Cultivar Description

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Grasslands Trophy — a new white clover (*Trifolium repens* L.) cultivar with tolerance of summer moisture stress

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Abstract. A breeding project has developed a new synthetic white clover (*Trifolium repens* L.) cultivar, Grasslands Trophy, that possesses tolerance of summer moisture stress in dryland pasture environments in the 850–1250 mm average annual rainfall temperate perennial pasture zone in eastern Australia. The breeding strategy used to develop Grasslands Trophy was an *in situ* breeding cycle for: (i) identifying and selecting superior genotypes, (ii) crossing elite germplasm and (iii) progeny testing derived breeding lines for the expression of key agronomic and grazing value traits. The primary selection criteria were early vigour, herbage yield, persistence and seed yield potential. Parental selection was also applied for seed yield, uniformity of leaf size, uniform flowering pattern and freedom from disease and virus symptoms. Grasslands Trophy is medium–large in leaf size with stable leaf size, combines intermediate stolon density with intermediate stolon thickness, and expresses high stolon survival and strong autumn regrowth following summer moisture stress. Grasslands Trophy has mid-season flowering maturity, intensive flowering prolificacy and high seed yield capability. Agronomic results from trials in northern New South Wales and New Zealand indicate that Grasslands Trophy has broad adaptation, expresses high summer and winter growth activity, and is reliably persistent for at least 4 years.

Introduction

Under Australian conditions of extensive grazing, legume-based pastures are the most important feed resource supporting the grazing industries because of their contribution to feed quality and to the sustainability of soil fertility, water resources and crop production where ley farming is practised (Archer 1995). White clover is the main perennial pasture legume in high rainfall temperate regions (Pearson *et al*. 1997) due to its broad adaptation. The white clover zone in Australia encompasses about 8 million ha (Clements 1987) with the potential to extend to 16 million ha (Ayres and Caradus 2002).

Although pasture improvement based on white clover and superphosphate in Australia during the 1950s and 1960s was very successful (Ayres and Lloyd Davies 2000), by the early 1980s poor clover persistence and high herbage yield fluctuations emerged as major problems, especially in summer rainfall environments (McDonald 1988). This lack of reliability, particularly in marginal environments with commercially available white clover cultivars, is attributed to poor persistence (Gillard *et al*. 1989; Kenny and Reed 1984; Robinson and Lazenby 1976). Sensitivity to summer moisture stress is identified as the major factor limiting persistence in dryland environments (Archer and Robinson 1989; Hutchinson *et al*. 1995; McCaskill and Blair 1988). Stolon survival through periods of soil moisture stress is a pre-requisite for persistence because white clover in the sward is slow to recover full production from seedling recruitment (Archer and Robinson 1989). Accordingly, improvement of stolon survival through periodic moisture stress in summer–autumn is the primary breeding objective for white clover improvement for Australian dryland pastures (Ayres *et al*. 1996).

Knowledge of the adaptative characteristics, mechanisms of regeneration and limitations of contemporary white clover cultivars (Lane *et al*. 1997, 2000), in conjunction with heritability estimates and analysis of genotype × environment interaction (Jahufer *et al*. 2002), have provided more accurately defined breeding objectives and a sound breeding strategy for white clover improvement in Australia. The application of this knowledge has led to the development of Grasslands Trophy. A summary of the breeding history, key characteristics and agronomic performance of Grasslands Trophy is outlined below.

Origins and breeding

Grasslands Trophy is a synthetic variety developed by conventional breeding. An overview of the breeding history is presented below, and details of key activities within each stage of the breeding cycle are provided under phases 1–4 that follow.

Breeding history

A diverse set of 140 white clover breeding lines was evaluated at Glen Innes, New South Wales (NSW), Australia (phase 1). Nineteen lines with superior growth and persistence under

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Table 1. Morphological characteristics of Grasslands Trophy white clover and check cultivars under field conditions at Armidale, New South Wales, Australia in spring 1999

Leaf area was calculated from width and length measurements of the middle leaflet of the third or fourth fully expanded leaf. Stolon thickness was measured halfway between the third and fourth node distal from the stolon growing point. Stolon density and stolon growing point density were measured by counting the number of stolon segments and the number of stolon apices respectively in a 10 by 10 cm quadrat frame. Flower maturity was measured as number of days to peak floristic expression from 1 September. Values are the mean ± s.e.

Characteristic	Grasslands Trophy	Haifa	Grasslands NuSiral	Irrigation	Grasslands Prestige
Leaf area (mm^2)	188 ± 21	200 ± 22	182 ± 22	167 ± 22	103 ± 22
Flowering maturity (days)	54	51	51	54	54
Flower density (number/m^2)	546 ± 115	418 ± 118	744 ±102	328 ± 118	685 ± 114
Stolon thickness (mm)	2.4 ± 0.16	2.6 ± 0.16	2.6 ± 0.14	2.3 ± 0.16	1.8 ± 0.14
Stolon density (number/m^2)	560 ± 83	380 ± 56	400 ± 59	447 ± 66	767 ± 113
Growing point density (number/m^2)	480 ± 72	333 ± 50	347 ± 52	413 ± 62	680 ± 102

grazing were identified. Stolons collected from surviving plants were used to develop 17 breeding lines. [One of these breeding lines, Grasslands Trophy, was developed (phase 2) as a polycross of four families that originated from maternal half-sib families from Portugal, Southern France, USA and the Mediterranean.] The 17 breeding lines (including Grasslands Trophy) were subsequently evaluated (phase 3) at two sites in NSW (Glen Innes and Armidale) and two sites in New Zealand (Manawatu and Lincoln). Grasslands Trophy was identified as having broad adaptation and being suitable to progress to commercialisation (Jahufer *et al*. 2005) based on winter growth, autumn recovery following summer moisture stress and persistence under grazing in NSW.

In summer 2000–01, 200 plants from the Trophy germplasm were screened for seed yield and flowering pattern at Lincoln. The final 24 parents of Grasslands Trophy were selected on the basis of uniform flowering pattern, absence of foliar diseases and outstanding seed production potential (phase 4). The parental genotypes were polycrossed in isolation in summer 2001–02 to produce pre-nucleus seed. Nucleus seed was then produced in 2002–03.

Key phases of the breeding cycle

Phase 1 (1994–97)

A 3-year field evaluation (mixed sward culture: tall fescue, *Festuca arundinacea*; grazing with sheep) of 140 promising lines (five replications) compared with 10 check cultivars (five replications) was undertaken at Glen Innes (29°44′S, 1057 m elevation, 853 mm AAR) in northern NSW, Australia. The geographic origins of the 140 lines were accessed from all major white clover zones that experience moisture stress including Southern USA, Southern Europe, Eastern Mediterranean, North Africa and Australia. The promising lines were chosen from AgResearch's ~25 000 and NSW Department of Primary Industries ~1000 line working collections of white clover on the basis of morphological characteristics, parentage and region of origin indicative of tolerance of summer moisture stress. Nomination of the best lines took place on the basis of early vegetative spread, herbage yield (spring growth, autumn re-growth, winter growth) and clover presence in the third growth cycle.

Phase 2 (1997–98)

Selected genotypes from the best 10% of lines were used to develop 17 elite breeding lines at Palmerston North [40°23′S, 33 m elevation, 960 mm average annual rainfall (AAR)], New Zealand through polycrossing the selected genotypes and best performing cultivars within leaf size and maturity groups. Crossing groups included three to eight selected lines. Seed from half-sib families was bulked within each crossing group to produce each of the 17 breeding lines for subsequent evaluation.

Phase 3 (1998–2001)

The resulting 17 breeding lines and check cultivars were evaluated at two field sites (Glen Innes and Armidale in northern NSW) under mixed sward culture. All plots were grazed by sheep at 6-weekly intervals and evaluated for early spread, herbage yield (spring growth, autumn re-growth, winter growth), clover presence in the third growth cycle, and

Table 2. Leaf size stability of Grasslands Trophy white clover and check cultivars under field conditions at Armidale, New South Wales, Australia

Leaf size was rated by two independent scorers under field conditions in conjunction with measurement of clover yield and presence. S, small; MS, medium–small; M, medium; ML, medium–large; L, large

Cultivar	May 1999	June 1 1999	July 1999	Sept. 1999	Nov. 1999	Jan. 2000	Mar. 2000	May 2000	June 2000	Sept. 2000	Nov. 2000	Feb. 2001	Apr. 2001
Grasslands Trophy	ML	ML	ML	ML	ML	ML	ML	L	ML	ML	ML	M	ML
Haifa	ML	ML	ML	ML	ML	ML	ML	L	L	L	L	M	ML
Grasslands NuSiral	L	ML	ML	ML	ML	M	M	ML	ML	ML	ML	MS	ML
Irrigation	ML	ML	MS	M	M	ML	ML	ML	ML	M	ML	M	M
Grasslands Prestige	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	M	MS	M

flowering pattern and prolificacy. The breeding lines were also screened for morphological characteristics (leaf size stability, stolon thickness, stolon density, stolon growing point density), cyanogenic glucosides and digestibility.

Data from the 3-year (1998–2001) field evaluation at Armidale (30°31′S, 1050 m elevation, 793 mm AAR) are presented to show the morphological characteristics and agronomic merit of Grasslands Trophy in comparison with the commercially relevant cultivars Haifa, Grasslands NuSiral, Irrigation and Grasslands Prestige Early. Data for the Glen Innes site are incomplete because of the incidence of severe and protracted drought.

The experimental unit was a 2-m row containing 20 clover transplants with the potential to spread to form a 2 by 1 m plot. There were five blocks each containing 25 lines (17 breeding lines and eight cultivar checks). Each replicate contained 26 row-plots arranged in two rows of 13 traverses. In each block, check cultivars were placed at every fifth plot and breeding lines were allocated at random to the remaining plots. This design allows for adjustment of responses which might be influenced by spatial effects. The estimation of treatment (breeding line) effects for herbage yield and characterisation data was by mixed model analysis which corrects for spatial trends and local plot effects. Clover presence data (ratings) were analysed as binomial responses and other variables were assumed to be normal. The analysis was done using the ASREML computer program (Gilmour *et al*. 2002) and results presented as plots of the fitted values and their 95% confidence intervals. The systematic trend over time was modelled by a spline regression.

Phase 4 (2001–02)

This phase of the breeding cycle involved selection of persistent genotypes from the elite breeding lines; testing for seed yield potential and freedom from symptoms of virus and disease; parental selection for seed yield, uniformity of leaf size, and uniform flowering pattern; and freedom from virus and disease symptoms. The selection environment at Lincoln (43°23′S, 11 m elevation, 689 mm AAR), New Zealand had regular exposure to white clover mosaic virus, alfalfa mosaic virus and powdery mildew. Any genotype exhibiting virus or

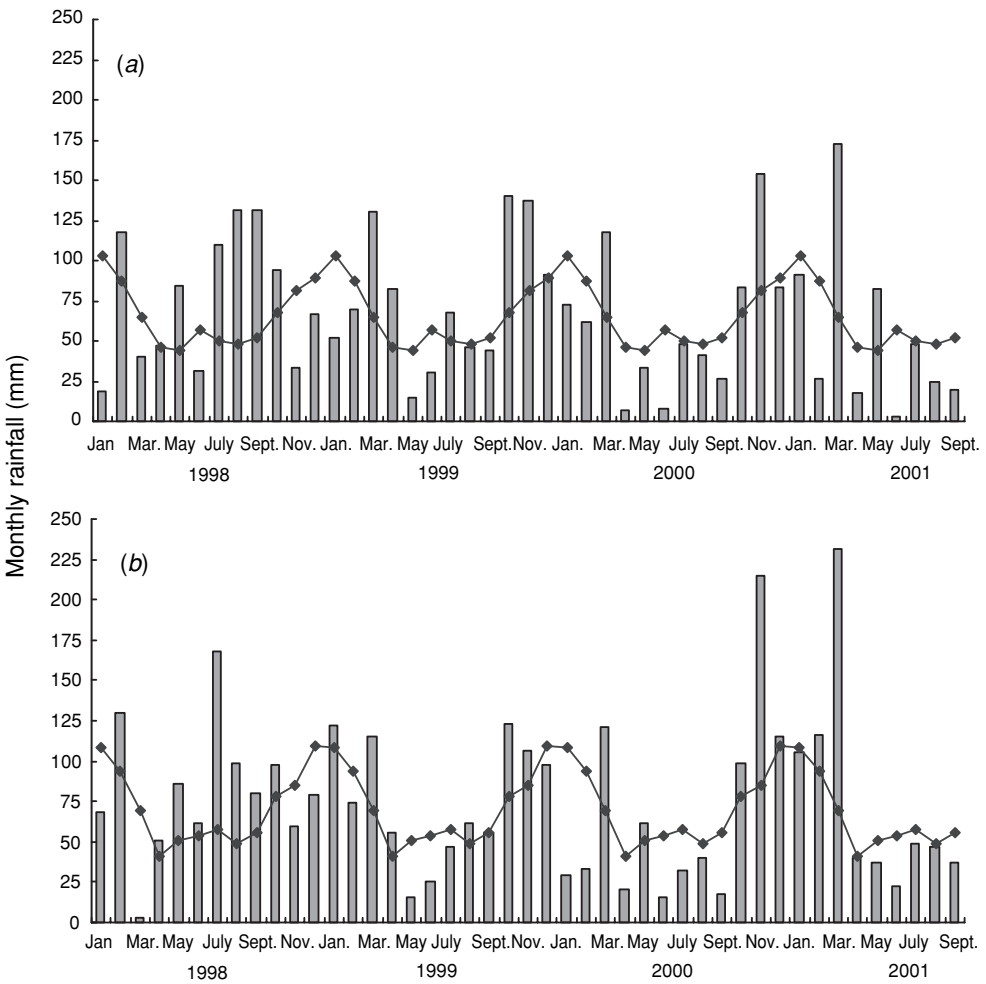

Fig. 1. Seasonal rainfall distribution (grey bars**,** 1998–2001; ◆, long-term average) at (*a*) Armidale and (*b*) Glen Innes, New South Wales, Australia.

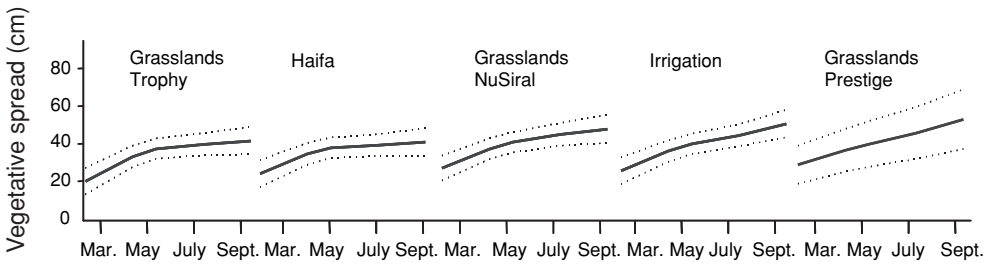

Fig. 2. Early vigour in the establishment year of Grasslands Trophy white clover compared with check cultivars Haifa, Grasslands NuSiral, Irrigation and Grasslands Prestige. Vigour was assessed as lateral vegetative spread (cm) of clover from the planted row. The dotted lines denote the confidence interval of the spline curve.

mildew symptoms during parental selection or in the pre-nucleus generation was eliminated.

Cultivar characteristics

Morphological characteristics of Grasslands Trophy are presented in Table 1. Grasslands Trophy expressed medium–large leaf size, similar to Grasslands NuSiral (Table 1). Grasslands Trophy also maintained stable leaf size across strongly contrasting seasonal conditions, reverting to medium leaf size only during severe moisture stress (Table 2). By contrast, most of the check cultivars were relatively more 'plastic' in leaf size, although it is noteworthy that Grasslands Prestige also maintained relatively stable leaf size. Grasslands Trophy displayed strongly defined white crescent leaf markings, although these were generally not as pronounced as those observed in Haifa.

Stolon thickness of Grasslands Trophy was intermediate between that of large leaf types like Haifa and medium leaf types like Irrigation. Stolon density and growing point density of Grasslands Trophy were markedly greater than Haifa. This was also evident under winter rainfall conditions in New Zealand, where the stolon growing point density of Grasslands Trophy was very high (1500 stolons/m^2) compared with Grasslands Sustain (1313 stolons/m^2), Grasslands Huia (1219 stolons/m^2), Grasslands NuSiral (1000 stolons/m^2) and Grasslands Kopu (438 stolons/m^2) (D. R. Woodfield and J. L. Lord, unpublished data).

White clover breeding for dryland pasture environments in Australia requires these strong stolon characteristics for perennation, expressed by Grasslands Trophy, for two reasons. Firstly, stolon characteristics of white clover are closely associated with the longevity of the parent plant and regenerating clone, colonising ability of the population, and persistence in the sward. Secondly, seedling survival from recruitment from soil seed-bank reserves in a developed sward is problematic. Where seedlings successfully establish in autumn, there is a protracted lag-time before production of significant clover herbage mass in the following spring.

Table 3. Seasonal herbage yield of Grasslands Trophy white clover compared with check cultivars in the second growth cycle under field conditions at Armidale, New South Wales, Australia

Herbage (kg clover DM/ha) was harvested to ground level at the end of spring 1999, summer 1999–2000 and winter 2000 using an autoscythe following 6 weeks rest from defoliation. Values are the mean ± s.e.

Season	Grasslands Trophy	Haifa	Grasslands NuSiral	Irrigation	Grasslands Prestige
Spring (1999)	854 ± 142	464 ± 77	831 ± 138	1021 ± 170	721 ± 120
Summer (1999–2000)	226 ± 61	176 ± 48	60 ± 16	108 ± 29	43 ± 12
Winter (2000)	231 ± 53	244 ± 56	140 ± 32	124 ± 28	106 ± 24

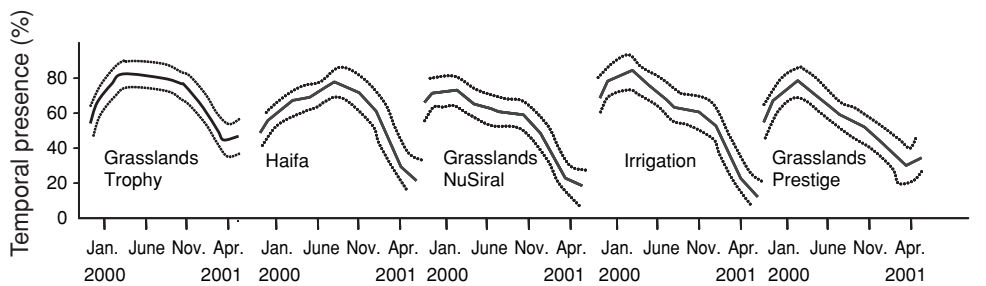

Fig. 3. The persistence of Grasslands Trophy white clover compared with check cultivars Haifa, Grasslands NuSiral, Irrigation and Grasslands Prestige. Persistence was assessed as temporal presence (%) of white clover. The dotted lines denote the confidence interval of the spline curve.

Table 4. Digestibility (%) and cyanogenic glucoside status [HCN concentration (µg HCN/g DM); HCN rating (% frequency of cyanogenic genotypes)] of Grasslands Trophy white clover and check cultivars at three stages of maturity

Clover forage sampled at vegetative, full bloom and ripe seed stages of maturity in the second growth cycle (Spring 1999) was assayed for *in vitro* digestibility using the procedure of Ayres (1991). HCN assays were undertaken on clover leaves sampled in November 2000 at mid-bloom stage of maturity by the procedure of Ayres *et al*. (2001).

Cultivar	Digestibility			Cyanogenesis	
	Vegetative stage	Full bloom	Ripe seed	HCN concentration	HCN rating
Grasslands Trophy	82.2	79.3	77.3	1219	100
Haifa	82.5	78.8	77.6	677	94
Grasslands NuSiral	81.1	78.4	78.6	758	82
Irrigation	82.1	79.8	77.8	846	100
Grasslands Prestige	81.7	78.6	76.1	1326	100

Flowering prolificacy is still required to maintain soil seed-bank reserves for natural recovery after severe episodic drought events. Grasslands Trophy with its high seed production capability (developed for successful commercialisation) addresses this requirement. Grasslands Trophy expressed mid-season flowering maturity at Armidale, and its flowering intensity (546 flowers/m^2) ranked higher than all cultivars except Grasslands NuSiral (744 flowers/m^2) and Grasslands Prestige (685 flowers/m^2). The flowering pattern at Lincoln, of the selected parents of Grasslands Trophy had similar peak flowering date to Grasslands Tribute and Grasslands Pitau. Grasslands Trophy has excellent seed yield potential with the nucleus generation yielding 975 kg/ha at Lincoln, in 2003.

Agronomic performance

Seasonal conditions during field evaluation were characterised by large fluctuations in monthly rainfall and severe moisture stress at the evaluation sites at both Glen Innes and Armidale (Fig. 1). Notably there were three periods (summer 1998–99, summer 1999–2000, spring 2000) when soil moisture declined to a dry profile, and moisture deficit was severely detrimental to white clover survival.

Agronomic performance was assessed in terms of early vigour (stolon spread in the first growth cycle, Fig. 2), seasonal herbage yield (Table 3) and persistence (Fig. 3) in the second and third growth cycles. Of the check cultivars, only Grasslands NuSiral, Grasslands Prestige and Irrigation attained the nominal early vigour benchmark of 50% plot cover in the first growth cycle. Grasslands Trophy expressed early vigour comparable with Haifa (Fig. 2). Grasslands Trophy showed consistently high spring growth and significantly better summer growth than the check cultivars, and winter growth was comparable to the highly winter active cultivar Haifa (Table 3).

It is noteworthy that at a winter-rainfall site at Palmerston North, Grasslands Trophy yielded 157% of the annual herbage production of Grasslands Huia in year 1 and 133% in year 2; seasonal herbage production was 145, 132, 135 and 157% of Grasslands Huia for spring, summer, autumn and winter, respectively (D. R. Woodfield and J. L. Lord, unpublished data).

Under the severely adverse seasonal conditions during the evaluation at Armidale, clover presence of all check cultivars declined in the third growth cycle. However, Grasslands Trophy showed exceptional persistence into the fourth growth cycle (Fig. 3). Grasslands Trophy was exceptional in retaining a high level of stolon survival through summer moisture stress; whereas the check cultivars on average suffered about 50% stolon demise over summer–autumn, the corresponding value for Grasslands Trophy was 21.4% stolon demise. The evaluation was terminated after 4 years.

Grasslands Trophy exhibited high digestibility while vegetative and a slight decline in digestibility with the onset of maturity (Table 4) that is generally characteristic of white clover (Ayres *et al*. 1998). Grasslands Trophy is moderately high in hydrogen cyanide, and at a cyanogenic level comparable with contemporary New Zealand cultivars like Grasslands Prestige.

In conclusion, in the absence of a clearly defined morphological ideotype for persistence, selecting *in situ* for tolerance of moisture stress (especially stolon survival through summer moisture stress), seasonal growth performance (especially winter growth because of the limiting influence of the winter feed-gap under conditions in eastern Australia), and competitive vigour under grazing provide a successful plant improvement strategy for white clover. Accessing, evaluating and conventional breeding from medium–large leaf and mid-season maturity types from Mediterranean environments provide breeding lines that possess superior moisture stress recovery characteristics and better cool season growth performance for Australian dryland environments.

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Seed released by: Agricom (New Zealand) Ltd, PO Box 539, Ashburton 8300, New Zealand.

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