EFFECTS OF PLANT NEMATODES AND *ACREMONIUM* ENDOPHYTE ON WHITE CLOVER ESTABLISHMENT WITH RYEGRASS OR TALL FESCUE

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SUMMARY

Ryegrass and tall fescue with and without *Acremonium* endophyte were grown with white clover in seed trays for 9 weeks, with and without plant nematodes. Nematodes had a major effect on clover seedlings and a minimal effect on grasses. In the presence of nematodes, clover was further suppressed by ryegrass with endophyte. This has important implications since developed pasture in New Zealand is largely based on endophytic ryegrass and white clover, in which clover feeding nematodes are almost always present.

INTRODUCTION

Acremonium lolii endophyte in perennial ryegrass (*Lolium perenne*) and *A. coenophialum* in tall fescue (*Festuca arundinaceae*) improved grass vigour, persistence and drought tolerance (Latch *et al* 1985; Arachevaleta *et al* 1989). Adverse effects on stock health are generally outweighted by the increased carrying capacity of *Acremonium* infected pasture (Prestidge *et al* 1985; West *et al* 1989). Increased plant vigour is partly due to suppression of insect pest attack by the endophyte (Barker *et al* 1983; Bacon *et al* 1986). Tall fescue endophyte may also suppress numbers of root feeding nematodes (Pedersen *et al* 1988; West *et al* 1988).

Grasses with endophyte may suppress clover to a greater extent in mixed swards than do non-endophytic equivalents (Sutherland and Hoglund 1989). This could reduce the long term productivity of endophytic pasture which is dependent on symbiotic N_2 fixation as its source of nitrogen. Possible reasons for clover suppression include (i) greater competitive ability of endophyte infected grass through pest suppression or enhanced nutrient uptake, (ii) exudates from the endophyte/grass relationship suppressing clover seed germination or plant growth, (iii) repellancy of endophyte infected grass towards herbivores, including pests, placing greater differential pressure on alternative hosts such as clover. The objective of this experiment was to determine interactions between endophyte in grass, plant pathogenic nematodes in soil and seedling vigour in grass and white clover mixtures.

METHODS

Ryegrass (cv 'Ellett' late 1989 harvest) or tall fescue (cv 'Kentucky 31' 1986 ex USA) seed with or without their respective endophytic infections were sown in November 1989 with white clover (cv 'Grasslands Huia' basic seedline A3965, NZ Seed Testing Station) in polystyrene seed trays (40 x 32.5 x 8 mm). Inverted turves of Bruntwood silt loam soil from dairy grazed pasture were placed into trays with minimum disturbance. Half of the trays were frozen (−20°C) for 48 hours prior to planting to reduce soil nematodes while retaining other beneficial or pathogenic microflora, e.g. rhizobia or root rotting fungi. Alternate clover and grass seeds were sown to an even depth in November 1989 in a 20 x 20 mm grid pattern. Treatments were thus: R and T (ryegrass or tall fescue without nematodes or endophyte; RN and TN (with nematodes only); RE or TE (with endophyte only) and RNE or TNE (with nematodes and endophyte). There were four replicates in randomised blocks. Trays were kept outdoors, hand weeded, and watered daily by automatic sprinklers.

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Assessments

All trays were destructively harvested after 9 weeks. Grass and clover plants were cut at ground level from a 10 x 5 plant-row area within each tray. Plants, grass tillers and clover stolons and leaves were counted before DM determination of the bulked foliage samples.

Nematodes in plant pathogenic genera in the soil were counted from two 5 cm diameter soil cores (312 ml soil) taken from each tray and extracted in Whitehead trays. Root material was washed from 10 grass and clover plants and stained in analine blue. Approximately 1 g of blotted, stained roots were examined microscopically and endoparasitic nematodes counted.

Statistical analysis

Where appropriate square root or $\log_e$ transformations were applied to clover and nematode counts. Untransformed plant data (Tables 1 and 2) are presented on a comparative basis with the ryegrass only treatment as a standard. LSD's are therefore average values and in reality differ for different treatment comparisons. Rhizobial counts are based on back-transformed ($\log_e$) data.

RESULTS

Tillers from endophyte free seedlines contained no endophyte, and 98% of tillers from endophyte infected ryegrass seed contained endophyte. The endophyte in tall fescue seed had lost viability (2% tillers infected) but as there were differences between infected and non-infected tall fescue the analyses were conducted as if endophyte was present.

Treatments with endophytic fungus had greater plant survival 9 weeks after sowing (+ endophyte = 34.5 plants; − endophyte = 30.4 plants; $P<0.01$). Dry matter yield and its components (no. tillers, tillers/plant, DM/tiller) are given in Table 1 relative to treatment R. Ryegrass had 60% more tillers/plant than tall fescue ($P<0.001$) but tall fescue had 30-50% greater DM/tiller ($P<0.001$), with the greatest difference in the "endophyte infected" tall fescue. There was significantly greater DM ($P<0.05$) and tillers/plant ($P<0.01$) on both grasses growing in the presence of nematodes. This

TABLE 1: Grass DM yield and components of yield 9 weeks after sowing on treatment R (ryegrass without nematodes or endophyte). Remaining treatments expressed relative to R = 100.

Treatment	No. plants	No. tillers/ plant	DM/tiller (mg)	Total DM (g)
Actual yield				
R[1]	29.3	5.56	41.8	6.79
Relative yield				
R	100	100	100	100
RN	103	128	97	128
RE	115	97	100	111
RNE	125	100	110	137
T	109	64	133	92
TN	103	66	137	92
TE	117	53	147	92
TNE	115	78	150	136
LSD	18	17	40	40
Significant effects (*, **, ***, differences significant at P<0.05, P<0.01 and P<0.001 respectively).				
grass (G)		***	***	
nematode (N)		**		*
endophyte (E)	**			
G x N x E		**		

[1]ryegrass (R) or tall fescue (T), with nematodes (N), with endophyte (E).

resulted from the markedly increased vigour of clover in the absence of nematodes (Table 2). The effect of nematodes on tiller numbers was more pronounced on ryegrass without endophyte and on tall fescue with endophyte (grass x nematode x endophyte interaction; $P<0.01$).

Clover growth

Nine weeks after sowing nematodes had markedly reduced clover plant density, and many surviving plants on nematode treatments were severely retarded. There was a greater number of larger clover plants (>2 leaves) with tall fescue than with ryegrass ($P<0.05$) and this difference was reflected in growth parameters (no. leaves and stolons) and DM yield (Table 2). More larger clover plants occurred with endophyte infected than endophyte free grasses when nematodes were absent but in the presence of nematodes, endophyte resulted in further suppression of clover (nematode x endophyte interaction; $P<0.05$). This nematode x endophyte interaction was also present in the other clover parameters and DM yield. In general where there were more clover plants surviving, clover growth/plant was also better. Clover growth on treatment R, however, was poor in comparison with other no-nematode treatments.

TABLE 2: Clover DM yield and components of yield 9 weeks after sowing with ryegrass and tall fescue, with or without endophyte and nematodes. Data provided for treatment R (ryegrass without endophyte or nematodes), remaining treatments expressed relative to R = 100.

Treatment	No. plants	No. plants with >2 leaves	No. leaves/ plant	Total no. stolons	DM/ plant (mg)	Total DM (g)	Rhizobial nodules/g stained root
Actual yield							
R[1]	26.7	23.0	4.29	5.3	51.1	1.43	81.0
Relative yield							
R	100	100	100	100	100	100	100
RN	67	49	87	128	106	73	19
RE	105	118	162	425	265	254	113
RNE	26	8	66	0	26	4	1
T	122	134	192	494	238	280	173
TN	39	34	113	123	116	44	22
TE	140	154	201	934	433	586	117
TNE	50	33	89	47	99	44	45
LSD	37	(32)[2]	(61)	(237)	(129)	(130)	LSR 4.9[3]

Significant effects (*, **, ***, differences significant at $P<0.05$, $P<0.01$ and $P<0.001$ respectively).

Grass (G)		*	**	**	**	**	**
nematode (N)***		***	***	***	***	***	***
endophyte (E)							*
G x N				*			
N x E		*	*	***	***	***	*
G x N x E		*					

[1] ryegrass (R) or tall fescue (T), with nematodes (N), with endophyte (E).

[2] ( ) analysis after square root transformation, raw data given. LSD averaged values only.

[3] analysis after $\log_e$ transformation, backtransformed data given.

Clover was better nodulated in the presence of tall fescue than ryegrass ($P<0.01$), and in the absence of nematodes ($P<0.001$). Nematode treatments therefore significantly affected clover nodulation, and there was a strong association between clover nodulation and clover yields.

Nematode assessments

Ectoparasitic genera *Helicotylenchus, Tylenchus* and *Paratylenchus* were present in very low numbers in soil and were not considered to have significantly influenced results. Endoparasites *Heterodera trifolii* and *Meloidogyne hapla* affect clover only while *Pratylenchus* spp affect both grass and clover. Soil freezing reduced numbers of soil mobile stages of *Pratylenchus* and *Tylenchus* by >75% and the remaining genera by >98% at planting. Root invasion by *Heterodera* on frozen soil was greater than for *Meloidogyne,* possibly as a result of survival by the encysted eggs in the soil.

At 9 weeks the low root population of *Meloidogyne* was maintained on frozen soil (− nematodes = 3.8; + nematodes = 340/g stained root; $P<0.001$). On non-frozen soil greater root knotting by *Meloidogyne* occurred in clover growing with tall fescue than with ryegrass (tall fescue = 127; ryegrass = 8.6 knots/g root; $P<0.001$) reflecting the numbers of *Meloidogyne* present in roots (931 and 123/g root respectively). This partly resulted from the very poor growth and survival of clover on treatment RNE as a result of initial nematode infection of seedlings. *Heterodera* were fewer on frozen treatments after nine weeks (− nematodes = 3.3; + nematodes = 13.2/g root; $P<0.05$) but there was no signficant grass or endophyte effect. *Heterodera* invade clover seedlings aggressively, often as soon as the radicle is produced, but root populations tend to decline relative to *Meloidogyne* during the summer (Yeates *et al* 1985). *Meloidogyne* and *Heterodera* populations were greater in roots of small retarded clover plants than in larger plants (*Meloidogyne* x 3.7; *Heterodera* x 7.0 nematodes/g root; $P<0.01$).

Numbers of *Pratylenchus* were negligible in all clover roots, and in grass roots from frozen soil. Numbers in grass roots on non-frozen soil were also very low relative to levels normally associated with plant growth responses. There were fewer in association with endophyte free compared with endophyte infected ryegrass, and endophyte infected compared with endophyte free tall fescue (RN = 3.0 ± 1.1; RNE = 8.6 ± 3.8; TN = 11.0 ± 6.4; TNE = 3.6 ± 0.8/root respectively).

DISCUSSION AND CONCLUSIONS

The major effect was the influence of *Meloidogyne* and *Heterodera* nematodes on clover growth. The resulting severe stunting and poor nodulation has been widely observed (Healy *et al* 1973). The disadvantage suffered by clover as a result of nematode attack may have been intensified by spring rather than autumn sowing. Nematodes did not have a direct influence on grass seedlings, supporting field trials in which establishing ryegrass gave minimal responses to nematicide (Watson *et al* 1986).

Endophyte effects were minor relative to those of nematodes. Insect pests did not affect grass or clover at any growth stage. Endophyte improved grass seedling survival but did not significantly affect seedling vigour. Such growth enhancement has been demonstrated in the absence of vertebrate pests in both grasses (Latch *et al* 1985; Clay 1987). Endophytic effects present in tall fescue seedlings from seed which contained non-viable endophyte, confirm similar observations in ryegrass (Stewart 1985). When nematode numbers were markedly reduced, clover grew better with endophyte infected than endophyte free grass showing there was no direct effect of endophyte on companion clover plants. There was no evidence to suggest that endophyte reduced nematodes feeding on ryegrass, but *Pratylenchus* numbers may have been reduced on tall fescue containing endophyte as shown by West *et al* (1988). Nematode numbers on both grasses were very low however.

Clover established better with tall fescue than with ryegrass. Compatibility of tall fescue with legumes has been recognised (Hay 1987; Hay and Hunt 1989). This may relate to lower tiller density providing a more open canopy during the establishment period. Conversely ryegrass may have other qualities which suppress establishing clover and which are not endophyte related.

Because of the highly competitive nature of mixed grass and clover swards a small shift in competitive balance may produce dramatic changes in grass:clover content. Where endophyte provides a slight advantage to grass vigour and nematodes a considerable disadvantage to clover, then endophyte infected grass appears able to suppress clover during pasture establishment. This has important implications since both endophyte infected ryegrass and clover nematodes are a feature of developed pastures in New Zealand.

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