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Attraction Behaviors of Entomopathogenic Nematodes (Steinernematidae and Heterorhabditidae) to Synthetic Volatiles Emitted by Insect Damaged Potato Tubers

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Abstract Entomopathogenic nematodes (EPNs) play a role in indirect defense of plants under attack by root herbivores. Several investigations have shown that EPNs are attracted or repelled by various volatile compounds (VOCs) released from insect damaged plant roots. We hypothesized that the directional responses of EPNs to the VOCs would be affected by foraging strategy and would vary among species, VOC type, and VOC concentrations. We tested the chemotactic responses of four commercial EPN species (*Steinernema feltiae*, *S. carpocapsae*, *S. kraussei*, and *Heterorhabditis bacteriophora*) to seven compounds released from insect (*Melolontha hippocastani*)-damaged (decanal, nonanal, octanal, undecane, 6-methyl-5-hepten-2-one, and 1,2,4-trimethylbenzene) and undamaged (2-ethyl-1-hexanol) potato tubers. Our results suggest that EPNs are able to distinguish herbivore-induced VOCs from those that are typical for healthy potato tubers. In our investigation, nonanal, octanal, and decanal had a greater influence on the movement of EPNs than other tested synthetic volatiles. Decanal was an attractant for *H. bacteriophora* and *S. kraussei* at both tested concentrations (as a pure compound and at a concentration of 0.03 ppm). The results suggest that the susceptibility to perception of chemical stimuli from the environment is a species-specific characteristic that prevails over the influence of the foraging strategy.

Keywords Attraction behavior · Entomopathogenic nematodes · Decanal · Nonanal · Octanal · Undecane · 6-methyl-5-hepten-2-one · 1,2,4-trimethylbenzene · 2-ethyl-1-hexanol

Introduction

Plants are attacked by insects that chew and suck on plant parts, diminishing their vitality. In response to attacks by herbivores, plants produce a wide variety of volatile compounds (VOCs) (Rasmann et al. 2005; Rasmann and Turlings 2008; Takabayashi and Dicke 1996). The release of VOCs by insect-damaged plants proved to be specific to the damaging insects and the plant species (Ali et al. 2010; Gosset et al. 2009; Rasmann et al. 2005; Rasmann and Turlings 2008). Ali et al. (2010) have demonstrated that citrus roots upon feeding by the root weevil *Diaprepes abbreviatus* emit several terpenes into the surrounding soil. Weissteiner (2010) reported that potato tubers, damaged by the larvae of the forest cockchafer *Melolontha hippocastani* release several compounds, such as decanal, nonanal, octanal, undecane, 6-methyl-5-hepten-2-one, and 1,2,4-trimethylbenzene. In contrast, undamaged potato tubers release 2-ethyl-1-hexanol.

The forest cockchafer is a European scarab beetle of the genus *Melolontha* (Scarabaeidae). The root-feeding larvae of scarab beetles are called white grubs, which are among the most destructive pests of horticultural plants, pastures, and turfgrasses in many parts of the world (Laznik and Trdan 2015). White grubs injure crops by feeding on the root systems after planting and significantly reduce crop quality (Jackson and Klein 2006).

Soil is the natural habitat of entomopathogenic nematodes (EPNs) (Steinernematidae and Heterorhabditidae) (Koppenhöffer et al. 2004; Shapiro-Ilan et al. 2006). In both

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Steinernema and *Heterorhabditis*, there is a single free-living stage, the infective juvenile (IJ), that carries in its gut bacteria of the genus *Xenorhabdus* and *Photorhabdus*, respectively (Boemare et al. 1993). Encountering a suitable host, IJs usually enter the insect via natural openings, and once inside, they release their symbiotic bacteria, which multiply and produce toxins that kill the insect within 24 to 72 h after infection (Stock 2015).

As a species-specific characteristic, different strategies to approach hosts have evolved in EPNs (Campbell et al. 2003; Lewis 2002). For instance, *Heterorhabditis bacteriophora* and *Steinernema kraussei* actively search for hosts (cruisers), while some species wait for a host in an ambush (ambushers) like *S. carpocapsae*. Some species such as *S. feltiae* combine various tactics in the search for a host and are categorized as intermediates (Campbell et al. 2003; Lewis 2002). However, previous research on their behavior did not consider the natural habitat of the nematodes. Kruitbos et al. (2009) suggested that EPNs may be habitat specialists, and they highlighted the difficulties of studying soil-transmitted parasites in non-soil media. Wilson et al. (2012) proposed that many species will show different behavior depending on the substrate in which they forage, and these differences may be related to different volatile signals (e.g., VOCs, CO_2) used by the EPN as foraging cues.

Tritrophic interactions, which include a host plant, a harmful organism, and a natural enemy have been documented only recently for the belowground parts of plants (Ali et al. 2010; Hiltpold et al. 2013; Rasmann et al. 2005; Turlings et al. 2012). Volatile secondary metabolites, emitted belowground enable plants to directly and indirectly influence the community of soil-dwelling organisms (Bais et al. 2006; Erb et al. 2013). Previous studies have shown that damaged roots of different plant species release VOCs into the environment that influence the movement of EPNs, both as attractants (Hallem et al. 2011; Laznik and Trdan 2013; Rasmann et al. 2005) and as repellents (Hallem et al. 2011; Laznik and Trdan 2016). Rasmann et al. (2005) reported that maize roots, damaged by larvae of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae, known commonly as western corn rootworm [WCR]), emit (E)-β-caryophyllene as a key attractant for EPNs. This sesquiterpene hydrocarbon proved to be a weak attractant for *H. megidis*, one of the most infectious nematode against WCR. Using VOCs, plants can defend themselves against herbivores and plant pathogenic fungi and bacteria, support beneficial symbiosis, and combat competitive plant species (Bais et al. 2006).

Chemotaxis is the main sensory mode that nematodes use to orient towards their hosts. Infective juveniles have been shown to respond to CO_2 and other cues (Dillman et al. 2012; Hallem et al. 2011; Turlings et al. 2012). There are reports that IJs move to or away from host excretory products, depending on changes in pH, temperature, bacterial symbionts, electrical fields, plant exudates, and various plant VOCs (Burman and Pye 1980; Grewal et al. 1993; Hiltpold et al. 2014; Rasmann et al. 2005; Shapiro-Ilan et al. 2012).

Here, we describe the chemotactic behavior of *Steinernema feltiae* (Filipjev), *Steinernema carpocapsae* Weiser, *Steinernema kraussei* (Steiner), and *Heterorhabditis bacteriophora* Poinar towards the following compounds: (1) decanal; (2) nonanal; (3) octanal; (4) undecane; (5) 1,2,4-trimethylbenzene; (6) 6-methyl-5-hepten-2-one; (7) 2-ethyl-1-hexanol. The choice of VOCs used in our investigation was based on the research of Weissteiner (2010). We note that trimethylbenzenes (Richnow et al. 2003) and 2-ethyl-1-hexanol (Vitali et al. 1993) also are known as widespread environmental contaminants.

The aims of our research were: (1) to study the response of EPNs with different foraging strategies (ambushers, intermediates, or cruisers) to synthetic VOCs; (2) to determine whether chemotaxis is species-specific; and (3) to assess whether the tested synthetic VOCs affect EPN behavior.

Materials and Methods

Source and Maintenance of Entomopathogenic Nematodes Four EPN species were tested in the experiments, the commercial preparations of Nemasys (a.i. *S. feltiae*), Nemasys C (a.i. *S. carpocapsae*), Nemasys L (a.i. *S. kraussei*), and Nemasys G (a.i. *H. bacteriophora*) were obtained from BASF. All preparations were reared using the last instar larvae of *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) (Bedding and Akhurst 1975). The IJs were stored at 4 °C at a density of 2000 IJ ml^{-1}. We only used IJs that were less than 2 wk. old (Laznik and Trdan 2013). The concentration of the EPN suspension was calculated according to Laznik et al. (2010). Nematode viability was determined prior to initiation of the chemotaxis experiment (Laznik and Trdan 2013), and only nematode stocks with >95 % survival were used (Laznik and Trdan 2016).

Tested Volatile Compounds The choice of VOCs used was based on the research of Weissteiner (2010). GC-MS analysis of VOCs, reported to be released by undamaged and damaged tubers was performed in order to show different volatile patterns induced by chewing grubs. We tested decanal (≥ 97 %, Sigma Aldrich), nonanal (97 %, Sigma Aldrich), octanal (≥ 95 %, Sigma Aldrich), undecane (≥ 99 %, Sigma Aldrich), 1,2,4-trimethylbenzene (98 %, Sigma Aldrich), 6-methyl-5-hepten-2-one (≥ 98 %, Sigma Aldrich), and 2-ethyl-1-hexanol (≥ 99 %, Sigma Aldrich).

Chemotaxis Assay The chemotaxis assay was based on an assay developed by Ward (1973) and O'Halloran and Burnell (2003), and modified by Laznik and Trdan (2013). The assay plates used were Petri dishes, 9 cm diam 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 $MgSO_4$. A sketch of the experimental arena is shown in Fig. 1. Each treatment included five replicates. All experiments were repeated 3 times. Volatiles were tested at two concentrations, (1) as pure compounds (Laznik and Trdan 2016) and (2) at 0.03 ppm (according to Laznik and Trdan (2016) and Weissteiner et al. (2012), the average concentration of the VOCs in soil at a distance of 10 cm from the root system); for details see Fig. 1. The concentration of 0.03 ppm was adjusted by dissolving a pure compound in distilled water. The mixture was agitated in a shaker and immediately used in the bioassay. Petri dishes were placed in a dark rearing chamber (RK-900 CH, Kambič Laboratory equipment, Semič, Slovenia) at 22 °C and 75 % RH. After nematodes had been allowed to move freely for 2 h or 24 h, the Petri dishes were placed in a freezer at −20 °C for 3 min to immobilize the nematodes. The number of individuals in the treatment and control areas were counted with a binocular microscope (Nikon C-PS) at 25 × magnification. The specific chemotaxis index (CI) (Bargmann and Horvitz 1991) was calculated as follows: (Number of nematodes in the treatment area – Number of nematodes in the control area)/Total number of nematodes in the assay.

The CI varied from 1.0 (perfect attraction) to −1.0 (perfect repulsion). In the experiments reported here, compounds with a CI are classified as follows: ≥ 0.2, as attractive; from 0.2 to 0.1, as a weak attractant; from 0.1 to −0.1, no effect; from −0.1 to −0.2, as a weak repellent, and ≤ −0.2, as a repellent to EPNs (Laznik and Trdan 2013).

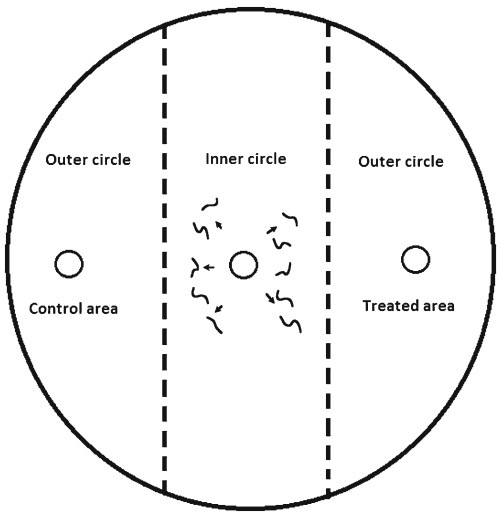

Fig. 1 Experimental arena (Laznik and Trdan 2013). Three circular marks (1 cm diam) were made on the bottom of the plate: first in the center, and then on each side of the Petri dish 1.5 cm from its edge. A 10 μl drop of the tested substance was placed on the right side of the agar surface (treated area), and 10 μl of distilled water (control area) (Laznik and Trdan 2013) was placed on the left side of the agar surface (both parts represent outer circles). The volatile organic compounds (VOCs) were immediately applied to the agar plates before the application of the nematodes (Bargmann and Horvitz 1991). A 50 μl drop of 100 infective juvenile's (IJs) was placed in the center of the agar surface (inner circle). In control treatment 10 μl of distilled water was applied in control and treated area and a 50 μl drop of 100 IJs was placed in the center of the agar surface

Statistical Analysis For all treatments and controls, the preferential movement of nematodes from the inner to the outer circle of the Petri dish (i.e., a directional response) was determined using a paired *t*-test comparing the number of IJs in the inner vs. the outer circle (Statgraphics Plus for Windows 4.0; Shapiro-Ilan et al. 2012; $\alpha = 0.05$). To compare response levels 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, $\alpha = 0.05$). In addition, an analysis of variance (ANOVA) was performed on the CI to compare the level of response to the tested VOCs among the different EPN species depending on the exposure time and concentration, the means were separated by Duncan's multiple range test with a significant level of $p \leq 0.05$ (Laznik and Trdan 2013). The data are presented as the mean ± S.E. All 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

Movement of EPN Species from Inner to Outer Circle The directional movement of nematodes in response to VOCs from the inner to the outer test circle was influenced by various factors (foraging strategy, EPN species, VOC, time of exposure, concentration of VOC), and their interactions (Table 1). Based on the *t*-test results, significant differences were observed between the average percentage of IJs in the inner (67.8 ± 0.9) and the outer (32.8 ± 0.3) circles after 24 h. However, after 2 h, only 2.5 ± 0.4 % of the EPNs moved from the inner to the outer circle. The concentration of VOCs significantly influenced the movement of IJs. Using a pure compound, the percentage of IJs moving from the inner to the outer circle was 19.9 ± 1.1, however, at a concentration of 0.03 ppm, only 15.7 ± 1.0 % of IJs showed this behavior. After 24 h, we observed significantly different host searching strategies in the movement of the IJs, especially in the average percentage of IJs in the outer circle among ambushers (pure compound: 34.2 ± 1.7; 0.03 ppm concentration: 23.9 ± 1.1), cruisers (pure compound: 34.4 ± 1.4; 0.03 ppm concentration: 34.4 ± 2.4), and intermediates (pure compound: 40.1 ± 3.0; 0.03 ppm concentration: 21.7 ± 1.4). We also observed significant differences among the EPN species (Figs. 2 and 3). *Steinernema carpocapsae* was the least mobile species, since only 13.5 ± 1.1 % of IJs moved from the inner to the outer

Table 1 ANOVA results for the directional movement of IJs from the inner to the outer circle

Source	*F*	*df*	*P*
Foraging strategy	11.71	2	<0.001[a]
Species	10.76	3	<0.001[a]
VOC	4.86	7	<0.001[a]
Time of exposure	895.56	1	<0.001[a]
Concentration of VOC	12.46	1	<0.001[a]
Temporal replication	0.85	4	0.872
Spatial replication	0.92	2	0.724
Foraging strategy × VOC	3.02	14	<0.001[a]
Foraging strategy × time of exposure	6.99	2	0.001[a]
Foraging strategy × concentration of VOC	3.34	2	0.036[a]
VOC × time of exposure	1.81	7	0.082
VOC × concentration of VOC	1.47	7	0.175
VOC × species	4.16	21	<0.001[a]
Species × time of exposure	16.83	3	<0.001[a]
Species × concentration of VOC	2.4	3	0.067
Foraging strategy × VOC × concentration of VOC	1.55	14	0.090
Foraging strategy × VOC × time of exposure	5.03	14	<0.001[a]
Species × VOC × concentration of VOC	0.62	21	0.907
Species × VOC × time of exposure	6.35	21	<0.001[a]

[a] The source of variation was significant at $\alpha = 0.05$

circle after 24 h. Among the other species, *H. bacteriophora* (20.01 ± 2.1 %), *S. feltiae* (18.4 ± 1.4 %), and *S. kraussei* (17.7 ± 1.2 %), no significant differences were observed in the movement from the inner to the outer circle. Nonanal (22.4 ± 3.0 %), octanal (19.8 ± 1.8 %), and decanal (18.2 ± 2.1 %) had a greater influence on the movement of EPNs than the other volatiles (Figs. 2 and 3). The influence of decanal on the movement of cruisers at both concentrations after 24 h was much greater than on ambushers and intermediates (Figs. 2 and 3). Pure 2-ethyl-1-hexanol, octanal, and undecane influenced the movement of *S. feltiae* to the outer circle much more than the lower concentration (Fig. 3).

Chemotaxis Index The CI values were influenced by different factors (time of exposure, VOCs, EPN species, foraging strategy) and their interactions (Table 2). The chemotactic response was influenced by various factors. There were differences among the EPN species; for example, the CI ranged from −0.03 ± 0.01 (*S. feltiae*) to 0.04 ± 0.01 (*S. carpocapsae*). Decanal was the VOC with the highest CI (CI = 0.06 ± 0.01), whereas the lowest movement was attributed to the control (CI = −0.02 ± 0.01). The movement was greater after 24 h (CI = 0.03 ± 0.01) than after 2 h (CI = 0.0 ± 0.0). Furthermore, the CI was higher for ambushers (CI = 0.04 ± 0.01) and cruisers (CI = 0.02 ± 0.01) than for intermediates (CI = −0.03 ± 0.01). Concentration of the VOCs did not statistically influence ($P = 0.194$) the CI values ranged from 0.0 ± 0.01 (pure compound) to 0.02 ± 0.01 (0.03 ppm concentration).

After 2 h, the only nematode species showing any behavior response to the tested VOCs was *S. feltiae*. At both concentrations, octanal was a repellent/weak repellent (pure compound: CI = −0.22 ± 0.03; 0.03 ppm concentration: CI = −0.15 ± 0.08) for *S. feltiae*, the only nematode

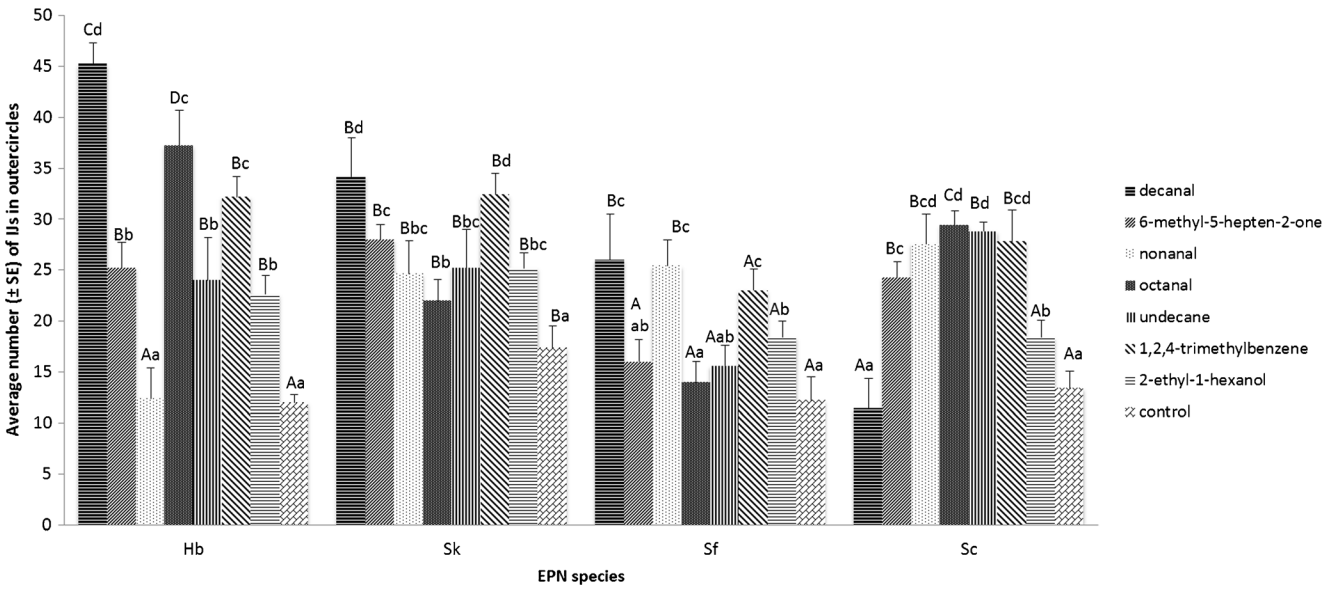

Fig. 2 Average number (±SE) of IJs that moved from the center to the outer sides of the agar plate after 24 h at a 0.03 ppm concentration of the volatile organic compounds (VOCs). Data with the same letter are not significantly different ($P > 0.05$). Capital letters indicate statistically significant differences among different entomopathogenic nematode (EPN) species and same VOCs. Small letters indicate statistically significant differences among different VOCs within the same EPN species. Hb – *Heterorhabditis bacteriophora*; Sk – *Steinernema kraussei*; Sf – *S. feltiae*; Sc – *S. carpocapsae*

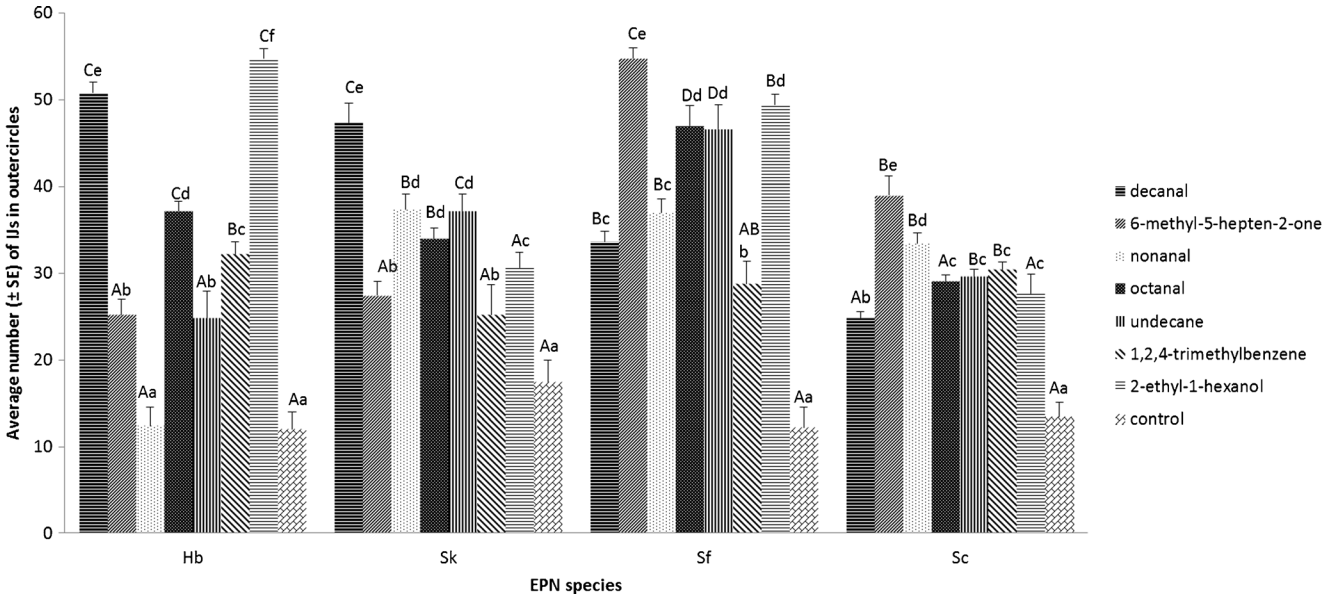

Fig. 3 Average number (±SE) of infective juveniles (IJs) that moved from the center to the outer sides of the agar plate after 24 h using pure compounds. Data with the same letter are not significantly different ($P > 0.05$). Capital letters indicate statistically significant differences among different entomopathogenic nematode (EPN) species and same volatile organic compounds (VOCs). Small letters indicate statistically significant differences among different VOCs within the same EPN species. Hb – *Heterorhabditis bacteriophora*; Sk – *Steinernema kraussei*; Sf – *S. feltiae*; Sc – *S. carpocapsae*

species that was categorized as intermediate. Cruiser and ambusher EPNs showed no behavior response to octanal, a VOC that is typical for grub damaged potato tubers. After 24 h at a concentration of 0.03 ppm, *S. feltiae* showed a behavior response only to 1,2,4-trimethylbenzene (CI = 0.12 ± 0.03) (Table 3). This VOC proved to be a weak attractant only at the lower concentration tested. After 24 h, pure 1,2,4-trimethylbenzene had no behavior effect on the movement of *S. feltiae* (Table 4). Pure undecane (CI = −0.35 ± 0.02), octanal (CI = −0.19 ± 0.06), and decanal (CI = −0.10 ± 0.03), typical for grub damaged potato tubers, proved to be a repellent/weak repellent for *S. feltiae* after 24 h (Table 4). *Steinernema feltiae* was weakly attracted (CI = 0.14 ± 0.01) only to pure 6-methyl-5-hepten-2-one after 24 h of exposure (Table 4).

Table 2 ANOVA results for the CI values

Source	*F*	*df*	*P*
Foraging strategy	21.70	2	<0.001[a]
Species	16.35	3	<0.001[a]
VOC	6.11	7	<0.001[a]
Time of exposure	30.99	1	<0.001[a]
Concentration of VOC	1.89	1	0.170
Temporal replication	0.74	4	0.755
Spatial replication	0.65	2	0.798
Foraging strategy × VOC	7.11	14	<0.001[a]
Foraging strategy × time of exposure	6.83	2	0.001[a]
VOC × time of exposure	6.17	7	<0.001[a]
VOC × species	7.03	21	<0.001[a]
Species × time of exposure	5.38	3	<0.001[a]
Foraging strategy × VOC × time of exposure	5.26	14	<0.001[a]
Species × VOC × time of exposure	6.32	21	<0.001[a]

[a] The source of variation was significant at $\alpha = 0.05$

At a concentration of 0.03 ppm after 24 h, *S. carpocapsae*, the representative of ambusher nematode species, was weakly attracted to several compounds released from grub damaged tubers (6-methyl-5-hepten-2-one, nonanal, octanal, and 1,2,4-trimethylbenzene). Interestingly, none of the tested VOCs at a concentration of 0.03 ppm after 24 h showed any repellent effect on the tested EPN species. Both representatives of cruisers (*S. kraussei* and *H. bacteriophora*) responded differently to VOCs at a concentration of 0.03 ppm after 24 h (Table 3). None of the tested VOCs showed any effect on the movement of *S. kraussei*. In contrast, decanal (CI = 0.30 ± 0.08) and octanal (CI = 0.21 ± 0.04) were attractants for *H. bacteriophora* (Table 3). Interestingly, similar CI values were also confirmed for *H. bacteriophora* for decanal and octanal, when pure compounds were tested after 24 h (Table 4). *Steinernema carpocapsae* was weakly repelled (CI = −0.11 ± 0.06) by 2-ethyl-1-hexanol, a VOC released from undamaged potato tubers (Table 4).

Discussion

We investigated the influence of synthetic samples of VOCs excreted by damaged (injuries were caused by larvae of *M. hippocastani*) and undamaged potato tubers (Weissteiner

Table 3 Effect of different volatile organic compounds (VOCs) on the chemotactic response of the entomopathogenic nematode (EPN) species at a concentration of 0.03 ppm after 24 h. Each data point represents the mean CI ± S.E. Data with the same letter are not significantly different ($P > 0.05$). Capital letters indicate statistically significant differences among different EPN species and the same VOCs. Small letters indicate statistically significant differences among different VOCs within the same EPN species. *Sc* – *Steinernema carpocapsae*; *Sf* – *S. feltiae*; *Sk* – *S. kraussei*; *Hb* – *Heterorhabditis bacteriophora*

	2-ethyl-1-hexanol	Decanal	6-methyl-5-hepten-2-one	Nonanal	Octanal	Undecane	1,2,4-trimethylbenzene	Control
Sc	−0.04 ± 0.06 ABa	0.03 ± 0 Bb	0.16 ± 0.02 Ccd	0.19 ± 0.02 Bd	0.15 ± 0.06 Bcd	0.04 ± 0.04 Aab	0.1 ± 0.05 Bc	−0.02 ± 0.06 Aab
Sf	−0.06 ± 0.05 Aa	0 ± 0.02 Aa	0.04 ± 0.04 Bb	−0.01 ± 0 Aa	0 ± 0.04 Aab	0.02 ± 0.03 Aab	0.12 ± 0.03 Bc	−0.06 ± 0.05 Aa
Sk	0.06 ± 0.05 Babc	0.08 ± 0.05 Bbc	0.03 ± 0.03 Bbc	0 ± 0.04 Aab	−0.02 ± 0.03 Aa	0.04 ± 0.03 Abc	0.08 ± 0.03 Bc	−0.07 ± 0.04 Aa
Hb	−0.09 ± 0.06 Aa	0.3 ± 0.08 Cb	−0.02 ± 0.01 Aa	0 ± 0.06 Aa	0.21 ± 0.04 Bb	0.02 ± 0.08 Aa	−0.03 ± 0.07 Aa	0 ± 0.05 Aa

CI are classified as follows: ≥ 0.2, as attractive; from 0.2 to 0.1, as a weak attractant; from 0.1 to −0.1, no effect; from −0.1 to −0.2, as a weak repellent and ≤ −0.2, as a repellent to EPNs (Laznik and Trdan 2013)

Table 4 Effect of different volatile organic compounds (VOCs) on the chemotactic response of the entomopathogenic nematode (EPN) species to pure compounds after 24 h. Each data point represents the mean CI ± S.E. Data with the same letter are not significantly different ($P > 0.05$). Capital letters indicate statistically significant differences among different EPN species and the same VOCs. Small letters indicate statistically significant differences among different VOCs within the same EPN species. *Sc* – *Steinernema carpocapsae*; *Sf* – *S. feltiae*; *Sk* – *S. kraussei*; *Hb* – *Heterorhabditis bacteriophora*

	2-ethyl-1-hexanol	Decanal	6-methyl-5-hepten-2-one	Nonanal	Octanal	Undecane	1,2,4-trimethylbenzene	Control
Sc	−0.11 ± 0.06 Aa	0.06 ± 0.03 Bbc	0.23 ± 0.03 De	0.16 ± 0.03 Cd	0.12 ± 0.04 Bcd	0.06 ± 0.04 Bbc	−0.04 ± 0.03 Aa	−0.02 ± 0.06 Aab
Sf	−0.05 ± 0.07 ABc	−0.1 ± 0.03 Abc	0.14 ± 0.01 Ce	0 ± 0.02 Acd	−0.19 ± 0.06 Ab	−0.35 ± 0.02 Aa	0.05 ± 0.09 Ade	−0.06 ± 0.05 Ac
Sk	0.09 ± 0 Cc	0.2 ± 0.03 Cd	−0.06 ± 0.02 Aa	0.14 ± 0.08 BCcd	−0.08 ± 0.05 Aa	0.21 ± 0.04 Cd	0.03 ± 0.04 Ab	−0.07 ± 0.04 Aa
Hb	0.02 ± 0.02 Ba	0.35 ± 0.08 Dc	−0.02 ± 0.01 Ba	0 ± 0.07 ABa	0.21 ± 0.04 Cb	0.02 ± 0.08 Ba	−0.03 ± 0.08 Aa	0 ± 0.05 Aa

CI are classified as follows: ≥ 0.2, as attractive; from 0.2 to 0.1, as a weak attractant; from 0.1 to −0.1, no effect; from −0.1 to −0.2, as a weak repellent and ≤ −0.2, as a repellent to EPNs (Laznik and Trdan 2013)

2010) on the movement of the EPN species *S. feltiae*, *S. kraussei*, *S. carpocapsae*, and *H. bacteriophora*. The results indicate that all tested EPN species exhibited low chemotaxis to synthetic volatiles mimicking the release of damaged or undamaged potato tubers. The highest value of CI (0.35 ± 0.08) was reached when the IJs of *H. bacteriophora* were exposed to decanal. In several related studies (Dillman et al. 2012; Hallem et al. 2011; O'Halloran and Burnell 2003), many CIs above 0.5 have been reported for *H. bacteriophora* and *S. carpocapsae*, which were included in our investigation. In a related study (Laznik and Trdan 2013), the same strains of EPN species (obtained from the company BASF, former Becker Underwood) as in our investigation were tested. The values of CI of EPNs towards (E) β-caryophyllene, linalool, and α-caryophyllene (VOCs released from mechanically damaged maize roots) similarly were low, as in our current investigation towards other synthetic VOCs. One possible explanation of low chemotaxis to volatiles can be a strain-specific characteristic of EPNs. Laznik and Trdan (2013) suggested that the response to different volatile cues is more a strain-specific characteristic than host foraging strategies. Since strains, used in other related studies (Dillman et al. 2012; Hallem et al. 2011; O'Halloran and Burnell 2003), were different from ours, we confirm from our previous (Laznik and Trdan 2013) and the current results that the chemotaxis to volatiles is a strain-specific characteristic. On the other hand, a Petri dish assay is sub-optimal as compared to the use of natural or autoclaved soil, and it prevents nematodes from exhibiting natural behavior. Our results demonstrate that EPNs have evolved a specialized olfactory system that is able to distinguish herbivore-induced chemicals from chemicals that are typical for healthy potato tubers. Similar conclusions also have been reported by Ali et al. (2011) in which the cruiser *H. indica* (Lewis 2002), the ambusher *S. carpocapsae* (Lewis 2002), and intermediates *S. diaprepesi* and *S. riobrave* (Lewis 2002) were all attracted to roots of the Swingle rootstock, damaged by the root weevil *D. abbreviatus*. These current results suggest that responsiveness to different volatile cues is a species-specific characteristic. The findings support a recent study by Willet et al. (2015), where it is concluded that EPNs display interspecific social behavioral plasticity.

Nonanal, octanal, and decanal had a greater influence on the movement of EPNs than the other tested synthetic volatiles. Nonanal was a weak attractant for *S. carpocapsae*. Decanal and octanal were attractants for *H. bacteriophora* and weak attractants for *S. carpocapsae*. Interestingly, *S. feltiae* was the only nematode species that was repelled by octanal. Nonanal and decanal are indicator substances for processes of degradation (Weissteiner 2010), and decanal also is induced by mechanical and herbivore damage (Schütz et al. 1997; Weissbecker et al. 1999). These results suggest that EPNs are able to distinguish herbivore-induced chemicals from chemicals that are typical for healthy potato tubers.

Laznik and Trdan (2016) discovered that undamaged roots of carrot produce the VOC terpinolene, which has a repellent effect on the movement of EPNs. This suggests that healthy plant roots release specific VOCs into the soil, signaling to natural insect enemies (EPNs) to keep away. In our current investigation, we tested only one VOC (2-ethyl-1-hexanol) that was released from undamaged potato tubers (Weissteiner 2010). As a pure compound, this VOC was a weak repellent to *S. carpocapsae*. However, we are aware that such a concentration does not reflect natural conditions in which the concentrations of VOCs are much lower. Weissteiner et al. (2012) reported that average concentrations of VOCs in soil at a distance of 10 cm from the root system is approximately 0.03 ppm. At the concentration of 0.03 ppm, 2-ethyl-1-hexanol had no effect on the movement of EPN species in our investigation.

This research also showed that the movement of EPNs toward the selected VOC was substantially affected by their foraging strategy. In the experiments, decanal was an attractant for *H. bacteriophora* and *S. kraussei*, which are classified as cruisers. We found that cruisers and intermediates were more mobile than species *S. carpocapsae* (ambusher). Bell (1991) reported that the movement of cruisers was conditioned at greater distances by perception of chemical stimuli, which was not, however, a characteristic of nematode ambushers. In related studies, *Heterorhabditis bacteriophora* was susceptible to chemical stimuli perceived from the environment (Grewal et al. 1993; Rasmann and Turlings 2008). This result also was confirmed in our research for decanal and octanal, which were attractants to *H. bacteriophora*. In comparison with the other species, the ambusher *S. carpocapsae* was highly susceptibile to 6-methyl-5-hepten-2-one. Thus, results support the idea that the susceptibility to perception of chemical stimuli from the environment is a species-specific characteristic that prevails over the influence of the foraging strategy (Laznik and Trdan 2013, 2016).

In this investigation, two concentrations of VOCs were used. As a pure compound, which does not reflect the concentration found near plant roots (Köllner et al. 2004), it had a greater influence on IJ movement than the concentration of 0.03 ppm, which was the average concentration of VOCs found in soil 10 cm from the root system (Weissteiner et al. 2012). The difference in response of the EPNs in this study to the concentration of VOCs was most substantially expressed with undecane. As a pure compound, undecane was an attractant for *S. kraussei* and a repellent for *S. feltiae*. However, at 0.03 ppm, undecane had no influence on the movement of EPNs. We also found that the duration of exposure of an EPN to the VOCs was decisive for the perception of chemical stimuli. After 24 h, we detected movement of EPNs in 32 % of the trials, whereas the movement after 2 hr was detected only

in 3 %. Similar results were found in our previous research (Laznik and Trdan 2016).

Plant roots are known to emit an incredible variety of compounds, which affect interactions between plants and other organisms (Rasmann et al. 2012). Non-volatile compounds have a close-range effect on other organisms and may be classified as stimulants that elicit feeding or oviposition or in contrast may inhibit both activities (deterrents) (Turlings et al. 2012). Here, we focused our attention on VOCs. Most VOCs that are involved in belowground tritrophic interactions remain unknown, but an increasing effort is being made in this field of research. Understanding more of the complex interactions would not only allow a better understanding of the rhizosphere but could also offer ecologically sound alternatives in pest management of agricultural systems (Hiltpold et al. 2013; Rasmann et al. 2012).

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