PLANT-PARASITIC NEMATODES ASSOCIATED WITH PERENNIAL RYEGRASS AND TALL FESCUE WITH AND WITHOUT *ACREMONIUM* ENDOPHYTE

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ABSTRACT

Pure species plots of perennial ryegrass and tall fescue were established using clonal material with and without infection by *Acremonium* endophyte. Plots were sampled in the third year to determine nematode populations in soil. Seven times more nematodes in the genus *Pratylenchus* were found in association with ryegrass than tall fescue and plant parasitic nematodes formed a greater proportion of total soil nematodes under ryegrass. *Pratylenchus*, *Helicotylenchus* and *Tylenchus* numbers were not significantly reduced by endophyte-infected grass. A higher proportion of *Pratylenchus* and *Helicotylenchus* were found at 5-10 cm than 0-5 cm soil depth indicating greater nematode stress on deeper roots.

Keywords: *Lolium perenne*, *Festuca arundinacea*, *Acremonium* endophyte, nematodes, *Pratylenchus*

INTRODUCTION

Acremonium coenophialum endophyte infection in tall fescue (*Festuca arundinacea*) has been shown to reduce numbers of associated ecto- and endoparasitic nematode species (Kimmons *et al.* 1990; West *et al.* 1990). The reduction in nematode burdens is considered to be a possible contributing factor to the better performance and persistence of endophyte containing (E+), compared with endophyte free (E-), tall fescue (West *et al.* 1988). Evidence of similar nematode effects have been less readily demonstrated in field stands of perennial ryegrass (*Lolium perenne*) (Cook *et al.* 1991; Prestidge *et al.* 1993), although lower numbers of the grass endoparasite *Meloidogyne naasi* were found on E+ compared with E- ryegrass grown in pots (Stewart *et al.* 1993). In E+ and E- comparisons in ryegrass/clover swards, a rapid change in pasture species composition makes it difficult to quantify changes in nematode populations which are directly attributable to endophyte (Watson *et al.* 1993). This paper discusses differences found in plant parasitic nematode numbers in soil samples taken from small plots of ryegrass and tall fescue ($\pm$ E) grown as pure species swards for 3 years (Hume *et al.* 1993).

METHODS

Trial establishment and early management details are given by Hume *et al.* (1993). In brief the trial was established in May 1991 at Palmerston North using clonal single tillers planted at 10 cm spacing into a cultivated Karapoti brown sandy loam soil. Main plots (4 m²; three replicates) consisted of $\pm$ watering (summer 1991/92 and 1992/93, and a single application of 25 mm on 9 February 1994), and $\pm$ insecticide for Argentine stem weevil (*Listronotus bonariensis*) control (500 g/ha oxamyl sprayed every 10-14 days during late spring until early autumn of both years). Subplots (1 m²) consisted of two grass species $\pm$ their respective endophytes. Ryegrass cv. Grasslands Pacific contained its own wild strain of *Acremonium*. Tall fescue consisted of plots of cv. Grasslands Roa inoculated with strain AR506 and Grasslands Advance inoculated with the strains AR506 or AR1002.

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Nematode sampling was conducted on 7 April 1994, 1 year after the last application of pesticide. Five 2.5 cm diam. soil cores were taken from selected plots and bulked for 0-5 and 5-10 cm depths. Plots sampled included all replicates of the - water, - pesticide main treatment and tall fescue subplots on one replicate each of the + pesticide treatment, + and - water. Nematodes were extracted from soil using a standard tray method. All plant feeding nematodes by genera, and total soil nematodes which include microbivores and carnivores, were counted. Data were analysed by ANOVA after log_e transformation of nematode counts. Main effects are presented in Table 2 and any significant interactions within the text.

RESULTS

Pratylenchus sp. and *Tylenchus* (*sens.lat.*) gave the highest counts of plant parasitic nematodes and were recovered from the soil of all plots sampled (Table 1). *Helicotylenchus pseudorobustus* occurred in much lower numbers while *Paratylenchus* sp. and *Longidorus elongatus* were present in numbers too low to be analysed for treatment effects. *Heterodera trifolii* and *Meloidogyne* sp. were, as expected, virtually absent without the presence of white clover or other non-graminaceous plants.

TABLE 1: Nematode numbers in plant parasitic genera and total nematodes in soil of 28 plots of three year old ryegrass and tall fescue.

	Mean No./250ml soil	No. plots with nematodes
<i>Pratylenchus</i> sp.	359	28
<i>Tylenchus sens. lat.</i>	178	28
<i>Helicotylenchus pseudorobustus</i>	34	26
<i>Paratylenchus</i> sp.	4.3	15
<i>Longidorus elongatus</i>	0.3	3
<i>Heterodera trifolii</i>	0.1	2
Total soil nematodes	2948	28

Grass species

Numbers of *Pratylenchus* sp. in ryegrass plots were seven times greater than those in tall fescue ($P < 0.001$) and there was a suggestion that *H. pseudorobustus* may also prefer ryegrass ($P = 0.077$) (Table 2). Plot to plot variability was considerable for *Tylenchus* and numbers did not differ significantly between the two grasses. Mean counts of total soil nematodes for the two grasses were very similar. Counts of total soil nematodes were low, reflecting dry soil conditions through the summer and up to the time of sampling. A greater proportion of nematodes under ryegrass were plant pathogenic species compared with those under tall fescue (33 vs 9%; $P < 0.001$).

Endophyte

Numbers of *Pratylenchus* sp. in plots with E+ grasses were greater than those without endophyte ($P < 0.05$; Table 2). *H. pseudorobustus* and *Tylenchus* were not significantly affected by endophyte but the numbers of total nematodes in soil were 26% lower under grasses which were endophyte infected ($P = 0.055$). Tall fescue/endophyte strain combinations in E+ plots produced both infected and non-infected tillers and very low amounts of endophyte alkaloids (Hume and Tapper, unpubl. data) indicating that the strains were unstable (M. Christensen, pers. comm.). Although the endophyte alkaloid levels were very low, significant differences for Argentine stem weevil damage were expressed between E+ and E- tall fescue plots (Hume, unpubl. data).

Sample depth

A lower proportion (31%) of the total number of *Pratylenchus* sp. ($P < 0.001$) and *H. pseudorobustus* ($P < 0.05$) occurred in the upper, 0-5 cm soil depth compared with the 5-10 cm depth (Table 2). *Tylenchus* distribution was not significantly stratified. Of total soil nematodes, 71% under tall fescue and 50% under ryegrass occurred in the 0-

5 cm soil depth (depth x grass interaction $P=0.013$). Thus the percentage of total nematodes which were pathogenic was greater in soil at 5-10 cm than in the upper 5 cm (26 vs 12% respectively; $P<0.001$).

Watering

Numbers of *Pratylenchus* sp. were 54% lower ($P=0.013$; Table 2) in autumn 1994 in plots that had been well watered during the summers 1991/92 and 1992/93 and had received one small application of water in summer 1993/94. The water application in February 1994 had no effect on grass growth so that population differences may have carried over from watering effects that occurred 12 months earlier as was evident with the pesticide treatment. There were no significant effects on the other nematodes, or on the proportion of soil nematodes which were plant pathogens.

Pesticide spraying

Analysis of spray effects on the tall fescue plots of block 1 depended on the assumption that there was no spray x water interaction. On this basis pesticide application reduced *Pratylenchus* by 82% on E- and 63% on E+ plots (spray x endophyte interaction $P<0.05$). A greater proportion of *Pratylenchus* on sprayed plots occurred in the 0-5 cm soil zone than in unsprayed plots (7 of 20 per plot compared with 17 of 102 respectively; spray x depth interaction $P<0.05$). Pesticide application did not significantly affect *H. pseudorobustus*, *Tylenchus* or *Paratylenchus* sp. but reduced numbers of total soil nematodes by 60% on E+ and 37% on E- plots from approximately 2300 nematodes per 250 ml soil (spray x endophyte interaction $P<0.05$).

TABLE 2: Effect of four factors on three genera of plant parasitic nematode and total nematodes in three year old plots of ryegrass and tall fescue.

Variable	<i>Pratylenchus</i>	Mean No/250 ml soil ¹ <i>Helicotylenchus</i>	<i>Tylenchus</i>	Total soil nematodes	% parasitic nematodes
Grass species					
Ryegrass	5.53 (252)	2.40 (11.0)	2.61 (14)	6.94 (1030)	3.47 (32.3)
Tall fescue	3.54 (34)	1.68 (5.4)	3.31 (27)	6.93 (1022)	2.23 (9.3)
S.E.D.	0.18	0.37	0.39	0.14	0.20
Significance ²	***	+	ns	ns	***
Endophyte status					
- Endophyte	4.30 (74)	2.29 (9.9)	3.13 (23)	7.09 (1194)	2.79 (14.4)
+ Endophyte	4.77 (118)	1.79 (6.0)	2.79 (16)	6.78 (883)	3.00 (20.1)
S.E.D.	0.18	0.37	0.39	0.14	0.20
Significance	*	ns	ns	+	ns
Sample depth					
0-5 cm	4.12 (62)	1.65 (5.2)	3.07 (22)	7.16 (1287)	2.46 (11.7)
5-10 cm	4.95 (141)	2.43 (11.4)	2.85 (17)	6.71 (819)	3.24 (25.5)
S.E.D.	0.21	0.29	0.24	0.17	0.13
Significance	***	*	ns	*	***
Water application					
-Water	4.92 (137)	1.65 (5.2)	2.53 (13)	6.95 (1012)	2.91 (18.4)
+ Water	4.15 (63)	2.43 (11.4)	3.39 (30)	6.95 (1041)	2.79 (16.3)
S.E.D.	0.09	0.57	0.59	0.04	0.34
Significance	*	ns	ns	ns	ns

¹ log_ex transformed data with back transformed means in brackets

² ***, *, +, : means different at $P<0.001$, 0.05 and 0.1 respectively

DISCUSSION

Pratylenchus sp. was the main plant parasitic nematode present under both grasses but greater numbers were associated with ryegrass. *Pratylenchus* spp. are widely distributed throughout New Zealand pasture and there is an urgent need to identify the species present. The nematode invades root tissues and is associated with promotion of root rotting fungi in many plants. It is usually the only truly endoparasitic genus affecting grasses in New Zealand and has been associated with damage to pasture grasses during dry periods (Yeates 1989). In year one of the trial described, a response to the pesticide treatment was present only on non-watered plots of ryegrass during summer, in a season in which the incidence of Argentine stem weevil was low (Hume *et al.* 1993). The pesticide regime used for controlling stem weevil has also been demonstrated to effectively reduce nematode populations in pasture soil (Yeates and Prestidge 1986). The profile distribution in this trial suggests that *Pratylenchus* sp. applies greater pressure to roots below 5 cm depth. This may be a contributing factor to the poor performance of ryegrass during drought compared with tall fescue and to the autumn pulling by stock sometimes observed in ryegrass. Root pressure applied by *Pratylenchus*, and the effects of endophyte, may be better indicated by extracting the nematode directly from roots than from soil samples.

H. pseudorobustus present in this trial is the most widely distributed species of the genus in New Zealand pastures, although *H. labiatus* can predominate in some districts, particularly in Waikato and Bay of Plenty (Yeates and Wouts 1992). Three other species have been shown to have much more restricted distribution. Host preferences, at both species and cultivar level, may vary for different nematode species. *Tylenchus* probably consists of a complex of species which can have fungal and/or plant feeding habits, and are not considered to be major pasture pathogens in New Zealand. *Paratylenchus* is relatively widely distributed nationally and can build to very high numbers on free draining or very dry soils (Yeates and Prestidge 1986; Watson *et al.* 1994). *Longidorus* spp. are very rare in pasture but would probably occur in greater abundances below the 10 cm sampling depth used in this study.

There was no indication of a suppressive effect on the plant parasitic nematodes by endophyte infection. Greater numbers of *Pratylenchus* found with E+ grasses may possibly have reflected greater root mass in these treatments. The strains of endophyte used in tall fescue may have affected the expression of nematode activity in this species through their specific alkaloid profiles. Development of nematode responses in relation to endophyte infection may also require environmental conditions, such as heat or moisture stress, which were not satisfied on the trial site. It is possible that *Acremonium* endophyte may have a more direct effect on sedentary endoparasitic nematodes of grasses as shown for *Meloidogyne naasi* and *M. graminis* on ryegrass and tall fescue respectively (Stewart *et al.* 1993; Kimmons *et al.* 1990). This may result from plant-endophyte responses, such as elevated production of chitinase (Roberts *et al.* 1992), which have yet to be fully understood.

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