An investigation on the chemotactic responses of different entomopathogenic nematode strains to mechanically damaged maize root volatile compounds

Ž. Laznik *, S. Trdan

Dept. of Agronomy, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1111 Ljubljana, Slovenia

**Highlights**

- Chemosensation of IJ varied depending on different factors.
- Time of exposure proved to be a strain specific characteristic.
- Chemosensation is strain specific characteristic.
- Different host searching strategy has no influence on chemotactic response of IJ.
- IJ strains in our experiment showed only a weak attraction to α-caryophyllene.

**Abstract**

Entomopathogenic nematodes (EPNs) respond to a variety of stimuli when foraging. In a laboratory investigation, we tested the chemotactic responses of 8 EPN strains (Steinernema and Heterorhabditis) to three mechanically damaged maize root compounds (linalool, α-caryophyllene and β-caryophyllene). We hypothesized that the EPN directional response to the tested volatile compounds would vary among the species and volatile compound and may be related to foraging strategies. The nematodes with an intermediate foraging strategy (Steinernema feltiae) proved to be less active in their movement toward volatile compounds in a comparison with the ambushers (Steinernema carpocapsae) and cruisers (Steinernema kraussei and Heterorhabditis bacteriophora); β-caryophyllene was found to be the most attractive substance in our experiment. The results of our investigation showed that the cruisers were more attracted to β-caryophyllene than the ambushers and intermediates. The foraging strategy did not affect the movement of the IJs toward the other tested volatile compounds or the control. Our results suggest that the response to different volatile cues is more a strain-specific characteristic than a different host-searching strategy. Only S. carpocapsae strain B49 displayed an attraction to linalool, whereas S. kraussei showed a retarded reaction to β-caryophyllene and α-caryophyllene in our experiment. The EPN strains showed only a weak attraction to α-caryophyllene, suggesting that this volatile compound could not have an important role in the orientation of IJs to the damaged roots of maize plants. These results expand our knowledge of volatile compounds as the cues that may be used by EPNs for finding hosts or other aspects of navigation in the soil.

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**1. Introduction**

Since the domestication of maize, *Zea mays* (L.) approximately 5000–7000 years ago (Sluyter and Dominguez, 2006), this crop has been targeted by a variety of arthropod pests, often causing tremendous losses in yield (Oerke, 2006). In nature, plants have evolved various defense strategies to fend off herbivorous attackers either directly or indirectly (Kessler and Baldwin, 2002). Many plants release volatiles in response to herbivore attack (Paré and Tumlinson, 1997), and such volatile organic compounds (VOCs) can attract predatory arthropods (Turlings et al., 1995), entomopathogens (Rasmann et al., 2005; Ali et al., 2011) and/or repel herbivores (Heil and Silva-Bueno, 2007). Among the proposed
inducible defenses is the production and release of volatile chemicals that could serve as signals to attract the natural enemies of the herbivores (Dicie and Sabelis, 1988; Turlings et al., 1990).

Entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae are effective biological control agents of soil and above-ground pests (Koppenhöffer et al., 2004; Lazenik et al., 2010b). The broad host range and high host virulence of EPNs make them amenable for inundative insect pest control (Koppenhöffer et al., 2004; Lazenik et al., 2010a, 2010b; Lazenik and Trdan, 2011). Only the resistant, third-stage, infective juveniles (IJ$s) of these EPNs are free living and non-feeding in the soil, and it is during this stage of development that the animal seeks a host insect. Having located a potential host, these nematodes usually enter the insect through a natural opening, such as the mouth, anus, or spiracles, after which host death usually occurs within 24–48 h (Wouts, 1991). The IJ host-finding strategy differs from species to species (Lewis, 2002), and the foraging strategies used by IJs to find a host vary along a continuum between ambush (Steinernema carpocapsae), intermediate (Steinernema feltiae) and cruise foraging (Heterorhabditis bacteriophora) (Lewis, 2002).

The cruisers allocate much of their time scanning for resource-associated cues as they move through their environment, exhibiting only brief pauses, and are, therefore, more effective at finding sedentary and cryptic hosts (Lewis, 2006; Ali et al., 2011). In contrast, the ambush foragers allocate little time to active movement and scan for volatile cues during long pauses (Campbell and Gaugler, 1997; Ali et al., 2011); they are thought to wait for resources to come to them, thus increasing their effectiveness at finding highly mobile prey. H. bacteriophora IJs are cruisers that move through the soil, actively approaching their host by chemotaxis, whereas S. carpocapsae IJs are ambushers that remain relatively stationary and rise up on their tails, a behavior known as nictation, to facilitate their attachment to passing hosts (Lewis et al., 2006). S. feltiae has an intermediate foraging strategy, and these nematodes raise their bodies off the substrate more frequently than other non-nictating species (Campbell and Gaugler, 1997). Steinernema kraussei is thought to adopt a cruiser foraging strategy (Campbell et al., 2003) that is particularly suitable for finding subterranean sedentary insects, such as Hyllobius abietis (Torr et al., 2007).

Chemosensation and chemotaxis are essential processes in the survival of both free-living and parasitic animals. Animals rely on chemical signals in their environment to detect food sources, potential hosts, noxious compounds, reproductive partners and, occasionally, to enable them to choose between alternative developmental stages (Prasad and Reed, 1999). Chemosensation is the main sensory mode used by nematodes to orient themselves to their hosts. IJs have been shown to respond to both CO$_2$ and other cues (Lewis, 2002). There are reports that IJs move toward or away from host excretory products (Grewal et al., 1993), bacterial symbionts (Pye and Burman, 1981), changes in pH (Pye and Burman, 1981), temperature (Burman and Pye, 1980), electrical field (Shapiro-Ilan et al., 2012) and various plant volatile compounds (Boff et al., 2002; Rasmann et al., 2005; Ali et al., 2011).

Here, we describe our study of the chemotactic behavior of S. feltiae (Filipjev) (strain B30, strain C76, and strain 3162), S. carpocapsae Weiser (strain B49, strain C67, and strain C101), S. kraussei (Steiner) (strain C46) and H. bacteriophora Poinar (strain D54) toward linalool, α-caryophyllene and β-caryophyllene, compounds released from the mechanically damaged root systems of different Zea mays hybrids (Lazenik et al., 2011). In a related study, Ali et al. (2010) reported that mechanically damaged citrus roots attracted less nematodes than insect-damaged roots. The aims of our research were (1) to study the effect of different foraging strategies (ambush, intermediate or cruise) of EPNs to the tested volatile compounds, (2) to determine whether chemotaxis is species and strain specific and (3) to assess whether the volatile compounds from mechanically damaged maize roots have any behavioral effect on the studied entomopathogenic nematodes.

2. Material and methods

2.1. Source and maintenance of entomopathogenic nematodes

Eight strains of EPNs were included in the experiment. All of the strains of EPNs were isolated from the soil. S. feltiae strains (B30 and C76), S. carpocapsae strains (B49, C67, and C101), S. kraussei strain C46 and H. bacteriophora strain D54 were isolated in Slovenia (Lazenik and Trdan, 2011), and S. feltiae strain 3162 was isolated in Hungary (Tóth, 2006). All of the strains used in the experiments were tested against different insect pests in our previous work (Lazenik and Trdan, 2011). All of the EPN strains were reared using the final-instar larvae of Galleria mellonella (L.) (Lepidoptera: Pyralidae) (Bedding and Akhurst, 1975). G. mellonella production was executed in a rearing chamber (RK-900 CH, Kambič Laboratory equipment, Semič, Slovenia) at 28 ± 2 °C and 60% relative humidity (RH) and 12 h photoperiod (Lazenik and Trdan, 2011). The IJs were stored at 4 °C at a density of 2000 Jl ml$^{-1}$. We used only IJs that were less than two weeks old (Gutiérrez et al., 2008). The concentration of the EPN suspension was according to Lazenik et al. (2010a, 2010b). The nematode viability was determined prior to the initiation of the chemotaxis experiment (Lazenik et al., 2012), and only nematode stocks with >95% survival were used (De Nardo and Grewal, 2003).

2.2. Chemotaxis assay

The chemotaxis assay was based on the assay developed by Ward (1973) and O’Halloran and Burnett (2003). The assay plates used were 9 cm diameter Petri dishes containing 25 ml of 1.6% technical agar (Biolife, Milano, Italy), 5 mM potassium phosphate (pH 6.0), 1 mM CaCl$_2$ and 1 mM MgSO$_4$. Three circular marks (1 cm in diameter) were made on the bottom of the plate: first in the center, then on the right and last on the left side of the Petri dish, 1.5 cm from its edge. A 50 µl drop of 100 IJs was placed in the center of the agar surface. A 10 µl drop of linalool (95% pure; Fluka), α-caryophyllene (98% pure; Fluka) or β-caryophyllene (98% pure; Fluka) (O’Halloran and Burnett, 2003; Kollner et al., 2008) was then placed on the right side of the agar surface, and 10 µl of M9 buffer (control) (Zwilling, 1998) was placed on the left side of the agar plate. Each treatment included five replicates. All of the experiments were repeated 3 times. The Petri dishes were sealed with PARAFILM® and placed in a rearing chamber (RK-900 CH, Kambič Laboratory equipment, Semič, Slovenia) at 25 °C and 75% RH, without light. The nematodes were allowed to move freely for 3 h or 22 h, and the Petri dishes were then placed in a freezer at −20 °C for 3 min to immobilize the nematodes. The number of nematodes in the treatment and control areas were counted using a binocular microscope (Nikon C-PS) at 25× magnification. The volatile compounds were applied to the agar plates immediately prior to the application of the nematodes (Bargmann et al. 1993). The specific chemotaxis index (CI) (Bargmann and Horvitz, 1991) was calculated as follows:

\[
\text{Number of nematodes in the treatment area} - \text{Number of nematodes in the control area} \div \text{Total number of nematodes in the assay}
\]

The chemotaxis index could vary from 1.0 (perfect attraction) to −1.0 (perfect repulsion). In the experiments reported here, the compound with a chemotaxis index are described as follows: ≥0.2, attractive; from 0.2 to 0.1, a weak attractant; from 0.1 to −0.1, no effect; from −0.1 to −0.2, a weak repellent and ≤−0.2, a repellent to entomopathogenic nematodes.
2.3. Statistical analysis

For all of the treatments and controls, the preferential movement of the nematodes from the inner to outer circle of the Petri dish (i.e., a directional response) was determined through a paired t-test comparing the number of IJs in the inner versus the outer circle (Statgraphics Plus for Windows 4.0; Shapiro-Ilan et al., 2012; \( \alpha = 0.05 \)). Additionally, to compare the level of response among the foraging strategies, the average number of IJs that moved to the outer circle or stayed in the inner circle was calculated for each dish, and average numbers were compared through an analysis of variance (ANOVA, \( \alpha = 0.05 \)). Additionally, an analysis of variance (ANOVA) was performed on the chemotaxis index to compare the level of response to the tested volatile compounds among the different EPN species depending on the exposure time, and the means were separated by Duncan’s multiple range test with a significant level of \( p \leq 0.05 \). The data are presented as the mean ± S.E. All of the statistical analyses were performed using Statgraphics Plus for Windows 4.0 (Statistical Graphics Corp., Manugistics, Inc., Rockville, MD, USA), and the figures were generated using MS Office Excel 2010.

### Results

#### 3.1. Diversity of movement among EPN strains and their foraging strategies

The analyses of the pooled results showed that directional movement in response to volatile compounds from the inner to outer test circles were influenced by different factors and their interactions (Table 1). Based on the t-test results (\( t = 9.64; \)

<table>
<thead>
<tr>
<th>Source</th>
<th>F</th>
<th>df</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foraging strategy</td>
<td>26.45</td>
<td>2</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Volatile compound</td>
<td>12.99</td>
<td>3</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Time of exposure</td>
<td>32.95</td>
<td>1</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Temporal replication</td>
<td>0.38</td>
<td>4</td>
<td>0.8264</td>
</tr>
<tr>
<td>Spatial replication</td>
<td>0.48</td>
<td>2</td>
<td>0.7501</td>
</tr>
<tr>
<td>Foraging strategy × volatile compound</td>
<td>2.67</td>
<td>6</td>
<td>0.0142*</td>
</tr>
<tr>
<td>Foraging strategy × time of exposure</td>
<td>6.97</td>
<td>2</td>
<td>0.0010*</td>
</tr>
</tbody>
</table>

\* The source of variation was significant at \( \alpha = 0.05 \).

Fig. 1. Average number of infective juvenile nematodes (IJs) that moved from the center of the agar plate to the outer side of the plate during 22 h. Graph A shows the movement of the different EPN strains independent of the tested volatile compound. Graphs B through E show the movement of different EPN strains depending on the tested volatile compound. B30, C76, 3162 = Steinernema feltiae; B49, C67, C101 = Steinernema carpocapsae; C46 = Steinernema kraussei; D54 = Heterorhabditis bacteriophora. Intermediate = Steinernema feltiae; Ambusher = Steinernema carpocapsae; Cruiser = Steinernema kraussei and Heterorhabditis bacteriophora.


3.2. Chemotaxis index

The analyses of the pooled results showed that the chemotaxis index values were influenced by the EPN strain (\(F = 13.62; \text{df} = 7, 63; p < 0.0001\)), volatile compound (\(F = 36.16; \text{df} = 3, 63; p < 0.0001\)), and interaction between the EPN strain and volatile compound (\(F = 10.91; \text{df} = 21, 63; p < 0.0001\)). The time of exposure (\(F = 3.05; \text{df} = 1, 63; p = 0.0951\)), interaction between EPN strain and time of exposure (\(F = 2.39; \text{df} = 7, 63; p = 0.0574\)), and interaction between the volatile compound and time of exposure (\(F = 1.98; \text{df} = 3, 63; p = 0.1471\)) did not have a statistically significant influence on the chemotaxis index values.

The chemotactic response of the EPN strains was statistically significant for the different volatile compounds. The general analysis showed that \(\beta\)-caryophyllene (\(CI = 0.14 \pm 0.02\)) had a weak attraction for the EPN strains, however there were no chemotactic responses of the EPN strains to \(\alpha\)-caryophyllene (\(CI = 0.06 \pm 0.03\)), linalool (\(CI = 0.05 \pm 0.02\)) or the control (\(CI = 0.02 \pm 0.03\)) (Figs. 2–5). The chemotaxis index values were influenced by the different EPN strains: S. carpocapsae strain B49 (\(CI = 0.15 \pm 0.02\)) and H. bacteriophora strain D54 (\(CI = 0.12 \pm 0.01\)) were considered to display a weak attraction to the tested volatile compounds. In contrast, the other tested strains did not show any chemotactic response to the tested volatiles, with the values of the chemotaxis index ranging from 0.02 \pm 0.01 (S. feltiae strain C76) to 0.07 \pm 0.03 (S. kraussei strain C46). The time of exposure did not have an influence on the chemotactic response of the EPN strains, with the values of the chemotaxis index ranging from 0.06 \pm 0.02 (3 h) to 0.07 \pm 0.03 (22 h).

3.3. The chemotactic response of entomopathogenic nematode strains to \(\beta\)-caryophyllene

\(\beta\)-caryophyllene proved to be an attractant to H. bacteriophora strain D54 (\(CI = 0.30 \pm 0.04\)) and S. carpocapsae strain B49 (\(CI = 0.29 \pm 0.03\)), whereas S. kraussei strain C46 (\(CI = 0.17 \pm 0.02\)) demonstrated only a weak attraction to \(\beta\)-caryophyllene (Fig. 2). The other tested strains did not show any chemotactic response to \(\beta\)-caryophyllene, and the values of the chemotaxis index ranged from 0.03 \pm 0.04 (S. feltiae strain 3162) to 0.09 \pm 0.02 (S. feltiae strain C76). Among the S. feltiae strains, there were no significant differences in the attraction to \(\beta\)-caryophyllene after 3 h (\(p = 0.6721\)) and 22 h (\(p = 0.8541\)). The time of exposure (\(p = 0.0012\)) proved to have an influence on the attraction of S. kraussei strain C46 to \(\beta\)-caryophyllene: strain C46 showed a weak attraction after 3 h (\(CI = 0.1 \pm 0.03\)), whereas the attraction after 22 h (\(CI = 0.27 \pm 0.02\)) proved to be stronger. Among the different S. carpocapsae strains, statistically significant differences (3 h, \(p = 0.0012\); 22 h, \(p = 0.0008\)) in the attraction to \(\beta\)-caryophyllene were found only for strain B49 (3 h, CI = 0.28 \pm 0.03; 22 h, CI = 0.30 \pm 0.03), whereas strains C67 and C101 showed no chemotactic response (Fig. 2).

3.4. The chemotactic response of entomopathogenic nematode strains to \(\alpha\)-caryophyllene

The results showed that \(\alpha\)-caryophyllene was only a weak attractant to H. bacteriophora strain D54 (\(CI = 0.16 \pm 0.03\)), S. feltiae strain B30 (\(CI = 0.11 \pm 0.03\)) and S. kraussei strain C46 (\(CI = 0.10 \pm 0.03\)), with the other tested strains not showing any chemotactic response; the values of chemotaxis index ranged from −0.04 \pm 0.03 (S. feltiae strain 3162) to 0.08 \pm 0.03 (S. carpocapsae strain C67) (Fig. 3). Statistically significant differences (3 h: \(p = 0.0132\); 22 h: \(p = 0.0092\)) among the different S. feltiae strains in the attraction to \(\alpha\)-caryophyllene were found only for strain B30 (3 h, CI = 0.09 \pm 0.03; 22 h, CI = 0.13 \pm 0.03); strains C76 and 3162 showed no chemotactic response. The time of exposure (\(p = 0.0231\)) demonstrated an influence on the attraction of S.
kraussei strain C46 to α-caryophyllene. After 3 h, strain C46 showed no attraction (CI = 0.06 ± 0.03), whereas the chemotactic response after 22 h (CI = 0.16 ± 0.03) was weak. Among the different S. carpocapsae strains, statistically significant differences (22 h: \( p = 0.0432 \)) in the attraction to α-caryophyllene were found only for strain B49 (CI = 0.12 ± 0.01), whereas strains C67 and C101 showed no chemotactic response (Fig. 3).

3.5. The chemotactic response of entomopathogenic nematode strains to linalool

As shown in Fig. 4, linalool proved to be an attractant to S. carpocapsae strain B49 (CI = 0.23 ± 0.03), whereas S. carpocapsae strain C101 (CI = 0.12 ± 0.03) and S. feltiae strain 3162 (CI = 0.11 ± 0.02) were only weakly attracted by linalool. The other tested strains did not show any chemotactic response to linalool, and the values of the chemotaxis index ranged from −0.03 ± 0.03 (S. feltiae strain B30) to 0.04 ± 0.03 (S. carpocapsae strain C67). Only strain 3162 (22 h: CI = 0.13 ± 0.01) among the different S. feltiae strains showed a weak attraction to linalool; strains B30 and C76 showed no chemotactic response. Among the different S. carpocapsae strains, only strain B49 (3 h, CI = 0.24 ± 0.04; 22 h, CI = 0.23 ± 0.03) showed an attraction to linalool, with the other strains, C67 and C101, showing no chemotactic response (Fig. 4).

4. Discussion

The results of our current laboratory investigation showed that the movement and chemosensation of IJs toward and away from damaged maize root volatile compounds (Laznik et al., 2011) varied depending on the species, strain, foraging strategy, volatile compound and interaction between the EPN strain and volatile compound. The intermediate foragers (S. feltiae) proved to be less active in their movement toward the volatile compounds in comparison with the ambushers (S. carpocapsae) and cruisers (S. kraussei and H. bacteriophora); α-caryophyllene proved to be the most attractive compound of the three substances tested in our experiment. The results of our investigation...
showed that the cruisers were more attracted to β-caryophyllene than the ambushers and intermediates. The foraging strategy did not influence the IJ movement toward the other tested volatile compounds and the control. Similar conclusions were also reported in the recent research of Ali et al. (2011) in which the ambusher *S. carpocapsae* (Lewis, 2002), the cruiser *Heterorhabditis indica* (Lewis, 2002), and two species thought to exhibit an intermediate foraging strategy (Lewis, 2002) were all attracted to *Diaprepes abbreviatus*-damaged roots of *Swingle* rootstock. Some related studies on the foraging strategies of EPNs have been conducted in non-soil systems (O’Halloran and Burnett, 2003), however we are aware that such studies do not reflect the nematode’s true behavior in nature, whereby they are exposed to a myriad of conflicting chemical signals (Hui and Webster, 2000; Hiltpold and Turlings, 2008). In our experiment, pure compounds were applied to agar (O’Halloran and Burnett, 2003; Köllner et al., 2008), which does not reflect the concentration near the roots of plants (Köllner et al., 2004). Köllner et al. (2004) reported that the total sesquiterpene hydrocarbon content in the herbivore-damaged roots of *Zea mays* was 81 ng g⁻¹, whereas the control plants contained only 25 ng g⁻¹, and the relative amount of β-caryophyllene among several other different terpenes in the maize roots was less than 5%. Moreover, Ali et al. (2010) reported that roots damaged by insect larvae attracted more nematodes than mechanically damaged roots and sand controls. The speed of the nematode’s response to the chemical stimuli in its natural environment largely depends on the diffusion rate of the chemical compound and on the soil structural heterogeneity (Anderson et al., 1997). When a foraging nematode is confronted with an array of signals originating from the same general area, the response may depend on the strength and exposure time and on the nature of the stimuli (Hui and Webster, 2000).

Lewis (2002) reviewed the literature on foraging and host recognition in *Heterorhabditis* and *Steinernema* IJs and proposed that ambusher nematodes respond to host (insect) cues in a hierarchical order, with the volatile cues only becoming important after the IJ had made contact with the insect cuticle, whereas remote volatile cues are more important for cruiser nematodes. Several related studies have also shown that IJs exhibit a preference for different volatile root compounds (Hui and Webster, 2000; Boff et al., 2002; Rasmann et al., 2005; Ali et al., 2010, 2011). β-caryophyllene is a common compound and has been identified from various plant species (Rasmann et al., 2005; Helmsg et al., 2007; Hiltpold and Turlings, 2008; Laznik et al., 2011), however its function, as for most plant volatiles, remains unclear. As Rasmann et al. (2005) reported that β-caryophyllene strongly attracted *Heterorhabditis megidis*, attraction has been confirmed for all of the tested species, with the exception of *S. feltiae*.

Our results suggest that the response to different volatile cues is more a strain-specific characteristic than a different host-searching strategy. Similar conclusions were also made in the research of Ali et al. (2010, 2011). Indeed, *H. bacteriophora* and *S. carpocapsae* strain B49 showed strong chemotaxis to β-caryophyllene, whereas the other two isolates of *S. carpocapsae* hardly reacted. A similar conclusion can be made with regard to linalool, with only *S. carpocapsae* strain B49 showing an attraction to this volatile compound from damaged maize roots. One reason for the attraction of *S. carpocapsae* strain B49 to linalool and β-caryophyllene may relate to its origin, as this strain was isolated in a grassland near a maize field (Laznik et al., 2009), supporting the theory of Stuart and Gaugler (1996) who concluded the possible genetic adaptation of EPNs to different biotic and abiotic factors. In related work, Nguyen and Duncan (2002) reported that specialization rather than the foraging strategy may better explain the attraction of EPNs to different volatile compounds. The EPN strains in our experiment showed only a weak attraction to α-caryophyllene, suggesting that this compound could not have an important role in the orientation of IJs to the damaged roots of maize plants. *S. kraussei* showed a retarded reaction to both β-caryophyllene and α-caryophyllene in our experiment, suggesting a different host (insect) cue hierarchical order than the other cruisers (*H. bacteriophora*), with the volatile cues only becoming important after a long exposure. Reflecting an increasing interest in the belowground plant-mediated interactions and their effects on various trophic levels (Cutler and Webster, 2003; Rasmann et al., 2005; Hiltpold and Turlings, 2008; Ali et al., 2011), our results suggested several questions to be further addressed for the understanding of the plant volatile-induced signal orientation of different EPN species and strains. In addition, further knowledge is necessary to determine whether the roots of surrounding plants elicit chemical repellents that may adversely affect the host-finding ability of foraging nematodes.

**Fig. 5.** Effect of the time of exposure on the chemotactic response of the EPN strains to M9 buffer. Each data point represents the mean chemotaxis index ± S.E. The bars with the same letter are not significantly different (P > 0.05). The small letters indicate statistical significant differences among the different EPN strains at the same time of the exposure. The capital letters indicate statistically significant differences among the different times of exposure for the same EPN strain. B30 = *Steinernema feltiae*, C76 = *S. feltiae*, 3162 = *S. feltiae*, B49 = *S. carpocapsae*, C67 = *S. carpocapsae*, C101 = *S. carpocapsae*, C46 = *S. kraussei*, D54 = *Heterorhabditis bacteriophora*.  

![Effect of time exposure on chemotactic response of EPN strains to M9 buffer](image_url)
Acknowledgments

This work was conducted within Horticulture Project P4-0013-0481, a program funded by the Slovenian Research Agency. Part of the research was funded within the national project CRP V4-1067, which is funded by the Slovenian Research Agency. Ministry of Agriculture, Food, and Forestry of the Republic of Slovenia, and Ministry of Environment and Spatial Planning of the Republic of Slovenia, and within Professional Tasks from the Field of Plant Protection, a program funded by the Ministry of Agriculture, Forestry, and Food of Phytosanitary Administration of the Republic of Slovenia. Special thanks are given to Dr. Támas Lakatos and Dr. Timea Tóth for providing the Steinernema feltiae strain 3162 and Melita Strukelj for technical assistance.

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