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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.,