THE IMPACT OF TALL FESCUE (*FESTUCA ARUNDINACEA*) ENDOPHYTE (*NEOTYPHODIUM* SPP.) ON NON-TARGET SOIL NEMATODES

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ABSTRACT

Non-target effects of *Neotyphodium* endophyte infection in field-sown tall fescue were studied as a model for investigating non-target effects of genetically-modified plants. Two strains of *Neotyphodium* endophyte (AR501 and AR542) in tall fescue at a site near Palmerston North (Aorangi) and one strain (AR501) at a site near Lincoln were sampled in March and April 2002 respectively. The only consistent effect of either endophyte strain on nematode abundance was an increase of large omnivorous nematodes beneath AR501 tall fescue at both sites. This was reflected in omnivorous nematodes constituting a significantly greater proportion of the nematode fauna over the two sites. The nematode results show that relatively minor effects on this component of soil biota were observed as a result of plant changes associated with endophyte-infection, even after long-term growth in field plots.

INTRODUCTION

There has been much attention in recent times on the possible effects of genetically-modified (GM) plants on the New Zealand environment (Conner et al. 2003). From a practical viewpoint these are difficult to study as the strict requirements for containment of GM plants limits opportunities to sample and measure any changes in field plots. This is particularly so for forage plants as there are few GM examples developed in New Zealand and none in field trials, with containment trials currently approved only for GM sugarbeet, potatoes, petunias and maize (Environmental Risk Management Authority 2004). Until such trials eventuate for forage plants it is possible to provide some baseline comparisons on the environmental effects of plants that have been altered by more conventional techniques.

In this study the non-target impacts of tall fescue (*Festuca arundinacea*) plants that were infected with selected strains of a fungal endophyte were compared against those that contained no endophyte. Until recently, tall fescue seed containing endophytes has not been commercially available because of the mammalian toxicity associated with wild-type fungi. The AR542 endophyte strain (Max P™) that has been introduced into New Zealand, lacks the mammalian toxins. Establishing that there were no environmental consequences to having endophyte in tall fescue was an important consideration prior to its commercial release.

There is evidence from overseas that damage by insects (Popay & Bonos 2005) and root parasitic nematodes (Kimmons et al. 1990) is reduced by endophyte-infected compared to endophyte-free tall fescue, and that endophyte-infection can improve tolerance to drought (West et al. 1993) and soil aluminium (Malinowski & Belesky 1999). Endophyte infection apparently alters root morphology and exudation in plants from which they are found compared to endophyte-free plants (Malinowski & Belesky 2000) and as such is likely to affect soil biota. To investigate any such effects, two field sites, which form part of the AgResearch National Endophyte Evaluation trial, were sampled to determine the impact of endophyte infection in tall fescue on the soil biota. Soil and root samples

were examined for microbes (Sayer et al. 2004), mycorrhizal fungi and insects (Popay & Jensen 2005), as well as the nematode populations reported here.

MATERIALS AND METHODS

The two sites used in this study were at Aorangi near Palmerston North (Kairanga silt loam soil) and Lincoln near Christchurch (Wakanui silt loam). Plots (5 m × 3 m) at each site had been sown with tall fescue 5 years before sampling. Ten plots were sampled from Aorangi on 11 March 2002. These were four replicates of endophyte-free (E-) tall fescue (cv. Advance), four replicates of Advance tall fescue infected with the endophyte designated AR501 and two replicates of "breeding pool" tall fescue infected with the AR542 endophyte. Eight plots were sampled from Lincoln on 8 April 2002. These were four replicates each of Advance E- and AR501. In each plot 10 soil samples to 100 mm depth were taken over tall fescue plants using a 100 mm diameter corer.

The endophyte infection levels were determined in tillers taken from the cores using a tissue immunoblot procedure. More than 500 tillers per treatment were tested for Aorangi samples and more than 900 for Lincoln. In addition nematodes were extracted from 100 g hand-crumbled soil from each plot by the method described by Bell & Watson (2001). The total number of nematodes and Enchytraeids were counted from the extract under a stereo microscope at 40–80 × magnification. The extracts were then fixed in hot 4% formalin. After fixing, a 100 μl subsample of each extract was mounted on a 50 × 76 mm glass slide, sealed under a 45 × 45 mm cover glass with wax and nematodes discriminated to genera using a compound microscope at 100–400 × magnification. Nematode feeding types follow Yeates et al. (1998). Calculation of diversity and species richness follow Yeates (2003).

Data from Aorangi were analysed by REML to allow for uneven replication among treatments, while data from Lincoln and for the combined sites (where AR542 data was excluded) were analysed by ANOVA.

RESULTS

Endophyte infection levels at Aorangi were 4% in E- plots, 70% in AR501 and 97% in AR542. In E- plots at Lincoln 1% of tillers contained endophyte, while AR501 plots were 80% infected.

Of the plant parasitic nematodes identified from Aorangi, there was more than twice as many *Pratylenchus* under AR542 than E- plants (Table 1). Fungal feeders dominated the remaining nematode fauna at Aorangi. The dominant bacterial feeding genera identified was *Eucephalobus*, which was less ($P<0.10$) abundant under AR542 ($36.7 \times 10^3/m^2$) than E- plants ($149.3 \times 10^3/m^2$). There were more Enchytreaids under AR542 plants then under either E- or AR501 plants.

TABLE 1: Abundance of nematodes (thousands/m^2) and Enchytraeids from soil beneath tall fescue at the Aorangi site. Plant feeders are identified to family or genera. Note: * denotes P<0.001.**

Feeding group/Genera	E-	AR501	AR542	SED
Total nematodes	1658.3	1555.4	1332.3	294.5
Plant associated	232.9	261.0	219.5	70.9
Plant feeders:				
Heterodera	10.6	0.0	0.0	12.0
Helicotylenchus	29.2	20.9	20.4	22.1
Pratylenchus	80.9	160.1	196.4	62.0
Fungal feeders	911.6	752.1	613.1	177.3
Bacterial feeders	347.4	267.8	182.0	110.7
Predators	9.8	14.4	13.7	10.8
Omnivores	35.9	79.0	35.4	27.4
Enchytraeids	3.3	1.5	7.5	1.3 ***

The Lincoln site had fewer total nematodes than the Aorangi site but, as with the Aorangi site, the free-living fauna was dominated by fungal feeders (Table 2). Omnivorous nematodes were at least twice as abundant under AR501 as E- plants at both sites (Tables 1 & 2) and this was significant (P<0.05) at the Lincoln site.

TABLE 2: Abundance of nematodes (thousands/m^2) and Enchytraeids from soil beneath tall fescue at the Lincoln site. Plant feeders are identified to family or genera. Note: * denotes P<0.05.

Feeding group/Genera	E-	AR501	SED
Total nematodes	810.8	877.0	184.4
Plant associated	199.3	244.0	115.6
Plant feeders:			
Helicotylenchus	9.4	2.1	7.8
Pratylenchus	150.8	112.0	39.2
Fungal feeders	318.8	329.0	80.3
Bacterial feeders	112.0	120.2	54.4
Predators	7.1	37.7	31.5
Omnivores	12.6	31.8	4.2 *
Enchytraeids	2.5	3.1	2.4

Over both sites combined, a significantly greater percentage of the fauna consisted of omnivorous nematodes under AR501 than E- plants (Table 3).

TABLE 3: Percentage contributions of nematode feeding types to the total nematode fauna from soil beneath endophyte free (E-) or AR501 infected tall fescue. Data is combined from Aorangi and Lincoln sites. Note: ** denotes P< 0.01.

Feeding type	E-	AR501	SED
Plant associated	19.1	22.5	4.9
Plant parasitic	14.1	12.7	3.9
Fungal	47.2	42.5	3.6
Bacterial	16.7	15.3	3.3
Predators	0.8	2.8	1.8
Omnivorous	2.1	4.2	0.6**

The ratio of bacterial to bacterial+fungal feeding nematodes indicates the predominant decomposition pathway at both sites was fungal mediated (data not shown). Both Shannon-Weaver diversity and species richness were greater in the AR501 (1.77 and 1.99) compared to endophyte-free treatments (1.68 and 1.89) over both sites, but this was not significant (P>0.10).

DISCUSSION

The high numbers of *Pratylenchus* under AR542 relative to E- plants at Aorangi is equivalent to previous results for *Helicotylenchus* beneath AR542 from Kerikeri and Ruakura (N.L. Bell, unpubl. data). Investigations of this difference and the possibility that it is related to greater root mass are being conducted in pot trials and will include assessment of the abundance of nematodes within both soil and roots.

Overall, the only consistent difference between AR501 and E- treatments was the increased abundance and proportion of disturbance-sensitive omnivorous nematodes. This indicates that some stress was present in the E- environment (Bongers 1990). It has been observed that E- plants are exposed to increased insect herbivory compared to

endophyte-infected plants, which can lead to a weakening and opening up of E- pastures. It may be that such an effect is being reflected here by the omnivorous nematode feeding group, possibly through a stressor effect of increased soil temperatures and lowered soil moisture in the E- plots.

The indication that both taxon diversity and richness increased with endophyte infection should be treated with some caution as some taxonomic diversity was "hidden" within the omnivore and unidentified plant feeder groups. There was no impression gained during the course of nematode assessments that there was a greater degree of diversity within these groups for any of the treatments.

Despite large differences in plant chemical production between the endophyte-infected and E- plants it appears most of the effect on nematodes was mediated through increased plant growth rather than directly. The results indicate that relatively minor effects on non-target soil biota can be expected from the sort of plant changes found with endophyte-infection, even after long-term growth in field plots. This finding has implications for the possible impacts of GM forage plants on soil biota. It appears that even quite large differences in plant root exudates may have small non-target impacts, but increased root growth is likely to have a larger impact, and that this may not necessarily be deleterious. Sequential sampling of field plots over a longer time period is needed to confirm such results.

The microbial fauna of pasture soils are usually found to be dominated by bacteria, hence bacterial-feeding nematodes dominate that fauna. In the case of the two sites here, the nematode community indicated that the decomposition pathway in these soils was predominantly fungal. This was borne out by the populations of fungi detected by Sayer et al. (2004) from the same soil samples. Although bacterial populations still dominated these soil samples there were higher populations of fungi detected than is normally expected from pastoral soils and this is perhaps explained by the dry soil conditions prior to, and at, the time of sampling. The close link between nematode and microbial populations, observed even from a single sampling, encourages the joint approach to environmental impacts research conducted in this and other studies (e.g. Brimecombe et al. 2001).

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REFERENCES

Bell NL, Watson RN 2001. Optimising the Whitehead and Hemming tray method to extract plant parasitic and other nematodes from two soils under pasture. Nematology 3: 179-185.

Bongers T 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83: 14-19.

Brimecombe MJ, De Leij FAAM, Lynch JM 2001. Nematode community structure as a sensitive indicator of microbial perturbations induced by a genetically modified *Pseudomonas fluorescens* strain. Biology and Fertility of Soils 34: 270-275.

Conner AJ, Glare TR, Nap JP 2003. The release of genetically modified crops into the environment. Part II. Overview of ecological risk assessment. Plant Journal 33: 19-46.

Environmental Risk Management Authority 2004. New Organisms - Summary Applications Table. http://www.ermanz.govt.nz/no/no-decisions-summary.asp [accessed 18 May 2005]

Kimmons CA, Gwinn KD, Bernard EC 1990. Nematode reproduction on endophyte-infected and endophyte-free tall fescue. Plant Disease 74: 757-761.

Malinowski DP, Belesky DP 1999. Tall fescue aluminium tolerance is affected by *Neotyphodium coenophialum* endophyte. Journal of Plant Nutrition 22: 1335-1349.

Malinowski DP, Belesky DP 2000. Adaptations of endophyte-infected cool-season grasses to environmental stresses: Mechanisms of drought and mineral stress tolerance. Crop Science 40: 923-940.

Popay AJ, Jensen JG 2005. Soil biota associated with endophyte-infected tall fescue in the field. New Zealand Plant Protection 58: 117-121.

Popay AJ, Bonos SA 2005. Biotic responses in endophytic grasses. In: Roberts C, West C, Spiers D ed. *Neotyphodium* in cool season grasses. Blackwell Publishing. Pp. 163-185.

Sayer ST, Burch G, Sarathchandra SU 2004. The impact of tall fescue (*Festuca arundinacea*) endophyte (*Neotyphodium* spp.) on non-target soil micro-organisms. New Zealand Plant Protection 57: 329-336.

West CP, Izekor E, Turner KE, Elmi AA 1993. Endophyte effects on growth and persistence of tall fescue along a water-supply gradient. Agronomy Journal 85: 264-270.

Yeates GW 1998. Feeding in free-living soil nematodes: A functional approach. In: Perry RN, Wright DJ ed. The physiology and biochemistry of free-living and plant-parasitic nematodes. CAB International, Wallingford, UK. Pp. 245-269.

Yeates GW 2003. Nematodes as soil indicators: functional and biodiversity aspects. Biology and Fertility of Soils 37: 199-210.