IMPACT OF THE NEMATOPHAGOUS FUNGUS *POCHONIA CHLAMYDOSPORIA* ON NEMATODE AND MICROBIAL POPULATIONS

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SUMMARY

The microbial and nematode populations associated with two plants (tomato and cabbage) inoculated with the nematophagous fungus, *Pochonia chlamydosporia* var. *chlamydosporia* or root knot nematode (*Meloidogyne incognita*), or both, were compared with those in unplanted controls. The dominant factor affecting culturable microbial populations was found to be the presence or absence of tomato plants. Generally microbial colony counts were lowest in unplanted soil, small increases were associated with cabbage and significantly greater numbers with tomato plants. Differences in microbial diversity (estimated from community profiles of carbon substrate utlisation, using Biolog) were observed between planted and unplanted soils, however, there were few differences between soils with either of the two plants. The presence of *P. chlamydosporia* was associated with a reduction in the numbers of plant parasitic nematodes (51%-78%) including the migratory ectoparasites, whereas free-living nematodes, culturable bacteria and bacterial populations assessed by Biolog were unaffected by the application of fungus.

INTRODUCTION

Root knot and cyst nematodes are major agricultural pests causing annual yield losses estimated at \$100 billion (Sasser & Freckman, 1987). The nematophagous fungus *P. chlamydosporia* is a facultative parasite of nematode eggs. It has potential as a biological control agent (BCA) against both root knot and cyst nematodes (Kerry and Bourne 1996; Kerry 2000). Extensive work has been done on the colonisation and the impact of various factors on the growth and parasitism of *P. chlamydosporia* (Crump and Kerry, 1986; Irving and Kerry, 1986; Kerry *et al.*, 1986 and, Dackman and Baathe, 1989). Furthermore, O'Flaherty *et al.* (2003) demonstrated that reductions in populations of *M. incognita* caused by application of *P. chlamydosporia* resulted in changes in the structure of the rhizosphere bacterial community. However, the effect of the fungus on non-target nematode and other microfauna populations in the soil and rhizosphere must be investigated in order to register it as a biological control agent with minimal environmental impact. This study is a subset of experiments in progress investigating the long and short-term impact of *P. chlamydosporia* on the populations of plant parasitic and free-living nematodes and the microbial communities on which they depend.

The present paper gives a comparative account of the nematode and culturable microbial populations in the rhizosphere of a host and a poor-host plant for *M. incognita* in the presence or absence of the fungus, *P. chlamydosporia*.

MATERIALS AND METHODS

A pot experiment was set up with tomato and cabbage plants grown in soil inoculated either with *P. chlamydosporia* or *M. incognita* or both organisms. Soil for the experiment was obtained from Great Field, Rothamsted, air-dried at room temperature, sieved through 4-mm mesh and 500 g placed in 10-cm dia pots. *P. chlamydosporia* (5000 chlamydospores g^{-1} soil) was added to selected pots using the method of Kerry *et al.* (1993). Seedlings of cabbage (*Brassica oleracea* cv. Greyhound) and tomato (*Lycopersicum esculentum* cv. Alicante), which are poor hosts and fully susceptible hosts of *M. incognita* were planted singly in each pot after application of the fungus and two weeks after transplantation, 5000 second stage juveniles of *M. incognita* were inoculated per pot, as required. The different combinations were untreated soil (S), *P. chlamydosporia* alone (SP), tomato (ST) or cabbage (SC), soil with *P. chlamydosporia* and tomato (STP) or cabbage (SCP), soil with nematodes and tomato (STN) or cabbage (SCN), soil with *P. chlamydosporia* and nematodes and tomato (STPN) or cabbage (SCPN). There were four replicates for each treatment including untreated controls. The experiment was left for 10 weeks after which the microbial and nematode populations were estimated. The bacterial populations were assessed by counting colony- forming units (cfu) on selective media: PSA (Pseudomonas Selective Agar) was used for pseudomonads, 1/10th TSA (Tryptic Soy Agar) for heterotrophic bacteria and MA (Mac Conkey Agar) for enteric bacteria. For estimation of bacterial populations, three cores were taken from each pot, pooled and mixed thoroughly to weigh out one g from each factorial treatment. Soil was suspended in 10 ml Phosphate Buffered Saline (PBS) and dilutions were plated on selective media. Colonies were counted after 24 and 48 h of incubation at 28 °C. Shifts in bacterial populations were assessed by comparing 95 carbon utilisation profiles with Biolog™ GN plates. Each microplate has 96 wells containing tetrazolium dye, 95 with different carbon substrates and one control well with no substrate. The dye is reduced as the carbon substrate is oxidised, colour development being proportional to the degree of substrate utilisation. The utilisation rate is determined by comparison with the average well colour development (AWCD) (Garland, 1997). The diluted soil suspension inoculum, 140 µl well^{-1}, was adjusted to contain approximately 10^4 bacterial cfu (estimated from the heterotrophic bacterial counts made on 1/10th TSA after 48 h). The plates were sealed using a porous film and incubated at 28 °C for seven days. The observations were recorded immediately after inoculation and then at 12 h intervals on a Labsystems Multiskan RC version 4.0 plate reader (Labsystems Basingstoke, UK). The data were analysed and interpreted using a Genstat programme (Genstat 5 Release 4.2, Lawes Agricultural Trust, Rothamsted Research).

For nematode counts, the plants were uprooted and the soil around roots was removed and mixed in the bulk soil of the pot. The nematodes were extracted from 200g of soil by sieving and decantation and modified Baerman's funnel techniques (Christie and Perry, 1951) and their counts made.

RESULT

The bacterial population densities were lowest in unplanted soil. The pseudomonads (Figure 1) were most abundant (5.7-9.9×10^6 cfu) in tomato treatments compared to cabbage (0.9-3.5×10^6 cfu). In general, tomato plant treatments also supported greater populations of heterotrophic (1.4-3.5×10^9) (Figure 2) as well as enteric bacteria (6.5-9.5×10^6) than those treatments applied to cabbage plants (Figure 3).
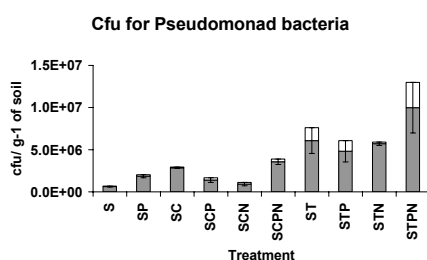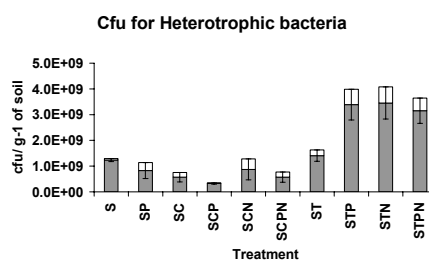
Cfu for Pseudomonad bacteria

Cfu for Heterotrophic bacteria

Figure 1

Figure 2

Cfu for Enteric bacteria

Nematode Counts

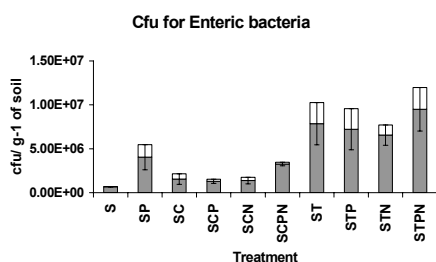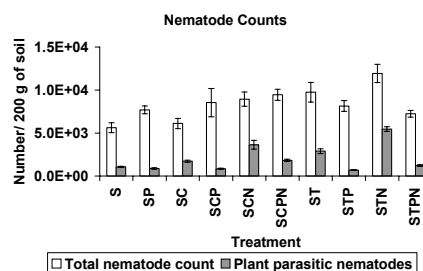☐ Total nematode count ■ Plant parasitic nematodes

Figure 3

Figure 5

The cfu levels of enteric bacteria were greater in soil treated with *P. chlamydosporia* (4×10^6) than in control soil (6.7×10^5). Total fungal counts were 24-times greater in soil from tomato alone and 61-fold increase in combined tomato treatments when compared to the unplanted controls (data not shown).

PCA analysis (Figure 4) of the microbial carbon utilisation profiles using Biolog™ GN plates showed that the major influence on microbial populations was associated with the presence or absence of plants, with only a small difference observed between the two plant species. No significant effect was observed with the addition of either *P. chlamydosporia* and/or *M. incognita.*
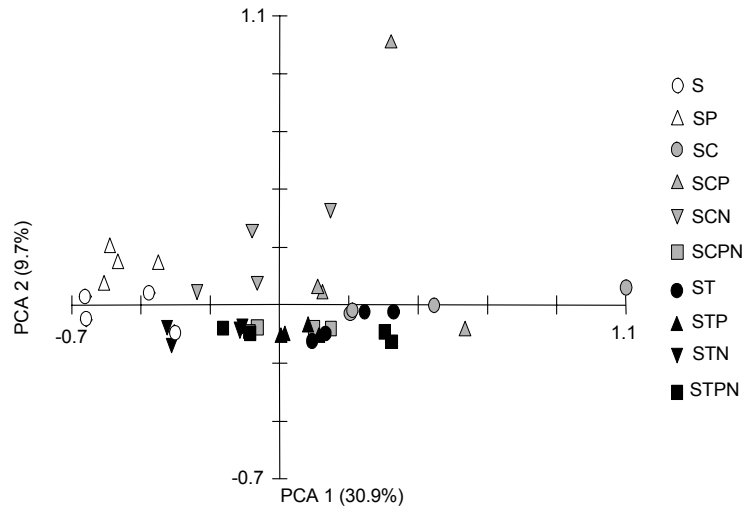
Figuur 4. Principal component analysis of microbial diversity as assessed by Biolog analysis (36 hr, -con/AWC)

The total nematode counts showed no significant difference in relation to plant species or to the application of the biological control agent *P. chlamydosporia* or the plant pathogenic nematode *M. incognita* (Figure 5). Total plant parasitic nematodes (PPN), however, increased in both the presence of the host plants, cabbage (S: SC 1.6 fold) and tomato (S: ST 2.7 fold), and also with the addition of *M. incognita* (S: SCN 3.4 fold increase, S: STN of 5.1 fold increase). The addition of *P. chlamydosporia*, however, greatly reduced plant parasitic nematodes by a maximum of 78% in the host plant tomato compared to cabbage where a 51% reduction in population was observed (Figure 5).

DISCUSSION

Application of *P. chlamydosporia* did not affect the culturable bacterial or non-target nematode populations. However, an increase in bacterial populations was observed in all tomato treatments when compared to cabbages or the controls indicating the major impact on microbial populations being plant species. Despite the differences in number, the bacterial diversity seems to be largely the same in the two plants when compared to unplanted soil, contrary to the results obtained by O'Flaherty *et al.* (2003) where nematode-infected tomatoes supported a changed microbial profile in the rhizosphere. In the current experiment *P. chlamydosporia* effectively colonised the rhizosphere, reducing the population of root-knot nematodes along with the migratory ecto-parasitic nematodes. Increased populations of pseudomonad species, reportedly antagonistic to plant parasitic nematodes (Gallagher and Manoil, 2001; Hendrickson *et al.*, 2001; Yorgey *et al.*, 2001; Ali *et al.*, 2002; Krechel *et al.*, 2002; Siddiqi and Shaukat, 2002), in the tomato plants indicate some correlation with nematode infections.

From the above study *P. chlamydosporia* was proved to be an effective biological control agent by reducing the numbers of plant parasitic nematodes including *M. incognita,* within both crops by 51 to 78%. These results have further confirmed that *P. chlamydosporia* has potential for development as a BCA against root-knot nematodes and have shown that integration into a pest control strategy would not affect beneficial and indigenous micro-fauna.

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