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The role of root architecture in foraging behavior of entomopathogenic nematodes

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ABSTRACT

As obligate parasites, entomopathogenic nematodes (EPN) rely on insect hosts to complete their development. In insect pest management, EPN infectiousness has varied a lot. A better understanding of their host-finding behavior in the rhizosphere is therefore crucial to enhance EPN potential in biological control. As previously demonstrated, roots can be used as a pathway to insect hosts by EPN, but this interaction and its impact on EPN foraging remain poorly documented. Three artificial model-roots with different degrees of complexity and connectivity were designed to investigate the impact of root architecture on foraging behavior of the EPN *Heterorhabditis megidis*. Insect baits were placed at the bottom of each model-root that was subsequently buried in moist sand. After injection of the EPN, the number of EPN-infected baits as well as the number of mature nematodes inside each individual carcass was recorded. The influence of insect-induced root volatiles was also evaluated by spiking the baits with a synthetic version of a natural insect-induced root cue. The ecological relevance of the results was tested in soil with two maize genotypes each exhibiting broadly different root architectures. *H. megidi* performed better in presence of model-roots. Foraging performances of *H. megidis* declined with the increasing model-root complexity. Adding the synthetic root volatile dramatically changed this pattern and favored the EPN on the most complex model-roots. *H. megidis* also moved in the vicinity of maize roots to find the insect baits in soil, and natural root architecture also tended to shape *H. megidis* foraging behavior. This study adds to the scarce body of literature characterizing physical and chemical interactions between EPN and roots. The present data illustrate that root architecture not only modifies plant quality but also shapes upper trophic levels' ecology.

1. Introduction

Entomopathogenic nematodes (EPN) are obligate parasites purposely infecting and killing insects. They differ from parasitic or necromenic nematodes as their hosts are killed within a relatively short period of time, usually within 24–48 h (Dillman et al., 2012). Their potential in controlling agricultural insect pests has been shown in many systems with various effectiveness (Grewal et al., 2005). Even if EPN can control up to 80% of an insect pest population under certain field conditions (Hiltpold et al., 2010), spatial constraints, and EPN sensitivity to abiotic factors may help explain their varying efficacy and consequently their limited use in large-scale biological control or integrated pest management strategies (Denno et al., 2008; Georgis et al., 2006). Sensitivity to abiotic factors such as desiccation and temperature can be addressed by selective breeding (Anbesse et al., 2013; Ehlers et al., 2005; Grewal et al., 1996; Griffin and Downes, 1994; Strauch et al., 2004). Although EPN can be bred to survive harsh conditions, these obligate parasites eventually have to find insect hosts in the constraining milieus that are soils (Barnett and Johnson, 2013). This complex 3-dimensional matrix impacts EPN ability to forage and encounter insect hosts (e.g., Choo and Kaya, 1991). For many years, EPN specific foraging behavior has been classified over a continuum from "cruiser" to "ambusher" (Lewis et al., 1992). Despite some intermediate behavior, EPN are often either highly mobile (cruiser) or standing on their tail and waiting for passing-by hosts to jump on (ambusher) (Campbell and Gaugler, 1997). Cruisers are therefore often used against sessile insects whereas highly mobile insects are better controlled with ambushers (Gaugler et al., 1997). Yet, this broad behavior continuum could controversially depend more on environmental factors than on EPN species (Wilson

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et al., 2012) therefore rendering knowledge on EPN behavior in their natural environment critical in their application as biological control agents.

As roots are the primary source of food for soil-dwelling herbivore insects, EPN are likely to use roots and rhizospheric cues to find insect hosts. Several EPN species were shown to be more effective in the presence of insect and root cues both on agar plates (Hui and Webster, 2000; Kanagy and Kaya, 1996; Wang and Gaugler, 1998) and in soil (Choo and Kaya, 1991; Choo et al., 1989; Van Tol et al., 1998). More recently, plant roots induced by insect pests were shown to emit specific volatiles that attract EPN to the zone of damage where the herbivore is present (Ali et al., 2010; Hiltpold et al., 2011; Rasmann et al., 2011, 2005). Most of these studies emphasized the effect of root volatiles (or chemicals emitted by root-associated biota) on EPN. Whereas the number of studies on root-derived volatiles and their impact on EPN has recently increased (reviewed by Turlings et al., 2012), little is known about the effect of root presence and architecture on foraging EPN. Ennis et al. (2010) tested the hypothesis first proposed by Van Tol et al. (1998) that foraging EPN use roots as paths through the soil matrix. They documented that in a soil–sand mix, the EPN species *Steinernema carpocapsae* followed pine twigs (presumably mimicking roots) and this resulted in a higher infection rate of the pine weevil, *Hylobius abietis* as compared to the controls without twigs (Ennis et al., 2010). Whereas pine twigs were used by EPN as pathways to find the insect host, they also conducted vibrations generated by the feeding insects (Ennis et al., 2010) and used by EPN as a physical signal to locate hosts (Torr et al., 2004). Nevertheless, in this first and only assessment supporting the root-routeway hypothesis, Ennis et al. (2010) used linear twigs whereas roots usually display more complex patterns in a 3-dimensional environment.

The objectives of this study were to assess the impact of root complexity and connectivity on host-finding abilities of the EPN *Heterorhabditis megidis* under controlled conditions, in presence or absence of root cues. In soil, the behavior of *H. megidis* was evaluated in presence of maize roots with distinct root architecture.

2. Material and methods

To disentangle the effect of root complexity and connectivity on the ability of the EPN *H. megidis* to find insect hosts, a series of three distinct experiments were conducted. The first experiment consisted of an evaluation of the movement of EPN in sand in the vicinity of artificial root-models with various complexities. Based on this bioassay, the second experiment comprised the addition of a volatile organic compound emitted by insect damaged roots and attractive to the tested EPN species. The last set of experiment consisted of the assessment of the previous observed behaviors in more natural conditions using maize plants grown in soil and exhibiting strong divergence in their root architecture. In all experiments, insect baits were used to trap EPN.

2.1. Insect and nematode handling

Galleria mellonella L. larvae were used both to rear the EPN and in the reported bioassays. This insect was grown by Timberline Live Pet Foods (Marion, IL, USA) and purchased at Columbia Pet Center (Columbia, MO, USA). Larvae used to rear EPN were stored at ca. 8 °C whereas insects used in bioassays purchased on the experimental days.

To rear *H. megidis*, *G. mellonella* larvae were individually placed in wells of 24-well plates (Greiner Bio-One North America Inc., Monroe, NC, USA) and covered with quartz sand (Unumin Corporation, Pevely, MO, USA) previously moistened with water (9:1 wt./wt.). A suspension containing ca. 20 *H. megidis* in maximum 50 μl of water was pipetted on top of each well and the plate stored in the dark at 25 °C. After 48 h, infected *G. mellonella* were placed on White traps (White, 1927) and again stored in the dark at 25 °C until EPN emergence. In response to ascarosides, emerging EPN quickly disperse from the insect carcass probably to avoid intraspecific competition (Kaplan et al., 2012). To prevent this particular behavior to influence the present experiments, newly emerged nematodes were stored in water at 8 °C for a period of 5–7 days prior the experiments. Only fresh batches of nematodes were used (3-week old maximum) in the bioassays.

2.2. Artificial model-root systems

In order to evaluate the impact of root architecture on the behavior of *H. megidis* under controlled conditions, three artificial model-roots were designed and were constructed using stainless steel wire (0.5 cm diameter) (Fig. 1). The simplest model-root consisted in a primary root only made of a straight wire (Fig. 1A). Three pairs of lateral roots (stainless steel wire, 0.5 cm diameter) were added to the second model-root; 6 cm from the bottom, two lateral roots (8 cm long each) were welded to the primary root with a 45° angle toward the bottom. With the same angle, a second lateral root set (11 cm long each) was attached to the primary root 6 cm above the first, on a vertically perpendicular plan. A third set of lateral roots (14 cm long each) was soldered to the central primary root at 6 cm above the previous lateral roots in the same vertical plan as the first (Fig. 1B). Using this second model-root as a basis, lateral roots were connected to each other from their center with stainless steel wire (0.5 cm diameter) resulting in the third, most complex, model-root (Fig. 1C). For each model, the primary root extended above-ground and was defined as the stem of the devices. To avoid chemical effect of the wire on the EPN behavior, each model-root was wrapped in several layers of thin Teflon tape (Mil Spect T-27730A, Merco Threadmaster, NY, USA).

2.3. Effect of root complexity on EPN host-finding

G. mellonella larvae were used as insect baits to trap nematodes possibly moving in the vicinity of the different artificial model-roots. The larvae were individually enclosed in cylindrical plastic tubes (1 ml Kartell plastic vial, 8 mm diameter, 30 mm height, Dynalon Labware, NY, USA) previously drilled with 13 holes (1 mm diameter) allowing gas exchange and guaranteeing EPN access to the baits.

Three baited tubes were attached at the bottom of each model-root and these devices were individually buried in 12 kg of 10% moist quartz sand (Unumin Corporation, Pevely, MO, USA) (sand:-water, 9:1 wt./wt.) in cylindrical plastic pots (24 cm diameter, 23 cm height). The sand was finally lightly compressed to ensure a good contact with the model-root. Controls consisted of pots with three baits deposited on the bottom of the pot and filled with sand but in which no model-root was buried.

Approximately 1500 EPN were injected 2 cm-deep in 1.5 ml of water in a single hole located 10 cm from the stem of the model-roots or from the center of the pots (controls). Except for the simplest model-root, EPN were injected in the vertical plan of the first lateral branch, ca. 2 cm above it. In total, four batches of fresh *H. megidis* were tested over time. Each trial consisted of 12 pots (3×3 model-roots + 3 controls) resulting in a total of 12 replications per treatment.

After 36 h in a growth chamber (Conviron, 24 ± 3 °C; 16L:8D), *G. mellonella* baits were removed from the pots, rinsed with water to remove potentially adhering nematodes, and individually placed in wells of 24-well plates (Greiner Bio-One North America Inc.,

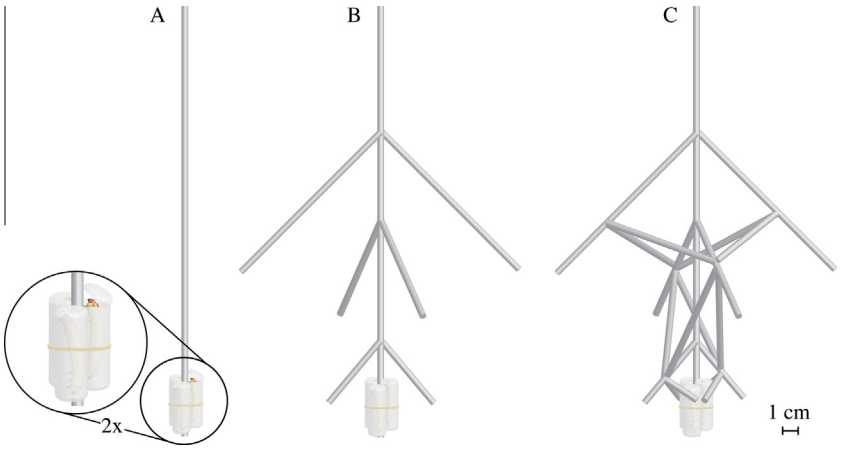

Fig. 1. Schemes of the model-root systems designed to assess the impact of root complexity and connectivity on EPN host-finding ability. The model-roots were ranging from (A) a simple straight primary root, (B) a straight primary root with one branching level, and (C) two levels of branching. Used as insect baits, *Galleria mellonella* larvae were inserted in punctured plastic tubes and attached at the bottom of each model-root system. Detailed description in the text. (Drawings: Dr. I. Hiltpold (roots and plastic tubes), Dr. T. Degen (insect larvae, http://www.thomas-degen.ch)).

Monroe, NC, USA). Plates were incubated in the dark at 25 °C for four additional days.

Under a dissection scope (M-32, Wild, Switzerland, 160× magnification), *G. mellonella* larvae were beheaded and ventrally dissected. The number of insect baits killed by *H. megidis* was recorded. To estimate the initial number of EPN that infected the baits, the number of mature nematodes in each EPN-killed insect was recorded.

2.3. Combined effect of root complexity and root volatile on EPN host-finding

Insect-damaged roots of various plant species have been shown to emit volatile organic compounds recruiting EPN (Ali et al., 2010; Hiltpold et al., 2011; Rasmann et al., 2011, 2005). To evaluate the effect of such an alarm signal in presence of distinct root architectures, the *H. megidis* were allowed to migrate along the model-roots while exposed to a volatile usually emitted by some insect-damaged maize roots (Rasmann et al., 2005). A small cotton-ball (previously washed with pentane) imbibed of 200 ng of synthetic (*E*)-β-caryophyllene (EβC) (98% pure, Sigma–Aldrich, St Louis, MO, USA) (dissolved in 20 µl pentane), and was placed in one of the three plastic tubes containing insect baits before the model-root was buried in sand. Following the same experimental procedure as described in the previous section, the number of *G. mellonella* killed by EPN and the number of mature *H. megidis* per larva was recorded for 12 replicates per treatment.

2.4. Natural root system

EPN are living in natural ecosystems more complex than what sand and wired-roots can simulate, therefore an experiment was conducted using two maize genotypes with very distinct root architectures. Maize seeds (genotypes PHG47 × PHJ40 (GEN1) and PHG39 × PHZ51 (GEN2), respectively showing condensed and wide-spread root systems (Table S1 and Fig. S1), M. Bohn, University of Illinois, IL, USA) were planted in 2:1 (v/v) autoclaved soil (topsoil from Putman Silt Loam soil) and peat (ProMix™, Premier Horticulture Inc., Quakertown, PA, USA) in plastic pots (24 cm diameter, 23 cm height) of which a 5 cm disk was previously drilled from bottom center. The holes allowed later access to the bottom of the pots and were sealed with the remaining plastic disk and duct tape (ShurTech Brands, OH, USA) prior to the start of the experiment. As controls, holed-pots were filled up with the same soil mix at the same time but were not planted with maize. Pots were watered as needed.

After 5 weeks under greenhouse conditions, when root systems nearly filled the pots, the plastic disks were removed and three *G. mellonella* larvae in plastic tubes (see above) were placed in the soil at the bottom of the root systems. Pots were again sealed with the disks and ca. 3000 EPN, suspended in 2 ml of water, were injected in each pots, 2 cm deep, and 10 cm from the plant stems. After 48 h, the insect baits were recovered from the pots, and *G. mellonella* larvae were individually placed in wells of 24-well plates (Greiner Bio-One North America Inc., Monroe, NC, USA) after being rinsed of possibly adhering nematodes. Following the same procedure as described above, the number of *G. mellonella* killed by EPN and the number of mature *H. megidis* per larva were recorded over 12 replicates per treatment using 3 different batches of fresh EPN.

2.5. Impact of natural root system on EPN host-finding

Once the insect baits were removed, maize plants were cut 10 cm from the soil surface, the root balls removed from the pots, and soaked for 1 h in water to soften the soil. Remaining pieces of soil were delicately washed from the roots using high-pressure water. Washed root systems were sprayed with 70% ethanol to prevent bio-degradation.

Following the methodology developed by Grift et al. (2011), one top and four lateral digital images of each root system were acquired. Using dedicated software written in Mathlab®, the fractal dimension and the root top angle were measured from processed images and recorded as two root topology indicators. Fractals are objects that are irregular, but self-similar at various scales (Eshel, 1998; Mandelbrot, 1983). Besides maize roots, many structures in nature appear fractal-like, such as trees, ferns, snowflakes, clouds, sponges, and mountains. Fractals are based on a mathematical definition and since the fractal dimension (FD) is a proven indicator of the level of complexity, the assumption was made that the FD of an assumed complex object such as a maize root is an indicator of its complexity. Results presented by Bohn et al. (2006) and Grift et al. (2011) demonstrate the usefulness of FD to accurately describe root system complexity and to study its genetic basis.

2.6. Statistical analysis

When following a normal distribution, data from the bioassays were analyzed using ANOVA and *t*-test. If normality was not met, ANOVA on Ranks and Mann–Whitney Rank Sum Test were conducted. Tukey's Post-Hoc tests were performed when ANOVA and ANOVA on Ranks were significant.

The fractal dimension and root angle of both maize genotypes were analyzed with *t*-tests and Mann–Whitney Rank Sum Tests.

3. Results

3.1. Effect of root complexity on EPN host-finding

The root complexity had an overall effect on *H. megidis* foraging behavior. The increasing complexity and connectivity of the model-roots significantly impacted the number of EPN-infected *G. mellonella* (Fig. 2A, ANOVA on Ranks, $H = 2.727$, $P = 0.018$) and the number of *H. megidis* adults found in the insect carcasses (Fig. 2B, ANOVA, $F_{3;140} = 5.154$, $P = 0.002$). Significantly more *G. mellonella* larvae were infected by *H. megidis* in pots planted with the model-roots showing a straight pattern and one level of branching (Fig. 2A). When *H. megidis* were provided with the most complex model-root, the number of infected bait larvae did not differ from the infection level measured in the controls (Fig. 2A).

Following the same pattern, the number of adult *H. megidis* found in the bait cadavers gave a more detailed indication of the effect of root complexity and connectivity on EPN host-finding. Very few adults were found in the cadavers collected in the control pots, whereas 4-fold more mature *H. megidis* were recorded in baits from pots planted with straight model-roots (Fig. 2B). This number was decreasing with the increasing complexity of the model-root to finally reach levels comparable to the controls with the most complex model-root (Fig. 2B).

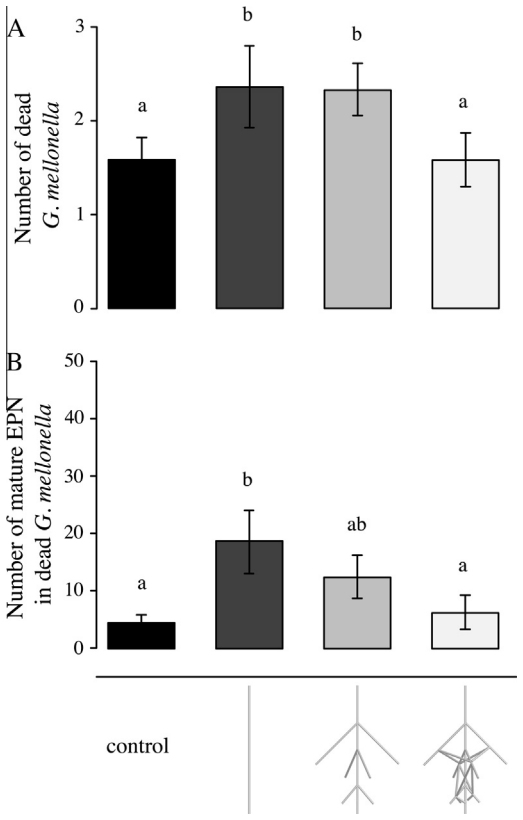

Fig. 2. Root complexity and connectivity impact EPN host finding. (A) Significantly less *Galleria mellonella* were infected by the EPN *Heterorhabditis megidis* in the control pots (black) in comparison to the insect baits placed at the bottom of the straight model-roots (dark gray) and the artificial root system with one level of branching (gray). *H. megidis* reached an ecological threshold with the most complex model-roots (light) as the number of insect baits significantly decreased. (B) The numbers of adult EPN recovered from the infected larvae were significantly higher when the pots received both straight (dark gray) and model-roots with one level of branching (gray). The increase of root complexity and connectivity negatively influenced the number of EPN initially infecting the insect baits. Equivalent counts of EPN were recorded in the presence of the most complex model-root (light) than in the control pots (black). Letters indicate significant differences and bars represent the SEM.

3.2. Combined effect of root complexity and root volatile on EPN host-finding

The addition of EβC had a profound impact on *H. megidis* behavior. Significantly more insect baits were infected at the bottom of mode-roots treated with EβC (2.5 ± 0.1 *G. mellonella*) as compared to the number of *G. mellonella* killed by EPN in the trials without the synthetic compound (1.96 ± 0.14 *G. mellonella*) (Mann–Whitney Rank Sum Test, $P = 0.012$). Also, the overall number of mature nematodes per larva was significantly higher when the volatile was applied (30.9 ± 2.62 nematodes) as compared to the number of nematodes counted in the trials where no EβC was spiked (9.37 ± 1.85 nematodes) (*t*-test, $T = -6.905$, $P < 0.001$).

Although the number of EPN-killed *G. mellonella* was not different among the treatments (Fig. 3A ANOVA on Ranks, $H = 4.071$, $P = 0.254$), the number of adult *H. megidis* recovered from bait insects was significantly increasing with the root complexity (Fig. 3B, ANOVA, $F_{3;140} = 5.935$, $P < 0.001$).

Following an opposite pattern than observed when no EβC was applied, significantly more *H. megidis* were recovered in cadavers

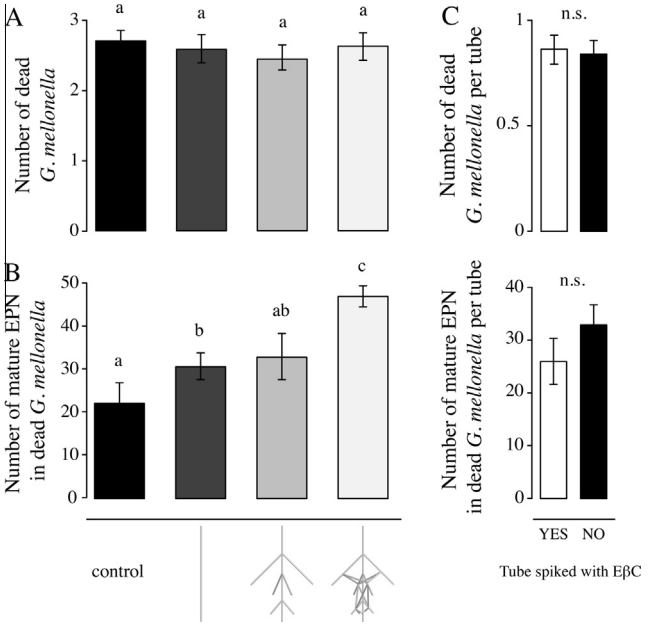

Fig. 3. The addition of a dilution of pure synthetic (*E*)-β-caryophyllene (EβC) dramatically reversed the EPN behavior along the model-root systems. (A) The counts of *Heterorhabditis megidis* infected insect baits were not different among all treatments. (B) The addition of the volatile cue emitted by some insect damaged plants influenced the number of adult EPN recovered from the carcasses, resulting in significantly more nematodes in the most complex model-roots (light gray) than in any other treatments. (C) No significant differences were found in the numbers of dead insect baits nor in the number of mature EPN between the tubes spiked with EβC and the adjacent tubes. Letters indicate significant differences (n.s. = not significant) and bars represent the SEM.

located at the bottom of the most complex model-root exhibiting two levels of branching as compared to all other treatments (Fig. 3B). Numbers of nematodes recovered from pots planted with straight and 1-level branched roots were similar but higher than those found in cadavers from the control pots (Fig. 3B).

At the bottom of a model-root that received the synthetic volatile, the average number of dead *G. mellonella* and the number of mature EPN per larva were not different whether measured in the tube spiked with EβC or in the adjacent tubes that did not receive the treatment (Fig. 3C, number of dead *G. mellonella*: *t*-test, $T = 0.247$, $P = 0.806$; number of EPN in dead *G. mellonella*: *t*-test, $T = 1.644$, $P = 0.103$).

3.3. Impact of natural root system on EPN host-finding

In soil, the number of insect baits infected by *H. megidis* was significantly higher in presence of maize roots (Fig. 4A, ANOVA, $F_{2;35} = 13.564$, $P < 0.001$). However, no significant differences were measured between GEN1 and GEN2 maize (Fig. 4A).

The presence of roots also significantly affected the number of nematodes infecting the insect baits (Fig. 4B, ANOVA, $F_{2;35} = 16.251$, $P < 0.001$) as 5 to 7-fold more mature EPN were recovered in the baits placed at the bottom of the pots with maize root systems as compared to controls. The counts of mature EPN in insect carcasses between GEN1 and GEN2 were marginally significant (Fig. 4B, $P = 0.054$), yet 1.5-fold more mature *H. megidis* were found in GEN1 as compared to GEN2.

3.4. Root structure analysis

Average fractal dimension did not differ between GEN1 and GEN2 (Mann–Whitney Rank Sum Test, $P = 0.577$), whereas the root angle of GEN1 was significantly lower than GEN2 root angle (Fig. 4C, Mann–Whitney Rank Sum Test, $P = 0.003$). GEN1 roots were more condensed in the center of the pot, targeting to the insect baits, than GEN2 roots.

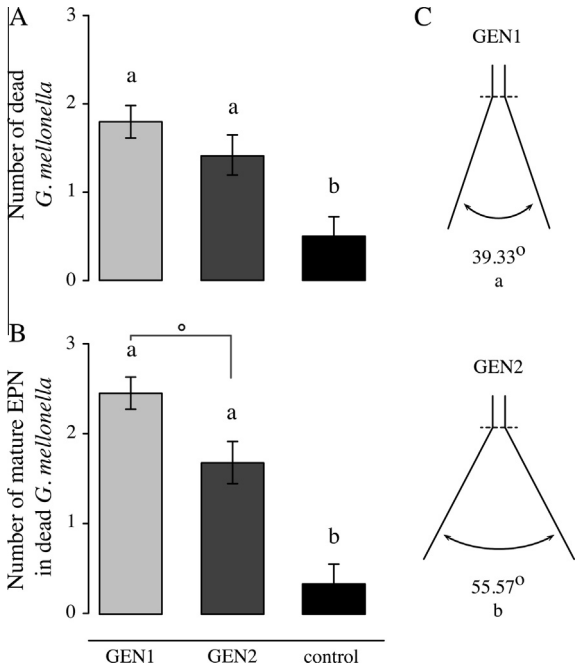

Fig. 4. The architectural topology of natural root systems impact EPN host-finding in soil. (A) Significantly less *Galleria mellonella* were infected in the control pots (black) in comparison to the insect baits placed at the bottom of pots where maize plants were grown (gray and dark gray). No differences were measured between GEN1 (dark gray) and GEN2 (gray). (B) The numbers of adult EPN recovered from the infected larvae were significantly higher when *Heterorhabditis megidis* had the opportunity to move in the vicinity of maize roots (gray and dark gray) than in pots where roots were absent (black). (C) Sketches depicting average root top angles of GEN1 and GEN2 maize genotypes. Letters indicate significant differences and marginal significant differences are shown by °. Bars represent the SEM.

4. Discussion

Van Tol et al. (1998) first suggested that foraging EPN use roots as paths through the soil matrix. Our data support this hypothesis. Root structure affected *H. megidis* behavior and ability to find and kill insect hosts in the rhizosphere. The increasing complexity of the model-roots resulted in lower numbers of insect baits infected by EPN as well as in reduced numbers of EPN recovered from these carcasses. Mimicking root volatile emission, usually occurring after the induction of root upon insect-herbivore feeding and signaling the presence of hosts, counterbalanced the negative impact of root complexity on EPN host-finding and overall increased the number of nematodes migrating toward the insect baits and infecting it. Supporting these results obtained with artificial root systems, the topology of natural root systems also influenced EPN behavior in soil.

Randomly moving through soil in search of a cryptic insect host is conceivably costly for foraging EPN. Consequently, they adopt various behaviors ranging from ambusher, waiting for a host to pass by, to cruiser, actively moving in the ground (Campbell and Lewis, 2002). Those EPN opting for an active foraging behavior, such as *H. megidis*, respond to various chemical and physical host cues in order to locate insects in the soil matrix (Gaugler and Campbell, 1991; Gaugler et al., 1989; Torr et al., 2004) as well as to root emitted signals (Ali et al., 2010; Rasmann et al., 2005; Turlings et al., 2012). In addition, roots themselves have been supposed to have an impact on EPN insect infection rate (Van Tol et al., 1998). *H. megidis* strain UK-H-211 displayed differences in host-finding abilities in presence or absence of roots, suggesting an EPN movement along the roots toward insect host rather than a random behavior in the soil column (Van Tol et al., 1998). This hypothesis was later confirmed using pine twigs as a model-root (Ennis et al., 2010). In the presence of the twig, the EPN *S. carpocapsae*, usually behaving as an ambusher nematode, was migrating toward larvae of the pine weevil *H. abietis* whereas the infection rates observed in absence of twigs were significantly lower.

As demonstrated here, not only the presence or absence of roots but also the complexity and connectivity of the root system affect EPN ability to find and kill hosts. The presence of model-root generally increased the effectiveness of the EPN whereas increasing complexity limited this positive effect of roots on EPN host-finding. Consequently, when provided with the most complex model-roots, *H. megidis* host-finding was as low as in trials where no model-root was dug. Nonetheless, the present data well support the importance of roots in EPN foraging and that this plant organ is presumably used by various EPN species to migrate in the soil matrix. However, it is not yet clear if EPN move on the root surface, so depending on the root surface morphology, or benefit from the cracks in the media resulting from the root growth, thereby being rather dependent on root size. It can be hypothesized that both root surface morphology and cracks surrounding the roots play a role in nematode foraging but this needs to be further tested.

Root volatiles emitted in response to insect feeding (Ali et al., 2010; Rasmann et al., 2011, 2005) also influence the behavior of nematodes along the model-roots. The overall number of EPN found in the larvae that received synthetic EβC, a volatile emitted by certain insect-induced maize varieties (Köllner et al., 2008), was approximately 3-fold higher than when no EβC was applied. In addition, the EPN response to the root complexity was the

opposite as when no volatile was spiked, with more individual per larva at the bottom of the most complex model-root system.

Volatile signaling is pivotal in the darkness of the soil environment. As primary producers, plants shape rhizospheric interactions via the exudation of bio-active molecules (e.g., Hassan and Mathesius, 2012; Hiltpold and Turlings, 2012; Junker and Tholl, 2013; Rasmann et al., 2012; Zhuang et al., 2013) and various groups of subterranean organisms use volatile cues in the processes of host location and non-host avoidance. Insects (Johnson and Nielsen, 2012), plant-parasitic nematodes (Bird, 2004), bacteria-feeding nematodes (Anderson et al., 1997a, 1997b) or EPN (Hallem et al., 2011; Turlings et al., 2012) can exploit volatile cues to locate hosts or food under-ground. However, the diffusion of volatile organic compounds in soil has received scant attention to date. Depending on soil composition and moisture (Hiltpold and Turlings, 2008), diffusion of certain volatile can be extensive. Recent sampling detected volatiles typically emitted by damaged citrus trees as far as 10 m from the trunk base (Ali et al., 2012), suggesting a long range of influence of root emitted volatile organic compounds. The results reported here suggest that volatile organic compounds diffuse along the roots and subsequently facilitate host location by foraging EPN over a certain distance. Differences measured between trials that received model-root and trials free of any root path suggest that EβC was not only diffusing randomly in the sand matrix but also followed roots and/or cracks around roots. Although such phenomenon still needs to be demonstrated, this hypothesis likely explains the dramatic shift of EPN behavior in the vicinity of the model-roots in presence of EβC. If volatile gradients are indeed stronger along roots than in distant soil, EPN moving in the root zone may encounter stronger volatile gradients than in the adjacent soil matrix, especially as the emission of root volatiles is stronger on sites of insect wounding than systemically (Hiltpold et al., 2011). The similar number of nematodes recovered from carcasses spiked or not with the synthetic volatile support the hypothesis that EPN use certain cues for long-range insect-damaged root location, such as EβC (Turlings et al., 2012), but also rely on complementary cues to locate and identify insect hosts on a shorter scale, as demonstrated in other soil-dwelling organisms (Erb et al., 2013; Hiltpold et al., 2013).

Plants also transmit physical cues such as vibrations (e.g., Cocroft and Rodríguez, 2005; De Groot et al., 2011) eventually impacting upper trophic levels (Joyce et al., 2014). Below-ground, roots have been supposed to transmit vibrations produced by feeding insects (Ennis et al., 2010), a cue to which EPN respond (Torr et al., 2004). Although not tested in the present experiments, root-transmitted insect vibrations resulted in higher infection of insect larvae by EPN in previous experiments (Ennis et al., 2010) supporting the hypothesis that root can serve not only as a pathway for chemical but also for physical cues eventually facilitating EPN foraging toward their insect host. In this context, root complexity and connectivity as well as vibration conductance are likely to play a major role. In addition to vibrations, EPN respond to electrical fields (Shapiro-Ilan et al., 2009, 2012) with electrical potential comparable to those observed on the surface of some insects (Colin et al., 1992; Warnke, 1976) or to some membrane potentials of roots (Ivashikina et al., 2001; Toko et al., 1990; Weisenseel and Meyer, 1997). As stressed roots exhibit variations in their electric potential (Filek and Kościelniak, 1997), EPN possibly react to electrical variations in soil, signaling the presence of potential hosts, as first hypothesized by Shapiro-Ilan et al. (2012). Therefore, it is conceivable that root connectivity also impacts the transmission of this physical signal and subsequently EPN orientation below-ground.

Similar to other organisms, nematodes certainly desire the shortest possible route to reach their food source because it increases the chances to find it and limits energy investment in foraging to minimal needs. There is empirical evidence that, in presence of a bacterial food source (e.g., *Esherichia coli* Escherich), the bacteria-feeding nematode *Cenorhabditis elegans* Maupas follows an approximate straight line toward the bacterial cells whereas spreading randomly in the absence of cues (Anderson et al., 1997b). Reynolds et al. (2011) documented that plant-parasitic nematodes also prefer the shortest ways to their sustainable hosts. It was therefore surprising to measure differences in EPN effectiveness, as the shortest distance from the injection point to the insect baits, following the model-roots, were equivalent, and not depending on the root complexity. However, in absence of root volatile, the root complexity possibly led nematodes away from their host, whereas, in the same timeframe, many EPN infected insect baiting simpler model-roots. *H. megidis* weak navigation in complex model-root was overbalanced by the addition of synthetic EβC.

From an applied perspective, EPN behavior cannot only be assessed under highly controlled environments where the complexity of the soil matrix and of the rhizosphere is simplified to emphasis the influence of the model-roots on their behavior. It is therefore important to note that the presence of natural root systems strongly impacted the ability of *H. megidis* to migrate toward the insect baits in soil. Barely any bait was infected nor EPN were found in the few infected baits from the pots where no roots were grown in the soil. The presence of root of any architecture significantly facilitated the movement of the EPN toward the baits, suggesting that roots serve as pathways for EPN in natural condition also. This assumption is supported by the higher numbers of EPN recovered from the baits placed at the bottom of root systems exhibiting the lowest root top angles as these root were more targeting toward the insects than the root having wider root top angles.

Being the first study to evaluate the effect of natural root structure on EPN behavior, this experiment however only scratches the surface of this process. Soil type and texture influence EPN behavior (e.g., Alekseev et al., 2006; Campos-Herrera and Gutiérrez, 2009; El-Borai et al., 2012; Kaspi et al., 2010; Koppenhöfer and Fuzy, 2006; Kruitbos et al., 2009; Toepfer et al., 2010). Judging whether soil type impact EPN behavior around roots will require addition experiments, but this could explain the very low number of nematodes per larva recovered in soil as compared to those in sand. An alternate explanation may be the high biological activity in the rhizosphere and the root surface where more interspecific and/or interphyletic competition may occur (Ali et al., 2013) and mask the beneficial impact of root structure on their host-finding ability.

5. Conclusions

Plants have different root architectures (Weaver, 1926; Weaver and Bruner, 1927), possibly influenced by abiotic (e.g., Gagliano et al., 2012; Sharp et al., 1990; Weaver and Bruner, 1927) and biotic (e.g., Bais et al., 2003; Bertin et al., 2003; Callaway and Aschehoug, 2000; Ridenour and Callaway, 2001) factors and this study first documents that root topology affect EPN foraging behavior and consequently their effectiveness. Therefore root architecture is a parameter to consider when implementing EPN in pest control programs, in addition to other local factors such as soil characteristics (Koppenhöfer and Fuzy, 2006), root volatile production (Degenhardt et al., 2009; Hiltpold et al., 2010), and related communities of antagonists (Ali et al., 2011, 2013).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jip.2014.08.002.

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Table S1. Selected maize hybrids and their mean performance for various root complexity and architectural characteristics as well as agronomic traits.

			Phenotype[†]										
Genotype	CROSS	RCG	FDV	FDH	RTA	STD	MF	FF	SPAD	PHT	EHT	STG	YLD
PHG39×PHZ51	SN	+	0.045	0.031	9.0	2.9	71.33	73.0	59.45	269.17	127.00	5.17	202.31
PHG47×PHJ40	SN	-	-0.062	-0.041	-4.4	-36.9	65.00	65.5	58.52	206.67	96.17	1.33	188.73

[†]RCG-Root Complexity Group where + is high complexity and - is low complexity; FDV- Fractal Dimension Vertical; FDH- Fractal Dimension Horizontal; RTA- Root Angle; STD-Stem Diameter; SPAD-Spadmeter Reading; PHT-Plant Height; EHT-Ear Height; STG-Stay Green; YLD-Yield; SS-Stiff Stalk *Stiff Stalk; SN-Stiff Stalk*Non-Stiff Stalk.

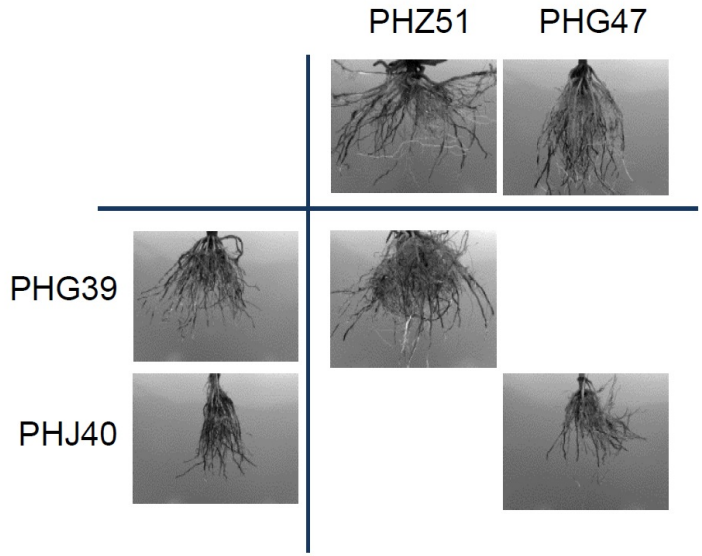

Figure S1. Images obtained from adult root systems of maize inbred lines PHG30, PHJ40, PHG47, and PHZ51 and their hybrids PHG39×PHZ51 and PHG47×PHJ4