Plant Roots Increase Bacterivorous Nematode Dispersion through Nonuniform Glass-bead Media

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Title

PLANT ROOTS INCREASE BACTERIVOROUS NEMATODE DISPERSION THROUGH NON-UNIFORM GLASS-BEAD MEDIA

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Roots increase dispersion through glass beads: *Trap* et al.

Abstract: Dispersion of bacterivorous nematodes in soil is a crucial ecological process that permits settlement and exploitation of new bacterial-rich patches. Although plant roots, by modifying soil structure, are likely to influence this process, they have so far been neglected. In this study, using an original three-compartment microcosm experimental design and PVC bars to mimic plant roots, we tested the ability of roots to improve the dispersion of bacterivorous nematode populations through two wet, non-uniform granular (glass bead) media imitating contrasting soil textures. We showed that artificial roots increased migration time of bacterivorous nematode populations in the small bead medium, suggesting that plant roots may play an important role in nematode dispersion in fine-textured soils or when soil compaction is high.

Keywords: dispersion; ecology; glass-bead media; migration time; colonization time; plant roots.

Bacterivorous nematodes are widely distributed soil organisms involved in key terrestrial ecosystem functions such as soil fertility and plant productivity (Djigal et al., 2004, Irshad et al., 2011, Blanc et al., 2006, Anderson et al., 1978, Ferris et al., 1998, Bonkowski et al., 2009). By releasing nutrients (nitrogen and phosphorus) immobilized in bacterial biomass in the vicinity of plant roots, they largely contribute to soil nutrient availability (Ferris et al., 1998, Anderson et al., 1983) and plant nutrition and growth (Irshad et al., 2012, Bonkowski and Clarholm, 2012, Trap et al., 2015).

The positive effects of bacterivorous nematodes on soil and plant functions are conditioned by their ability to move within heterogeneous soils (Griffiths and Caul, 1993). Dispersion of nematodes from one bacterial site to new resource patches is a crucial ecological process facilitating ecosystem functions (Horiuchi et al., 2005, Hassink et al., 1993, Savin et al., 2001, Hassink et al., 1993, Rodger et al., 2004). It is strongly determined by soil conditions such as bulk density (Hunt et al., 2001, Portillo-Aguilar et al., 1999), soil water content (Young et al., 1998) or temperature (Hunt et al., 2001), soil texture and hence porosity (Young et al., 1998, Portillo-Aguilar et al., 1999, Georgis and Poinar, 1983, Prot and Van Gundy, 1981), bacterial species (Rodger et al., 2004, Young et al., 1998), salt gradients (Le Saux and Queneherve, 2002), or soil water run-off (Chabrier et al., 2009).

In most experiments, bacteria-nematode effects on soil nutrient availability have been studied in bulk soils and root exudates were mimicked by providing carbon as an energy source for bacteria, usually as glucose (Anderson et al., 1983, Cole et al., 1978, Coleman et al., 1978, Ferris et al., 1997, Ferris et al., 1998). Possible physical influences of roots on nematode dispersal, and the subsequent effects on soil nutrient availability, have thus not been represented. Moreover, in experiments with plants (Bjornlund et al., 2012, Djigal et al., 2004), shifts in both energy supply and porosity induced by roots are confounded, limiting our ability to decipher mechanisms by which roots impact nematode-driven ecological functions. In this

study, using an original three-compartment microcosm experimental design, we tested the ability of roots to improve the dispersion of nematodes and their associated bacteria through two wet granular media made from glass beads of different sizes in order to mimic two contrasting soil textures.

MATERIALS AND METHODS

The study was conducted in sterile three-compartment 90-mm Petri dishes (compartments labeled A-C). We designed six treatments (Figure 1). The first two treatments corresponded to negative (NC) and positive (PC) controls, respectively. In NC, compartments were not connected (compartments were independent) while in PC and for the four other treatments, short gates (~5 mm width) were opened between compartments A and B and between compartments B and C by melting the plastic walls separating compartments (Figure 1). In all treatments, compartments A and C were filled with 10 ml TSB-A (3 g L^{-1} Tryptic Soy Broth Fluka 22092 and 1% agar w/v supplemented with cholesterol 5 mg L^{-1}). The compartment B was filled with 10 ml TSB-A in NC and PC treatments, whereas in the other four treatments, it was filled with 15 g of non-uniform (polydisperse) glass beads (Abralis, France), either of small size (SB: mean diameter 130 µm, min-max diameters 60-260 µm, porosity 40%), or large size (LB: mean diameter 600 µm, min-max diameters 300-1100 µm, porosity 32%). Bead size was measured using a laser granulometer (Mastersizer APA2000, Malvern Instruments Ltd., United Kingdom) while the distribution of pore size was approximated using the Finney Model (Frost, 1978, Finney, 1970) for uniformly sized (monodisperse) granular media with the average size of beads as the most representative bead size for each medium.

Before use, glass beads were acid-washed using HCl 1M, rinsed with sterile deionized water and saturated at 100% of their holding capacity. In two of the glass-bead treatments (5 and 6), a flexible PVC bar (2 mm diameter, 4 cm length), previously sterilized in bleach and washed with sterilized deionized water, was used to mimic roots and placed in compartment B (Figure 1). The ends of the PVC bar were inserted through the gates, thus linking compartment A with C (Figure 1). In compartment B, the PVC bar was placed inside the bead medium. This PVC bar was used to test physical effects of roots on nematode dispersion without interfering with carbon supply by rhizodeposition. Each treatment was replicated 5 times (30 microcosms).

For all microcosms, compartment A was inoculated with 100 µl of fresh gram-positive *Bacillus subtilis* (strain 111b) culture and 15 adult bacterial-feeding nematodes belonging to *Rhabditis* sp., together as a spot dropped from the corner of the compartment at the center of the Petri dish (Figure 1). Bacteria and nematodes for experiments were isolated from an ectomycorrhizal root tip and the soil collected in a maritime pine forest, respectively (Irshad et al., 2011). Nematodes were maintained in our laboratory by transferring individuals onto new TSB-A plates containing *B. subtilis* (Irshad et al., 2011). Nematodes multiplied in the dark at 20 °C. Nematodes used in the inoculation experiments were prepared by removing them from the breeding TSB-A plates by washing the surface with a sterile NaCl solution (1%). They were washed from most *B. subtilis* by centrifugation (1000 rpm, 5 min) and re-suspended in sterile deionized water.

Every morning for three weeks, microcosms were carefully inspected using a binocular microscope and the number of individuals in compartment C was counted. We defined the “migration time” as the number of days required to observe one individual (juvenile or adult) in compartment C. We also assessed the “colonization time” as the number of days required for nematodes to exploit the whole compartment C and reach the maximal

carrying capacity set at 400 individuals (corresponding to a homogeneous distribution of nematodes in compartment). Means and standard deviation were calculated for each treatment and significant differences were tested using one-way ANOVA and Tukey HSD tests. Normality of residuals was checked using Shapiro test. All tests were computed with the R freeware (R, 2008) and statistical significance was set at $P < 0.05$.

Results and Discussion

After three weeks of incubation, no nematode was observed in compartment C in the negative control (Figure 2.A), confirming that nematodes were not able to cross the walls separating compartments. In the positive control (PC), around 8 days were required to observe individuals in C while 10 days were required in both large- and small-bead treatments. Our findings are in agreement with those obtained by Wallace (1958) that showed that the pore size in a saturated 75- to 150-µm soil fraction (similar to our small-bead medium) approaches that through which *Heterodera schachtii* larvae are unable to pass. In his study, a maximum of 10% of the nematodes migrated farther than 5 cm from the inoculation site for this soil fraction while ~35% of the population migrated farther than 5 cm in the 150-200 µm fraction.

When an artificial root was added across compartment B, the mean migration time decreased to 9 days and 8 days for large and small beads, respectively. The effect of the artificial root on nematode migration time was thus observed for both bead sizes, but the effect was significant for small beads only. By creating macropores (Angers and Caron, 1998), roots increased soil porosity for these free organisms and their dispersal rate. Nematodes can also move in the water film formed around the root as a “highway” towards a new site. Here, we did not provide glucose to mimic root exudates because our aim was to discriminate energy supply from physical effects of roots on nematodes. In natural

rhizospheres, the presence of root exudates is known to improve soil structure and increase aggregation, especially in clay soils (Angers and Caron, 1998, Bertin et al., 2003). It is possible that in natural conditions, the improving effect of roots on nematode dispersion could be modified by rhizodeposition rate and soil clay content (Hassink et al., 1993).

Interestingly, the colonization time of nematode populations growing in C varied according to treatments (Figure 2.B). The lowest values were observed for PC and for small beads with an artificial root (mean ~2.5 days of colonization time) while the highest values were observed for small beads without an artificial root (mean ~4.2 days). Intermediate values were found in large beads, with or without artificial roots. In PC and beads with an artificial root, adults moved easily from A to C. In contrast, for the treatment with small beads and without AR, the first individuals observed in C were juveniles. This pattern can be explained by the diameter of adult nematodes after 14 days of growth oscillating around 35 µm (n = 30). Individuals with a diameter superior to ~30 µm were highly constrained by the beads (Figure 3). In consequence, in treatments with small beads, only juveniles could move easily from A to C. Several hours and days were thus needed for juveniles to grow in C before becoming adults and reproducing, explaining why colonization was slower.

It is important to note that we did not inoculate compartments B and C with *Bacillus subtilis* cells. The colonization time of nematode populations was thus based on their ability to transport bacteria (or spores) from compartment A to C. Several studies observed phoretic transport of bacteria by nematodes (Hallmann et al., 1998, Knox et al., 2004, Knox et al., 2003) or defecation of living bacterial cells or spores after their passage through the nematode gut (Laaberki and Dworkin, 2008, Rae et al., 2012). For instance, Laaberki and Dworkin (2008) showed that ingested *B. subtilis* spores were resistant to *Caenorhabditis elegans* digestion. Some studies showed that nematodes can act as vectors of rhizobium (Jatala et al., 1974, Sitaramaiah and Singh, 1975, Horiuchi et al., 2005) or plant pathogenic bacteria

(Kroupitski et al., 2015). Once in C, living *B. subtilis* cells or spores attached on nematode cuticles or excreted by nematodes can proliferate rapidly on TSB-A before nematode population growth.

In conclusion, this microcosm experiment showed that the presence of small beads severely constrained adult but not juvenile dispersion. An artificial root increased bacterivorous nematode populations and associated-bacterial food dispersion in wet polydisperse media, especially in small-bead media. These results suggested that plant roots can play an important role in assisting nematode dispersion in fine-textured soils or when roots penetrate in compacted soils (Queneherve and Chotte, 1996, Iijima et al., 1991). Nematode effects on nutrient cycling are known to vary according to soil texture (Hassink et al., 1993), but our study suggests that the presence of roots may alleviate the effect of small soil pore size, enhancing local population connection and probably soil nutrient cycling (Clarholm, 1985). Our results also suggested that besides root exudates and active attraction, differences in root architecture among plant species can also explain why nematode population abundance or biomass in plant rhizospheres vary according to plant species (Griffiths, 1990, Horiuchi et al., 2005). Further studies using similar designs could be used to disentangle physical and nutritional impacts of roots on nematode-driven transport of nutrients or organic compounds such as enzymes or pollutants.

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Figure 1. Experimental setup with three-compartment Petri dishes used to assess the effect of roots and medium porosity on nematode dispersal and colonization. In all treatments but the negative control (treatment 1), compartments A and C were connected by gates opened through the wall, with or without an artificial root "AR" (2 mm diameter PVC bar) added to cross the compartment B. Compartments A and C were filled with TSB-A (see text for composition). Depending on the treatment, B was filled with TSB-A (treatments 1 and 2) or with small beads (SB) (treatments 3 and 5), or large beads (LB) (treatments 4 and 6) in sterile deionized water. Only compartment A was inoculated with *Bacillus subtilis* and 15 bacterial-feeding adult nematodes belonging to the *Rhabditis* sp., as a spot dropped from the corner of the compartment at the center of the Petri dish (closed circle).

Figure 2. Migration time (A) and colonization time (B) in days according to treatments. NC: negative control; PC: positive control (white); LB: large beads (light grey); SB: small beads (dark grey); -AR: without artificial root (solid line); +AR: with artificial root (dotted line). Different letters (a and b) indicate significance among treatments according to one-way ANOVA and Tukey HSD post hoc tests ($P < 0.05$, n = 5).

Figure 3. Size distribution of beads (A) and approximation of pore size distribution (B) for small (dotted line) or large (solid line) beads. $P(k)$ is the frequency of the radius (k) of pores. The blue solid line indicates mean nematode diameter size of adults (n = 30).

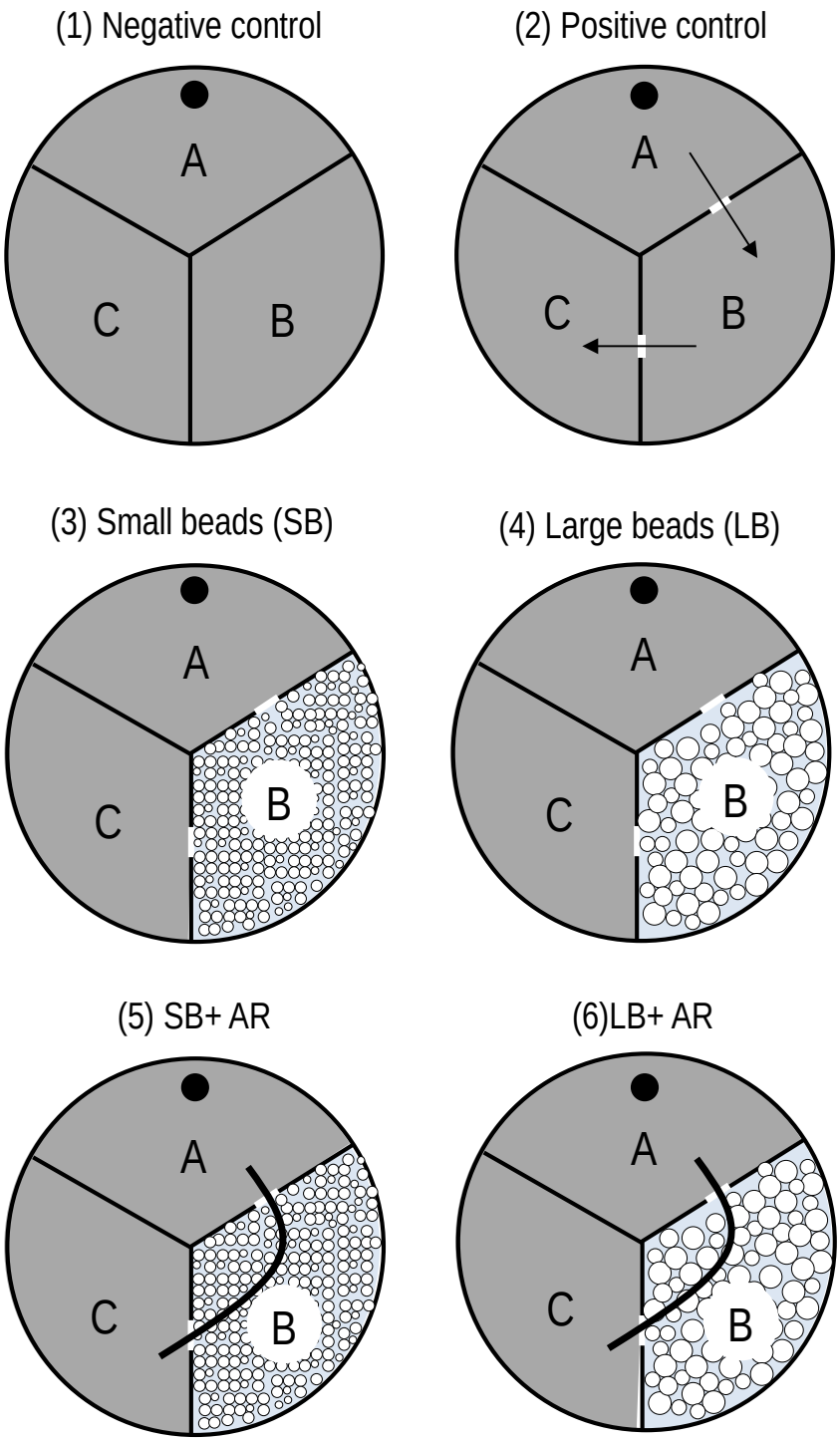

Figure 2