EVALUATION OF CULTIVAR PURITY IN WHITE CLOVER (TRIFOLIUM REPENS L.) SEED LOTS

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ABSTRACT

The recent introduction of overseas white clover cultivars for seed multiplication and re-export under the New Zealand seed certification scheme necessitated the compilation of cultivar morphological data bases to assist in checking seedlot cultivar purity. With characters recorded under a controlled glasshouse environment and using significant differences in data for stolon number, leaf size score, stolon width and leaf length/width ratio, it was possible to produce a key for separating eleven cultivars (Grasslands Huia, Grasslands Pitau, Grasslands Tahora, Grasslands Kopu, Aran, Olwyn, Kersey, Menna, Donna, Sonja and Barbian).

Morphological data from the careful assessment of 25 plants per block in a glasshouse showed no more variation than that recorded from 80-480 plants per block in field plots. Field plot testing for white clover cultivar purity is costly, labour intensive, time consuming and inconsistent because of environmental variables. Glasshouse evaluation of breeders and basic seedlots may provide better cultivar quality assurance than the present compulsory plot testing.

Additional Key Words: Trifolium repens; cultivar purity, seed multiplication, plot testing, morphological characters, seed certification.

INTRODUCTION

Until 1984, production of certified white clover seed in New Zealand was restricted to that of New Zealand bred cultivars, *viz,* Grasslands Huia, first certified in 1936; Grasslands Pitau, first certified in 1975; and Grasslands Tahora, first certified in 1982. Because of the known leaf size differences (Williams, 1983), cultivar purity of seedlots within the certification scheme could be maintained through field inspection and field plot testing (Scott and Hampton, 1985).

In 1984 at the request of the New Zealand seed industry, MAF allowed the importation of seed of overseas white clover cultivars for seed multiplication and re-export (Crump, 1985). For the 1987/88 season, seed of nine overseas white clover cultivars was produced in New Zealand. However, production of seed which meets all cultivar purity requirements is complicated by the presence of buried seed (Lancashire, Rolston and Scott, 1985; Hampton, Clifford and Rolston, 1987), and there is a worldwide recognised problem of genetic contamination of white clover seedlots (Lancashire *et al.,* 1985).

The New Zealand seed industry, through the introduction of certification requirements more stringent than that of the OECD herbage and oil seed certification scheme (Crump, 1985), buried seed testing, and new production technology (Hampton *et al.,* 1987) is confident it can produce white clover seedlots which meet all cultivar purity requirements. Under the OECD seed certification scheme, the first and last assessment of cultivar purity is carried out by means of plot testing (OECD 1982), whereby seedlots are checked to determine whether the plants produced conform to the description of the cultivar and published standards for cultivar purity. Characters to be recorded include plant height, date of flowering, leaf size, leaf length/width ratio (third or fourth leaf on a rapidly growing stolon), petiole thickness, stolon thickness and flower heads per plant (OECD 1971).

There are, however, a number of problems:

1. Published cultivar descriptions are often very brief and inadequate to allow similar cultivars to be distinguished.
2. Cultivars of the cross-fertilised white clover are populations of individual plants which may exhibit much less plant to plant uniformity than in some self-fertilised crops. Therefore individual plants from different cultivars may have identical characters although the cultivars themselves are quite distinct. To establish differences between cultivars it is necessary to measure the plant to plant variation within each cultivar (Hawkins, Horne and Kelly, 1964).
3. Growth and development of the white clover plant can be influenced by temperature (Mitchell and Lucanas, 1960), photoperiod (Mitchell and Lucanas, 1962), light intensity (Eagles and Othman, 1985), pest and disease status and soil fertility (Hawkins, 1959) under which the plants are grown.
4. Field plot testing is costly, labour intensive and time consuming (Scott and Hampton, 1985).

The production of new white clover cultivars under the New Zealand seed certification scheme necessitated the compilation of cultivar morphological data bases. We used these data to determine which plant characters could best be used to identify different cultivars, and also to compare data variation obtained from plants grown in a glasshouse and field plot test environment.

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MATERIALS AND METHODS

1. Glasshouse

A range of morphological characters was studied in 11 white clover cultivars which differed in origin and leaf size (Table 1). Seedling studies were conducted on plants transplanted 30 days after sowing in flats, into 11 cm diameter pots filled with a mixture of sterilized peat (60%) and sand (40%) plus osmocote(T) (573 gm^{-3}), superphosphate (380 gm^{-3}), trace elements (48 gm^{-3} of FTE) and dolomite (1148 gm^{-3}). The plants were maintained in controlled glasshouse environment conditions at 20 ± 2°C with a 16 hr photoperiod provided by irradiance with 40 W Phillips (T) Gro-Plus fluorescent tubes.

TABLE 1: Origin, seedlot status and leaf size classification of white clover cultivars.

Leaf size[1]	Cultivar	Origin[1]	Seedlot[2]
	Grasslands Kopu	New Zealand	Breeders
	Olwyn	United Kingdom	Basic
'Large'	Sonja	Sweden	Basic
	Donna	United Kingdom	Basic
	Grasslands Pitau	New Zealand	
	Grasslands Huia	New Zealand	Breeders
'Medium'	Kersey	United Kingdom	Basic
	Menna	United Kingdom	Basic
'Small'	Grasslands Tahora	New Zealand	Breeders
	Barbian	Netherlands	Basic

[1] from Caradus (1986).

[2] Position within the seed certification scheme; seedlots supplied by the breeder or breeder's agent as standards for the cultivar.

Each cultivar was replicated in each of four randomised blocks by 25 plants/cultivar/block. Each block of plants and plants within each block were alternated in position at regular intervals within the glasshouse and within the block respectively, to minimise possible position within-the-environment effects.

Six weeks after potting up, petiole length of the third open trifoliate leaf from the tip of the longest stolon, and the width and length of the terminal leaflet of the same leaf were measured, and the width/length ratio (Hawkins, 1959) calculated. Two weeks later the number of stolons originating from the crown, the number of leaves on the longest stolon and the number of leaves per plant were recorded. All plants were then defoliated to approximately 5 cm from the crown.

At 16 weeks after potting up the size of the largest leaf (Hawkins, 1959) was scored using an estimation of leaf area key (Williams, Evans and Ludwig, 1964). Plants were then defoliated. At 21 weeks the longest stolon was identified, its length measured from the tip to the centre of the plant crown, the size of the three youngest fully opened leaves at the end of the stolon scored, and the width of the stolon (Hawkins, 1959) measured at a point midway between the second and third leaf petiole of the scored leaves.

2. Field

Seed from 36 lots of certified white clover (Grasslands Huia, Grasslands Pitau, Grasslands Tahora, breeders, basic and first generation) was sown in flats in a glasshouse. Six weeks later (30.9.85), seedlings were transplanted into a prepared field site in a Tokomaru silt loam soil which had been fallowed the previous season. The plantings were at 60 cm spacings, with each seedlot represented by two replicates of four rows of 12 plants arranged as in-line blocks. Once established, the plants were defoliated by flail mower on 6.1.86 to stabilise growth patterns and prevent seed formation.

At 16 weeks after cutting, leaf size was scored (Williams *et al.*, 1964) using average per plant leaf size rather than any one individual leaf. Growth scoring (OECD 1971) was also carried out every two weeks. For each assessment the outside plants of each block were disregarded, leaving a population of 20 plants per block.

TABLE 2: Cultivar means for largest leaf size score at 16 weeks and stolon number at 8 weeks for glasshouse grown plants.

	Largest Leaf Score			Stolon Number		
Cultivar	mean	SD	% cv	mean	SD	% cv
G. Kopu	23.75	1.36	5.7	2.00	0.27	9.9
Aran	23.36	1.98	8.5	1.48	0.44	12.7
Sonja	23.25	2.19	9.4	2.68	0.38	15.9
Olwen	22.80	1.83	8.0	2.08	0.37	11.4
Donna	22.50	1.79	7.9	2.44	0.33	13.9
G. Pitau	22.25	1.48	6.7	3.04	0.32	15.2
Menna	21.20	1.53	7.2	2.72	0.24	11.2
Kersey	21.08	2.40	11.5	2.20	0.34	13.2
G. Huia	20.61	1.41	6.9	2.56	0.27	15.1
G. Tahora	19.58	1.44	7.4	2.84	0.26	15.6
Barbian	19.20	1.91	9.9	3.76	0.22	11.9
SE	0.54			0.33		
LSD 0.05	0.99			0.60		
0.01	1.49			0.79		

RESULTS

1. Glasshouse

Of the morphological characters recorded, significant differences in stolon number at 8 weeks, the size of the largest leaf at 16 weeks and stolon width at 21 weeks existed between many, but not all of the cultivars (Tables 2 and 3). These data are further summarised (Table 4) and show that it is possible to use one or more of these characters to distinguish between most of the cultivars at $P<0.01$, and all except Aran and Olwen, and Sonja and Donna at $P<0.05$. These latter four cultivars were separated using leaf length/width ratio data (Table 3).

Although characters such as petiole length, leaf number and leaf score for the three youngest fully opened leaves at the end of the largest stolon did differ between

(T) Trade name

TABLE 3: Cultivar means for stolon width and leaf length/width at 21 weeks for glasshouse grown plants.

Cultivar	Stolon Width mean	SD	% cv	3rd trifoliate leaf on longest stolon length	width	ratio
G. Kopu	2.75	0.27	10.31	14.36	12.96	1.10
Aran	3.30	0.44	13.33	11.48	10.36	1.11
Sonja	2.40	0.38	15.82	14.20	13.52	1.05
Olwen	3.26	0.37	11.35	14.76	13.56	1.06
Donna	2.34	0.33	14.08	13.88	12.44	1.12
G. Pitau	2.12	0.32	15.40	12.80	12.12	1.06
Menna	2.18	0.24	11.01	13.04	12.32	1.07
Kersey	2.63	0.34	12.95	12.16	10.92	1.12
G. Huia	1.85	0.29	15.16	13.24	12.00	1.10
G. Tahora	1.65	0.26	15.74	11.76	10.76	1.09
Barbian	1.87	0.22	11.80	11.36	11.16	1.01
SE	0.10			0.62	0.57	
LSD 0.05	0.178			1.13	1.04	
0.01	0.235			1.49	1.37	

TABLE 4: Cultivars between which differences in stolon number and/or leaf size score and/or stolon width were not significant at $P<0.01$ and $P<0.05$.

Cultivar	$P<0.01$	$P<0.05$
G. Huia[1]	G. Tahora	—[2]
G. Pitau	Menna, Donna	—
G. Tahora	G. Huia	—
G. Kopu	—	—
Aran	Olwen	Olwen
Menna	G. Pitau, Donna	—
Donna	Menna, G. Pitau, Sonja	Sonja
Sonja	Donna	Donna
Barbian	—	—
Kersey	—	—
Olwen	Aran	Aran

eg.

[1] G. Huia could be distinguished from all cultivars except G. Tahora for one or more of the morphological characters recorded at $P<0.01$ and,

[2] from all cultivars at $P<0.05$.

some cultivars (eg between large and small leaved types), coefficients of variation of over 25% were recorded, suggesting that either the sample size was too small, or that the character was inherently variable.

2. Field

Significant differences in growth score were recorded for Grasslands Huia, Grasslands Pitau and Grasslands Tahora, but coefficients of variation ranged between 20-40%. Coefficients of variation for leaf size score were less than 12% (Table 5), and did not differ from those recorded from glasshouse grown plants.

TABLE 5: Coefficient of variation for leaf size score for glasshouse and field grown plants.

Cultivar	Glasshouse grown plants No. of plants	cv %	Field grown plants No. of plants	cv %
G. Huia	25	6.8	480	6.3
G. Pitau	25	6.7	220	7.9
G. Tahora	25	7.4	80	12.0

DISCUSSION

By using data for stolon number, leaf size score, stolon width and leaf length/width ratios, it was possible to separate the eleven white clover cultivars (see Figure 1). Under a controlled glasshouse environment, data variations within cultivars for these characters were acceptable, and data means for some or all of these characters differed significantly between cultivars. These results confirm earlier recommendations (Hawkins *et al.*, 1964; M. Forde, pers. comm.) for the use of leaf size, stolon width and leaf length/width ratio for separating white clover cultivars. Further glasshouse evaluations (eg Table 6) showed that under controlled conditions, data for the characters recorded did not differ widely between sowings.

Systems of quality assurance in the New Zealand seed industry must be able to keep pace with industry development in a cost effective and efficient way. Annual field plot testing of white clover seedlots is a costly and time consuming exercise of limited value, particularly when resources are limited and cultivars cannot be separated easily using characters which are also strongly influenced by fluctuating environments (Hawkins, 1959). The ideal would be some form of rapid laboratory test for cultivar purity, and it is possible that an electrophoretic technique (Scott and Hampton, 1985) may be able to provide this (S. Gardiner, pers. comm.). However, in the interim we suggest that testing under controlled glasshouse environment is of more value than field plot testing for the following reasons.

1. The smaller plant numbers involved allow increased precision of character recording.
2. Data are available within 4-6 months cf 6-16 months for field plots.
3. Testing can be year long and not seasonal only.
4. Environmental variables are substantially reduced, allowing for more consistent results from reduced plant numbers.
5. The establishment of a data-base for individual cultivar characters which do not differ significantly between sowings under a controlled and constant environment would negate the necessity for always assessing 'standard' or 'control' plants.
6. Cultivar purity testing costs could be substantially reduced.

Obviously the number of seedlots which could be assessed under this sytem would depend on the glasshouse space available and the number of seeds per seedlot tested.

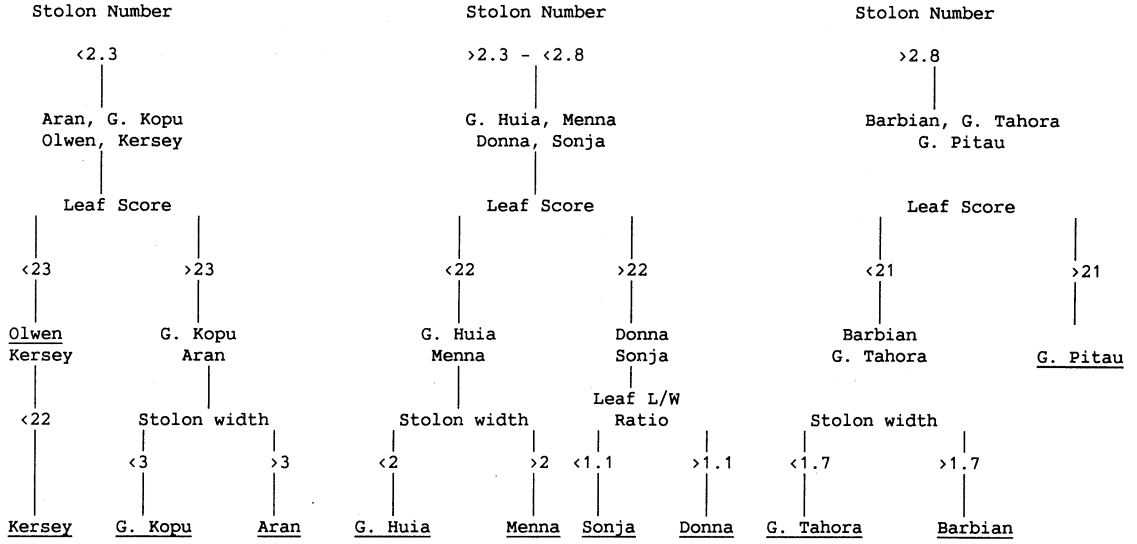

Figure 1: White Clover Indentification Key.

TABLE 6: Leaf size score and stolon width data for two glasshouse sowing dates.

Leaf Score	Olwen		Aran		Menna	
	S1	S2	S1	S2	S1	S2
mean	22.80	22.72	23.36	23.92	21.20	20.02
SD	1.83	1.09	1.98	0.94	1.53	1.36
range	22-24	20.5-24.5	20-26	22.0-26.0	18-24	18.0-23.0
% cv	8.0	4.8	8.5	3.9	7.2	6.8
Stolon Width	S1	S2	S1	S2	S1	S2
mean	3.26	2.24	3.30	3.09	2.18	2.02
SD	0.37	0.25	0.44	0.37	0.24	0.11
range	2.5-4.0	1.6-3.0	2.75-4.5	2.5-4.1	1.75-2.75	1.8-2.2
% cv	11.41	11.3	13.3	11.9	11.0	5.6

Olwen	Aran	Menna
[1]sown 4.6.85	[1]sown 4.6.85	[1]sown 4.6.85
[2]sown 3.6.86	[2]sown 6.6.86	[2]sown 3.6.86

Whatever the method of cultivar purity testing used, it is relatively simple to detect complete cultivar substitution or gross cultivar contamination. The identification of a small percentage of 'off-type' plants within a seedlot is more difficult, and the chances of doing so decrease as the sample size decreases. If all plants for every seedlot in a field plot test could be individually assessed for all the necessary characters, then the risk of wrongly accepting or rejecting a seedlot would be substantially less than an assessment of the same seedlot in the glasshouse, because the number of plants assessed would be greater. However in practice it is not possible to record accurately all data for all plants in field plots. Providing a seedlot has been sampled according to International Seed Testing Association rules, we contend that careful assessment in a glasshouse of the cultivar purity of all 'breeders' and 'basic' white clover seedlots in the New Zealand seed certification scheme is of more value to seed certification than field plot testing of large numbers of seedlots from all certification classes.

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