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Short communication

Do bacterial-feeding nematodes stimulate root proliferation through hormonal effects?

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Abstract

We prepared soil with greater populations of bacterial-feeding nematodes either by stimulating the native populations of the soil, adding an additional mixed community of nematodes, or by adding *Caenorhabditis elegans,* to investigate the effects of bacterial-feeding nematodes on root morphology, soil auxin (indolyl-3-acetic acid—IAA) concentrations and microbial community structure. In the presence of enhanced bacterial-feeding nematode populations, tomato plants had a more highly branched root system with longer and thinner roots. Root system development was greater with native nematodes than *C. elegans*. The changes of root morphology were accompanied by an increase of soil IAA content and an altered microbial community structure. Bacterial-feeding nematodes may have affected plant growth by stimulating hormone production through grazing-induced changes to the soil microbial community.

Keywords: Bacterial-feeding nematodes; Root morphology; Microbial community structure; IAA

Several experiments have shown that protozoa-stimulated root growth (Jentschke et al., 1995; Bonkowski et al., 2000, 2001; Kreuzer et al., 2006), with an increased proportion of auxin producing bacteria in the presence of protozoa (Bonkowski and Brandt, 2002) leading to the conclusion that grazing-induced changes in the composition and function of rhizosphere bacterial communities (Bonkowski, 2004). Tomato seedlings grown in soil with an enhanced abundance of bacterial-feeding nematodes similarly developed a more branched root system with longer and thinner roots (Mao et al., 2006). Nematodes use chemical clues to sense and discriminate between bacterial species (Grewel and Wright, 1992) and alter soil microbial community structure, as do protozoa (Griffiths et al., 1999). We hypothesized that the bacterial-feeding nematodes altered microbial community structure and affected root growth in a similar manner to protozoa (Mao et al., 2006). We further tested this hypothesis by determining soil auxin concentration and microbial community structure, but also by adding additional nematodes either from culture or extracted from soil.

The selective mesh technique, as described by Mao et al. (2006) and using the same soil and substrate types, was used to obtain soil with an enhanced abundance of native bacterial-feeding nematodes. Briefly, 400 g of soil mixed with 14 g of pig manure (inner soil) in bags of 5 μm or 1 mm pore diameter nylon mesh were placed in a pot and surrounded by 650 g of unamended (outer) soil. This allowed nematodes and soluble nutrients to migrate through the 1 mm diameter mesh to the outer soil, while the 5 μm diameter mesh prevented migration of nematodes but still allowed soluble nutrients to pass into the outer soil. Moisture content was adjusted to field capacity (20.8%, w/w) and six replicate pots incubated at 20 °C in the dark. After 4 weeks nematodes were extracted from the outer soil (Goodfriend et al., 2000) counted, preserved (Griffiths et al., 2002) and identified to trophic group (Yeates et al., 1993; Liang et al., 2002).

Two nematode inocula were also used. Firstly, *Caenorhabditis elegans* was cultured in Petri dishes on potato sucrose agar with *Bacillus subtilis*. Secondly, a mixed population of nematodes extracted from the outer soil of

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the 1 mm mesh treatment (SM1) as described above. The nematode inocula were collected on a nylon mesh (15 μm pore diameter), washed twice with sterile distilled water and added to outer soil from the 5 μm mesh treatment (SM5) to give the same abundance as the outer soil of the SM1 treatment, 135 nematodes g^{-1} dry soil. The soil treatments used for tomato growth were: (1) the outer soil of the SM1 treatment (SM1), (2) the outer soil of the SM5 treatment (SM5), (3) the outer soil of the SM5 treatment inoculated with *C. elegans* (SM5C), (4) the outer soil of the SM5 treatment inoculated with nematodes isolated from the SM1 treatment (SM5N).

For each treatment, 20 replicate pots were filled with 50 g soil, planted with a single tomato seedling (*Lycopersicon esculentum* Mill, var. Shanghai 906) as before (Mao et al., 2006). After 5 and 10 days growth, six replicates of each treatment were sampled for root analysis and three replicates for analysis of auxin and microbial community structure.

Images from individual washed roots were acquired by scanner (LA1600+ scanner, Canada) and root related parameters (total length, average diameter, total surface area and number of tips) were analysed using Win-rhizo software (Winrhizo2003b, Canada). Indolyl-3-acetic acid (IAA) in soil was measured using high performance liquid chromatography (HPLC) (Hu et al., 2001). Microbial community structure was analysed from a community-level physiological profile (CLPP) using Biolog ECO microplates (Biolog, Hayward, CA, USA) (Garland, 1997). Nematode data was analysed by *T*-test. Root and IAA data were analysed with a two-way ANOVA (days and soil treatment), with the root total length, total surface area and average diameter were transformed (natural logarithm) prior to analysis. Comparisons between means were performed with a Tukey's HSD test. The Biolog data were analysed using principal component analysis (PCA) of the area under the colour development profile (Hackett and Griffiths, 1997). All statistical analyses were carried out using the statistical package SPSS11.0.

The 1 mm mesh (SM1) gave more total and bacterial-feeding nematodes in the outer soil than the 5 μm mesh (SM5) (Table 1). Root length, surface area and number of root tips significantly ($P<0.001$) increased between days 5 and 10 (Fig. 1), while average root diameter not significantly differed (Fig. 1). Roots were significantly ($P<0.05$) longer, had more root tips, smaller diameter (only day 10) and greater surface area (only day 5) in SM1 than SM5 (Fig. 1). Roots in the outer soils of SM5 inoculated with nematodes (SM5C and SM5N) were also significantly ($P<0.05$) longer, had more root tips (only day 10), larger surface area and smaller diameter (only day 5), than in SM5 (Fig. 1). Roots grown in soil with mixed nematodes (SM5N) had a significantly ($P<0.05$) larger surface area and more root tips, but smaller diameter, than in soil inoculated with *C. elegans* (SM5C) on day 5 (Fig. 1). The IAA content of the soil also showed significant ($P<0.001$) effects of days with IAA content increasing from day 0 to day 5 to day 10 (regardless of soil treatment). IAA content was significantly ($P<0.05$) greater in the SM1 treatment than in SM5, while the outer soils of SM5 inoculated with nematodes (SM5C, SM5N) also contained significantly ($P<0.05$) more IAA than the SM5 treatment except on day 0. Soil inoculated with mixed nematodes contained more IAA than soil inoculated with *C. elegans* (Table 2). The CLPP gave strong evidence for grazing-induced shifts in microbial community structure between the SM5C and SM5N treatments, which were different from the SM5 treatment and from each other (Fig. 2). There was also a shift in microbial community structure from day 5 to day 10 in the soils inoculated with nematodes, more so when soil extracted nematodes were added (SM5N) than *C. elegans* (SM5C), but no temporal change in the control soil (SM5) (Fig. 2).

Our results confirmed that tomato seedlings grown in soils containing more bacterial-feeding nematodes (either from stimulation of the native population, addition of a mixed population of extracted nematodes or by adding a single species of bacterial-feeding nematode) developed a more highly branched root system with longer and thinner roots. At the same time, we found an increase in the IAA content of the soil accompanied by changes to microbial community structure. These results are comparable to the investigations of Bonkowski and Brandt (2002) and Kreuzer et al. (2006) in soil-free systems on the root systems of watercress and rice. In our experimental design, concentrations of soil NO_3^- would be similar between the treatments at planting (Mao et al., 2006). Although we cannot rule out an effect of N mineralized by nematodes during plant growth, the observed changes in root morphology in the presence of bacterial-feeding nematodes could be linked to grazing-induced changes in the composition and function of bacterial communities to

Table 1
Nematode abundance (individuals g^{-1} dry soil) and percentage composition (±standard error, $n = 3$) of trophic groups in outer soils of SM1 and SM5 treatments, see text for details, at the end of the incubation period but before planting with tomatoes

	Nematodes				
	Abundance	Bacterial feeders	Fungal feeders	Plant feeders	Omnivores/predators
SM1	135.1±13.1	97.9±0.9	1.5±0.4	0.5±0.3	0.1±0.1
SM5	27.8±2.8	73.3±4.5	13.1±4.7	10.1±1.8	3.5±1.0

The soil had an initial abundance of 12.1 nematodes g^{-1} dry soil.