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

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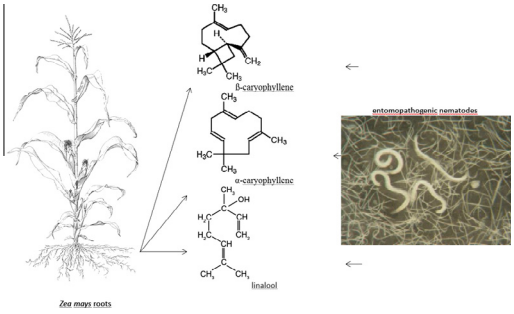
## HIGHLIGHTS

- 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.

## GRAPHICAL ABSTRACT

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## 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.



## 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

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inducible defenses is the production and release of volatile chemicals that could serve as signals to attract the natural enemies of the herbivores (Dicke 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; Laznik 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; Laznik et al., 2010a, 2010b; Laznik and Trdan, 2011). Only the resistant, third-stage, infective juveniles (IJs) 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 et al., 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 *Hylobius 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 (Laznik 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 (Laznik 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 (Laznik 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 (Laznik and Trdan, 2011). The IJs were stored at 4 °C at a density of 2000 IJ $ml^{-1}$. We used only IJs that were less than two weeks old (Gutiérrez et al., 2008). The concentration of the EPN suspension was according to Laznik et al. (2010a, 2010b). The nematode viability was determined prior to the initiation of the chemotaxis experiment (Laznik 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 Burnell (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 Burnell, 2003; Köllner 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:

(Number of nematodes in the treatment area
− Number of nematodes in the control area)/
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.