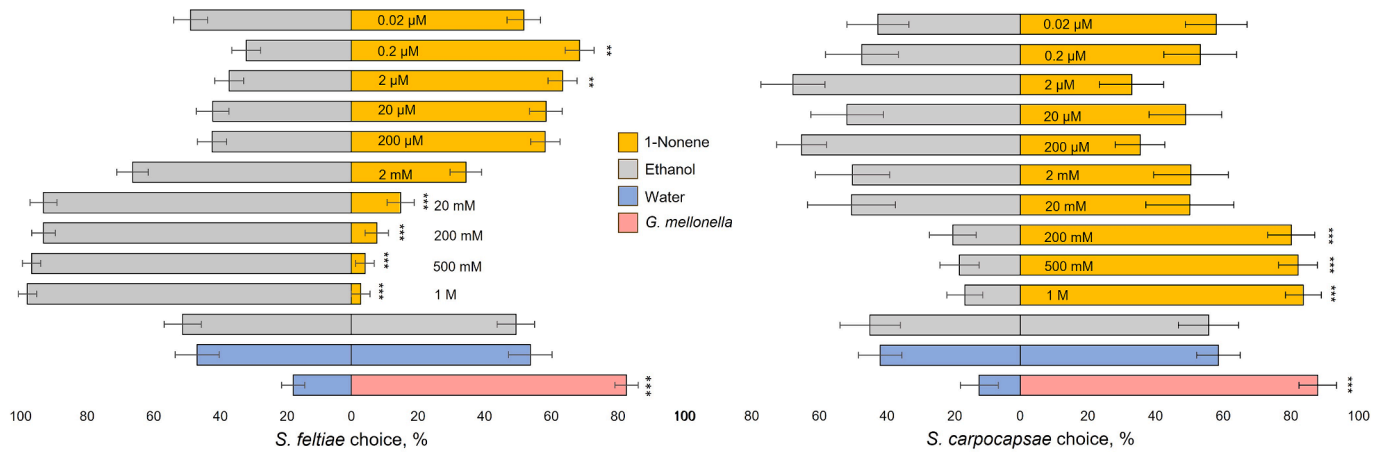

Fig. 2. Response of *Steinernema feltiae* and *S. carpocapsae* infective juveniles (IJs) to different concentrations of 1-nonene. Percentages (mean ± SEM) represent the ratio of IJs choosing the control (undiluted ethanol) and stimulus scoring circles. The supernatant of crushed *Galleria mellonella* larva *vs.* water, water *vs.* water, and ethanol *vs.* ethanol served as controls. Statistically significant differences were assessed using the Wilcoxon signed-rank test, $^{**}p < 0.01$, $^{***}p < 0.001$, SEM – standard error of the mean.

Table S2). *Steinernema carpocapsae* EPNs did not show any preference for lower concentrations of 1-nonene (from 20 µM to 0.02 µM) over ethanol control, as no statistically significant differences were recorded. Thus, the attractiveness was concentration-dependent and only the highest concentrations of 1-nonene were attractive to *S. carpocapsae* IJs.

4. Discussion

The results of the behavioral assay we carried out revealed that ethanol (often used as a polar solvent) is attractive for *S. feltiae* IJs even at high concentrations. To our knowledge, the evidence that undiluted ethanol can induce the same level of behavioral reactions in IJs as a prey cadaver significantly advances the knowledge of the effects of this compound on EPNs, as so far only one case is known where ethanol has a very weak attraction to EPN *H. bacteriophora* (O'Halloran and Burnell, 2003). Besides, ethanol was known as a slight attractant for a single species of free-living nematodes, *Caenorhabditis elegans* (Maupas 1899) Dougherty 1953 (Hallem et al., 2011; Bargmann et al., 1993). The chemical was not recorded among behaviorally active compounds within a big group of nematodes that feed on plants (plant parasitic nematodes) (Čepulytė and Būda, 2022). Data on the fact that ethanol does not affect *S. carpocapsae*'s behavior has been published (Hallem et al., 2011). However, the study was conducted while testing undiluted ethanol only. Our study has demonstrated that the behavioral reaction of IJs of this species does not change in the broad range of ethanol concentrations, from undiluted (before presentation on water-containing substrate) to 100 times diluted. Thus, bioassay carried out in a wide range of concentrations allows us to confirm the results previously published (Hallem et al., 2011) and to state that ethanol is not attractive to IJs of this species. Hence, the effect of ethanol depends on the EPN species: it was attractive for IJs of one species (*S. feltiae*), but completely neutral for another one (*S. carpocapsae*). Whether such an interspecific difference reflects different adaptations to the environment remains unknown. However, it can be assumed that for one species this compound might be an important environmental signal, and not for another.

EPNs in search of prey can detect either an uninfected larva or already infected by certain EPNs (Zhang et al., 2019). Among the chemical compounds that are both released into the environment by nematode-infected insect larvae and are behaviorally active for EPNs are prenol (3-methyl-2-buten-1-ol), 3-hydroxy-2-butanone (AMC) (Kin et al., 2019; Baiocchi et al., 2017), butylated hydroxytoluene (BTH) (Zhang et al., 2019), and dimethyl disulfide (DMDS) (Fu et al., 2021). All compounds are repellent except BTH which is attractive for EPN IJs. Thus, 1-nonene is an extra EPN attractant, which supplements a group of compounds that indicate a prey as already an EPN-infected dead insect larva. Compounds that are secreted by a dead organism and that are beneficial to perceiving organisms are classified as apneumones (Nordlund and Lewis, 1976). However, it is not yet possible to assign 1-nonene to this ecological group of compounds, because its actual source remains unknown: larval cadaver, nematode, bacteria, or even the combination of all three of them. However, it is indirectly evidenced that at least two species of bacteria, namely *Comamonas sediminis* Subhash et al., 2016 and *Pseudomonas monteilii* Elomari et al., 1997 produce and secrete 1-nonene (Wolfgang et al., 2019).

Besides the two types of bacteria mentioned above, it is known that 1-nonene (it has a strong fungi smell) is released by mold fungi *Penicillium chrysogenum* Thom (Wilkins et al., 2000; Matysik et al., 2008) and *P. palitans* Westling (Wilkins et al., 2000), as well as by some plants which include 1-nonene in their essential oils: coltsfoot *Tussilago farfara* L. (Ferrer et al., 2016), common rue *Ruta graveolens* L. (Chaaban et al., 2019), and southern yarrow *Achillea ligustica* All. (Bader et al., 2022). In the future, it would be interesting to check whether bacteria and fungi related to plant root rot can indicate EPNs (by emitting 1-nonene) in the presence of insect larvae suitable for food in their vicinity.

It is noteworthy, that although both EPN species tested respond to 1-nonene, their dose/response profiles differ significantly. When comparing the reactions of the two species of IJs, we assume it is important to carry out the tests under optimal (or close to optimal) conditions for each of them, and we have followed this principle. Only under such conditions one can reveal the potential of the species to respond to test (or biological) stimuli. *Steinernema carpocapsae* juveniles were attracted to high concentrations of 1-nonene, while *S. feltiae* to those up to 10^6 times lower (Fig. 2). It is a considerable difference. Moreover, the concentrations that were attractive to *S. carpocapsae* IJs were repellent to those of *S. feltiae* (Fig. 2). It should be noted that the ethanol used as a control was not attractive for one species (*S. carpocapsae*) and highly attractive for another one (*S. feltiae*) tested. Hence, *S. carpocapsae* was given a choice between 1-nonene and a neutral (behaviorally meaningless) control, but *S. feltiae* was asked to choose between a highly attractive "control" and the same substance in a blend with 1-nonene. The question could be whether it can modify the result? It is important to note that only the lowest concentration of 1-nonene dissolved in ethanol compared to just ethanol (attractive "control") was not significantly attractive to *S. feltiae* IJs (Fig. 2). Therefore, here a "masking" effect was possible, however in the rest of the range of attractive concentrations tested no such effect could have occurred because the IJs moved in the direction of 1-nonene (in ethanol) (Fig. 2).

To find out whether such differences in behavioral responses lead to any differences in prey-seeking in nature, quantitative and temporal characteristics of 1-nonene released from a cadaver are needed as well as extra data on attractivity/temperature effect modification (*e.g.* Lee et al., 2016) if any. In the absence of comprehensive data yet, however, it might be assumed that the concentration of 1-nonene released from cadavers increases after EPN infection. This assumption is supported by measurements before infection, and those a few days after infection when emissions were already quite abundant (Grunseich et al., 2021). Data from our behavioral tests indicate that insect larvae at the early stage of infection could be detected earlier by *S. feltiae* IJs than by *S. carpocapsae* IJs because the former are attracted to much lower concentrations of 1-nonene than the latter. In addition, the optimized conditions in our assays (*e.g.* increased temperature, prolonged assay duration) for a less mobile species *S. carpocapsae* should make the differences in the soil (in the absence of these optimized conditions) even more pronounced.

Furthermore, it was demonstrated that IJs of *S. carpocapsae* were able to colonize older cadavers compared to *S. feltiae* (San-Blas and Gowen, 2008), and assuming that aging cadavers release more 1-nonene, our results on reactions to high concentrations of the compound could provide an explanation for the phenomenon. After a while, when the concentration of 1-nonene increases, the infected cadaver would cease to attract *S. feltiae*, and even at higher concentrations the same infected cadaver would become repellent to them and only then it would become attractive to *S. carpocapsae* IJs. In this situation, *S. carpocapsae* IJs would either be very competitive with juveniles of the other EPN species that had earlier detected and colonized the larva or scavenge the insect larva that is no longer suitable or even become repellent for such EPN species as *S. feltiae*. Since scavenging is suggested as an alternative life strategy for EPNs (Blanco-Pérez et al., 2019; San-Blas and Gowen, 2008), based on our data, one can conclude that *S. carpocapsae* is more likely to scavenge than *S. feltiae*. Although 1-nonene is the only common compound emitted by all tested insect-EPN combinations (Grunseich et al., 2021), the role of the rest compounds co-emitted as blends remains to be investigated.

5. Conclusions

Novel behaviorally active compounds for EPN IJs were revealed. 1-Nonene was attractive to *S. carpocapsae* and ethanol to *S. feltiae,* each compound at high concentrations. Meanwhile, 1-nonene was both attractive and repellent for *S. feltiae* depending on the concentration: low were attractive while high were repellent. Different responses of IJs of two EPN species to compounds secreted by already infected prey larvae indicate interspecific difference in foraging. The attraction of *S. carpocapsae* IJs to the high concentration of volatiles released by larval cadavers of late-stage infection is consistent with the scavenger's feeding strategy. EPN behavior peculiarities could be taken into consideration for EPN application in sustainable pest control.

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CRediT authorship contribution statement

Rasa Čepulytė: Writing – original draft, Supervision, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Deimantė Tiškevičiūtė:** Writing – review & editing, Software, Methodology, Formal analysis, Data curation. **Evelina Osinska:** Methodology, Investigation, Formal analysis. **Vincas Būda:** Writing – review & editing, Validation, Supervision, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2024.105505.

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