

ADAPTATION OF WHITE CLOVER TO MOISTURE STRESS

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Abstract

In a series of four experiments, the w-seeding ability and shoot and root characteristics of **dryland** white clover populations (*Trifolium repens* L.), and the effect of selection for root characteristics within white clover were examined. There was little evidence that **dryland** populations were better at re-seeding than **Huia**, but **dryland** populations were more taprooted than populations collected from moister environments. Root morphologies of **dryland** populations were similar to that of **Huia** but were smaller leaved, although not as small leaved as **Tahora**. Evidence that selection for more taprooted, smaller-leaved types of white clover would be successful is also presented.

Keywords: root type, drought, re-seeding, plant breeding, survival.

INTRODUCTION

Increased survival and production of white clover in drought-prone areas may be achieved by breeding for (a) drought avoidance, through re-seeding prior to onset of moisture stress (O'Brien 1970), (b) improved moisture conservation through reduced leaf size, **stomatal** closure and high cuticular resistance (Parsons 1982), and (c) improved moisture uptake, through a more extensive, deeper or denser root system, and increased root to shoot ratio (Smith & Morrison 1983, Stevenson & Laidlaw 1985).

'Grasslands Huia', which has been used during the past 40 years to **oversow** dry hill country pastures in NZ, persists poorly in dry hill country (Charlton 1984), having been bred specifically for use in high fertility lowland.

Previous white clover root studies (Caradus 1977, 1981) have shown a strong correlation between root and shoot type, such that predominantly taprooted genotypes are larger leaved than fibrous rooted genotypes. A preliminary study investigating the effect of selection for smaller leaved, more taprooted types is also reported.

MATERIALS AND METHODS

Experiment 1: Vegetative persistence and reseedling in North Canterbury

In winter 1984, 106 lines of white clover including 61 lines from a NZ dry hill country ecotype collection, 5 lines of Whatawhata early flowering types, 24 lines of New Zealand x Mediterranean crosses and other lines of diverse origin were established at a dry hill country farm (Carvossa) near Waikari, North Canterbury. Each line was represented by 60 genotypes, planted in 6 replicates as 10 plant rows.

The site was on a Tipapa hill soil, at 540 m **a.s.l.**, with a 15° slope and 550 mm annual rainfall. The area was regularly grazed after establishment, but closed during summer 1985/86 to allow re-seeding. In autumn 1986 the number of vegetatively surviving plants and the number of seedlings established around each planted row were counted.

Root type experiments

Plants for Experiments 2-4 were grown in Palmerston North in field tiles (9 cm diameter x 37 cm deep) containing a **B** horizon Egmont loam to which 30% potassic superphosphate had been added at a rate of 1 g P/kg soil. Genotypes were propagated from stolon tips and pre-rooted in potting mix before transplanting into the tiles. Measurements were made in all experiments of leaflet width as a measure

of shoot type, largest taproot diameter, number of taproots (basal diameter >1 mm), dry weight of taproots and fibrous roots, and shoot dry weight.

Experiment 2: The evaluation of 30 white clover populations

A total of 600 genotypes (20 genotypes of 30 populations in Table 3) were evaluated. Each genotype was replicated twice. Plants were grown for 14 weeks in autumn-winter 1985.

Experiment 3: The evaluation of drought-surviving genotypes

125 white clover genotypes that had survived vegetatively through a very dry summer at Carvossa, North Canterbury were evaluated, along with six genotypes each of Huia, Tahora, Kopu, and Kent wild white. Each genotype was replicated twice. Plants were grown for 10 weeks in summer 1985.

Experiment 4: The evaluation of selections for root and shoot type

The root types of 220 genotypes from 45 populations of white clover were measured. Plants had been grown as spaced plants, at Palmerston North for 2 years prior to measurement. Plants were excavated to a depth of 10 cm, soil was washed from roots, and measurements made. Plants which differed significantly for leaf size, number of taproots and percentage fibrous root were selected and crossed to produce eight selections (Table 1). Forty genotypes from each selection were evaluated for root type. Each genotype was replicated twice. Plants were grown for 9 weeks in summer 1985.

Table 1: Description of selections 1-8 used in experiment 4.

Leaf size	No. of taproots		% Fibrous roots	
	High	LOW	High	LOW
Large	1	2	5	6
Small	3	4	7	8

Table 2: The vegetative survival of planted material, the number of seedlings established per row and the area of population collection.

Collection area	No. of populations	Vegetative survival (%)	Mean number of seedlings/row
Whatawhata	5	2.4	3.0
Taupo	4	1.7	1.6
Poverty Bay	3	2.2	1.7
Hawke's Bay	9	2.0	2.1
Southern Hawke's Bay	9	3.8	2.1
Wairarapa	10	3.7	1.6
Marlborough	8	3.3	1.6
North Canterbury	6	6.7	2.3
Canterbury	5	7.3	2.4
Central Otago	6	4.2	1.2
NZ x Mediterranean	24	1.6	1.5
Other NZ lines	12	2.0	1.6
cv. Huia		1.7	3.8
cv. Pitau	1	2.0	2.3
cv. Kopu	1	0.0	2.0
cv. Tahora	1	0.0	1.0
CV. Kent Wild White	1	0.0	0.0
LSD...		2.1	0.7

RESULTS

Experiment 1

Re-seeding. Lines selected for early flowering and high seed yields at Whatawhata and populations collected from Canterbury and North Canterbury had greater seedling establishment than those derived from crosses between New

Zealand and Mediterranean material (Table 2). The high reseedling of Huia and low reseedling of Tahora are also of interest.

Vegetative persistence. Persistence was very low for all lines following the summer drought of 1984/85, with only 3% of all plants surviving. However, two populations originally collected from North Canterbury (28% and 20%) and 10 populations from Canterbury, Marlborough, Wairarapa and Southern Hawke's Bay had over 10% survival. Overall, collections from North Canterbury and Canterbury showed superior vegetative persistence (Table 2). No plants of Kopu or Tahora survived.

Root type — Experiments 2-4

Comparison of populations. Populations collected from **dryland** sites either had a similar or significantly smaller leaf size than Huia, but only one population (number 28 from Wither Hills) had a significantly smaller leaf size than Tahora (Table 3). Only two **dryland** populations (numbers 27 and 28, both from Wither Hills) had significantly smaller **taproot** diameters and fewer **taproots** than Huia, the remainder were not significantly different (Table 3).

Table 3: Description of populations and mean leaflet width, **taproot** diameter, **taproot** number and total fresh weight (FW) after 14 weeks growth in field tiles (Experiment 2).

NO.	C-cultivar S-selection E-ecotype	Description	Leaflet width (mm)	Taproot diameter (mm)	Number of taproots per plant	Total F.W. (g) per plant
1	c	Huia	11.9	1.96	6.9	14.9
2	c	Tahora	17.3	1.93	6.1	16.2
3	c	Kopu	9.9	1.83	6.3	13.6
4	S	G.23	17.1	2.17	6.2	15.6
5	C	Dusi, South Africa	17.7	2.22	6.4	15.7
6	S	Root knot nematode tolerant	15.2	2.15	0.2	20.6
7	S	Southland selection	8.6	1.62	6.1	10.4
8	E	Sheep farms, Northland	11.5	1.69	5.7	14.4
9	E	Dairy farms, Northland	11.6	1.65	4.0	7.5
10	E	Whatawhata HCRS‡	10.4	1.75	5.3	14.6
11	S	Whatawhata early flowering	12.1	2.01	4.6	13.0
12	E	Bailantrae HCRS‡	6.6	1.42	3.6	5.6
13	E	Devon, England	a.3	1.58	4.0	6.2
14	E	Cheviot Downs, Canterbury	11.2	1.94	5.9	12.9
15	S	High yields at low-P	7.7	1.39	3.0	6.1
16	S	Low yields at low-P	0.7	1.42	2.6	5.2
17	E§	Pukewhinau, Sth. Hawkes Bay	10.3	1.74	6.4	14.0
18	E§	Horoeka, Sth. Hawkes Bay	10.7	1.73	6.2	13.3
19	E§	Akitio, Sth. Hawkes Bay	11.6	2.00	6.5	15.5
20	E§	Ruakawa, Hawkes Bay	9.6	1.91	5.3	11.9
21	E§	Castlepoint, Wairarapa	11.6	2.08	7.3	19.5
22	E§	Summerhill, Wairarapa	11.6	1.99	5.3	11.3
23	E§	Weber, Sth. Hawkes Bay	11.0	2.08	6.4	15.3
24	E§	Hawarden, Nth. Canterbury	7.5	1.02	5.4	6.3
25	E§	Cheviot 1, Nth. Canterbury	6.4	1.64	4.6	8.0
26	E§	Cheviot 2, Nth. Canterbury	7.4	1.74	5.9	6.1
27	E§	Wither Hills 1, Marlborough	6.2	1.50	4.4	7.3
26	E§	Wither Hills 2, Marlborough	7.2	1.55	2.6	5.9
29	E§	Tara Hills, Central Otago	9.4	1.60	6.1	11.2
30	E§	Mt. Stoker, Central Otago	9.7	1.93	7.5	11.2
		P	***	***	***	***
		\$ MSD _{0.05} or MSR _{0.05}	1.6	0.40	2.5	x1.9

§ Dryland collection ‡ Hill country research station
 \$ MSD= minimum significant difference
 MSR= minimum significant ratio (Sokal and Roife 1961)

Four dryland populations (Summerhill, Hawarden, Cheviot 2 and Mt. Stoker) were characterised by having a high proportion of taproot as well as a high proportion of root compared with populations collected from wetter environments (Figure 1). Dusi, a South African cultivar bred specifically for drought tolerance, had a high proportion of root but a low proportion of taproot.

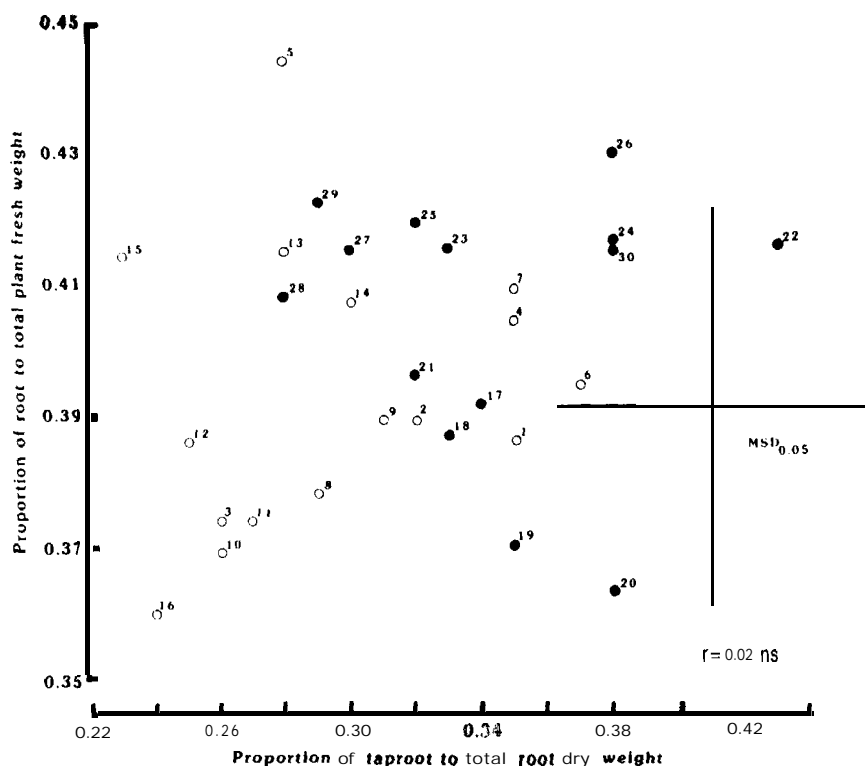


Figure 1: The relationship between proportion of taproot to total root dry weight and proportion of root to total plant fresh weight for 30 white clover populations. Populations collected from dryland sites are denoted by ●; other populations by ○. Population description are given in Table 3.

In experiment 1, genotypes that survived vegetatively at Carvossa were smaller leaved, but had a similar root morphology compared with Huia (Table 4). These genotypes were, however, more taprooted, and had a higher proportion of root than Tahora.

Effect of selection for root and shoot type. The selection for plants with different root and shoot type was a partial success (Figure 2): lines 7 and 8 were both small leaved and line 8 more taprooted than line 7; lines 1 and 2 were both large leaved and line 1 more taprooted than line 2; and line 5 was large leaved and had few taproots. However, the other three selections were less satisfactory; line 6 was too small leaved and was not taprooted enough; line 3 was taprooted but its leaves were too large and line 4 was small leaved but more taprooted than expected.

Table 4: Leaflet width and root morphology of cultivars Huia, Tahora, Kopu and Kent Wild White compared with genotypes which survived a dry summer at Carvossa. Standard errors are given.

Line	Leaflet width (mm)	Taproot diameter (mm)	% Taproot weight	% root weight
Huia	16.3 ± 0.4	2.93 ± 0.19	27 ± 3	28 ± 2
Tahora	12.3 ± 0.6	2.07 ± 0.14	21 ± 1	26 ± 2
Kopu	20.0 ± 0.7	3.03 ± 0.17	26 ± 3	35 ± 1
Kent Wild White	12.6 ± 0.6	1.94 ± 0.30	12 ± 2	31 ± 2
Survivors	15.0 ± 0.2	2.65 ± 0.06	29 ± 1	30 ± 1

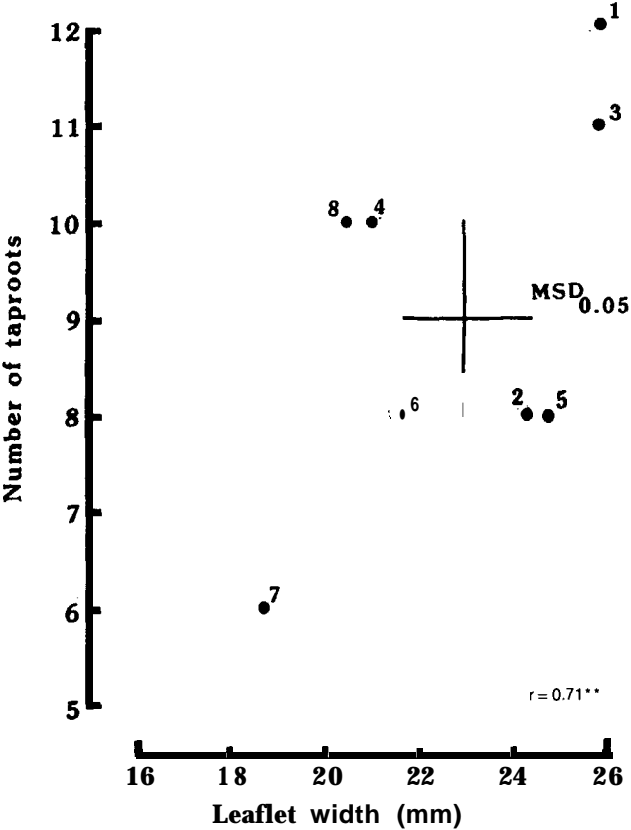


Figure 2: The leaf size and number of taproots of eight lines selected for root and shoot morphology (Table 1).

DISCUSSION

Reseeding and vegetative persistence

In previous studies there is evidence that dryland populations of white clover may be better at reseeding than Huia, Pitau, Kopu or Tahora (MacFarlane & Sheath 1984). However, in the present study, Huia had the highest reseeding ability, although the reseeding abilities of collections from Whatawhata and several other dryland regions were also high (Table 2).

The significantly better vegetative survival of populations originating from North

Canterbury and Canterbury (Table 2) indicates that selecting for regional cultivars using adapted ecotypes is likely to give an improvement in drought tolerance. Populations from Canterbury and North Canterbury also showed reasonable seedling re-establishment, indicating that unless reseeded ability can be shown to be the main mechanism of persistence it will not be necessary to consider reseeded separately in breeding a cultivar for this environment.

Chapman (1966) showed that seedling regeneration plays a minor role in white clover persistence in moist hill country compared to vegetative persistence through high stolon densities and high rates of stolon initiation. If these results apply to dry hill country also, then breeding for better reseeded ability will not be worthwhile.

Neither Tahora, bred for moist hill country (Williams *et al.* 1982) nor Kopu, bred for intensive lowland farming (van den Bosch *et al.* 1986) survived the moisture stress at Carvossa and showed only moderate reseeded ability. Neither cultivar could be recommended as a replacement for Huia in North Canterbury dry hill country.

Root type

Dry hill country populations appear to be more taprooted than populations (2, 7, 12, 15, 16) collected from moist hill country environments (Table 3). This is partly due to the larger leaf size of dry hill country populations but also to a larger taproot diameter and higher number of taproots at this leaf size. The correlation between leaf size and taproot diameter, and leaf size and taproot number is strong (Caradus 1977, 1981; Caradus & Woodfield 1986). However the dryland populations from Hawarden (24) and Cheviot 2 (26) had a larger taproot diameter and more taproots, and the population from Mt Stoker (30) more taproots than expected from their leaf size.

The lack of a linear relationship (Figure 1) between the proportion of root weight to total plant weight, and the proportion of taproot to total plant weight suggests that these characters could be selected independently to improve root type in white clover for dryland environments. The use of the root:shoot and taproot:total root dry weight ratios as selection criteria does have limitations, since plants with high or low shoot yields (and root yields) can have high root:shoot ratios and a high proportion of taproot. When using these ratios to select plants, it is important to use shoot or total plant yield as a covariate. Of the populations which had both a high proportion of root and taproot, Summerhill (22) and Mt Stoker (30) have reasonable shoot yields (Figure 2, Table 3).

In general, dryland populations (Table 3) and the survivors after drought (Table 4) had similar root morphologies to that of Huia, but were smaller leaved. There is evidence that smaller leaved white clovers are more persistent under sheep grazing (Davies & Levy 1931; Williams & Caradus 1979). Dryland populations appear to have adapted to moisture stress by retaining the root structure of a Huia type while having a smaller leaf size and lower shoot yield. This is not surprising as many of these dryland populations were collected from sites where only Huia or Ni! Certified Mother had been sown.

Ennos (1985) also found that there was significant genetic variation in root growth within a single collected population of white clover, and showed that the heritability of root length is high (0.42-0.84). The heritability will vary between populations depending on the homogeneity or heterogeneity of the population, but heritabilities of this order indicate that selection for root type independent of leaf size will be successful, as in Experiment 3 (Figure 2).

These results will be used by breeders to select small and medium leaved lines with larger and more frequent taproots for further testing in dryland environments.

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References

- Caradus J.R. 1977. Structural variation of white clover root systems NZ Journal of Agricultural Research 20: 213-219.
- Caradus J.R. 1981. Root morphology of some white clovers from NZ hill country. NZ Journal of Agricultural Research 24: 349-351.
- Caradus J.R., Woodfield D.R. 1986. Root type evaluation of white clover genotypes and populations. *Proceedings of Plant Breeding Symposium* Lincoln, N.Z. 1986. (In press).
- Chapman D.F. 1986. Natural re-seeding and *Trifolium repens* demography in grazed hill pastures. III. Seedling appearance and survival. *Journal of Applied Ecology* (In press).
- Chapman D.F., Sheath G.W., MacFarlane M.J., Rumball P.J., Cooper B.M., Crouchley G., Hoglund J.H., Widdup K.G. 1986. Performance of subterranean and white clover varieties in dry hill country. *Proceedings NZ Grassland Association* 47: 53-62.
- Charlton J.F.L. 1984. Persistence of Grasslands Huia white clover (*Trifolium repens* L.) in hill country pastures. *Proceedings NZ Grassland Association* 45: 131-139.
- Davies W., Levy E.B. 1931. Strain investigations of grasses and clovers. 4. White clover (*Trifolium repens* L.). *NZ Journal of Agriculture* 42: 75-89.
- Ennos R.A. 1965. The significance of genetic variation for root growth within a natural population of white clover (*Trifolium repens* L.). *Journal of Ecology* 73: 615-624.
- MacFarlane M.J., Sheath G.W. 1964. Clover — What types for dry hill country. *Proceedings NZ Grassland Association* 45: 140-150.
- O'Brien A.D. 1970. White clover (*Trifolium repens* L.) in a subtropical environment on the east coast of Australia. *Proceedings XI International Grassland Congress*: 165-168.
- Parsons L.R. 1982. Plant responses to water stress, pp 175-192. In Christiansen M.N.; Lewis C.F. (Eds). *Breeding Plants for less Favourable Environments*. Wiley and Sons, New York.
- Smith A., Morrison A.R.J. 1983. A deep rooted white clover for South African conditions. *Proceedings Grassland Society of South Africa* 18: 50-52.
- Sokal R.R., Rolfe F.J. 1981. Biometry. 2nd Edition. W.H. Freeman and Co, San Francisco.
- Stevenson C.A., Laidlaw A.S. 1985. The effect of moisture stress on stolon and adventitious root development in white clover (*Trifolium repens* L.). *Plant and Soil* 85: 249-257.
- van den Bosch J., Lancashire J.A., Cooper B.M., Lyons I.O., Williams W.M. 1986. G18 white clover — a new cultivar for intensive lowlands. *Proceedings NZ Grassland Association* 47: 173-177.
- Williams W.M., Caradus J.R. 1979. performance of white clover lines on New Zealand hill country. *Proceedings NZ Grassland Association* 40: 162-169.
- Williams W.M., Lamberl M.G., Caradus J.R. 1982. Performance of a hill country white clover selection. *Proceedings NZ Grassland Association* 43: 166-195.