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White clover cultivars developed for temperate regions of South America

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Abstract. White clover is an important forage legume in temperate areas of South America but the common cultivars used are winter-active, profuse flowering types and only persist for 2-3 years due to frequent moisture stress and hot summer conditions. A breeding programme was initiated to develop new types for intensive dairy and extensive cattle grazed pastures. Wide scale screening and crossing of germplasm from international and local sources revealed the potential of Ladino and ladino x local cultivar material for use in intensive pastures with good soils and grazing management.

Improved persistence was a major objective for the extensive pastures and ecotype material collected from local farms together with crosses involving ladino and SE USA ecotypes were the best combinations. Experimental selections were developed for the two target environments and evaluated in 'scope' trials at three contrasting sites to determine herbage production and persistence potential. A ladino x local cultivar selection with large leaves, high herbage yields, extended winter to summer growth and ability to persist for at least 3 years was released as the cultivar 'Goliath' for intensive dairy-type pastures. A local ecotype selection with medium leaf size, slower establishment but high stolon density aiding persistence, and high seed yields was released as the cultivar 'Aquiles' for use in extensive pastures.

Introduction

White clover (*Trifolium repens)* is widely used in temperate pastures across Argentina, Uruguay, Chile and southern Brazil but is short-lived and fails to persist more than 3 years (Garcia 1993). Frequent moisture stress and high summer temperatures are the most important environmental factors affecting growth and vegetative persistence in these regions. The most commonly used cultivars are El Lucero in Argentina and Zapican in Uruguay, both characterised by medium-large leaves, active winter growth and early, profuse flowering. Natural reseeding does occur, but is not considered a reliable mechanism for persistence (Garcia, 1993) due to unpredictable rainfall and strong competition from resident grasses.

A breeding programme was initiated in 1997 to develop white clovers better adapted to: (1) the intensive dairy-type pastures situated on better soils and (2) the extensive cattle grazed pastures more commonly found on poorer soils in temperate regions of South America. Initial screening trials in Uruguay (La Estanzuela and Glencoe) and Argentina (Pergamino) using a wide range of local and overseas genetic material, identified white clover genotypes showing good genetic potential for improvement of dry matter production and vegetative persistence . The large-leaved Ladino based material showed good summer-autumn growth. This material together with the local cultivars such as Zapican and Bayucua, showing rapid establishment vigour and good winter-spring growth, were considered the most useful germplasm for the intensive-dairy situation. Garcia (1993) suggested that the strong growth of the Ladino and other large-leaved material observed in Uruguay could be due to their

deeper root system enhancing survival under drought conditions. In contrast, naturalised ecotype clover material collected from farms in south east USA (Widdup, 1995) and local types collected on Argentinean farms in areas from 32^0S – 39^0S (J. Amadeo, unpublished data) had medium-small sized leaves and high stolon density which is known to aid persistence in stress environments (Caradus and Williams, 1989, Bouton *et al*, 1998). These clover types were considered more appropriate for the extensive cattle grazed pastures.

Elite plant material was selected from these initial trials and either combined in polycrosses or used in paircrosses aimed at combining clovers with desirable traits. This paper describes the performance of the progeny from crosses used to develop improved white clover selections, and the productivity of these selections in contrasting environments in Argentina and Uruguay.

Material and methods

Screening clover progeny

Eighty progeny lines were generated from crosses between Ladino, South American cultivars and ecotypes, USA ecotypes and Mediterranean material. These progeny together with South American ecotypes and control cultivars (Zapican, El Lucero, Regal and Brown loam) were evaluated in randomised complete block experiments with five replications at La Estanzuela, Glencoe and Pergamino from 2000-2003. Clover plants were established in trays in the greenhouse during April 2000 and planted during June into tall fescue (*Festuca arundinacea*) swards or into natural grasslands at Glencoe. Each plot consisted of 10 plants in a 1.2-2.0 m row with 1m spacing between rows. Traits such as seasonal herbage growth, stolon density in spring and flowering intensity were visually scored (0-9). The trials were grazed with sheep with five to eight grazings annually.

Herbage and seed production trials

Three experimental selections GC124, GC125 and GC126 developed from the crosses were evaluated in 'scope' trials at sites across Argentina and Uruguay to determine their adaptation to different environments. Three sites reported here were: La Estanzuela (34^0S) characterised by hot summers (mean maximium temperature of 29^0C), 310mm mean summer rainfall on a reasonable water retentive soil, Pergamino ($33^0$44' S) with hot summers (28^0C), 325mm summer rainfall on a good retentive soil, and the harsher Gral Villegas site ($35^0$02'S) with very hot summers (35^0C) and 320mm summer rainfall on a drought prone soil. In addition to the experimental lines, cultivars Zapican, Lucero, Diablo and Kanopus from South America, Haifa from Australia and Regal from USA, were included as controls in each trial. At each site, the white clover was sown at 6 kg/ha together with tall fescue at 10 kg/ha into 2.5 m x 5 m plots with 4 replications during April 2003. Seasonal DM herbage yield was assessed by either cutting a 0.5 m x 0.5 m quadrat or using a rotary mower to cut a 0.5 m x 5 m strip and visually estimating clover and grass composition. After sampling, the areas were grazed by sheep or cattle with about seven grazings annually. Number of stolon tips/m^2 was assessed in the second and third years of the Pergamino trial. Seed production potential was measured from a second set of experiments where clover lines were established alone in 2 m x 2 m plots with four replications at La Estanzuela and Pergamino in 2003-2004. The herbage and seed production trials were analysed using ANOVA in Genstat 8.

Results and discussion

Clover breeding lines

A group of Ladino x South American cultivar lines were identified with an extended seasonal growth pattern through a combination of strong summer growth typical of Ladino types with the good winter-spring growth of locally adapted cultivars such as Zapican (Table 1). Elite genotypes from the best seven Ladino x local cultivar lines were polycrossed and the harvested seed designated, GC124. A group of locally collected ecotypes were slower establishing in the first year compared with Zapican but had better performance by the third year (Table 1). These ecotypes had significantly greater stolon numbers than Zapican and also showed good seed yield potential. Elite genotypes from three ecotypes were polycrossed and the seed harvested as GC126. A third group consisted of Ladino x ecotype lines which were similar in vigour and habit to the ecotypes but had lower seed yield potential (Table 1). Elite genotypes from the best eight lines were polycrossed and the seed designated as GC125.

Scope trials

Clover herbage yields from all cultivars and selections were high in the first two years at the three sites but declined markedly in the third year (Table 2). This decline in yield usually occurs during the second summer in hot temperate regions (Garcia 1993) and is associated with the decay of clover taproots and the dependence on stolon nodal roots for ongoing growth and persistence (Brock 2001).

At La Estanzuela, the ladino based selections GC124, GC125 and Kanopus (a recently bred ladino-type cultivar in Uruguay) consistently out-yielded the winter active cultivars Lucero, Haifa and Diablo in all years (Table 2). The new selections were similar to Zapican during the first two years but had superior growth in the third year (Table 2). The superiority of the new ladino-based material over the local cultivars was also evident at the two Argentinean sites but the differences were not always significant. The better persistence shown by the ladino based selections may be linked to the deeper nodal roots of these types (Garcia 1993) aiding survival in hot summer conditions.

The ecotype GC126 selection produced similar clover yields in all years to the GC124 and GC125 at La Estanzuela, but together with GC124 showed improved yields in the third year at Pergamino and the harsher Gral Villegas site (Table 2). The improved persistence of the ecotype selection is probably linked to its high stolon density shown in the third spring (Table 2) which was significantly greater than the local cultivars.

Table 1: Mean annual and seasonal performance (relative to Zapican=100), stolon and flower density of the top 25% of white clover lines under sheep grazing in the La Estanzuela and Pergamino screening trials.

Clover lines	Annual performance (Zapican=100)			Seasonal performance (Zapican = 100)				Stolon density[1]	Flower density[1]
	Year 1	Year 2	Year 3	Spring	Summer	Autumn	Winter		
Zapican	100	100	100	100	100	100	100	100	100
Brown Loam (Ladino)	97	115	101	102	130	113	85	85	56
Ladino x Local cvs. (7)[2]	105	132	115	108	124	128	116	100	90
Ladino x Ecotypes (8)	95	117	109	97	111	120	104	107	85
Sth. American Ecotypes (6)	94	107	117	97	107	115	107	115	110
LSD (0.05)	ns	20	18	ns	22	23	18	14	20

[1]Visual estimate of stolon numbers/m^2 and flower numbers/m^2 (relative to Zapican=100)
[2]Number of lines in the group mean

Table 2: Annual white clover herbage yield (kgDM/ha) of clover selections and cultivars from tall fescue based pastures at La Estanzuela, Pergamino and Gral Villegas. Stolon tips/m^2 was measured at Pergamino in the third spring. Percentage white clover in the pasture for the main control cultivar is shown in parenthesis.

	La Estanzuela,Uruguay			Pergamino, Argentina				Gral Villegas, Argentina		
Cultivar/ selection	Year 1	Year 2	Year 3[1]	Year 1	Year 2	Year 3[1]	Stolon tips/m^2	Year 1	Year 2	Year 3[1]
GC124	8745	6670	1850	5505	3165	1540	825	9105	7490	1260
Kanopus	9880	7640	2015	5930	3310	1105	690	8185	7470	1280
Regal	9155	6430	1410	2390	2730	970	780	NA	NA	NA
GC125	8790	6255	2045	5215	3295	815	1105	6960	6870	560
GC126	7665	5170	1780	6565	2885	1525	1245	8105	7895	1250
Zapican	9725 (81%)	5425 (54%)	1335 (42%)	NA[2]	NA	NA		NA	NA	NA
Lucero	7135	3245	800	3430 (43%)	2670 (63%)	1020 (68%)	650	4750 (43%)	3530 (67%)	260 (12%)
Diablo	6135	2120	610	6445	2700	1060	620	8125	5560	540
Haifa	4725	3670	850	4685	3425	1280	690	NA	NA	NA
LSD (0.05)	1575	1385	765	2050	ns	400	185	2200	1900	525

[1]April – November 2005
[2] NA = not sown

In the south-east USA region which has similar climatic conditions to temperate Argentina and Uruguay, the improved persistence of the ecotype based cultivar Durana compared with Ladino types was associated with a higher stolon density (Bouton *et al*, 1998). There is some contradiction between the South America and SE USA results regarding persistence of Ladino types and the scope trials will continue so as to determine the long-term persistence of these contrasting clover selections in South America.

When examining the seasonal pattern of growth (Table 3), the GC124, GC125 and Kanopus based pastures had similar clover yields to local cultivars in the winter but made up a significantly greater portion of the pasture during the summer and autumn. The GC126 had a seasonal growth pattern similar to Zapican with significantly less summer growth than the GC124 and GC125 selections.

Table 3: Mean seasonal percentage of white clover herbage (2003-2005) in the tall fescue based pasture of the selections and cultivars at La Estanzuela.

Cultivar/selection	Clover proportion in the sward (%)			
	Spring	Summer	Autumn	Winter
GC124	79	57	60	60
Kanopus	83	72	66	67
Regal	76	64	60	66
GC125	77	62	62	61
GC126	74	47	58	60
Zapican	**69**	**47**	**49**	**61**
Lucero	61	31	28	49
Haifa	50	19	34	53
Diablo	52	25	20	50
LSD (0.05)	9	10	10	ns

Seed production trials

The winter-active and early flowering cultivars, Zapican, Haifa and Diablo produced high seed yields compared to the Ladino based cultivars and selections (Table 4). On average the GC124 and GC125 produced about 85% of the seed yield of Zapican whereas the GC126 ecotype produced significantly greater seed yields than Zapican at La Estanzuela. The high seed yield potential of GC126 is an important trait when considering the commercialisation of a new selection.

Table 4: Seed yield (kg/ha) of clover selections and cultivars in pure plots at La Estanzuela and Pergamino in 2003-2004.

Cultivar/selection	Seed yield (kg/ha)	
	La Estanzuela	Pergamino
GC124	453	256
Kanopus	529	201
Regal	-	147
GC125	453	183
GC126	626	243
Zapican	**506**	**302**
Haifa	-	375
Diablo	680	582
LSD (0.05)	107	65

Conclusion

Overall, the large-leaved GC124 had high growth potential throughout the year, persisted for at least 3 years and had only slightly lower seed yields than current local cultivars. The GC124 has been released as the cultivar 'Goliath' for use in intensive, high producing pastures. The small-medium leaved GC126 with high stolon density and improved persistence together with excellent seed yield, has been released as 'Aquiles' for use in the more extensive pasture areas of Argentina and Uruguay.

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