Effect of mineral nitrogen on spring growth and nitrogen fixation of white clover lines

H. B. Berthelsen[1], J. Ford, J. Evans and J.R. Caradus

AgResearch Grasslands, Private Bag 11008, Palmerston North, New Zealand.
[1] Current address: Department of Agricultural Sciences, The Royal Veterinary and Agriculture University, Denmark

Abstract

The impact of mineral nitrogen (N) applied at 0, 100 and 200 kg N/ha, on the N-fixation of a range of white clover cultivar and breeding lines grown in mixed species plots, was investigated.>>Measurements were made of N-fixation (acetylene reduction), N content and herbage yield at two weekly intervals during mid- to late-spring, when demand by grasses for N is often greatest.>>Applied N reduced N-fixation *per se.* more than it did clover yield or content in the sward.>>There were no N treatment x breeding line interactions for any of the characters measured.>>However, applied N significantly decreased N-fixation, but increased clover percent nitrogen, grass percent nitrogen and total sward nitrogen content.>>Date and breeding line differences were significant for most characters measured.>>There were significant N x date interactions for N-fixation.>>Cultivar x date interactions were significant for proportion of clover in sward, and N-fixation efficiency.

It may not be possible to identify white clover cultivars able to either maintain high levels of N-fixation or conversely, completely cease N-fixation at levels of up to 200 kg N/ha.

Additional key words: Trifolium repens, *acetylene reduction, herbage yield, breeding lines*

Introduction

White clover (*Trifolium repens* L.) can assimilate nitrogen either through biological nitrogen (N) fixation or uptake of mineral N. These are not mutually exclusive. However, white clover persistence in mixed species swards supplied with mineral N is often poor due to aggressive grass growth. Several studies have compared the growth of different white clover cultivars in grass swards supplied with N (see review by Caradus 1991). However, only a few studies (Laidlaw 1984; Eltilib & Ledgard 1988) have investigated the direct impact of mineral N on N-fixation of white clover cultivars. The aim of this study was to determine the direct and immediate effect of applied N on N-fixation of a range of white clover cultivars grown in a grazed mixed species sward during mid- to late-spring.

Materials and Method

Thirty plants of each of 10 white clover cultivars and breeding lines (Table 1) were transplanted into 0.5 x 0.5 m plots in a four year old perennial ryegrass (*Lolium perenne* L.) and browntop (*Agrostis capillaris* L.) sward at Palmerston North, from which all 'native' white clover had been removed using a selective herbicide (dicamba).

Table 1. Description of white clover cultivars and breeding lines.

Cultivars (c) or Breeding line (*l*)		Origin
Grasslands Huia	c	Selection for high yield and persistence from Hawkes Bay and Nth Canterbury ecotypes
Grasslands Demand	c	Southland adapted cultivar
G.23	*l*	Northland adapted cultivar
G.49	*l*	Stem nematode resistant seln from within Pitau
Grasslands Sustain	c	Medium-large leaf, high stolon growing point density
Irrigation selection	*l*	Selection for persistence in New Zealand within Victorian Irrigation
Southern Europe II	*l*	Selection for high yield from early flowering Spanish and Portuguese populations
Will	c	Ladino from North Carolina, USA
Al-tolerant selection	*l*	Selection from within Huia
Dryland selection	*l*	Selection from population from Waiteko grown at Dannevirke

The sward was grazed to a height of 2 cm before planting the clover in July 1991. There were six replicates of each cultivar/breeding line arranged in a randomised block design. Plots were separated by 0.5 pathways in all directions and blocks separated by 1 m pathways. Pathways were kept free of clover by use of dicamba (10 ml/*l*) 3 or 4 times per year. The trial was mob-stocked by sheep eight times a year.

The trial site was planted so that there were two replicates of each cultivar/breeding line at each of three applied N levels, which were applied in spring 1993. The rates were 0, 50 and 100 kg N/ha applied on 7 October (after acetylene reduction assay) and again on 1 November to give cumulative rates of 0, 100 and 200 kg N/ha.

On 8 October 1993 acetylene reduction was used to measure relative N-fixation of the 60 plots. Acetylene reduction analyses were conducted by taking seven 2.5 cm diameter soil cores (7.5 cm depth) from each plot. Sealed in a 0.5*l* glass jar, 30 ml of acetylene gas was injected through a rubber stopper in the seal. Jars were incubated for 60 minutes in shaded white boxes at the field site. At that time a sample of gas was removed from each jar for determination of ethylene content.

Further acetylene reduction assays were carried out on 18 October, 1, 15 and 29 November, and 13 December. These were taken systematically across plots so that no area was sampled twice. Quadrats (400 cm^2) were cut using electric shearing pieces to a height of 1 cm on 8 October, 2, 16 and 30 November and 13 December. White clover was separated from other species and both components weighed dry. The N content of white clover leaf material harvested was determined for harvests made on 8 October, 2, 30 November and 13 December. The trial was grazed on 29 September and 3 November by mob-stocking with sheep.

Since means and variances were correlated all data were square root transformed before analysis of variance using GENSTAT. Data were combined for all sampling dates and analysed using a split-plot in time analysis.

Results and Discussion

Meteorological data

Meteorological data were available from a station 300 m west of the trial site. Daily maximum, minimum and grass minimum temperatures of the day and two days prior to each acetylene reduction assay are given in Figure 1. Daily minimum and grass minimum temperatures were generally increasing toward each sampling day except for the sharp drop in temperatures leading up to the 15 November sampling which culminated in a -2.6°C frost. Daily maximum temperatures were more stable for days preceding sampling except for 1 November and to a lesser extent 29 November when daily maximum temperatures decreased to the sampling day.

Dry weight yields

There was a significant ($P<0.05$) difference among cultivars for proportion of clover in the sward (Table 2). Grasslands Sustain, Grasslands Demand and Irrigation selection had the highest clover content over the trial period. G.23 and G.49 had the lowest clover content.

Nitrogen treatment had no significant effect on proportion of clover or total sward dry weight. There was a significant ($P<0.001$) effect of harvest date on proportion of clover in the sward, with a gradual reduction from 12% to 3% in clover content from 8 October to 30 November, with recovery to 11% by 13 December. The significant ($P<0.01$) cultivar x harvest date interaction came as a result of differences among cultivars in the extent of this reduction and recovery of clover content as exemplified in Figure 2.

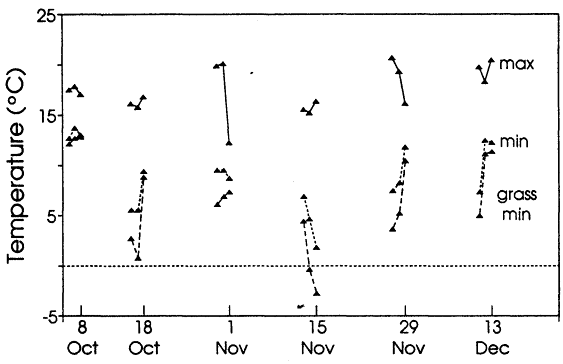

Figure 1. Maximum and minimum daily temperatures, and grass minimum temperature on each sampling day and two days prior to each sampling day.

Table 2. Comparison of white clover cultivars and breeding lines for proportion of clover in the sward, N-fixation and N-fixation efficiency, and percent N in leaf tissue. Each value is the mean of 3 nitrogen treatments and 4 or 5 harvest dates.

Cultivar / breeding line	Proportion of clover	N-fixation (kg N/ha/d)	N-fixation efficiency (kg N fixed/*t* clover DM)	Clover % N
Grasslands Huia	0.06	0.15	25	4.29
Grasslands Demand	0.11	0.21	30	4.26
G.23	0.04	0.05	10	4.05
G.49	0.05	0.09	15	3.78
Grasslands Sustain	0.12	0.15	12	3.79
Irrigation selection	0.11	0.10	6	3.95
Sth Europe II	0.10	0.15	14	3.98
Will	0.06	0.12	16	3.94
Al-tolerant selection	0.06	0.13	16	4.09
Dryland selection	0.07	0.14	14	4.17
P	*	**	*	*
$LSD_{0.05}$	0.05	0.03	6	0.30

There was no significant cultivar x N treatment interaction for any measure of dry matter yield or clover content. This is a result often obtained in such studies examining the effect of applied N on white clover yield in grass swards (see review by Caradus 1991; Caradus *et al.*, 1993).

Nitrogen fixation

There was a significant ($P<0.01$) difference among cultivars for N-fixation (acetylene reduction) (Table 2). Grasslands Demand had the highest values and G.23 and G.49 the lowest. There was more than a four-fold ($P<0.05$) range for N-fixation efficiency (N fixed per unit clover dry matter) (Table 2) among cultivars with Demand and Huia the highest and Irrigation selection the lowest.

Nitrogen treatment had a significant ($P<0.05$) effect on N-fixation (Table 3), such that 100 kg N/ha reduced N-fixation by 41% and 200 kg N/ha reduced N-fixation by 52%. Preferential utilisation of mineral N by legumes over fixed-N is commonly observed. The energy requirement for legumes fixing their own N is higher than for those supplied with mineral N since 11-13% more fixed carbon is respired by N-fixing plants (Ryle *et al.*, 1979). There was also a significant N treatment x date ($P<0.01$) interaction for N-fixation such that on 16 November 200 kg N/ha depressed N-fixation by 86% but on 13 December by only 54%.

There was no significant cultivar x N treatment interaction for either N-fixation or N-fixation efficiency. West *et al.* (1985) concluded from a pot trial, however,

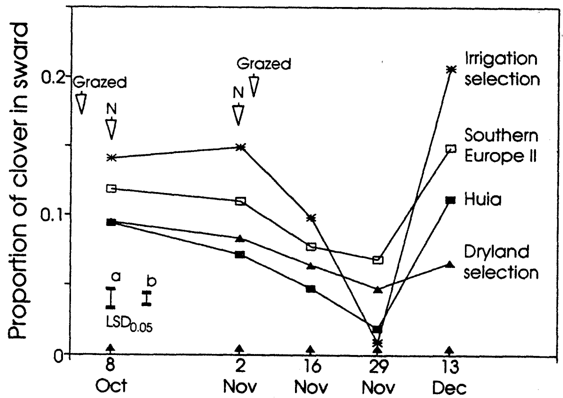

Figure 2. Proportion of clover in the sward at each harvest date for Huia, Irrigation selection, Southern Europe II and Dryland selection. Each value is the mean of three nitrogen treatments. a) $LSD_{0.05}$ for comparison within dates, and b) $LSD_{0.05}$ for comparison within cultivars.

Table 3. Effect of applied N on N-fixation of white clover and grass percent leaf N concentration and total sward N content. Each value is the mean of four harvest dates and 10 white clover cultivars/breeding lines.

Nitrogen treatment (kg N/ha)	N-fixation (kg N/ha/d)	Clover % N	Grass % N	Total sward N (kg/ha)
0	0.19	3.88	2.54	35
100	0.11	3.95	2.95	46
200	0.09	4.25	3.31	58
P	*	*	*	**
$LSD_{0.05}$	0.02	0.06	0.25	5

that Kopu was able to maintain a more effective N-fixing system in the presence of applied N compared with Huia. Additionally, genetic variability has been shown for the ability of white clover cultivars to nodulate under high N (Rys 1986).

A significant (P<0.001) cultivar x date interaction was due largely to the high early N-fixation efficiency of Huia and Al-tolerant selection (which was selected from within Huia) and the high later (29 November) N-fixation efficiency of Demand and Will (Figure 3).

Clover and grass nitrogen concentration

Applied N significantly (P<0.05) increased clover and grass percent N such that total sward N content per unit area increased by 30% with 100 kg N/ha and 60% with 200 kg N/ha (Table 3). The absolute difference of 1%, in percent N in harvested shoots of clover and grass was maintained irrespective of N level applied (Table 3). In simulated swards with low N applied (equivalent of 28 kg N/ha over 88 d period) the absolute difference between clover and grass percent N was 2.2% but with N applied (equivalent of 616 kg N/ha over 88 d period) the difference was only 0.5% (Davidson & Robson 1985).

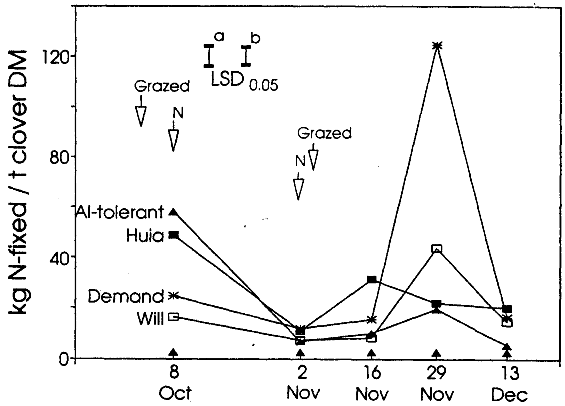

Figure 3. Nitrogen fixation efficiency at each sampling for Huia, Demand, Will and Al-tolerant selection. Each value is the mean of three nitrogen treatments. a) $LSD_{0.05}$ for comparison within dates, and b) $LSD_{0.05}$ for comparison within cultivars.

Conclusion

Applied N at the rate of 100 kg N/ha caused a rapid reduction in N-fixation although neither absolute clover dryweight yield nor proportion of clover in the sward was significantly affected by N applied up to 200 kg N/ha. Longer term trials have shown a similar effect (Crush *et al.*, 1982). Preferential uptake of applied N by clover resulted in an increased clover % N with additions of 100 and 200 kg N/ha. There were significant differences among cultivars for yield, proportion of clover in the sward, shoot nitrogen concentration, N-fixation and N-fixation efficiency. However, there were no cultivar x N interactions suggesting that all cultivars reacted similarly to the applied N. It may not be possible to identify cultivars in the field with an ability to maintain high levels of N-fixation in the presence of applied N. Conversely, no cultivars completely ceased N-fixation at levels of up to 200 kg N/ha.

References

Caradus, J.R. 1991. Mechanisms improving nutrient use by crop and herbage legumes. *In:* Crops as enhancers of nutrient use (eds. V.C. Baligar and R.R. Duncan), pp. 253-311. Academic Press Inc., San Diego.

Caradus, J.R., Pinxterhuis, J.B., Hay, R.J.M., Lyons, T. and Hoglund, J.H. 1993. Response of white clover cultivars to fertiliser nitrogen. *New Zealand Journal of Agricultural Research* ***36***, 285-295.

Crush, J.R., Cosgrove, G.P. and Brougham, R.W. 1982. The effect of nitrogen fertiliser on clover nitrogen fixation in an intensively grazed Manawatu pastures. *New Zealand Journal of Experimental Agriculture* ***10***, 395-399.

Davidson, I.A. and Robson, M.J. 1985. Effect of nitrogen supply on the grass and clover components of simulated mixed swards grown under favourable environmental conditions II. Nitrogen fixation and nitrate uptake. *Annals of Botany* ***55***, 697-703.

Eltilib, A.M. and Ledgard, S.F. 1988. Production and nitrogen fixation by 'Grasslands Kopu' and 'Grasslands Huia' white clovers under different nitrogen regimes. *New Zealand Journal of Agricultural Research* ***31***, 325-330.

Laidlaw, A.S. 1984. Quantifying the effect of nitrogen fertiliser applications in spring on white clover content in perennial ryegrass-white clover swards. *Grass and Forage Science* ***39***, 317-321.

Ryle, G.J.A., Powell, C.E. and Gordon, A.J. 1979. The respiratory costs of nitrogen fixation in soyabean, cowpea and white clover. *Journal of Experimental Botany* ***30***, 145-153.

Rys, G.J. 1986. Cultivar variation in the nodulation of white clover seedlings provided with nitrogen. *In* Plant Breeding Symposium DSIR 1986 (eds. T.A. Williams and G.S. Wratt), pp.326-329. Agronomy Society of New Zealand Special Publication No. 5.

West, C.P., Steele, K.W. and Halligan, E.A. 1985. Competition effects on nitrogen fixation and mineral N uptake in *Trifolium repens* L. - *Lolium perenne* L. associations. Proceedings of the 15th International Grasslands Congress, Kyoto, Japan. pp. 4-0-6.