Nitrogen fixation by caucasian clover and white clover in irrigated ryegrass pastures

K.H. WIDDUP1, R.G. PURVES2, A.D. BLACK3, P. JARVIS3 and R.J. LUCAS3
1AgResearch, PO Box 60, Lincoln
2148 Fisher Avenue, Beckenham, Christchurch
3Soil, Plant and Ecological Sciences Division, PO Box 84, Lincoln University
keith.widdup@agresearch.co.nz

Abstract

The N₂ fixation ability of caucasian clover was compared with that of white clover in irrigated ryegrass pastures over years 2 and 3 of a grazing experiment, using the ¹⁵N enrichment technique. ‘Endura’ caucasian clover was inoculated with the specific Rhizobium strain ICC148. The N concentration in clover herbage and the proportion of clover N derived from N₂ fixation (PN) were similar for both clovers at averages of 4.6%N and 50–60% respectively over the 2 years. The amount of N₂ fixed per hectare was directly related to the amount of clover dry matter (DM) produced by the two clover species. Caucasian clover produced four times the DM yield of white clover in year 2 (5400 cf. 1450 kg DM/ha) and four times the amount of N₂ fixed in herbage (136 cf. 36 kg N/ha) in year 3, Caucasian clover produced 50% more clover DM (3450 cf. 2370 kg DM/ha) and N₂ fixed (98 cf. 66 kg N/ha) than white clover. The increased N input from caucasian clover increased grass %N and N uptake from soil in caucasian clover pastures resulting in higher total pasture production compared with white clover pastures (15.7 cf. 14.2 t DM/ha) by year 3. In this study, caucasian clover demonstrated greater potential than white clover to meet the N demands of high-yielding perennial ryegrass in an intensive pastoral system.

Keywords: clover content, Lolium perenne, nitrogen fixation, pasture production, Trifolium ambiguum, T. repens

Introduction

Biological nitrogen (N₂) fixation by legumes is both ecologically and economically important to pastoral farming in New Zealand (Walker 1995). White clover (Trifolium repens) is the predominant legume used and studies have shown that annual N₂ fixation levels averaged 185 kg N/ha/year over 10 New Zealand sites (Hoglund et al. 1979). At Kirwee, Canterbury, dryland and irrigated pastures fixed 120 and 190 kg N/ha/year respectively (Crush 1979). New Zealand’s white clover-based pastures, however, are not fixing sufficient nitrogen to support the demands of high-yielding grasses nor to provide enough quality feed for maximum animal production (Caradus et al. 1996; Chapman et al. 1995; Clark & Harris 1995).

Caucasian clover (Trifolium ambiguum) has been demonstrated recently to have potential in lowland, intensive farming systems where it has consistently produced greater total legume yields than white clover in mixed pastures (Black et al. 2000; Moss et al. 1996; Watson et al. 1996). The performance of caucasian clover improved after the selection of the specific Rhizobium strain ICC148. Inoculation with this strain resulted in improved establishment, more effective nodules and greater N₂ fixation especially in high country pastures (Pryor et al. 1998). The grazing experiment established at Lincoln University in December 1996 (Black et al. 2000) to compare caucasian- and white clover-based ryegrass swards provided the resource to measure the N₂ fixation ability of caucasian clover and compare it with that of white clover.

Material and methods

The layout and management of the grazing experiment at Lincoln University was described by Black et al. (2000). The experiment was sown in December 1996 with either ‘Endura’ caucasian clover or ‘Demand’ white clover at high or low soil fertility with eight replicates. The ‘Endura’ caucasian clover seed was inoculated with the specific Rhizobium strain ICC148 (Pryor et al. 1998). ‘Ruanui’ zero endophyte ryegrass was direct-drilled into the pure clover swards in March 1997. The N₂ fixation study was initiated in spring 1998. It used five replications of the two clover treatments under high soil fertility with eight replicates. The ‘Endura’ caucasian clover seed was inoculated with the specific Rhizobium strain ICC148 (Pryor et al. 1998). ‘Ruanui’ zero endophyte ryegrass was direct-drilled into the pure clover swards in March 1997. The N₂ fixation study was initiated in spring 1998. It used five replications of the two clover treatments under high soil fertility (Olsen P of 22). The experiment was irrigated over the summer period (December–March) of the 2 years.

The ¹⁵N enrichment method (Ledgard et al. 1985) was used to measure N₂ fixation. Commencing in October 1998, labelled ammonium sulphate (40 atom% ¹⁵N) at 0.05 g N/m² was applied to 1.45m² of pasture in the 10 plots (two clovers x five replicates) and enclosure...
cages were placed over the treated areas. The pasture in the treated areas was selected to ensure that adequate populations of the treatment clover species were present. In the caucasian clover plots, $^{15}$N was applied to areas with minimal amounts of volunteer white clover. A 0.25 m$^2$ quadrat was cut from each treated area approximately every 4 weeks and sub-samples from these cuts were dissected into clover and grass components before drying to determine dry matter (DM) yield and botanical composition. Separate additional sub-samples of the clover and grass herbage were dried and ground for $%N$ and $^{15}$N analysis using a mass spectrometer (Anca 20-20 stable isotope analyser). After every second cut, $^{15}$N was re-applied to all 10 plots. The $^{15}$N treated areas were not moved in 1998/1999 but new areas were selected and treated with $^{15}$N every second cut (8-weekly) in 1999/2000. The plots were sampled six times between November 1998 and June 1999 and then eight times between October 1999 and June 2000 (year 3).

The proportion (%) of N fixed by the clovers from atmospheric N$_2$ ($P_N$) was calculated by:

$$ P_N = \frac{100 \times (\text{atoms}^%_{15}N_{\text{grass}} - \text{atoms}^%_{15}N_{\text{clover}})}{(\text{atoms}^%_{15}N_{\text{grass}} - B)} $$

where $B=0.3663$, the atoms$^%_{15}$N of atmospheric N$_2$.

The amount of fixed N (kg/ha) in the cut clover herbage was then given by:

$$ \text{Fixed N} = \text{clover DM (kg/ha)} \times \text{clover}^%N/100 \times P_N/100 $$

### Results

#### Mean herbage $%N$, $P_N$ and N$_2$ fixation

Mean $%N$ in clover herbage was similar for both caucasian and white clover during 1998/1999 and 1999/2000 (Table 1). The proportion of the N derived from N$_2$ fixation ($P_N$) was also similar for caucasian and white clover. In both clover species, N$_2$ fixation contributed an average of between 50% and 60% of their herbage N over the 2 years (Table 1). The $P_N$ was highest in late spring–summer at about 75% and lowest in late autumn at 45% for both clovers.

In year 2, caucasian clover fixed more N than white clover throughout the whole growing season (Table 1, Figure 1). In year 3, the N fixed by caucasian clover was greater in the summer period but similar to white clover in the cooler autumn period (Figure 1). It is likely that the low December values in year 3 were owing to low soil moisture levels, as irrigation was not initiated on the pastures until mid-December. Mean $%N$ in grass herbage was significantly higher in the grass growing with caucasian clover than in the white clover based pastures during both years (Table 1).

#### Pasture DM production

In 1998/1999, the caucasian- and white clover-based pastures produced similar total DM (grass plus clover), however, clover DM production was greater from caucasian than white clover pastures (Table 2). DM production from the associated grass in caucasian clover pastures was lower than from white clover pastures (Table 2).

### Table 1

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean $%N$</th>
<th>$P_N$ (%)</th>
<th>Fixed N in clover Herbage (kg N/ha/yr)</th>
<th>Estimate of total fixed N$^F$ (kg N/ha/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clover</td>
<td>Grass</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>4.61</td>
<td>3.69</td>
<td>55.6</td>
<td>136</td>
</tr>
<tr>
<td>White</td>
<td>4.55</td>
<td>3.38</td>
<td>51.0</td>
<td>36</td>
</tr>
<tr>
<td>Isd (5%)</td>
<td>0.15*</td>
<td>0.14*</td>
<td>6.4 ns</td>
<td>13.4*</td>
</tr>
<tr>
<td>Caucasian</td>
<td>4.59</td>
<td>3.46</td>
<td>56.7</td>
<td>98</td>
</tr>
<tr>
<td>White</td>
<td>4.59</td>
<td>3.12</td>
<td>60.7</td>
<td>66</td>
</tr>
<tr>
<td>Isd (5%)</td>
<td>0.11*</td>
<td>0.13*</td>
<td>5.6 ns</td>
<td>7.7*</td>
</tr>
</tbody>
</table>

$^F$ Based on 1.7 x fixed N in legume herbage

* = significant at $P<0.05$

### Table 2

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Clover content (% pasture)</th>
<th>DM production (kg DM/ha/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clover</td>
<td>Grass</td>
</tr>
<tr>
<td>Caucasian</td>
<td>52.8</td>
<td>5400</td>
</tr>
<tr>
<td>White</td>
<td>15.1</td>
<td>1450</td>
</tr>
<tr>
<td>Isd (5%)</td>
<td>21.8*</td>
<td>2540</td>
</tr>
<tr>
<td>Caucasian</td>
<td>21.9</td>
<td>3450</td>
</tr>
<tr>
<td>White</td>
<td>16.8</td>
<td>2370</td>
</tr>
<tr>
<td>Isd (5%)</td>
<td>4.8*</td>
<td>940</td>
</tr>
</tbody>
</table>

* Based on 1.7 x fixed N in legume herbage

ns = not significantly different at $P>0.05$

* = significant at $P<0.05$
In 1999/2000, the caucasian clover-based pastures produced greater total DM compared with pastures sown with white clover (Table 2). The grass DM were similar from both pastures, but clover DM was greater from caucasian clover than white clover pastures (22% cf. 17% clover) resulting in the greater total pasture production. Volunteer white clover produced 340 kg DM/ha (10% of the total clover yield) in the caucasian clover pastures in year 3.

Discussion

Caucasian clover and white clover had similar proportions of fixed N$_2$ ($P_N$) in their herbage, which indicates that both clovers have a similar ability to fix N$_2$ in the presence of perennial ryegrass, under irrigation and in high fertility soil. The amount of N$_2$ fixed per hectare was directly related to the amount of DM produced by the two clover species. Caucasian clover produced four times more DM than white clover in year 2 (5400 cf. 1450 kg DM/ha) and this was associated with four times the amount of N fixed (136 cf. 36 kg N/ha). In year 3, caucasian clover produced 50% more DM than white clover; the amount of N fixed (kg N/ha/yr) from N$_2$ fixation in the caucasian clover pastures was therefore 50% greater than that in the white clover pastures.

Mean %N in clover herbage was similar for both clover species and was constant throughout the year, but the clovers showed variation in $P_N$ across seasons of the year. The $P_N$ was highest in late spring–summer (75%) and lowest in autumn (45%) for both clover species. Ledgard et al. (2001) attributed lower $P_N$ levels over autumn and early spring to low temperatures reducing clover growth, and/or elevated soil inorganic N during these periods. The level of soil inorganic N has a major modifying action on clover N$_2$ fixation – as soil inorganic N availability increases, N$_2$ fixation decreases (Hoglund et al. 1979). High $P_N$ occurred in late spring–summer when high temperatures increased clover growth rates and soil inorganic N levels are normally lower owing to rapid growth and high N uptake by grass.

The association between caucasian clover and the specific Rhizobium strain ICC148 was obviously very effective and productive in this experiment. The only other reported N$_2$ fixation values from caucasian clover under grazing in New Zealand indicated lower values than white clover (Watson et al. 1996), but they suggested the acetylene reduction technique and shallow
by the management of the N2 fixation plots. Four-weekly cutting with no excreta return for 8 months had a higher clover content and fixed more N2 than white clover under the repeated cutting regime.

In year 3, 15N was applied to a new caged area every 8 weeks, a management allowing greater exposure of plots to normal grazing and excreta return; the clover contents were lower than in year 2. The lower clover contents were also attributed to a heavy infestation of grass grub (Costelytra zealandica) in autumn 1999 (averaged 180 grubs/m2) which greatly reduced the shoot and root material of both clover species (Trevor Jackson, pers. comm.). In spring 1999, white clover had recovered from surviving stolon pieces and new seedlings whereas the recovery of caucasian clover depended solely on new rhizome development from surviving rhizomes and tap-roots. Despite this disadvantage, caucasian clover produced significantly more clover herbage than white clover in the third year.

High N fixation by legumes in mixed pastures leads to greater N input into soil organic N resulting in increased N availability to associated grasses. In this study, the grass %N and the total soil N uptake was greatest in caucasian clover-based pastures by year 3 (Table 1 and 3) which resulted in higher total pasture production during year 3 (Table 2). This response needs to be monitored further at the Lincoln University grazing experiment to determine whether caucasian clover can maintain higher clover and total production than white clover in an environment with high soil N.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Grass N</th>
<th>Legume N</th>
<th>Total Soil N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian clover</td>
<td>167</td>
<td>111</td>
<td>276</td>
</tr>
<tr>
<td>White clover</td>
<td>252</td>
<td>32</td>
<td>281</td>
</tr>
<tr>
<td>Caucasian clover</td>
<td>414</td>
<td>69</td>
<td>483</td>
</tr>
<tr>
<td>White clover</td>
<td>369</td>
<td>43</td>
<td>412</td>
</tr>
</tbody>
</table>

* Legume N uptake from soil = Legume DM * %N * (1-PN/100)

# Conclusions

1. Both caucasian clover and white clover had similar proportions of fixed N2 in their herbage. This indicates that both clovers had a similar N-fixing ability in the presence of perennial ryegrass, irrigation and high fertility soil.
2. The amount of N fixed per hectare by caucasian and white clover was directly related to the herbage DM produced by the two clover species. Caucasian clover produced four times the DM and total N fixed of white clover in year 2 of the grazing experiment and 50% more DM and N fixed in year 3. The total N fixed ranged from 167–232 kg N/ha for caucasian clover and 61–112 kg N/ha for white clover.

3. In the caucasian clover-based pastures, the increased N input from the clover had lead to greater soil N uptake by the associated grasses by year 3 resulting in higher total pasture production. Further study is required to better quantify soil N changes and changes in total pasture production over time.

ACKNOWLEDGEMENTS

The authors acknowledge funding from the Struthers Trust for the development of the grazing experiment at Lincoln University and FRST funding for provision of 15N and N analyses.

The assistance of Bruce Pownall for the supply of ewe lambs and maintaining animal health, Keith Pollock and Jo Amyes for field assistance and Stuart Larsen and Roger Cresswell for conducting the N analyses are thanked. We thank the C. Alma Baker and Struthers Trusts for providing A.D. Black with financial support from post-graduate scholarships.

REFERENCES


