

## IMPROVED PERFORMANCE FROM SCREENING AND HYBRIDISING OF WHITE CLOVER GERMPLASM IN SOUTHLAND

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### ABSTRACT

A range of white clover populations from the Mediterranean, Europe, and New Zealand together with ecotypes collected from Southland and North Island hill country were evaluated as spaced plants or small plots between 1975 and 1982. Plants were screened for seasonal productivity, persistence under grazing, morphology, and disease tolerance.

The clover lines with potential fell into two distinct types, persistent and productive. Persistent types (ecotype material) had small leaves, many stolons and an active spring/summer growth pattern suited to the climate of Southland. In a pasture, the prostrate morphology of these types was associated with an efficient transfer of nutrients from clover to grass. Productive types (Huia, Pitau, and French material) had larger leaves, fewer stolons, competed well with associated grasses, and were tolerant of leaf diseases, but were less persistent.

A hybridisation programme produced material combining desirable features of both persistent and productive types. Some of the best hybrids have a dense morphology, and produce 20% greater spring/summer and up to 40% greater annual yields than the cultivar Grasslands Huia. Final selections from these high-producing, persistent hybrids will be used to produce a clover cultivar suited to southern New Zealand.

### KEYWORDS

*Trifolium repens* L., germplasm, screening, hybridising.

### INTRODUCTION

The Otago-Southland region is an area of intensive pastoral farming. Pasture production from predominantly ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) swards have been measured at 9000 kg/ha per year (Cossens and Radcliffe, 1978) to 17 000 kg/ha per year (Harris *et al.*, 1973). The major factors limiting pasture production are temperature, and nitrogen (N) availability. Lower than optimum temperature, in particular, restricts active clover growth to the late spring-summer period when more than 80% of both clover yield and N fixation occurs

(Carran, 1979a; Widdup and Turner, 1983). Soil N availability limits grass production throughout the year but most severely during the summer and autumn (Carran, 1979b). Animal requirements match pasture production for most of the year but herbage shortfalls can occur in early spring and high quality feed is essential for fast lamb growth rates during spring/summer (Harris and Hickey, 1979).

Improved white clover performance is viewed as an effective method of providing greater levels of N for associated grasses through N fixation, and increasing the yield of high quality feed for grazing animals.

### EXTENT OF USEFUL VARIATION

More than 320 white clover populations from New Zealand and overseas were evaluated for seasonal productivity, morphology, persistence under grazing and disease tolerance, as spaced plants or small plots in the field at Gore. These were tested in five trials between 1975 and 1982.

In all trials, seedlings were raised in a glasshouse before being planted in a cultivated field. Each clover line consisted of 10 plants at 75 cm spacing, replicated six times. At regular intervals plants were visually scored on a 0-5 scale for herbage yield, growth habit, and leaf diseases. After assessment, plants were grazed by sheep with at least six grazings annually. Each trial lasted about three years which allowed some measure of persistence.

The good performance and adaptability of the standard cultivar Grasslands Huia was evident from the small percentage of other lines showing superior growth (Table 1). Most of the overseas lines showed poor agronomic performance. Mediterranean material had better cool season growth than Huia. However, improved performance at this time resulted in very little increase in total clover production in Southland because of the limitations to growth imposed by low temperatures.

Mediterranean material produced few, but large, stolons (Table 2) which were easily removed under intensive sheep grazing and led to poorer persistence. The good spring performance of the Mediterranean and Grasslands Pitau ((NZ X Spain) X NZ) material in the final year (Table 1) reflects tolerance to stem nematode (*Ditylenchus dipsaci* Filipjev). This pest infested spaced plant trials where stolon

**Table 1. Percentage of white clover lines with significantly ( $P < 0.05$ ) better growth than Grasslands Huia at Gore.**

Origin	Number of lines	Seasonal growth					
		Summer		Autumn/Winter		Spring	
		1st	Last	1st	Last	1st	Last
<b>Overseas</b>							
Mediterranean	13	0	0	69	15	0	23 <sup>1</sup>
Europe, USA, USSR	65	0	3	2	0	0	0
<b>Overseas x NZ</b>							
Mediterranean x NZ	33	27	12	61	39	6	9
Europe x NZ	24	0	4	12	8	0	8
<b>New Zealand</b>							
Pitau (selections)	28	18	7	86	71	4	53 <sup>1</sup>
Ladino x Pitau	3	0	0	0	0	0	0
Huia (selections)	38	0	0	29	21	3	3
<b>Ecotypes</b>							
North Island Hill Country	37	3	19	0	0	0	19
Southland	66	13	15	19	7	8	24
<b>Hybrids</b>							
Huia x Ecotypes	14	7	21	7	28	0	21

<sup>1</sup> Reflects stem nematode tolerance.

**Table 2. Variation in plant morphology and disease tolerance in a range of clover populations.**

Origin	Plant morphology <sup>1</sup>		Disease tolerance <sup>2</sup>	
	Mean	Range	Mean	Range
<b>Overseas</b>				
Mediterranean	1.4	1.0-2.0	1.0	0-2.2
Europe, USA, USSR	3.4	2.1-4.7	3.7	0-9.5
<b>Overseas x NZ</b>				
Mediterranean x NZ	2.0	1.8-2.5	1.5	0.3-4.5
Europe, USA x NZ	2.3	1.8-2.8	1.1	0-2.8
<b>New Zealand</b>				
Pitau (selections)	2.2	1.9-2.5	3.7	0.4-6.2
Ladino x Pitau	1.6		0.2	
Huia (selections)	2.3	1.7-2.9	1.8	0-5.5
Huia	2.8		4.3	
<b>Ecotypes</b>				
N.I. Hill Country	4.0	3.5-4.5	4.3	0.3-9.0
Southland	3.3	2.8-3.8	7.9	3.2-14.2
<b>Hybrids</b>				
Huia x Ecotypes	3.0	2.3-3.4	2.0	0.2-6.8

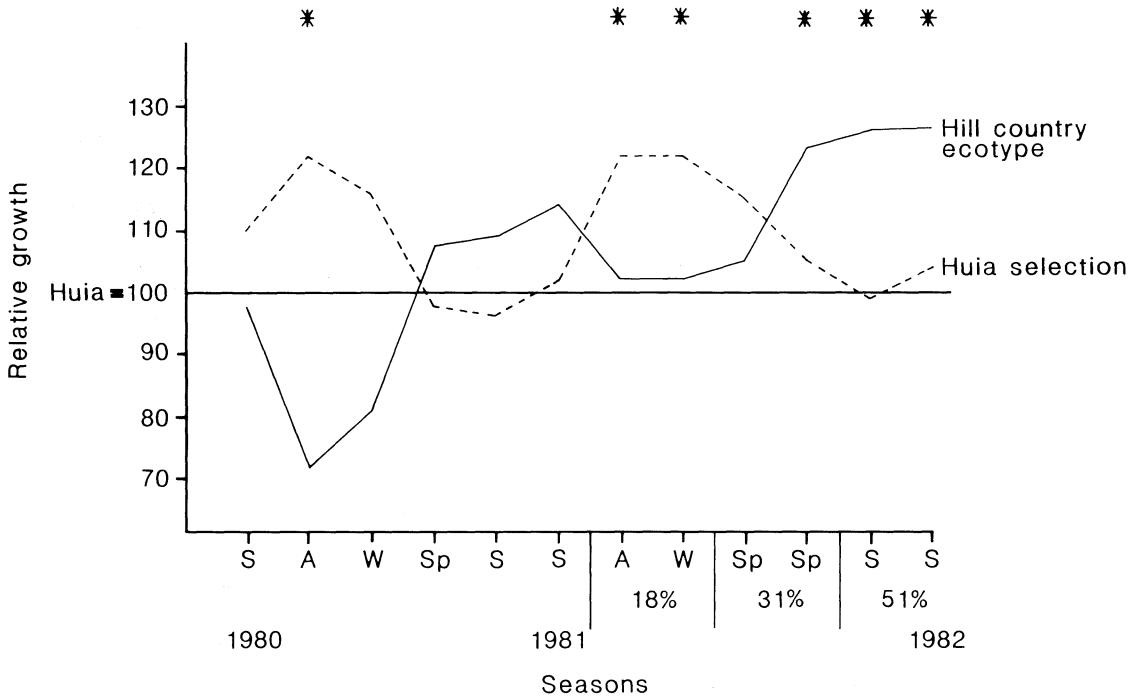
<sup>1</sup> 1 = erect, large leaved, few stolons  
5 = prostrate, small leaved, many stolons.

<sup>2</sup> 0-3 = little infection  
4-7 = moderate infection  
8 = heavy infection.

spread was hindered by interplant cultivation, but was masked in plots because healthy stolons filled the gaps left by dead stolons.

Clover germplasm could be divided into persistent and productive groups. The persistent group was small-leaved

ecotype material from Southland and the North Island hill country. This was collected from environments with low-moderate fertility, cool temperatures, and intensive continuous grazing by sheep. These conditions combined to shift emphasis from yield to persistence (Corkill *et al.*,



**Figure 1. Seasonal herbage production from two contrasting clover populations relative to Grasslands Huia. \*significantly better than Huia at  $P < 0.05$ . S=Summer, A=Autumn, W=Winter, Sp=Spring. Seasonal production as a percentage of annual production is indicated.**

1981). These surviving clovers are adapted to these conditions in having small leaves, short petioles, and densely branched stolons (Table 2).

An important feature associated with this morphology was high spring and summer growth (Fig. 1). During this period, climatic conditions are optimal for growth and N fixation and high quality feed is most needed. Ecotypes were generally slow to establish but in all trials showed improved performance in time (Widdup, 1984). Up to 24% of the ecotypes had significantly better spring/summer growth than Huia by the final year (Table 1).

The second promising group consisted of selections from Huia, Pitau, and French lines plus some hybrids between New Zealand and Mediterranean plants (Table 1). In contrast to the other group, these productive types are characterised by large leaves and long petioles (Table 2). This enables them to compete effectively for light and space when in association with erect growing grasses, particularly under conditions of high soil fertility and lenient grazing (Evans and Williams, 1982). They develop fewer but thicker stolons and have a lower level of branching than the ecotype material. Under hard continuous grazing they are less productive and persistent (Williams and Caradus, 1979).

The larger-leaved types have an extended growing season into autumn (Table 1, Fig. 1). This is an advantage

in mild climates such as Northland but of less significance in cool climates. However, some growth of high quality feed in the late summer/autumn period is desirable to meet sheep requirements (Harris and Hickey, 1979).

The foliar diseases sooty blotch (*Mycosphaerella killianii* Petr.) and rust (*Uromyces trifolii* Hedw.) are both prevalent during this period. The pathogens caused little damage to the large-leaved overseas types but caused moderate-heavy infections to New Zealand ecotypes (Table 2). The local pathogen biotypes showed specificity for Southland ecotypes. Some tolerance to these diseases is considered advantageous (Skipper and Lambert, 1984).

## PERFORMANCE FROM PASTURES BASED ON CONTRASTING CLOVER TYPES

Screening clovers in monoculture can indicate the genetic potential of a population but evaluation under grazed pasture conditions is necessary to indicate agronomic performance. The herbage production and nutrient cycling characteristics from three rotationally grazed pastures containing contrasting white clovers is shown in Table 3. Pitau is an example from the larger leaved group, Kent is representative of small leaved clovers, and Huia is intermediate.

**Table 3. Herbage production and soil biomass carbon from three pastures containing contrasting white clover types.**

	Kent	Huia	Pitau
Annual pasture production <sup>1</sup> (kg DW/ha per year)	13500	13160	13140
Ryegrass yield (kg DW/ha per year)	9460	8180	7660
White clover yield (kg DW/ha per year)	2370	3460	4000
Clover type <sup>2</sup>	4.4	2.8	2.2
Soil biomass (kg/ha per 50 mm) C <sup>3</sup>	720	580	590

<sup>1</sup> Data from Widdup and Turner (1983).

<sup>2</sup> From Table 2.

<sup>3</sup> Carran (unpub. data).

Total yield was similar for all pastures but the grass/clover proportions differed depending on the clover type used. The sward based on the large-leaved Pitau clover had the highest clover yield and the lowest associated ryegrass yield. The pasture containing the small-leaved dense Kent clover showed a converse effect (Table 3).

Differences in grass/clover proportion are caused by differences in the harvest index of the clover types (Rhodes and Harris, 1978). This affects the competitive ability of a clover in a pasture. Large-leaved clovers like Pitau have a high harvest index. Under infrequent defoliation, they are more effective in competing for light and space than small-leaved clovers with a low harvest index (Rhodes and Harris, 1978).

Differences in the harvest index of clovers imply that nutrient transfer pathways are changed. Field and Ball (1981) showed that N returned to pasture through animal excreta is subject to larger losses (volatilisation and leaching), than N cycling through death and decay. Large-leaved clovers have more of their nutrients in leaves and petioles which are returned to the soil via the animal. However, small-leaved clovers have a greater proportion of nutrients in stolon material than the large-leaved clovers. Consequently, a greater part of the N fixed symbiotically by small-leaved clovers is returned to the system by stolon

death and decay. The higher levels of soil biomass carbon measured from the Kent-based sward (Table 3) support this view. The trend for greater total production from the Kent pasture is further evidence of an efficient transfer of nutrients associated with this clover type. The resulting grass growth ensures a wide spread of herbage throughout the year.

## PERFORMANCE OF THE HYBRIDS

The objective was to produce hybrids which combined the desirable features of both clover types. As previously described, they complement one another in many important agronomic traits (Fig. 1, Table 2, 3).

Eighty F1 hybrid lines are being evaluated and the seasonal yields and plant morphology of five representative lines after 18 months are shown in Table 4. The five lines produce greater yields than Huia in all seasons, and more importantly are 15-25% better in spring/summer. Twenty five of the hybrids (32%) are significantly better than Huia in spring/summer which is better than any material in Table 1. The hybrids show a range in morphology and some productive, dense recombinants have been identified.

One of the hybrid lines (C5413), established in a trial at two altitudes (500 m and 750 m) on the East Otago Plateau, outyielded Huia in all seasons. In the second year after planting, this line produced 40% greater total clover yield than Huia (3135 kg/ha cf. 2240 kg/ha) (J.M. Keogh, pers. comm.). Together with results from Table 4, this suggests that the hybrid material has wide adaptability to both fertile lowland situations and low fertility hill country in the south of New Zealand.

As some segregation in the second generation is likely, all F1 hybrid plants have been open pollinated and seed representing the F2 populations collected. Eighty F2 lines are being evaluated in monoculture and in competition with grass. Selection of the most productive and persistent genotypes and open pollinating in a polycross will generate progenies to be evaluated. Data can be used to select parent genotypes as elite plants for a new synthetic cultivar.

Agronomic testing of a new perennial cultivar can take many years. For this reason, an experimental line based on

**Table 4. Performance of five hybrid white clover populations relative to Grasslands Huia at Gore.**

Hybrids	Summer	Autumn/ Winter	Spring	Plant type <sup>1</sup>
(Pitau x Huia) x Southland Ecotype	127	144	119	2.6
Masor x Southland Ecotype	120	129	118	2.7
Huia Selection x Hill Country Ecotype	123	117	119	2.6
Southland Ecotype x Hill Country Ecotype	115	111	123	3.1
(Hill Country x Huia) x Southland Ecotype	115	113	118	3.3
Pitau	119	141	103	2.2
Huia	100	100	100	2.8
LSD (P<0.05)	15	15	13	0.3

<sup>1</sup> 1 = erect, large leaved, few stolons

5 = prostrate, small leaved, many stolons.

superior genotypes selected from the F1 hybrids is being developed for testing under grazed pastures at several sites in Otago and Southland. Early evaluation of this experimental line will indicate its agronomic potential and avoid unnecessary delays in the release of a new white clover cultivar.

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## SYMPOSIUM DISCUSSION

Dr G.W. Burton, USDA, Georgia

How much of your increase in performance is due to heterosis?

Widdup

The F<sub>2</sub> populations at the moment do not look to be giving as high a value as the F<sub>1</sub> hybrids. But I am hoping to get F<sub>2</sub> segregates that will still be better than Huia.

Dr H.S. Easton, Grasslands Division, DSIR

How long was the experiment with the three clover types in a pasture run to get the differences in biomass?

Widdup

Four years. It is quite important to let trials run for a reasonable length of time to get these sort of differences.

Dr H.A. Eagles, Plant Physiology Division, DSIR

What is the potential in the long term of being able to produce seed of F<sub>1</sub> hybrids of white clover economically.

Widdup

I do not really know. There is a lot of variability in white clover and there are a lot of niches it fits into. I am not sure if that approach would be better than producing synthetics.

Dr S.A. Menzies, Plant Diseases Division, DSIR

In the northern part of the North Island root diseases and nematodes have quite an important effect on persistence and nutrient transfer. Have you looked at the root biomass for the presence or absence of root diseases in your selections? Do you think this would have any effect on differences?

Widdup

I showed the effects of stem nematode. This has a more obvious effect when plants are grown as spaced plants. The other two major nematodes in New Zealand are cyst nematode and root knot. Root knot is not found any further south than Nelson. There is cyst nematode in our soils. We get a depression in nitrogen-fixation during spring and we wondered if that was due to stem nematode invading the roots. We set up a small plot trial outside where we injected cyst nematode into some plots and not into others — the depressed nitrogen fixation was not due to the cyst nematode. It is more due to the rising amount of nitrogen in the soil in the spring period which is turning the fixation off. Our feeling is that cyst nematode is there but not really important. The Southland environment is moist so plants are not under that much stress, even though they are under quite heavy grazing sometimes. Whereas I think nematodes have a major effect in stressful environments, such as the dry country or hill country.