Grasslands Kopu II and Crusader: New generation white clovers

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Abstract

Two recently released white clover cultivars, Grasslands Kopu II and Crusader, were compared with existing cultivars for at least 3 years in each of five field trials under rotational grazing by either dairy cows or sheep in the Waikato and Manawatu. Kopu II, a large-leaved cultivar with intermediate cyanogenesis and high stolon density relative to its leaf size, performed strongly under dairy grazing with high clover yields in summer, autumn and winter, and improved persistence in year 3 of these trials. Kopu II, along with Kopu and Sustain, also had high soluble carbohydrate content. Crusader is comparable to Huia in leaf size, but has higher cyanogenesis and stolon density than Huia. Crusader outperformed all other cultivars under sheep grazing with high clover yields in autumn, winter and spring. Under dairy grazing, Crusader performed very well in years 1 and 2 but did not perform as well as Kopu II in year 3. Both of these cultivars have performed well in international testing with Kopu II having high yield and stolon density under cattle grazing in Northern USA, while Crusader has been nationally listed in the UK based on NIAB trial results. Kopu II and Crusader represent the first of a new generation of white clover cultivars bred to perform well in both national and international markets.

Keywords: breeding, cultivar, dairy, sheep, *Trifolium repens*, white clover

Introduction

White clover (*Trifolium repens*)-based pastures underpin most of New Zealand's milk, meat and wool production. The performance of white clover has been improved through breeding by between 6% and 15% per decade (Woodfield & Caradus 1994; Woodfield 1999). Some of this improvement has been due to accumulation of genes with better yield potential, but some has also been due to reducing the effects of yield-limiting factors such as pests and diseases. Despite these genetic improvements, the recent introduction of clover root weevil has seriously compromised white clover performance in Northern New Zealand (Eerens *et al.* 2001). In situations where clover root weevil is present, farmers have been forced to use higher rates of nitrogen fertiliser to replace the nitrogen normally fixed by clover. Similarly, the intensification of many dairy farm systems has included increases in nitrogen fertiliser use and increases in stocking rate, both of which can be detrimental to white clover performance (Clark & Harris 1995; Woodfield & Caradus 1996).

New Zealand produces more than 50% of the internationally traded white clover seed, and Grasslands Huia remains the dominant cultivar by volume traded internationally. The vast majority of this seed is exported but there is increasing pressure from overseas proprietary cultivars with better agronomic performance (Mather *et al.* 1995). Developing New Zealand proprietary cultivars that can match the performance of the best overseas cultivars in the major export markets has been a focus of collaborations between New Zealand seed companies and AgResearch for the past decade. This paper outlines the breeding history, morphology and performance of two new white clover cultivars, Grasslands Kopu II and Crusader, with potential for national and international markets.

Origin and breeding

Grasslands Kopu II, formerly known as Ranger (Woodfield *et al.* 1998), was developed from persistent genotypes identified in the fourth year of an evaluation of a world collection of white clover cultivars at Palmerston North under rotational sheep grazing (Woodfield & Caradus 1994). Twenty-four genotypes were selected from eight cultivars (Crau, SC-1, Tillman, Aran, Regal, Tahora, Pitau and Huia) and two experimental populations (C6531 and C6532) based on high clover yields, high stolon density, large-leaf size, and persistence (as indicated by high clover content) in the final year (Caradus *et al.* 1991) of this trial. These genotypes were polycrossed to produce F_2 progenies that were then screened as spaced plants at Lincoln for uniform flowering pattern, high seed yield and absence of foliar diseases. Twenty-four parents were selected and polycrossed in isolation to provide the pre-nucleus seed of Kopu II. The decision to commercialise Kopu II was based on its strong performance in the Northern USA (Woodfield *et al.* 1998) and in the Waikato (Woodward & Caradus 2000).

Crusader was developed from pair crosses among five Crau genotypes and six Syrian genotypes that had been selected for drought tolerance (Caradus *et al.* 1991; Barker *et al.* 1993) and high DM yield, and then screened for resistance to leaf rust, pepper spot and sclerotinia, and for increased seed yield. The eleven half-sib families were polycrossed and the resultant 11 seed lines rescreened for disease, uniform flowering pattern and seed yield. One half-sib family was eliminated due to susceptibility to leaf rust. Four plants of each of the remaining 10 half-sib families were cloned and polycrossed in isolation to provide the pre-nucleus seed of Crusader. The decision to commercialise Crusader was based on its performance and persistence in European trials. Crusader was added to the United Kingdom National list in May 2001 following NIAB trials.

Materials and methods

Agronomic Performance: Kopu II and Crusader were evaluated under grazing in five trials, with each trial running for a minimum of 3 years (Trials 1–5, Table 1). Individual plots contained 10 plants in a 1-m long row with 1-m intervals between plots in each direction. Each trial had six replicates and contained between 30 and 90 white clover lines; however, only the performance of cultivars present in all three sheep-grazed trials (12 cultivars) or in both dairy-grazed trials (nine cultivars) are reported here. Clover performance was assessed visually on a scale of 1=poor to 10=high yield prior to each grazing. Seasonal DM harvests were also collected. The ranking of cultivars was identical from visual and DM cuts and the visual data are presented. Stolon density was measured annually on all trials except the Waikato dairy-grazed trial. Data were analysed from each trial separately and combined and reanalysed based on whether the trial was grazed by dairy cows or sheep. To allow comparison among cultivars and trials, data were summarised by year and season and presented relative to Grasslands Huia. Fourth-year performance of the Waikato dairy trial (Woodward & Caradus 2000) is not presented individually but is included in the overall mean because the Manawatu dairy trial finished after 3 years.

Morphological measurements: Detailed morphological data were collected in separate Plant Variety Rights trials at Palmerston North (Trial 6, Table 1). These measurements were made on a minimum of 50 genotypes and included cyanogenesis (%), leaf size (cm^2), growth habit (1=prostrate to 3=erect), petiole length (mm), stolon internode length (mm), stolon diameter (mm), the number of florets per inflorescence, and days from first flower to 50% flowering.

Non-structural Carbohydrate: The non-structural carbohydrate content of 12 cultivars was measured using between 19 and 30 genotypes of each cultivar. To enable comparison among cultivars, plant material was harvested at the same time of day and the tissue placed directly into liquid nitrogen and then freeze-dried. Non-structural carbohydrate content was analysed by NIR spectrophotometry.

Results and discussion

Performance under dairy grazing

Kopu II, Pitau and Crusader were the best performed cultivars based on mean performance over the 3 years of testing in the Manawatu and 4 years in the Waikato, (Table 2). Pitau and Crusader established particularly well and performed very strongly in year 1 but declined in year 2 and 3. While the relative performance of Kopu II declined in the second year, its performance rebounded strongly in the third year to out-yield all other cultivars (Table 2). Both Crusader and Kopu II performed strongly in the fourth year of the Waikato trial, out-performing Huia, Kopu, Sustain and Aran (Woodward & Caradus 2000). The differing growth habit of Kopu II (large-leaved and erect) and Crusader (medium-leaved and intermediate habit) may make them complementary in dairy-farm systems. In general, the smaller leaved white clover cultivars, such as Demand and Prestige, are better suited to set-stocked

Table 1 Location and characteristics of five white clover trials under rotational grazing plus PlantVariety Rights (PVR) trial for morphological comparison.

Trial	Location	Stock class	Companion grass	Duration
1	Massey University, Manawatu	Dairy cows	Perennial ryegrass	1997–2000
2	Dexcel, Waikato	Dairy cows	Perennial ryegrass	1993–1997
3	AgResearch, Manawatu	Sheep	Perennial ryegrass	1996–1999
4	AgResearch, Manawatu	Sheep	Perennial ryegrass	1997–2000
5	AgResearch, Manawatu	Sheep	Cocksfoot	1997–2000
6	AgResearch, Manawatu	None	None, PVR trial	1999–2000

sheep systems. It is therefore not surprising that these two cultivars were not clearly superior to Huia (Table 2).

The strong overall performance of Kopu II and Crusader was based on better year-round growth (Table 2). These two cultivars, along with Pitau, had the best spring growth. Improved summer growth of white clover has been identified as a key to maintaining milk production when pasture quality is declining due to ryegrass flowering and accumulation of dead matter. The summer growth of Crusader and Kopu II was superior to that of the other nine cultivars. Similarly, the summer performance of Kopu II and Crusader in the Waikato trial was superior to Aran (Woodward & Caradus 2000), a cultivar reported to have good summer and autumn growth in northern New Zealand (Ledgard *et al.* 1990). Kopu II, Crusader and Pitau all had strong autumn growth, extending the warm-season (spring–summer) advantage (Table 2). Kopu II had a significant advantage over all other cultivars in winter, with the next best group (Pitau, Crusader, Kopu, Challenge and Sustain) all having superior winter performance to Huia (Table 2).

The stolon density of Kopu II was particularly high given its large leaf size. Some caution is needed since this was measured in only one dairy-grazed trial. However, after 3 years under beef cattle grazing in Northern USA, Kopu II had 56% higher stolon density than three large-leaved cultivars (Will, California Ladino and Tillman II) and 19% higher stolon density than Huia (Woodfield *et al.* 1998). This high stolon density under dairy grazing may in part, explain its high yield and persistence in later years of trials. Improved persistence of white clover is clearly important for New Zealand dairy systems.

Performance under sheep grazing

Crusader was the only cultivar under rotational grazing by sheep to have a significantly higher clover yield than Huia over the three 3-year trials (Table 3). As expected, the performance of the large-leaved cultivars was generally poorer than reported for dairy grazed trials. Kopu II was a slight exception to this trend, as its performance improved over the 3 years to finish among the three best performed cultivars in the third growth year (Table 3). This improved performance

Table 2 Annual and seasonal performance of nine white clover cultivars under rotational grazing by dairy cattle in the Waikato and Manawatu.

	Annual Performance (Huia = 100)				Seasonal Performance (Huia = 100)				Stolon
Cultivar	Year 1	Year 2	Year 3	Overall	Spring	Summer	Autumn	Winter	Density*
	%				%				(no./m^2)
Grasslands Huia	100	100	100	100	100	100	100	100	1007
Grasslands Pitau	166	113	114	131	145	116	121	168	1120
Grasslands Kopu	124	91	123	114	113	113	107	150	990
Grasslands Demand	113	86	108	105	111	104	96	119	1172
Grasslands Prestige	118	93	92	104	105	110	94	107	1259
Grasslands Challenge	130	93	116	112	112	102	106	149	1094
Grasslands Sustain	134	105	102	117	127	110	109	142	1120
Grasslands Kopu II	151	122	148	144	148	137	142	183	1502
Crusader	158	118	113	136	146	135	126	151	1312
LSD (0.05)	16	15	13	12	14	11	13	16	347

* Stolon density data collected only on the Manawatu trial

Table 3 Annual and seasonal performance of 10 white clover cultivars in three trials under rotational grazing by sheep in the Manawatu.

	Annual Performance (Huia = 100)				Seasonal Performance (Huia = 100)				Stolon
Cultivar	Year 1	Year 2	Year 3	Overall	Spring	Summer	Autumn	Winter	Density
	%				%				(no./m^2)
Grasslands Huia	100	100	100	100	100	100	100	100	2010
Grasslands Kopu	81	90	114	93	97	81	91	115	1868
Grasslands Tahora	104	105	131	110	123	98	109	118	2366
Prop	93	87	105	94	103	93	86	95	1819
Grasslands Demand	107	112	118	110	111	106	115	111	2329
Grasslands Prestige	99	105	119	105	113	100	105	104	2373
Grasslands Sustain	87	102	107	95	104	84	92	108	2038
Grasslands Challenge	87	95	116	96	105	80	98	119	1714
Grasslands Kopu II	95	105	131	108	116	93	107	131	1964
Crusader	128	128	130	128	139	108	127	156	2124
LSD (0.05)	14	19	18	16	18	15	18	22	381

relative to Huia over the three years was a general trend among all the other cultivars. This improvement is indicative of underlying genetic improvement in these cultivars which have all been released over the past 40 years based on their improved yield and persistence relative to Huia (Woodfield & Caradus 1999). Perhaps the most notable feature of the sheep-grazed trials was the consistently strong performance of Crusader, with a 28 to 30% relative yield improvement over Huia.

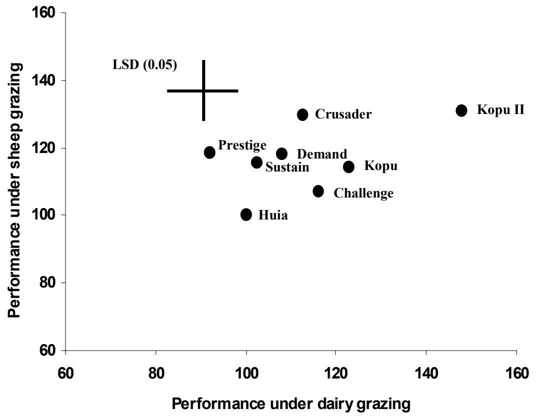
The seasonal performance of Crusader was as good as or better than all other cultivars (Table 3). Crusader and Tahora had better spring growth than Huia, but none of the cultivars had significantly better summer growth than Huia under sheep grazing. Crusader was the only cultivar with better autumn growth than Huia, while both Crusader and Kopu II had superior winter growth to Huia (Table 3).

The stolon density of the small-leaved (Tahora) and medium-small leaved cultivars (Demand and Prestige) was higher than that of the large-leaved cultivars (Table 3). These differences were not as great as observed in previous trials (Caradus & Williams 1989; Caradus *et al.* 1991; Cooper & Chapman 1993; Woodfield & Caradus 1994; Caradus *et al.* 1997).

Persistence

The ability to maintain a high clover content and high clover yields several years after sowing is the best indicator of persistence. When compared at a common age (3 years) across all trials, Kopu II exhibited good persistence in both dairy- and sheep- grazed trials (Figure 1; Tables 2 & 3). Crusader was as persistent as Kopu II under sheep grazing but less persistent under dairy grazing overall (Figure 1). Crusader (along with Kopu II) did, however, maintain 30% clover content in the fourth year of the Waikato trial

Figure 1 Comparative performance of eight white clover cultivars in the third year under rotational grazing by either sheep or dairy cattle (Huia = 100%).



(Woodward & Caradus 2000). None of these clover trials were affected by clover root weevil predation. The resistance/tolerance of these cultivars has been evaluated (Eerens *et al.* 2001), but the results have not been released by the Producer Boards who funded the research.

Selection for increased stolon growing point density while maintaining a particular leaf size has been important in breaking the negative association between yield and persistence in white clover. Cultivars such as Prestige and Sustain were the first "correlation breakers" at medium and medium-large leaf sizes (Caradus *et al.* 1997; Cooper & Chapman 1993). Kopu II appears to be a significant improvement over other large-leaf size cultivars particularly under dairy grazing (Table 2).

Morphological measurements

Cyanogenesis is an important characteristic in New Zealand. Cultivars with an intermediate to high frequency of cyanogenic plants and higher HCN production generally have better persistence and productivity than acyanogenic cultivars (Caradus & Williams 1989; Crush & Caradus 1995). The cyanogenesis level of white clover cultivars evaluated in these trials ranged from Kopu, with 48% cyanogenic genotypes, to Crusader with 93% cyanogenic genotypes (Table 4). Kopu II had a similar frequency of cyanogenic (63%) plants to Huia, Prestige, Sustain, Challenge and Aran.

Leaf size and erect growth habit contribute to the yield potential of white clover, while stolon growing point density is an important factor in its persistence (Caradus *et al.* 1997). Kopu II had a larger leaf size than the other large-leaved cultivars (Aran, Kopu, Challenge and Pitau), while its stolon growing point density was higher than the best of the large-leaved cultivars (Sustain) (Table 4). The leaf size of Crusader was equivalent to the medium-leaf size standard Huia, while its stolon growing point density is similar to Demand (Table 4). Based on both growth habit score and petiole length, Kopu II is most similar to the erect growth habit of Aran and Pitau, while the intermediate growth habit of Crusader was most similar to Huia and Demand (Table 4).

Kopu II had the thickest stolons along with Aran, Kopu and Challenge, and the longest stolon internode length, along with Sustain and Pitau. The stolon internode length of Crusader was more similar to that of the larger-leaved cultivars (Challenge, Aran, Pitau and Kopu) than to the other medium-leaved and medium-small leaved cultivars (Table 4). Similarly, the intermediate stolon thickness of Crusader was equivalent to that of Huia, Pitau and Sustain. The inflorescences of Kopu II have very high numbers of

Table 4 Cyanogenesis, morphology, flowering and stolon density of 12 white clover cultivars in PVR trials at Palmerston North.

Cultivar	Cyanogenesis	Leaf area	Internode length	Stolon diameter	Growth habit*	Petiole length	Flowering	Florets	Stolon density[1]
	(%)	(cm^2)	(mm)	(mm)	(1–3)	(mm)	(days)	(no./inflorescence)	(no. per m^{-2})
Huia	66	2.71	25.6	2.3	1.3	83.6	41	77	1805
Pitau	84	4.12	29.1	2.7	2.2	101.2	40	82	1620
Kopu	48	4.86	29.0	2.9	2.3	116.4	40	99	1620
Tahora	50	1.62	25.4	1.9	1.0	64.4	37	70	2119
Prop	77	1.32	23.1	1.9	-	19.5	27	70	1690
Demand	79	2.35	23.3	2.1	1.5	83.6	41	73	2052
Prestige	65	2.13	25.0	2.1	1.2	69.6	37	68	2109
Sustain	61	3.74	31.4	2.6	2.2	100.3	38	79	1739
Challenge	67	4.24	28.7	2.8	2.2	97.4	39	88	1579
Kopu II	63	5.60	32.2	3.0	2.5	104.6	41	97	1842
Crusader	93	2.62	28.7	2.5	2.0	75.4	36	85	1934
Aran	65	4.52	27.4	3.0	2.4	103.7	40	100	-
LSD (0.05)	-	0.66	3.2	0.2	0.2	11.1	2	10	321

* Growth habit score (1=prostrate to 3=erect).
[1] Mean stolon growing point density from four field trials.

florets, but its maturity (days from first to peak flowering) is similar to the majority of medium- and large-leaved cultivars evaluated. The maturity of Crusader was equivalent to Prestige and Tahora, but 2 to 5 days earlier than most of the other cultivars (Table 4).

Non-structural carbohydrate content

White clover and perennial ryegrass (*Lolium perenne*) are rich in protein but can often be relatively low in non-structural carbohydrates. Substantial variability for non-structural carbohydrate content was observed among white clover cultivars (Table 5). Large-leaved cultivars (Kopu, Kopu II, Sustain and Aran) tended to have higher levels of non-structural carbohydrates than medium- and small-leaved cultivars. When non-structural carbohydrate levels are low there is insufficient metabolisable energy to allow the animal to utilise all the ammonia produced from protein breakdown in the rumen. In these situations excess ammonia has to be transported to the liver, converted to urea and excreted. This represents a loss of nitrogen and a wastage of energy for the animal. Dietary supplementation with readily fermentable carbohydrates has led to demonstrable improvements in N metabolism in the rumen but is expensive. Increasing the level of non-structural carbohydrate within white clover through breeding should reduce the need for costly dietary supplements and may help to reduce methane emissions.

Table 5 Soluble carbohydrate levels (± SE) in 12 white clover cultivars at Palmerston North.

Cultivar	n	Soluble carbohydrate (% DM)
Aran	30	18.2 ± 0.5
Challenge	19	17.3 ± 0.5
Crau	25	15.6 ± 0.4
Demand	30	16.6 ± 0.3
Huia	20	13.4 ± 0.3
Kopu	21	20.9 ± 0.5
Kopu II	30	20.5 ± 0.5
Pitau	30	16.3 ± 0.3
Prestige	30	17.1 ± 0.4
Prop	20	15.5 ± 0.4
Sustain	30	19.0 ± 0.4
Tahora	20	14.4 ± 0.4

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