

## Evaluation of elite white clover germplasm under rotational cattle and sheep grazing

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### Abstract

Four white clover (*Trifolium repens* L.) cultivars, 4 pre-release cultivars and 24 breeding lines were evaluated in small plots in mixed species swards under rotational cattle and sheep grazing for 2 years. There was no significant stock class x line interaction for proportion of clover in the sward. There was no evidence that medium- and small-leaved types yielded better under sheep grazing while larger-leaved types yielded better under cattle grazing. Lines with the highest clover content tended to be large leaved and upright irrespective of stock class. They also had moderate to high stolon growing point densities. Breeding programmes have developed lines with high stolon growing point densities, ensuring vegetative persistence, while still maintaining a high proportion of clover in the sward. These pre-release cultivars and breeding lines gave significantly better clover contents than existing cultivars.

**Keywords** cultivars, selections, plant habit, cyanogenesis, stolon growing point density, clover content, cattle grazing, sheep grazing

### Introduction

White clover is grown in most New Zealand pastures predominantly as a nitrogen source, through nitrogen fixation, and to improve sward quality. The proportion of clover in swards is, however, often lower than the desired 30% proposed by Davies & Levy (1931). Standardised measurements of grazed pastures at 11 geographically dispersed sites throughout New Zealand showed clover contents to vary from 12 to 39% (Radcliffe 1974).

Overseas studies have shown that while grazing by sheep reduces clover content in mixed swards compared with cutting management (Newton & Davies 1987), cattle grazing has a relatively neutral effect (Frame & Newbould 1986). Sheep actively select clover in preference to grass (Lancashire & Keogh 1968), though cattle do not (Briseno de la Hoz & Wilman 1981). It is

therefore inferred that sheep exert greater selective pressures on clover than do cattle (Frame & Newbould 1986; Newton & Davies 1987). The present study cannot provide critical evidence for this but will rather evaluate the performance of a range of elite white clover germplasm under these two stock classes.

### Materials and Method

#### Plant material

The four white clover cultivars, 'Grasslands Huia', 'Grasslands Pitau', 'Grasslands Tahora' and 'Grasslands Kopu', were compared with 4 pre-release cultivars, G23, G26, G39 and G49, and 24 breeding lines (Table 1). The breeding lines included 12 selections made from a collection of 130 lines from south-western Europe (Caradus *et al.* 1990a). These selections were based on 12 geographical areas of origin, such that the best genotypes from the highest yielding populations within each geographical area were selected and polycrossed. Seed was bulked by geographical area to get the 12 breeding lines.

#### Trial design

The 32 cultivars and breeding lines were planted into grass swards in autumn 1989 at 2 sites. Palmerston North was mob-stocked with sheep. The other site at Aorangi Research Station, on the Kairanga Plains was located in a farmlet rotationally grazed by Friesian bulls at 7.4 per/ha.

Several months before planting volunteer white clover was removed from swards using a selective herbicide, Banvel, applied at the rate of 10 ml/l. Ten 2-month-old seedlings were replanted into 1-m rows, with 1 m spacing between rows and 0.5 m spacing between traverses. There were 5 replicates arranged in a randomised block design.

Trial sites were temporarily fenced for 6 weeks to allow plant establishment. Then trials were subjected to the grazing management of the surrounding paddock. At Palmerston North there were 7 grazings in the first year and 10 in the second year with intervals between grazings

**Table 1** Proportion of clover in sward, clover dry weight, leaf number density, stolon growing point density and leaflet width of lines. Values are means of 2 years and 2 sites.

Line No.	Name	Description of cultivar or line	Proportion clover	Clover dry weight (g/m <sup>2</sup> )	Leaf No. density (no/m <sup>2</sup> )	Leaflet width (mm)	Stolon growing point density (no/m <sup>2</sup> )
1	<b>Huia</b>	General purpose <b>cultivar for</b> a wide range of environments	0.28	26	4178	17.1	1850
2	Pitau	Winter active selection from <b>Huia x Spain</b> crosses	0.26	27	3330	23.0	1600
3	<b>Kopu</b>	Selected for improved summer growth and stem nematode resistance from <b>Pitau x ladino</b> crosses	0.26	23	2595	26.6	1780
4	Tahora	Selected from moist hill country <b>ecotypes</b>	0.17	15	3075	13.0	2760
5	G.23	Selection for improved cool season growth	0.37	37	4095	24.2	1340
6	G.26	Southland selection for good all year performance	0.30	29	4610	16.2	3260
7	G.39	Kaikohe selection from local sheep/beef farms	0.30	29	6295	16.6	<b>3810</b>
8	G.49	<b>Stem</b> nematode resistant selection from <b>Pitau</b>	0.40	37	5600	23.7	2120
9	Nematode. small	Bulk of glasshouse screen for root knot/clover cyst nematodes. small leaf	0.26	25	5343	16.7	3260
10	Nematode. large	Bulk of <b>glasshouse</b> screen for root knot/clover cyst nematodes. large leaf	0.31	35	4636	22.2	1520
11	NZ x USA	Selected for summer growth	0.39	31	4365	22.2	1930
12	Sthland	Bulk of local southland <b>ecotypes</b>	0.26	26	4366	16.8	2750
13	Frost. small	Selected for frost tolerance. small leaf	0.06	7	1223	13.1	1500
14	Frost. large	Selected for frost tolerance, large leaf	0.19	20	3060	14.5	1560
15	<b>Huia x Hill</b>	Selection for good all year production	0.30	30	5555	15.0	2940
16	<b>Huia x Sthland</b>	Very active spring/summer growth	0.32	22	5280	13.0	3630
17	N Line	Selected for tolerance to mineral nitrogen	0.25	21	2703	19.7	1660
18	<b>Huia re-sel<sup>n</sup></b>	Re-selection from <b>Huia</b>	0.33	33	4120	19.9	1610
19	<b>Stoloniferous sel<sup>n</sup></b>	Selection for high <b>stolon</b> densities in medium to large leaf type	0.40	39	<b>4880</b>	22.2	2610
20	Gene Pool A	Bulk of selection for good first year growth in spaced plants	0.46	52	6043	23.7	1900
21	<b>Sth Eur. I *</b>	Selected from very early flowering Portuguese material	0.30	26	3633	21.4	1620
22	<b>Sth Eur. II *</b>	Selected from early flowering Portuguese material	0.26	24	<b>3875</b>	<b>20.6</b>	2130
23	<b>Sth Eur. III *</b>	Selected from material collected in Central Italy	0.24	19	3790	16.6	1920
24	<b>Sth Eur. IV *</b>	Selected from material from north coastal Galicia. <b>Asturias</b> and <b>Cantabrian</b>	0.37	34	4670	19.0	2260
25	<b>Sth Eur. V *</b>	Selected from material collected from Lodi, Italy	0.32	27	2733	23.4	1500
26	<b>Sth Eur. VII *</b>	Selected from material collected from high altitude. north Italy	0.21	20	3266	15.4	2070
27	<b>Sth Eur. VI *</b>	Selected from material collected from low altitude. north Italy	0.24	24	3375	19.1	1920
28	<b>Sth Eur. VIII *</b>	Selected from material collected from unimproved farms, north Portugal	0.24	22	3493	17.7	2590
29	<b>Sth Eur. IX *</b>	Selected from material collected from improved farms. north Portugal	0.27	26	3285	21.5	1920
30	<b>Sth Eur. X *</b>	Selected from material collection from low altitude inland <b>Galicia</b> , Spain	0.30	26	<b>3480</b>	21.3	2060
31	<b>Sth Eur. XI *</b>	Selected from material collected from high altitude inland Galicia. Spain	0.28	24	3600	17.1	2070
32	<b>Sth Eur. XII *</b>	Selected from material collected on <b>Crau</b> Plains, southern France	0.26	25	2660	<b>21.0</b>	1190
P			...	...	...	...	...
<b>LSD<sub>0.05</sub></b>			0.09	10	1550	2.1	866

\* Selections made for high yield when grown as spaced plants.

ranging from 24 days in spring to 60 days in winter. At Aorangi there were 9 grazings in year 1 and 10 in year 2.

## Measurements

At the end of years 1 and 2, in autumn, a 400 cm<sup>2</sup> quadrat was harvested to 1 cm height, from each plot at each site. The entire sample was sorted into whiteclover and other species, which was predominantly perennial ryegrass. White clover leaves were separated from any harvested stolon and counted. White clover leaves, stolons and other species were weighed dry. At Palmerston North, before both harvests, aerial and surface stolon growing point densities were counted once per plot using a 100 cm<sup>2</sup> quadrat. Stolon growing points included all axillary buds with an open leaf. Leaf size was determined by the width of the terminal leaflet of the second open leaf along a stolon from the tip of 30 plants per line grown as spread plants at Palmerston North. Percentage of genotypes exhibiting a cyanogenic reaction was determined by testing 20 genotypes using the **picroate** method (Corkill 1940).

## Data analysis

Proportion of clover in the sward, total harvested clover dry weight, total dry weight harvested, and leaf number density (number/m<sup>2</sup>) were calculated and lines, sites and years compared by a split plot in time analysis of variance. Transformation, either log or **arcsin** square root, was used where appropriate. Lines were clustered using mean morphological and agronomic data into groups which minimised the total within-groups sums of squares. Analyses were conducted using **GENSTAT**.

## Results

### Description of lines

The leaf size of cultivars and pre-release cultivars were in the expected order with G.23, Kopu largest and Tahora smallest (Table 1). Other large-leaved lines included G.49, lines 10, 11, 19, 20 and 25. The small-leaved types included not only Tahora but also lines 13-

16 which included the frost tolerant selections and crosses between Huia and ecotypes from regions predominantly grazed by sheep. Stolon growing point density was generally negatively associated with leaf size ( $r=-0.50, P<0.01$ ).

### Agronomic performance

For all characters there was no significant ( $P>0.05$ ) variation among lines in their level of performance under either sheep or cattle grazing in both years. At a significance level of 10% there was a line x site x year interaction for proportion of clover. This was predominantly due to the exceptionally poor performance of line 32 in the second year at Aorangi, when it had only 18% of the clover content in year 1, whereas at Palmerston North it showed no change from year 1 to year 2.

There was no significant line x site interaction for any of the characters measured. The main effect of lines was significant ( $P<0.001$ ) for all characters with the exception of total clover and grass dry weight (Table 1). Lines with consistently high proportion of clover were G.49, large-leaf stoloniferous (19), and Gene Pool A (20), while those with consistently low proportion of clover were Tahora, and the small- and large-leaf frost tolerant selections (13 and 14). Comparison of lines grouped on the basis of proportion of clover showed that the best lines were large leaved and highly cyanogenic with a high leaf number density (Table 2). There was, however, no significant difference between the groups for stolon growing point density. White clover cultivars are often **characterised** by leaf size into small-, medium- and large-leaved groups (Caradus *et al.* 1989). In the present study lines with a leaflet width  $\leq 15$  mm were considered small leaved,  $> 15$  mm  $\leq 19$  mm medium leaved and  $> 19$  mm large leaved (Table 1). Among the small-leaved lines, line 15 and 16 had significantly ( $P<0.05$ ) greater clover contents than the current small-leaved cultivar Tahora. Among the medium-leaved lines none were significantly better than Huia, although line 24 gave a 32% increase in clover content. Among the large-leaved types, lines 5, 8, 11, 19 and 20 showed a significant improvement over either Pitau or Kopu.

**Table 2** Comparison of lines grouped on the basis of proportion of clover in sward.

category	Range of clover content	n	Proportion of clover	Leaflet width (mm)	Cyanogenesis (%)	Stolon growing point density (no/m <sup>2</sup> )	Leaf number density (no/m <sup>2</sup> )	Total dry weight (g/m <sup>2</sup> )
1	0.00 - 0.19	3	0.15 $\pm$ 0.03	13.5 $\pm$ 0.5	16 $\pm$ 12	1947 $\pm$ 407	2459 $\pm$ 616	99 $\pm$ 3
2	0.20 - 0.29	14	0.26 $\pm$ 0.01	19.3 $\pm$ 0.6	54 $\pm$ 6	2051 $\pm$ 139	3592 $\pm$ 193	99 $\pm$ 2
3	0.30 - 0.39	12	0.33 $\pm$ 0.01	19.6 $\pm$ 1.0	57 $\pm$ 5	2306 $\pm$ 253	4495 $\pm$ 265	101 $\pm$ 3
4	20.40	3	0.42 $\pm$ 0.02	23.2 $\pm$ 0.5	80 $\pm$ 7	2277 $\pm$ 274	5506 $\pm$ 339	101 $\pm$ 7
P			...	f..	**	ns	...	ns

Table 3 Lists of cultivars and lines in 6 groups derived using cluster analysis, with means and standard errors for each plant character of cultivars and lines within clusters.

Plant	Character	Cluster					
		A	B	C	D	E	F
	G. 26	G.49	Tahora	Huia	Pitau	Kopu	
	G. 39	Gene Pool A	Southland	G. 23	Frost, large	Frost, small	
	Nematode, small				Sth. Eur. VIII	Nematode, large	Sth. Eur. I N Line
	Huia X Hill					NZ x USA	Sth. Eur. II Sth. Eur. V
	Huia X Southland					Huia reseln	Sth. Eur. III Sth. Eur. XII
	Stoloniferous set <sup>n</sup>					Sth. Eur. IV	Sth. Eur. VI
							Sth. Eur. VII
							Sth. Eur. IX
							Sth. Eur. X
							Sth. Eur. XI
<b>Spaced plants</b>							
Leaflet width (mm)	16.6 ± 1.2	23.7 ± 1.2	15.6 ± 1.4	20.6 ± 1.1	19.2 ± 0.9	20.6 ± 2.2	
% cyanogenesis	64 ± 4	66 ± 6	50 ± 6	63 ± 4	44 ± 9	42 ± 13	
<b>Sward plants</b>							
Stolon growing point density (no/m <sup>2</sup> )	3266 ± 157	2010 ± 110	2700 ± 55	1765 ± 132	1889 ± 67	1526 ± 99	
Leaf number density (no/m <sup>2</sup> )	5377 ± 101	5633 ± 221	3645 ± 361	4376 ± 127	3490 ± 65	2423 ± 303	
Harvested clover dry weight (g/m <sup>2</sup> )	29 ± 2	44 ± 6	21 ± 3	33 ± 2	24 ± 1	20 ± 4	
Proportion of clover in sward	0.32 ± 0.02	0.43 ± 0.03	0.23 ± 0.03	0.34 ± 0.02	0.26 ± 0.01	0.23 ± 0.04	

### Classification of lines

Cluster analysis was used to group lines into 6 clusters (Table 3). There were significant differences among clusters for all characters except percent cyanogenesis. Cluster A contained lines characterised by a very high stolon growing point density and high clover content; cluster B was characterised by very high clover content and very large leaves but only moderate stolon growing point density; cluster C by small leaves and lower clover content but high stolon growing point density; cluster D by low stolon growing point density but high clover content; cluster E by low leaf number density; and cluster F by very low stolon growing point density and low clover content (Table 3).

### Discussion

Despite large differences among the lines studied for plant type (Table 1), there was no significant stock class x line interaction for whiteclover content. It is generally considered that of the three broad cultivar groupings, large-, medium- and small- leaved, the larger-leaved types are best able to withstand less frequent lax grazing systems whereas low-growing small-leaved cultivars are adapted to withstand frequent close defoliation (Harris 1987). There was no evidence that medium- and small-leaved types yielded better under sheep grazing while larger-leaved types yielded better under cattle grazing. Apparently the intensity of grazing was similar under both stock classes since both were rotationally grazed with no more than 10 defoliations per year. It was evident that lines with the highest clover content tended

to be larger leaved and upright irrespective of stock class. They also had moderate to high stolon growing point densities, for example, compare Pitau and Kopu with the highclovercontentlines G.49, NZ x USA, large leaf stoloniferous and Gene Pool A (Table 1).

Significant management system x white clover line interactions for clover content have been shown in other studies but comparisons in these cases were between set-stocking and rotational grazing (Brock 1988) or cutting versus continuous sheep grazing (Evans & Williams 1984), not between stock classes. Few studies have compared two or more white clover lines under different stock classes. Williams *et al.* (1982) compared four lines, Pitau, Huia, hill country selection and a 'wild' ecotype, under rotational cattle and sheep and set-stock sheep grazing. They found that line differences were greatest under set-stocking by sheep, smallest under rotational cattle grazing and intermediate under rotational grazing by sheep. The small-leaved stoloniferous hill country selection gave the best clover contents in all management systems, but the larger-leaved Pitau persisted only under rotational cattle grazing.

High stolon growing point densities are expected to confer an advantage in persistence since stolon production is essential for vegetative survival of white clover (Beinhart 1963). A number of studies have, however, shown that the 'penalty' for high stolon growing point density may be a reduction in harvested clover yield (Rhodes & Harris 1979). In the present study there was no correlation between stolon growing point density and either proportion of clover in the sward ( $r=0.11$ , n.s.) or absolute white clover yield ( $r=0.01$ , n.s.). This was due predominantly to high yields of lines (e.g. large leaf

stoloniferous, Huia x Hill, G26, G.39, Nematode -small and Huia x Southland) which also had high stolon growing point densities, and low yields of some lines (e.g. Frost tolerant-small and large, and Sth. Eur. XII) which also had low stolon growing point densities.

While it is often thought that year-to-year variation in clover content is more variable than site-to-site (Harris 1987) this was not necessarily the case in the present study. Both at Aorangi and Palmerston North year 1 and year 2 data were correlated ( $r=0.55$  and  $0.54$  respectively, ( $P<0.01$ )). In year 1, the 2 sites were better correlated than in year 2 ( $r=0.57$ ,  $P<0.01$  and  $r=0.38$ ,  $P<0.05$ , respectively), suggesting that it may be a matter of time before a site x line interaction becomes evident.

Selections for frost tolerance (lines 13 and 14), while successfully ensuring frost tolerance (Caradus *et al.* 1990b), have resulted in lines with relatively poor yields. This is in itself an adaptation, with a low leaf number (Table 1) resulting in a reduced leaf tissue mass exposed directly to frost. Low cyanogenesis of the frost tolerant selections is a further adaptation aiding frost-tolerance (Foulds & Young 1977).

Only two of the lines studied (25 and 30) could be classified as ladino type on account of having large leaves and being acyanogenic (Caradus *et al.* 1989). Both of these lines produced reasonable clover contents (Table 1) and were comparable with current large-leaved cultivars available. Ladino types are generally considered to be open in habit and intolerant of frequent grazing showing poor persistence after the first year (Williams 1987). It would, however, appear that selection5 can be made within the ladino type for lines which give consistently good clover yields.

It appears that at least in the first 2 years of growth white clover lines perform similarly under both rotational sheep and rotational cattle grazing. Breeding programmes have successfully identified and produced pre-release cultivars and breeding lines giving significantly better clover content5 than existing cultivars.

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