Productivity, persistence and nematode impact in pure and mixed swards of white clover and *Lotus corniculatus*

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Abstract

The effect on pasture productivity of sowing birdsfoot trefoil (*Lotus corniculatus*) and white clover (*Trifolium repens*) as pure and mixed swards was evaluated over two growing seasons. Different sowing techniques were used for mixed sward treatments: species sown in alternate rows, mixed within rows or broadcast. In year one, herbage yield ranged from 6.4 to 7.8 t DM/ha (the greatest yield was from the lotus monoculture, $P<0.05$). In year two, yields ranged from 11.4 to 13.6 t DM/ha, but were not affected by treatment. From year one to year two, the contribution of lotus to yield in mixed swards declined by 69%, while that of clover increased by 485% reflecting reduced density of lotus plants. Total nematode burden in the roots of lotus and clover seedlings during establishment was reduced in mixed swards compared with clover monocultures ($P<0.001$). Root knot nematode *Meloidogyne trifoliophila* invaded lotus roots, but could not initiate feeding sites and died within the root.

Keywords: birdsfoot trefoil, *Lotus corniculatus*, pasture production, plant nematodes, white clover

Introduction

White clover (*Trifolium repens*) is an essential component of dairy pastures in New Zealand. It is high quality forage for dairy cows (Rogers *et al*. 1982; Thomson 1984) that contributes to soil fertility through nitrogen fixation (Ball *et al*. 1979). However white clover (clover) yields can be unpredictable, varying from season to season (Harris 1997). One reason for this variation is the impact of plant nematodes on the competitive vigour and capacity of clover to fix nitrogen (Watson 1990; Watson *et al*. 1996). The main species of nematode affecting white clover in northern New Zealand are the root knot nematodes (*Meloidogyne trifoliophila* and *M. hapla*), the clover cyst nematode (*Heterodera trifolii*) and the lesion nematodes (*Pratylenchus penetrans* and *P. crenatus*). The ectoparasitic pin nematode (*Pratylenchus nanus*) is also common in pasture. Less is known about the effect of plant nematodes on lotus pastures. However, studies have shown that lotus plants are affected by *M. hapla* (Yeates *et al*. 1973; Townshend & Potter 1978), but are poor hosts for *M. trifoliophila* (Bernard & Jennings 1997) and *H. trifolii* (Yeates *et al*. 1973; Yanez *et al*. 1999). Little is known about the effects of plant nematodes on pastures containing both legumes.

This experiment evolved from observations on Dexcel farms that clover plants growing within crops of birdsfoot trefoil (*Lotus corniculatus* (lotus)) appeared healthier than those growing in neighbouring mixed swards with perennial ryegrass or in monoculture.

The aims of this experiment were to:

1. Determine whether growing lotus and clover in a mixed sward improves dry matter (DM) yield and persistence compared to that achieved from these legumes grown in monoculture;
2. Evaluate the effect of different sowing methods on productivity and persistence of lotus and clover in a mixed sward;
3. Evaluate the effect of the presence of lotus in a mixed sward with clover on plant nematode populations.

Materials and Methods

Experimental treatments consisted of different sowing methods of lotus and white clover in pure swards or in a mixture:

(1) Pure clover, drilled in rows
(2) Pure lotus, drilled in rows
(3) 50:50 mixture of lotus and clover, broadcast (broadcast treatment)
(4) 50:50 mixture of lotus and clover, with each species sown in alternate drill rows (alternate treatment)
(5) 50:50 mixture of lotus and clover, with each species mixed within drill rows (mixed treatment).

Plots were arranged in a randomised complete block design, with the five treatments replicated four times, giving a total of 20 plots, each measuring 6 x 2.4 m. The experiment was conducted from October 2004 to June 2006 at Dexcel's Scott farm near Hamilton, (37º47'S, 175º19'E). The soil type at the site was a Te Rapa humic silt loam (Singleton 1991).

Existing ryegrass/white clover pastures were sprayed in October 2004 with 4.5 L/ha Roundup® Renew Xtra and 25 g/ha Granstar. In November 2004, the area was chip-hoed, ploughed, power harrowed and rolled to prepare a fine seedbed. A pre-emergent herbicide spray (3 L/ha Triflur 480) was applied to the site and worked into the soil using a rota-tiller before rolling with a V-roller. Monocultures of bare lotus (cv. Grasslands Goldie) and coated clover (cv. Aran) seed were sown using an

Oyjord plot drill at a rate of 8.8 kg/ha. Lotus seed was inoculated with "Nodulaid" before sowing. Mixed swards of lotus and clover seed were sown with lotus at a rate of 4.4 kg/ha and clover at 4.1 kg/ha (based on equivalent seed numbers and germination percentage) using either the Oyjord plot drill or broadcast using an Earthway® EV-N-SPRED broadcaster. Seed was covered using a hand-operated harrow.

Pasture measurements

Average sward height of each plot was monitored weekly, using an automated sward stick, taking 20 random measurements per plot. Experimental plots were harvested when average sward height reached 20 cm. A period of "closing" was imposed over winter (April to August) to avoid depleting root carbohydrate reserves (Ayala *et al*. 2000).

Herbage yield was determined by harvesting three 2.4 m long strips from the left-hand side of each plot using a push mower set to a cutting height of 5 cm above ground. The weight of harvested material was recorded, and a sub-sample of 200 g was dried at 95ºC for 48 hours and weighed. The remainder of the plot was trimmed to 5 cm and the herbage removed.

Before harvesting, 10 herbage samples were hand-clipped to 5 cm from random locations within the right-hand side of the plot before bulking. A 100 g sub-sample was dissected into white clover stem, leaf and flowers, lotus stem, leaf, flowers and seed pods, all dead material and other species, and oven dried at 95ºC for 36 hours then weighed to estimate botanical composition on a DM basis. A second sub-sample of 150 g was frozen, freeze-dried, ground to pass a 1 mm diameter sieve, then analysed by near infrared spectroscopy for indicators of feed quality (Corson *et al*. 1999) and estimation of condensed tannin (CT) concentration.

Plant density was measured seasonally by removing 10 x 0.33 m^2 (15 cm deep) turves from pre-determined random locations (to avoid re-sampling the same area) within the right hand site of each plot. Soil was washed from each turf, and the number of whole lotus and white clover plants per turf was recorded.

Nematode sampling

Ten lotus and 10 white clover plants were collected at random locations from each treatment 2, 4 and 6 weeks after seedling emergence. Plants were stained in lactophenol blue to highlight nematodes within the roots for counting, and stored in glycerol. Roots were then blotted to remove glycerol and weighed to obtain a 'stained root weight'. The proportion of root knot, cyst and lesion nematodes per gram of root was determined by microscopic examination of whole root systems. Root length was measured using callipers. Numbers of branch roots and initiated rhizobial nodules per plant were counted and recorded. Data from mixed sward treatments were combined.

Statistical analysis

Analysis of variance was used to test for treatment differences using Genstat 8.1.

Results and Discussion

Pasture

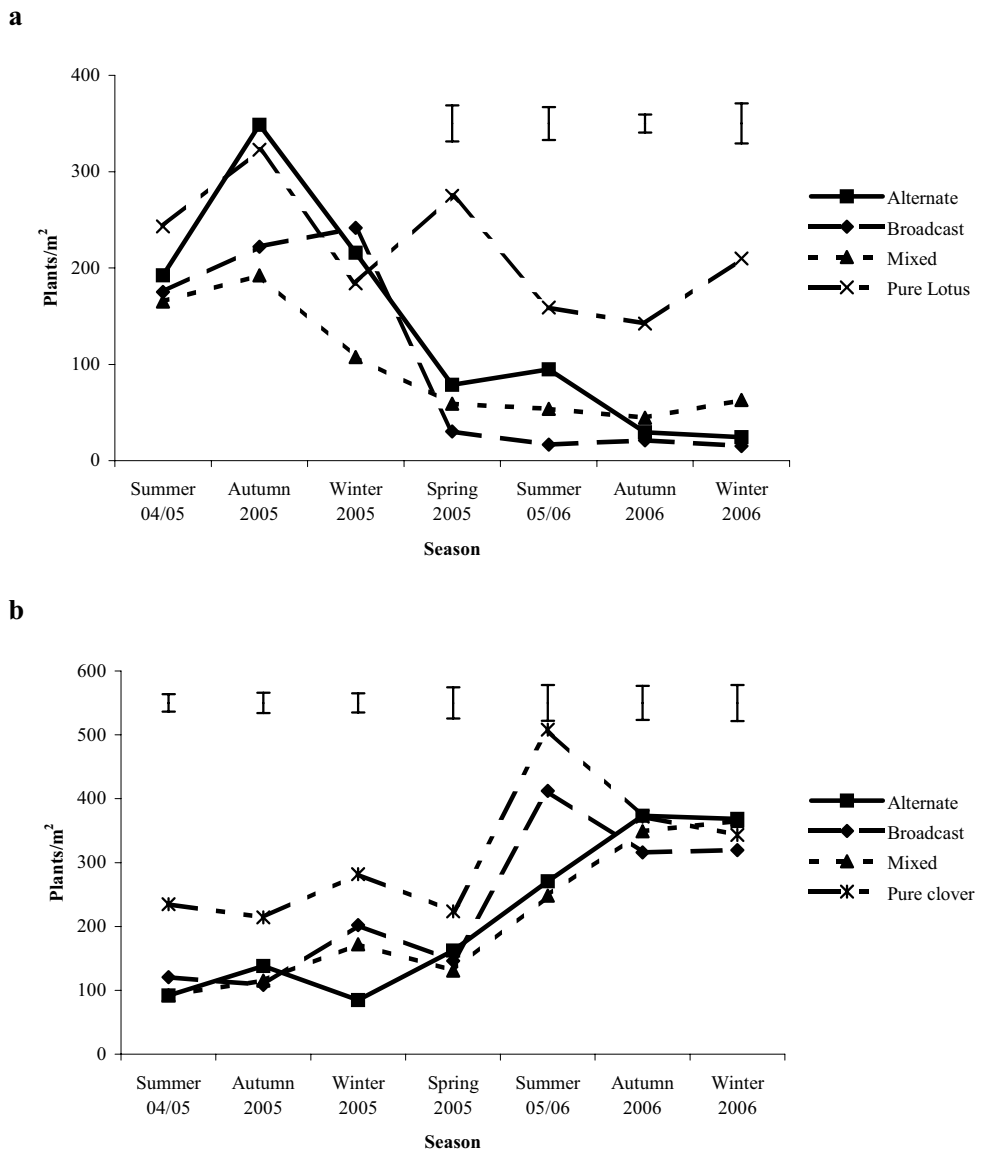
In year one, pure lotus produced the highest yield (7.8 t DM/ha), significantly more ($P<0.05$) than pure clover (6.4 t DM/ha; Table 1). However there was no difference in yield between mixed sward treatments and pure lotus or between mixed sward treatments and pure clover. In year two, treatment did not affect yield. In the mixed swards, clover's contribution to yield increased markedly from year one to year two (from av. 30% to 80%). Consequently, a reduction in lotus' contribution to yield was observed (from av. 70% to 13% in year two; Table 1). Compositional changes in mixed swards are likely to be due in part to competition between lotus and clover

Table 1 Herbage yield (t DM/ha) of pure and mixed swards of lotus and clover, and the effect of sowing method of mixed swards on yield over 2 years.

Treatment	Total yield		Total lotus yield		Total clover yield	
	2004/05	2005/06	2004/05	2005/06	2004/05	2005/06
Alternate [1]	7.6	12.1	5.2	1.5	2.4	9.8
Broadcast [2]	7.2	12.0	5.3	1.4	1.8	10
Mixed [3]	7.5	12.0	5.6	2.0	1.8	9.3
Pure lotus	7.8	13.6	7.7	8.6	0.1	3.4
Pure clover	6.4	11.4	0.1	0.2	6.3	10.9
SED	0.42	0.91	0.42	0.85	0.43	0.53
P value	0.043	0.233	<0.001	<0.001	<0.001	<0.001
P (excl clover)			0.001	<0.001		
P (excl lotus)					<0.001	0.085

[1] Species sown separately in alternate rows in sward.
[2] Species sown with broadcast spreader.
[3] Species sown as a mixture within drill rows.

Figure 1 Plant density in pure and mixed swards of (a) lotus and (b) white clover over 2 years (Bars represent SED).



plants, as clover stolons were observed to be growing over the lotus plant crowns. Since the crown is the primary site of regrowth for lotus plants, invasion by clover stolons may have reduced crown light interception and development of new shoots, leading to plant loss. In the mixed swards, a general decline in lotus plant density and an increase in clover plant density were observed over the 2 years (Fig. 1). Sown species maintained dominance throughout the experiment, with lotus and clover contributing at least 90% of sward DM.

Forage quality was not affected by treatment and levels of nutritional indicators were within the ranges regarded as good quality forage (Kolver 2000). Metabolisable energy levels ranged from 11.7 to 12.3 g/100 g DM; crude protein concentration ranged from 25.1 and 31.5 g/100 g DM across each year. Differences in condensed tannin concentration (CT) were observed in year two ($P<0.01$), with the lowest concentrations in pure clover, alternate rows and broadcast treatments (2.2, 1.7 and 2.3 g/100 g DM, respectively), and highest in mixed row and pure lotus treatments (3.2 and 3.4 g/100 g DM respectively, SED=0.22). This was associated with a higher proportion of clover in the pure clover (Table 1), alternate and broadcast treatments as clover typically has

Table 2 The percent composition of plant parasitic nematodes, *Meloidogyne*, *Heterodera* and *Pratylenchus*, present in roots of lotus and clover from pure and mixed swards at 2, 4 and 6 weeks after establishment, and the total number of nematodes per gram of root in week 6.

	Meloidogyne	*Heterodera*	*Pratylenchus*	Total nematodes/g root
Week 2				
Lotus	8.9	0.5	90.6	
Clover	16.3	2.8	80.9	
Mixed sward[a]	14.8	1.6	83.6	
SED	7.32	2.66	8.78	
P	NS	NS	NS	
Week 4				
Lotus	1.6	53.9	44.5	
Clover	1.4	34.5	64.2	
Mixed sward[a, b]	0.5	31.8	67.3	
SED	1.43	8.00	7.67	
P	NS	NS	0.05	
Week 6				
Lotus	0.0	14.6	85.4	1407
Clover	0.5	40.3	59.2	7801
Mixed sward[a]	0.1	41.7	58.2	5177
SED	0.32	4.09	4.15	
P	NS	0.001	0.001	0.001

[a] Data from all three mixed sward sowing treatments were combined.
[b] Numbers of other nematode species are included in calculations but details not presented in this report.

Table 3 Seedling performance after 6 weeks in plots sown in lotus, clover and mixed species.

Treatment	Lotus	Lotus in mixed sward	Clover	Clover in mixed sward	P (Species)	P (Mixed)	P (Interaction)	SED
Root length (mm)	54.4	56.1	56.2	61.8	NS	NS	NS	4.80
No. of nodules	1.4	1.6	3.2	3.0	0.01	NS	NS	1.14
No. of branch roots	10.8	10.4	18.2	17.9	0.001	NS	NS	1.17
Root wt (g)	9.2	5.9	4.9	5.1	0.001	0.05	0.01	0.73

lower CT concentrations than lotus (Waghorn *et al*. 1998). Concentrations of CTs above 3 g/100 g DM are considered nutritionally beneficial to ruminants (Aerts *et al*. 1999).

Nematode populations

Meloidogyne trifoliophila freely invaded lotus roots. However, this nematode could not initiate feeding sites and subsequently died within the root (Table 2). Thus lotus became a 'trap crop' for this species and reduced the soil inoculum that was available to infect clover. *Heterodera* populations were low initially, but increased rapidly probably from eggs hatching from the resistant cyst stage present in the soil, but ultimately declined in pure lotus (Table 2). *Pratylenchus* may have been able to exploit the opportunity presented by lowered *Meloidogyne* and *Heterodera* populations but total nematode root burden in 6-week-old lotus seedlings was still markedly reduced compared with white clover (Table 2).

Root length and nodulation of clover was not affected by the presence of lotus in the sward (Table 3). Root weight per plant of lotus was reduced in the mixed sward probably due to competition between lotus and clover plants rather than damage caused by few nematodes.

Conclusion

Total root nematode burden was reduced in mixed swards compared to clover monocultures over the first 6 weeks of establishment, though no long-term productivity gains were achieved by sowing lotus and clover in mixed sward. While sown species maintained high proportions within the swards, lotus plant density declined when mixed with clover. Sowing methods for mixtures of lotus and clover had similar productivity outcomes in this experiment. Any reduction in nematode burden is likely to be temporary, limited to when lotus is present in the sward with clover.

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REFERENCES

Aerts, A.U.; Barry, T.N.; McNabb, W.C. 1999. Polyphenols and agriculture: beneficial effects of proanthocyanidins in forages. *Agriculture, Ecosystems and Environment 75*: 1-12.

Ayala, W.; Hodgson, J.; Kemp, P. 2000. Changes in the morphology, production and population of *Lotus corniculatus* L. cv. Grasslands Goldie in response to seasonal defoliation regimes. *Agronomy New Zealand 30*: 93-100.

Ball, R.; Brougham, R.W.; Brock, J.L.; Crush, J.R.; Hoglund, J.H.; Carran, R.A. 1979. Nitrogen fixation in pasture. 1. Introduction and general methods. *New Zealand Journal of Experimental Agriculture 7*: 1-5.

Bernard, E.C.; Jennings, P.L. 1997. Host range and distribution of the clover root-knot nematode, *Meloidogyne trifoliophila*. *Journal of Nematology 28 (Supplement)*: 662-672.

Corson, D.C.; Waghorn, G.C.; Ulyatt, M.J.; Lee, J. 1999. NIRS: Forage analysis and livestock feeding. *Proceedings of the New Zealand Grassland Association 61*: 127-132.

Harris, S.L. 1997. The performance of clover in dairy pastures. *Dairyfarming Annual 49*: 142-149.

Kolver, E.S. 2000. Nutritional guidelines for the high producing dairy cow. *Proceedings of the Ruakura Farmers', Conference 51*: 78-87.

Rogers, G.; Porter, R.; Robinson, I. 1982. Comparison of perennial ryegrass and white clover for milk production. pp. 213-214. *In*: Proceedings of the Conference on Dairy Production from Pasture.

Singleton, P.L. 1991. Soils of Ruakura – a window on the Waikato. DSIR Land Resources Scientific Report No. 5. 122p.

Thomson, D.J. 1984. The nutritive value of white clover. pp. 78-92. *In*: Occasional Symposium of the British Grassland Society.

Townshend, J.L.; Potter, J.W. 1978. Yield losses among forage legumes affected with *Meloidogyne hapla*. *Canadian Journal of Plant Science 58*: 939-943.

Waghorn, G.C.; Douglas, G.B.; Niezen, J.H.; McNabb, W.C.; Foote, A.G. 1998. Forages with condensed tannins – their management and nutritive value for ruminants. *Proceedings of the New Zealand Grassland Association 60*: 89-98.

Watson, R.N. 1990. Effects of plant nematodes and *Acremonium* endophyte on white clover establishment with ryegrass or tall fescue. pp 347-351. *In:* Proceedings of the 43rd New Zealand Weed and Pest Control Conference.

Watson, R.N.; Dickie, G.; Neville, F.J.; Bell, N.L. 1996. Seedling invasion of three small leafed white clover cultivars by clover nematodes. pp. 239-243. *In*: Proceedings of the 49th New Zealand Plant Protection Conference.

Yanez, M.V.; Bohm, L.S.; Gonzalez, S.M. 1999. Evaluation of four populations of *Heterodera trifolii* Goffart in forage legumes. *Agronomy Survey 27*: 105-112.

Yeates, G.W.; Healey, W.B.; Widdowson, J.P. 1973. Screening of legume varieties for resistance to the root nematodes *Heterodera trifolii* and *Meloidogyne hapla*. *New Zealand Journal of Agricultural Research 16*: 81-86.