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Volume 38, 1998

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Seasonal variation in the herbage yield and nutritive value of perennial ryegrass (*Lolium perenne* L.) cultivars with high or normal herbage water-soluble carbohydrate concentrations grown in three contrasting Australian dairy environments

K. F. Smith[AB], *R. J. Simpson*[A], *R. N. Oram*[A], *K. F. Lowe*[C], *K. B. Kelly*[D], *P. M. Evans*[B] *and M. O. Humphreys*[E]

[A] CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia.
[B] Present address: Agriculture Victoria, Pastoral and Veterinary Institute, Private Bag 105, Hamilton, Vic. 3300, Australia; corresponding author; e-mail: smithk@hammy.agvic.gov.au
[C] Department of Primary Industries, Mutdapilly Research Station, MS 825 Peak Crossing, Qld 4306, Australia.
[D] Agriculture Victoria, Institute for Sustainable Irrigated Agriculture, Kyabram, Vic. 3620, Australia.
[E] Institute for Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, SY23 3EB, UK.

Summary. Two lines of perennial ryegrass (*Lolium perenne* L.), cv. Aurora and breeding line Ba 11351, from the United Kingdom with elevated concentrations of water-soluble carbohydrates in the shoot were compared with the standard cultivars, Ellett, Vedette and Kangaroo Valley, in pure grass swards under irrigation at Kyabram, Victoria, and Gatton, Queensland, and under natural rainfall at Condah, Victoria, during 1995–97. Near infrared reflectance spectroscopy was used to predict the water-soluble carbohydrate, crude protein, *in vitro* dry matter digestibility, neutral and acid detergent fibre, and Klason lignin concentrations of the perennial ryegrass herbage. Herbage yield and water-soluble carbohydrate differed between cultivars at each site at most harvests, with the high water-soluble carbohydrate lines usually yielding less and having higher water-soluble carbohydrate concentrations than the 3 standard cultivars. However, the high water-soluble carbohydrate lines also had higher water-soluble carbohydrate concentrations at harvests where their yield was equal to the standard cultivars. The other nutritive value traits differed significantly at more than half of the 32 harvests: the high water-soluble carbohydrate lines had higher crude protein and dry matter digestibility, and lower neutral detergent fibre, the neutral detergent fibre containing less acid detergent fibre and lignin than did the standard cultivars. The high water-soluble carbohydrate lines were more susceptible to crown rust during spring and summer than the standard cultivars at Kyabram and Gatton: heavy infections reduced yield, water-soluble carbohydrate, dry matter digestibility and crude protein. Higher water-soluble carbohydrate may depend on only a few genes, as does rust resistance and it seems likely that high yielding, high water-soluble carbohydrate cultivars can be developed by recombination and selection.

Additional keywords: cultivar comparisons, crown rust.

Introduction

Perennial ryegrass (*Lolium perenne* L.) is the most important grass species in dairy pastures in Australia. It is commonly sown in conjunction with either white clover (*Trifolium repens* L.) or subterranean clover (*T. subterraneum* L.). These ryegrass–clover pastures provide the major source of nutrients for grazing dairy cattle and are also used for sheep production.

The nutritive value of perennial ryegrass, and hence perennial ryegrass–clover pasture, varies throughout the growing season (e.g. Walsh and Birrell 1987). The relatively poor digestibility of perennial ryegrass pasture

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in summer leads to the use of feed supplements to maintain dairy production.

Water-soluble carbohydrates (WSC) provide the most readily available source of energy for grazing ruminants. Low concentrations of water-soluble carbohydrates in perennial ryegrass herbage may also decrease the efficiency of protein utilisation during autumn and winter (Dove and Milne 1994). Perennial ryegrass cultivars which accumulate high concentrations of WSC have been selected in the United Kingdom (UK) (Humphreys 1989*a*, 1989*b*, 1989c). Increased WSC concentrations in herbage could alleviate the seasonal deficiencies in the nutritive value of perennial ryegrass. Efficient microbial protein synthesis in the rumen depends on the synchronous supply of carbohydrates and amino acids (Beever 1993). When there is insufficient readily available energy for balanced fermentation, the acetate-dominant fermentation which occurs results in reduced supplies of both microbial protein and nett energy to the ruminant (Corbett *et al*. 1966; Blaxter *et al*. 1971). Low WSC concentrations in herbage have the potential to limit animal production even when the digestibility of herbage is high (Reed 1978; Dove and Milne 1994).

In grazing experiments in the UK, cultivars of perennial ryegrass with high WSC have supported higher stocking rates (Davies *et al*. 1989*a*, 1989*b*) and have lead to increased lamb production per hectare (Davies *et al*. 1989*b*, 1992; Munro *et al*. 1992). High WSC concentrations in herbage have also been associated with increased intake of pasture by grazing animals (Michell 1973; Jones and Roberts 1991). While there are no reports of the use of high WSC lines of perennial ryegrass in grazing experiments in Australia, simulations using decision support tools such as 'GrazFeed', indicate that high WSC lines have the potential to increase animal production by improving both digestibility and intake (Smith *et al*. 1998*c*).

Perennial ryegrass is sometimes poorly persistent under Australian conditions due to its relatively poor tolerance of summer drought and high temperatures. Improving the persistence of perennial ryegrass is a major goal of current breeding programs (Cunningham *et al*. 1994; Reed 1996). Water-soluble carbohydrate reserves in tiller bases over summer have been associated with persistence and survival in cocksfoot (Volaire and Lelièvre 1997). The influence of WSC reserves on persistence in perennial ryegrass is unclear although high molecular weight fructan concentrations are known to increase in the pseudostem of the perennial ryegrass during drought (Thomas 1991). The high WSC cultivar Aurora had high growth and yield stability during drought, and good regrowth after drought (Amin and Thomas 1996). Water-soluble carbohydrate reserves (g/plant) in the stubble (Alberda 1966; Fulkerson 1994) or the leaf blades (Fulkerson *et al*. 1994) have also been associated with differences in initial rates of regrowth among perennial ryegrass genotypes.

Perennial ryegrass genotypes which accumulate high concentrations of WSC could be of importance in Australia for both increased nutritive value and persistence. Perennial ryegrass cultivars from the UK which accumulate high concentrations of WSC were evaluated in a pot trial in Victoria by Radojevic *et al*. (1994). In that experiment the high WSC cultivars consistently had higher WSC concentrations in herbage than Ellett, and were more digestible over summer. However, the expression of the high WSC trait in Australian environments under field conditions, the effects of season and correlations with other traits are unknown. This paper describes a series of experiments in which the yield and nutritive value of 2 high WSC perennial ryegrass cultivars from the UK were compared with those of 3 New Zealand and Australian cultivars in 3 contrasting dairy environments.

Materials and methods

Germplasm

Five cultivars of perennial ryegrass were evaluated: Ellett, a cultivar developed from a naturalised population in New Zealand (Duder 1986) and widely sown in Australian dairy pasture; Vedette, a New Zealand cultivar which has performed well in Australia (Reed *et al*. 1995); Kangaroo Valley, a winter-active Australian ecotype from Kangaroo Valley, New South Wales (Cunningham *et al*. 1994); Aurora, a high WSC cultivar developed in the UK from germplasm collected in the Zürich uplands (Tyler and Jones 1982); and Ba 11351, a high WSC sister line to the cultivar Cariad developed from a cross between Aurora and Melle (Humphreys 1989*a*, 1989*b*).

Site details

The perennial ryegrass cultivars were sown in 3 Australian dairy environments: Gatton (Qld) irrigated; Kyabram (Vic.) irrigated; Condah (Vic.) rainfed, in the autumn of 1995. Plots at the Gatton site were resown in the autumn of 1996 as the 1995 sowing failed to survive a period of extremely hot, wet weather after harvest in December 1995. Harvest dates varied from site to site and the harvest dates at each site are listed in Table 1. These variable harvest dates were the result of variable growth conditions at the sites, all trials were managed according to the APPEC protocol for perennial ryegrass cultivar evaluation (APPEC 1996). Perennial ryegrass seed was sown as monoculture at 15 kg live seed/ha. Four replicates of each cultivar were sown at each site in a randomised complete block design. Plots were regularly harvested to a residual height of 50 mm and the yield of the plots measured. A representative subsample of perennial ryegrass from each plot was dried at 65°C before chemical analysis.

Table 1. Harvest dates for perennial ryegrass cultivars at Gatton (1995 and 1996 sowings), Kyabram and Condah

Harvest	Gatton (1995)	Gatton (1996)	Kyabram	Condah
1	5.vi.95	6.ix.96	30.viii.95	1.xi.95
2	3.vii.95	3.x.96	26.ix.95	11.i.96
3	31.vii.95	31.x.96	19.x.95	15.v.96
4	28.viii.95	27.xi.96	10.xi.95	11.ix.96
5	20.ix.95	23.xii.96	27.xii.95	30.x.96
6	19.x.95	20.i.97	16.ii.96	—
7	24.xi.95	20.ii.97	18.iii.96	—
8	—	20.iii.97	18.v.96	—
9	—	28.iv.97	5.vi.96	—
10	—	22.v.97	—	—

The incidence of crown rust (*Puccinia coronata* Corda f. sp. *lolii* Brown) was visually assessed at each site on a 1–9 scale, where 9 represented the most severe infection.

Chemical analysis

The dried herbage sample was ground to pass through a 1 mm screen in a Cyclotech mill. Near infrared reflectance (NIR) spectra were then collected for each of the samples using an NIRSystems model 5000 with a scanning monochromator. A subset of the samples (52) from the total of 660 were selected on the basis of spectral differences (Shenk and Westerhaus 1991*a*, 1991*b*) and analysed for the following nutritive value parameters. Water-soluble carbohydrates were extracted in 80% ethanol (80°C) and twice in water (80°C), for 60 min on each occasion, then measured using the anthrone method (Yemm and Willis 1954). Total nitrogen was determined using the Kjeldahl method, and crude protein estimated as N x 6.25. The *in vitro* dry matter digestibility (DMD) was determined using the pepsin–cellulase technique (McLeod and Minson 1978). Neutral detergent fibre (NDF) was determined by digesting a sample of herbage in neutral detergent solution (Van Soest and Robertson 1980) in an autoclave at 105°C for 1 h (Pell and Schofield 1993). The NDF was then further digested in acid detergent solution (Goering and Van Soest 1970) with the residue weighed to give acid detergent fibre (ADF), or in sulfuric acid according to the Klason lignin procedure. Acid detergent fibre and Klason lignin were expressed as a percentage of the NDF.

A further 20 samples were analysed using these standard chemical methods to serve as a validation for the NIR calibrations. Near infrared calibrations were then developed using modified partial least squares regression (Shenk and Westerhaus 1991*a*, 1991*b*) to predict WSC, CP, IVDMD, NDF, ADF and Klason lignin concentrations of the remaining samples. The statistics describing the NIR calibration equations and their performance on the 20 validation samples are given in Table 2.

Table 2. Regression statistics for near infrared (NIR) calibrations developed on 52 perennial ryegrass samples and tested on a further 20 samples from a total of 660 samples grown at Gatton, Kyabram and Condah

CP, crude protein (g/kg DM); WSC, water-soluble carbohydrate (g/kg DM); DMD, dry matter digestibility (% DM); NDF, neutral detergent fibre (% DM); ADF, acid detergent fibre (% DM); Lignin, Klason lignin (% NDF); SEC, standard error of calibration; R^2, squared multiple correlation coefficient between NIR and chemical values; SEP(C), standard error of performance, corrected for bias; Slope, slope of linear regression between NIR and chemical data; r^2, squared simple correlation coefficient between NIR and chemical data

Statistic	CP	WSC	DMD	NDF	ADF	Lignin
			Calibration			
SEC	12.19	19.45	2.03	2.31	1.95	0.85
R^2	0.95	0.94	0.87	0.84	0.92	0.91
			Validation			
SEP	11.80	18.49	1.95	2.24	1.90	0.92
Bias	0.07	0.13	0	0	0	0
SEP(C)	11.90	18.68	1.95	2.26	1.92	0.92
Slope	1.00	0.99	0.90	1.00	0.95	0.96
r^2	0.95	0.95	0.84	0.85	0.90	0.83

Statistical analyses

Analysis of variance at individual harvests. The data from the experiments described in this paper have previously been analysed for serial correlation effects associated with repeated measures on individual plots (Smith *et al*. 1998*a*). The data were analysed for antedependence using the GENSTAT 5 procedure ANTORDER (Ridout and Payne 1995) which utilises the profile method of Kenward (1987).

To overcome the effects of serial correlations data from harvests where first order antedependence was detected, the data were analysed using data from the previous harvest as a covariate. Sites and traits affected in this way were: Kyabram—yield, WSC, CP; Gatton (1996 sowing)—yield, WSC, CP; Condah—yield. Although other higher order antedependence effects were detected for some site/trait combinations (Smith *et al*. 1998*a*), these were not taken into account during data analysis and data from all other site and trait combinations were analysed using standard ANOVA.

Split-plot analysis of harvest and cultivar x harvest effects at each site. The effects of serial correlations were removed by using the GENSTAT 5 analysis of variance procedure for repeated measurements; AREPMEASURES (Payne 1995). This procedure is analogous to the split-plot over time ANOVA except that the degrees of freedom used in testing harvest and

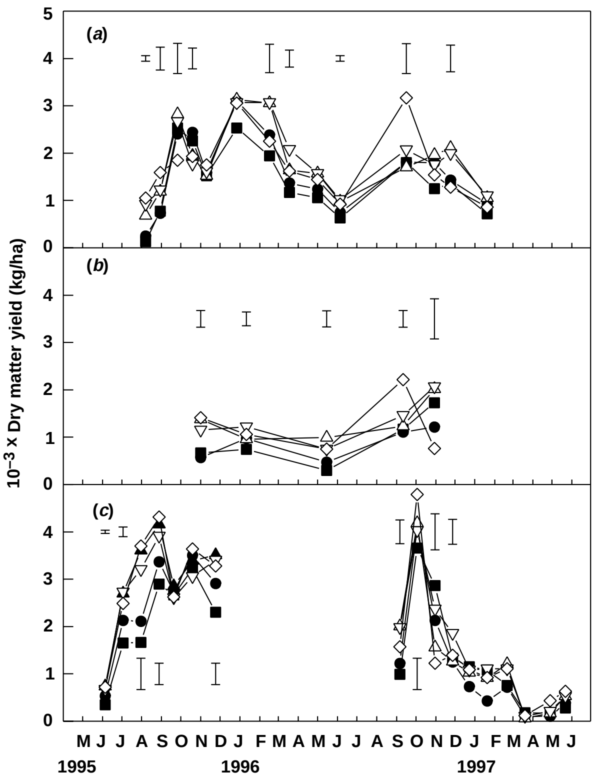

Figure 1. Seasonal variation in the dry matter yield (kg/ha) of perennial ryegrass cultivars, Aurora (■), Ba 11351 (●), Ellett (△), Vedette (▽) and Kangaroo Valley (◇), at (*a*) Kyabram, (*b*) Condah and (*c*) Gatton. Bars represent l.s.d. (P = 0.05); where no bar is plotted differences between cultivars were not significant.

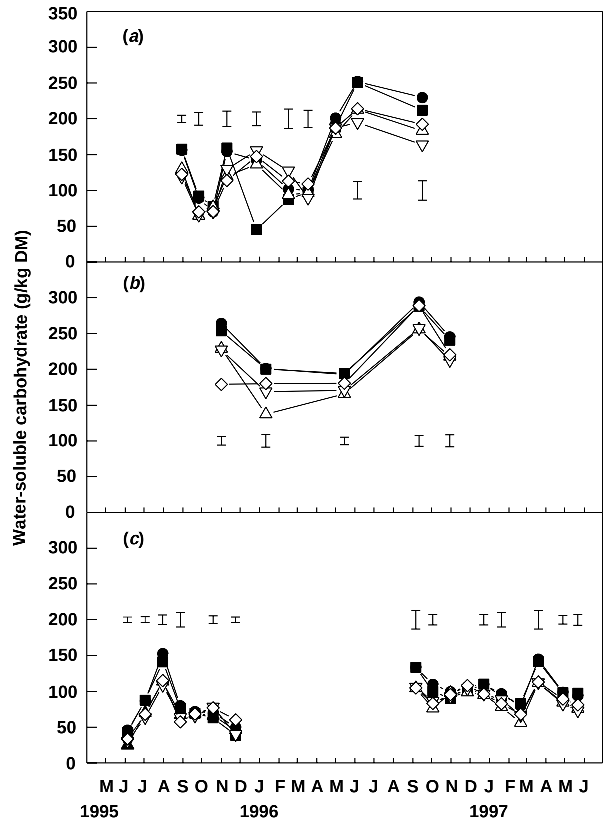

Figure 2. Seasonal variation in the water-soluble carbohydrate concentration (g/kg DM) of perennial ryegrass cultivars, Aurora (■), Ba 11351 (●), Ellett (△), Vedette (▽) and Kangaroo Valley (◇), at (*a*) Kyabram, (*b*) Condah and (*c*) Gatton. Bars represent l.s.d. (P = 0.05); where no bar is plotted differences between cultivars were not significant.

cultivar x harvest effects were modified by a factor ε (Greenhouse and Geisser 1959) to allow for the effects of serial correlation.

Results

Near infrared calibration and validation statistics

The NIR calibration and validation statistics for CP, WSC, DMD, NDF, ADF and lignin are given in Table 2. Satisfactory regressions were developed for all constituents, with standard errors similar to those reported previously for these constituents in forage (Smith and Flinn 1991; Smith *et al*. 1998*b*). Twenty additional random samples were analysed chemically to determine the simple correlations with the NIR estimates of chemical concentration. These varied between 0.83 and 0.85 for DMD, NDF and lignin, and 0.90 and 0.95 for WSC, CP and ADF. The slopes of these regression lines were between 0.9 and 1.0, and the standard errors between chemical and NIR values were sufficiently low to ensure the accuracy of the NIR estimates of composition.

Dry matter yield

Dry matter yield of all cultivars varied from site to site (Fig. 1); yields were lowest under natural rainfall at Condah and highest under irrigation at Gatton. Significant (P<0.05) differences in dry matter yield were detected in 9 of 14 harvests at Kyabram (Fig. 1*a*), all 5 harvests at Condah (Fig. 1*b*), and 5 of 7 in the 1995 Gatton sowing and 4 of 10 harvests in the 1996 Gatton sowing (Fig. 1*c*). In general the standard cultivars were higher yielding than the high WSC cultivars in all environments although there were harvests at all sites where the dry matter yield of the high WSC varieties was equal to, or better than, the yield of some of the local cultivars. All of the cultivars persisted poorly through the summer of 1996–97 at Gatton, with poor yields in the subsequent autumn, reflecting the poor persistence of perennial ryegrass in this environment.

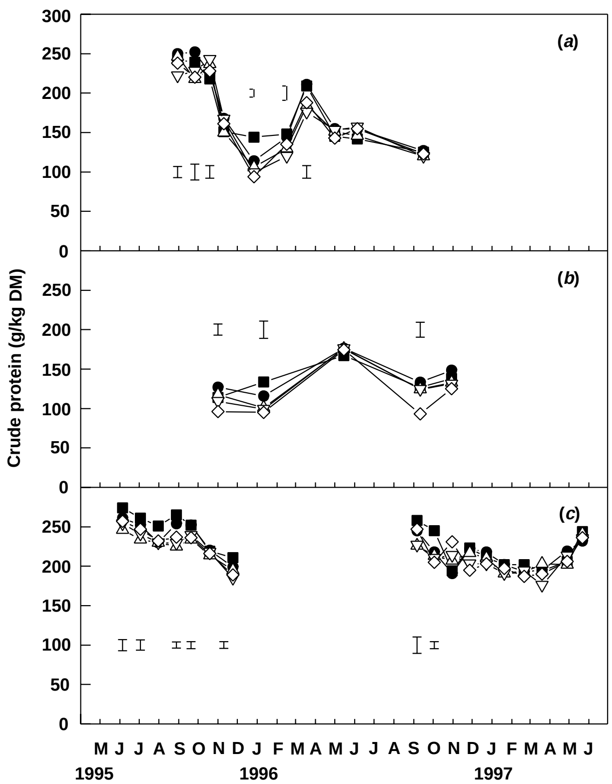

Figure 3. Seasonal variation in the crude protein concentration (g/kg DM) of perennial ryegrass cultivars, Aurora (■), Ba 11351 (●), Ellett (△), Vedette (▽) and Kangaroo Valley (◇), at (*a*) Kyabram, (*b*) Condah and (*c*) Gatton. Bars represent l.s.d. (P = 0.05); where no bar is plotted differences between cultivars were not significant.

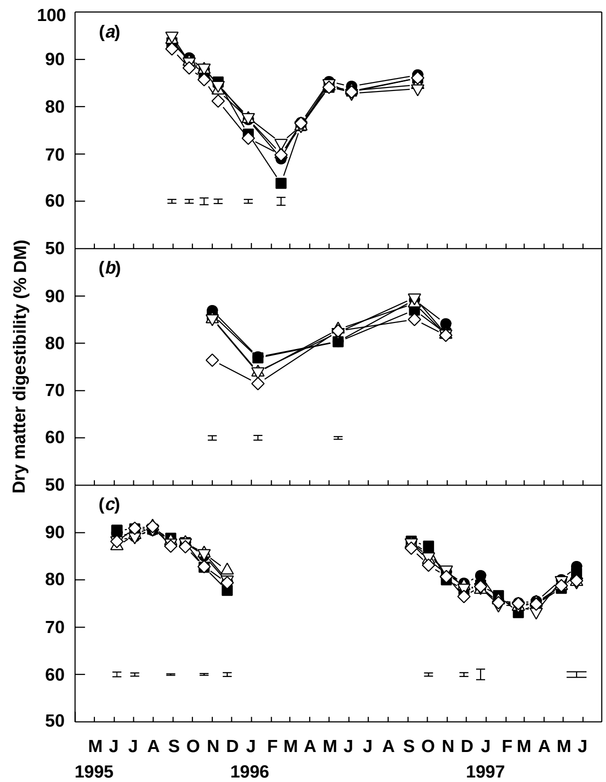

Figure 4. Seasonal variation in the dry matter digestibility (% DM) of perennial ryegrass cultivars, Aurora (■), Ba 11351 (●), Ellett (△), Vedette (▽) and Kangaroo Valley (◇), at (*a*) Kyabram, (*b*) Condah and (*c*) Gatton. Bars represent l.s.d. (P = 0.05); where no bar is plotted differences between cultivars were not significant.

Water-soluble carbohydrates

Water-soluble carbohydrate concentrations of Ba 11351 and Aurora, were often higher (P<0.05) than any of the Australasian cultivars at each harvest (Fig. 2). Thus the high WSC trait incorporated into Ba 11351 and Aurora was expressed across all seasons at these 3 contrasting Australian environments. The only exception to this trend was during the spring and summer of 1995 at Kyabram (Fig. 2*a*) and Gatton (Fig. 2*c*) where the UK varieties were heavily infected by crown rust (data not shown). The high WSC concentrations of Aurora and Ba 11351 were regained by the subsequent autumn at Kyabram (Fig. 2*a*).

Water-soluble carbohydrate concentrations at Gatton were generally lower than at the other sites, with the high WSC cultivars seldom reaching concentrations of 150 g/kg DM, while the standard cultivars never attained this WSC concentration. This is in contrast with the WSC concentrations at Condah, where all cultivars maintained WSC concentrations around, or above, 150 g/kg DM throughout the growing season.

Crude protein

Significant (P<0.05) differences between cultivars were often detected for CP concentration in each of the environments, with the exception of the 1996 sowing at Gatton where no differences were measured after the first 2 harvests (Fig. 3). When differences were detected, the high WSC cultivars usually contained higher CP concentrations than the standard cultivars.

Dry matter digestibility

The seasonal variation in DMD of the perennial ryegrass cultivars is plotted in Figure 4. At harvests where significant differences in DMD between cultivars were detected Aurora and Ba 11351 generally had higher DMD than some of the Australasian cultivars, although these effects were not as consistent as those detected for WSC.

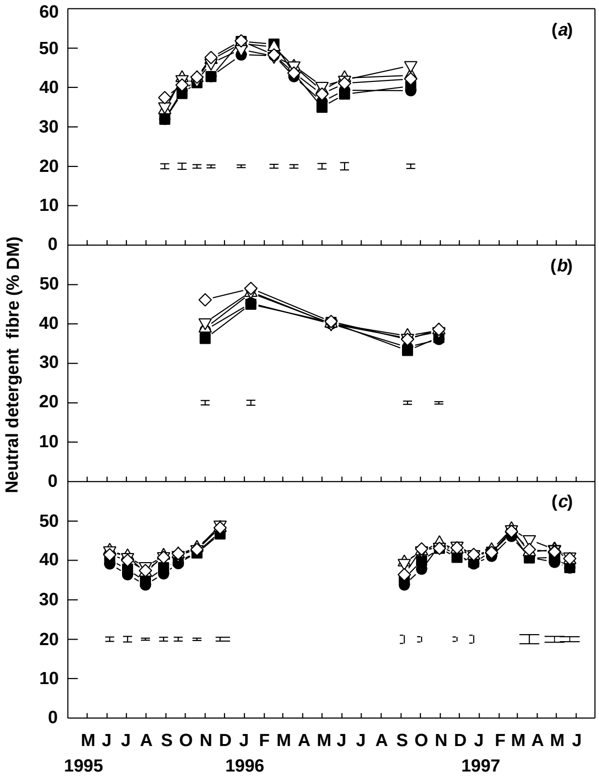

Figure 5. Seasonal variation in the neutral detergent fibre concentration (% DM) of perennial ryegrass cultivars, Aurora (■), Ba 11351 (●), Ellett (△), Vedette (▽) and Kangaroo Valley (◇), at (*a*) Kyabram, (*b*) Condah and (*c*) Gatton. Bars represent l.s.d. (P = 0.05); where no bar is plotted differences between cultivars were not significant.

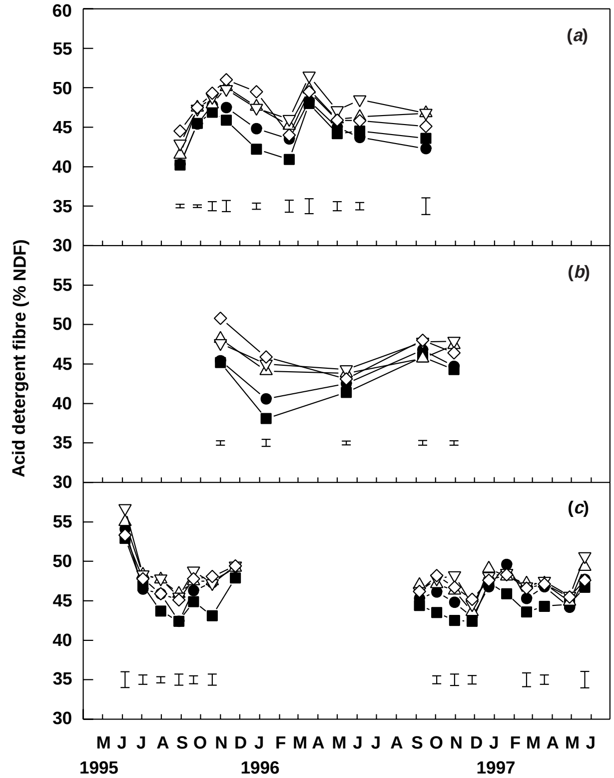

Figure 6. Seasonal variation in the acid detergent fibre concentration (% NDF) of perennial ryegrass cultivars, Aurora (■), Ba 11351 (●), Ellett (△), Vedette (▽) and Kangaroo Valley (◇), at (*a*) Kyabram, (*b*) Condah and (*c*) Gatton. Bars represent l.s.d. (P = 0.05); where no bar is plotted differences between cultivars were not significant.

Crown rust infection also reduced the DMD of Ba 11351 and Aurora at Kyabram and Gatton during the spring of 1995, and particularly in summer 1996, at Kyabram.

Neutral detergent fibre

Aurora and Ba 11351 herbage generally contained less (P<0.05) NDF than the other cultivars. This effect was consistent across environments and throughout the growing season (Fig. 5). Significant differences between cultivars were detected at almost every harvest.

Acid detergent fibre

Due to the differences in NDF detected between varieties, ADF is presented as a percentage of the NDF (cell wall) to avoid any correlation between NDF and ADF due to variation in the cell wall : cell contents ratio (Fig. 6). These data illustrate that Aurora and Ba 11351 also had altered cell wall composition compared with the Australasian cultivars, with less ADF as a percentage of the cell wall.

Lignin

The cell walls of Aurora and Ba 11351 also generally contained less (P<0.05) lignin than the cell walls of the other cultivars, although these effects were not consistent across all harvests and environments (Fig. 7).

Discussion

Dry matter yield

The yields of Aurora and Ba 11351 were generally lower than the locally adapted cultivars, reflecting the generally poor agronomic performance of European perennial ryegrass cultivars under Australian conditions. Dry matter yield and the distribution of yield for the cultivars Ellett and Vedette were similar, while the increased winter activity of Kangaroo Valley was evident in the high early spring yields of this cultivar in all environments (Fig. 1). The European cultivars Aurora and Cariad (a sister line of Ba 11351) of perennial ryegrass yielded poorly in Victoria, relative to Ellett

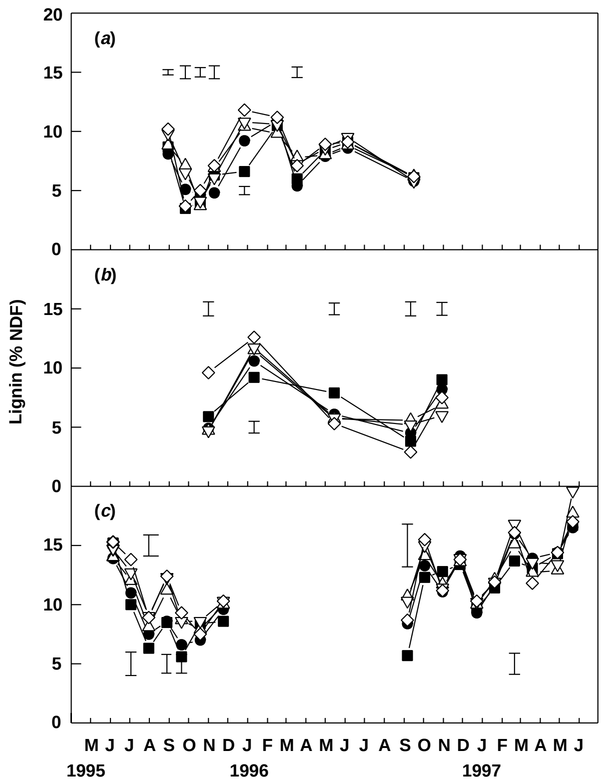

Figure 7. Seasonal variation in the lignin concentration (% NDF) of perennial ryegrass cultivars, Aurora (■), Ba 11351 (●), Ellett (△), Vedette (▽) and Kangaroo Valley (◇), at (*a*) Kyabram, (*b*) Condah and (*c*) Gatton. Bars represent l.s.d. (P = 0.05); where no bar is plotted differences between cultivars were not significant.

(Radojevic *et al*. 1994). The relatively poor adaptation of north European herbage grass cultivars and ecotypes to Australian conditions has been known for some time (e.g. Cooper 1964; Knight 1966). Cultivars from these environments tend to have poor winter production and poor summer persistence when grown in Australia or New Zealand.

Water-soluble carbohydrates

The concentration of WSC in all cultivars exhibited seasonal variation, with WSC concentrations increasing during spring at Kyabram and Condah. By contrast peak WSC concentrations were reached in either August (1995) or April (1996) at Gatton. Seasonal variation in WSC concentrations in perennial ryegrass has been detected previously (Waite and Boyd 1953; McGrath 1988; Humphreys 1989*c*; Radojevic *et al*. 1994). Peak WSC concentrations have been shown to vary from environment to environment with peaks either around floral initiation (McGrath 1988; Radojevic *et al*. 1994), head emergence (Humphreys 1989*c*) or in vegetative herbage in late summer (Radojevic *et al*. 1994). Although floral initiation and reproductive development were not monitored in this experiment, the seasonal peaks in WSC observed in this experiment are consistent with previous results (Radojevic *et al*. 1994).

The UK cultivars selected for high WSC concentrations were shown to have consistently higher WSC concentrations in all environments throughout the growing season. Such differences also have been observed when these varieties, or their relatives, have been evaluated in the UK (Humphreys 1989*a*) and Australia (Radojevic *et al*. 1994).

The only times at which Aurora and BA 11351 did not express their high WSC potential were when these varieties were heavily infected by crown rust. Crown rust infection has been shown to decrease the WSC concentration of both perennial and Italian ryegrass herbage in the UK (Potter 1987). Vedette has been categorised as being highly resistant to a range of Australian isolates of crown rust, while Ellett and Kangaroo Valley were moderately resistant (Clarke *et al*. 1997). Our results support this finding and suggest that these cultivars would provide genotypes with rust resistance for use in hybridisation with the susceptible high WSC cultivars.

Crude protein

The high WSC cultivars were shown to have CP concentrations generally as high or higher than the standard cultivars (Fig. 3), supporting the conclusions of Radojevic *et al*. (1994) that selection for high WSC concentrations has not reduced the CP content of these cultivars. Radojevic *et al*. (1994) used multiple linear regression analysis to demonstrate that negative correlations between WSC and nitrogen concentrations were mainly a result of divergent seasonal changes in WSC and nitrogen with time of year, which explained 72% of the variation of nitrogen content, whereas genotype and WSC concentration explained only 5 and 1% respectively.

The cultivar Aurora had been shown previously to combine high WSC concentrations with only a small decrease in CP when grown in the UK (Humphreys 1989*c*). It is likely that the strong negative relationship between WSC and CP which has been widely reported (Dent and Aldrich 1963; Thompson and Rogers 1971; Bugge 1978) was also influenced by environmental effects such as differences in maturity (Radojevic *et al*. 1994).

Structural carbohydrates and lignin

Aurora and Ba 11351 had less NDF and altered NDF composition compared with Ellett, Vedette and Kangaroo Valley. Neutral detergent fibre concentrations were generally 3–5% units lower for Aurora and Ba 11351 than the other varieties, suggesting that the extra WSC in these varieties was compensated by a reduction in NDF rather than any reduction in CP. Aurora and BA 11351 also appeared to have an increased proportion of hemicellulose (NDF–ADF) and decreases in both the cellulose (ADF–lignin) and lignin concentration in the cell wall (NDF).

Lignin concentrations of all cultivars were generally higher at Gatton than at the other sites probably reflecting the overall higher temperatures at Gatton. Lignin concentrations varied according to season at Kyabram and Condah, rising to the highest concentrations in summer.

Dry matter digestibility

Although differences between the DMD of cultivars were detected, with the high WSC cultivars also tending to have higher DMD, these differences were not as great, nor as consistent as those measured in previous experiments with high WSC cultivars in Australia (Radojevic *et al*. 1994) or the UK (Humphreys 1989*a*, 1989*b*).

The data from Condah are most comparable with those of Radojevic *et al*. (1994) as both experiments were conducted in southern Victoria. In both of these experiments differences in DMD were greatest during summer and early autumn while the DMD of the high WSC cultivars was not different to Ellett in winter and early spring. Radojevic *et al*. (1994) hypothesised that the difference in DMD between high and low WSC cultivars over summer was due to the the high WSC concentrations compensating for the seasonal decline in cell wall digestibility.

In this experiment DMD measurements in the summer at Gatton and Kyabram are likely to have been confounded by the severe infection of the UK cultivars by crown rust, which is known to decrease the DMD of perennial ryegrass (Potter 1987).

The relationship between cell wall composition and DMD of these high WSC genotypes under Australian conditions requires further investigation. However, further work with the high WSC trait will require the incorporation of rust resistance into the high WSC genotypes or thorough chemical control of rust infections.

Prospects for breeding perennial ryegrass cultivars with increased water-soluble carbohydrate concentrations for use in Australasia

The expression of the high WSC phenotype across a range of Australian dairy environments demonstrates that this is a trait that could be introduced into elite cultivars as part of existing perennial ryegrass breeding programs. The cultivars Aurora and Ba 11351 consistently exhibited enhanced nutritive value characteristics when grown at Condah, Kyabram and Gatton. These effects included increased WSC concentrations, decreased cell wall concentrations and reductions in the lignin content of the cell wall.

However, neither Aurora nor BA 11351 proved to be agronomically well adapted to Australian conditions; both had dry matter yields significantly lower than either Australian or New Zealand cultivars in all of the environments, and both were susceptible to crown rust and did not persist well.

Although the perennial ryegrass in these experiments was sown in monocultures, these results are applicable to perennial ryegrass–legume pasture. Water-soluble carbohydrate concentrations of perennial ryegrass cultivars were shown to be consistently lower when perennial ryegrass was grown with white clover than when the grass was grown in monoculture (Evans *et al*. 1996). Although this effect was consistent across all cultivars, the high WSC cultivar (Ba 10727) maintained a higher WSC concentration throughout the experiment.

The high WSC trait in Aurora and its relatives appears to be under relatively simple genetic control, with the consequence that significant advances could be made by crossing it to a range of useful cultivars (Humphreys 1989*a*). Thus, in the UK, Aurora was crossed with several cultivars to enable the development of high WSC cultivars with later maturity than Aurora. During these selections WSC differences were maintained over a generation of intense selection for uniformity of heading date (Humphreys 1989*b*). Therefore, it should be possible to introgress genes conferring high WSC accumulation into a range of cultivars adapted to Australian conditions.

Resistance to crown rust has been shown to be heritable in perennial ryegrass (Wilkins 1975; Hayward 1977; Reheul and Ghesquiere 1996) with both minor (Wilkins 1975) and major (Hayward 1977) resistance genes thought to be involved. Whilst little is known about the resistance genes present in Australian and New Zealand perennial ryegrass cultivars, variability in rust resistance has been identified both between and within cultivars (Clarke *et al*. 1997). Genotypes with resistance to crown rust should be hybridised with the high WSC genotypes to incorporate rust resistance with high WSC accumulation to avoid the loss of WSC associated with rust infection. The incorporation of genes conferring high WSC concentrations into adapted genotypes may also have a positive impact on the nutritive value and persistence of perennial ryegrass in a wide range of Australian environments.

Acknowledgments

The authors thank T. Bowdler, R. Slarke, D. Edwards and B. Clark for their assistance in maintaining the field sites. This research forms part of the postgraduate research by K. Smith at the University of Melbourne, and was financially supported by the Australian Dairy Research and Development Corporation.

References

Alberda, T. H. (1966). The influence of reserve substances on dry matter production after defoliation. *In* 'Proceedings of the 10th International Grassland Congress'. (Ed. A. G. G. Hill.) pp. 140–7. (Finnish Grassland Association: Helsinki.)

Amin, M. R., and Thomas, H. (1996). Growth and water relations of diverse populations of *Lolium perenne* exposed to drought in field, glasshouse and controlled environment. *Journal of Agricultural Science, Cambridge* **126,** 15–23.

APPEC (1996). APPEC; protocols and agreements (rules) for the conduct of merit testing trials for pastures. Australian Pasture Plants Evaluation Committee Inc., Department of Natural Resources and Environment, Ballarat, Victoria.

Beever, D. E. (1993). Ruminant animal production from forages: present position and future opportunities. *In* 'Proceedings of the 17th International Grassland Congress'. (Eds M. J. Baker, J. R. Crush and L. R. Humphreys.) pp. 535–42. (New Zealand Grassland Association: Palmerston North, NZ.)

Blaxter, K. L., Wainman, F. W., Dewey, P. J. S., Davidson, J., Denerley, H., and Gunn, J. (1971). The effects of nitrogenous fertilizer on the nutritive value of artificially dried grass. *Journal of Agricultural Science, Cambridge* **76,** 307–19.

Bugge, G. (1978). Genetic variability in chemical composition of Italian ryegrass ecotypes. *Zeitschrift für Pflanzenzüchtung* **81,** 235–40.

Clarke, R. G., Villalta, O. N., and Hepworth, G. (1997). Evaluation of resistance to five isolates of *Puccinia coronata* f. sp. *lolii* in 19 perennial ryegrass cultivars. *Australian Journal of Experimental Agriculture* **48,** 191–8.

Cooper, J. P. (1964). Climatic variation in forage grasses. I. Leaf development in climatic races of *Lolium* and *Dactylis*. *Journal of Applied Ecology* **1,** 45–62.

Corbett, J. L., Langlands, J. P., McDonald, I., and Pullar, J. D. (1966). Comparison by direct animal calorimetry of the net energy values of an early and a late season of growth of herbage. *Animal Production* **8,** 13–27.

Cunningham, P. J., Blumenthal, M. J., Anderson, M. W., Prakash, K. S., and Leonforte, A. (1994). Perennial ryegrass improvement in Australia. *New Zealand Journal of Agricultural Research* **37,** 295–310.

Davies, D. A., Fothergill, M., and Jones, D. (1989*a*). Frequency of stocking rate adjustments required on contrasting upland perennial ryegrass pastures continuously grazed to a sward height criterion from May to July. *Grass and Forage Science* **44,** 213–21.

Davies, D. A., Fothergill, M., and Jones, D. (1989*b*). Assessment of contrasting perennial ryegrasses, with and without white clover, under continuous sheep stocking in the uplands. 1. Animal production from the grass varieties. *Grass and Forage Science* **44,** 431–9.

Davies, D. A., Fothergill, M., and Jones, D. (1992). Assessment of contrasting perennial ryegrasses, with and without white clover, under continuous sheep stocking in the uplands. 4. Animal production in years 4–6. *Grass and Forage Science* **47,** 249–58.

Dent, J. W., and Aldrich, D. T. A. (1963). The interrelationships between heading date, yield, chemical composition and digestibility in varieties of perennial ryegrass, timothy, cocksfoot and meadow fescue. *Journal of the National Institute of Agricultural Botany* **9,** 261–81.

Dove, H., and Milne, J. A. (1994). Digesta flow and rumen microbial protein production in ewes grazing perennial ryegrass. *Australian Journal of Agricultural Research* **45,** 1229–45.

Duder, F. R. (1986). Ellett perennial ryegrass, origin, production, management and uses. Ministry of Agriculture and Fisheries, New Zealand, Aglink FPP 679, Wellington, NZ.

Evans, D. R., Humphreys, M. O., and Williams, T. A. (1996). Forage yield and quality interactions between white clover and contrasting ryegrass varieties in grazed swards. *Journal of Agricultural Science, Cambridge* **126,** 295–9.

Fulkerson, W. J. (1994). Effect of redefoliation on the regrowth and water soluble carbohydrate content of *Lolium perenne*. *Australian Journal of Agricultural Research* **45,** 1809–15.

Fulkerson, W. J., Slack, K., and Lowe, K. F. (1994). Variation in the response of *Lolium* genotypes to defoliation. *Australian Journal of Agricultural Research* **45,** 1309–17.

Goering, H. K., and Van Soest, P. J. (1970). 'Forage Fiber Analysis.' United States Department of Agriculture, Agriculture Handbook No. 379. (Agricultural Research Service: Washington.)

Greenhouse, S. W., and Geisser, S. (1959). On methods in the analysis of profile data. *Psychometrika* **24,** 95–112.

Hayward, M. D. (1977). Genetic control of resistance to (*Puccinia coronata*) in *Lolium perenne* L., and its implications in breeding. *Theoretical and Applied Genetics* **51,** 49–53.

Humphreys, M. O. (1989*a*). Water-soluble carbohydrates in perennial ryegrass breeding. I. Genetic differences among cultivars and hybrid progeny grown as spaced plants. *Grass and Forage Science* **44,** 231–6.

Humphreys, M. O. (1989*b*). Water-soluble carbohydrates in perennial ryegrass breeding. II. Cultivar and hybrid progeny performance in cut plots. *Grass and Forage Science* **44,** 237–44.

Humphreys, M. O. (1989*c*). Water-soluble carbohydrates in perennial ryegrass breeding. III. Relationships with herbage production, digestibility and crude protein content. *Grass and Forage Science* **44,** 423–30.

Jones, E. L., and Roberts, J. E. (1991). A note on the relationship between palatability and water-soluble carbohydrates content in perennial ryegrass. *Irish Journal of Agricultural Research* **30,** 163–7.

Kenward, M. G. (1987). A method for comparing profiles of repeated measurements. *Applied Statistics* **36,** 296–308.

Knight, R. (1966). The performance of hybrids between Mediterranean and northern European parents of cocksfoot (*Dactylis glomerata* L.) in a Mediterranean type environment. *Australian Journal of Agricultural Research* **17,** 105–17.

McGrath, D. (1988). Seasonal variation in the water-soluble carbohydrates of perennial and Italian ryegrass under cutting conditions. *Irish Journal of Agricultural Research* **27,** 131–9.

McLeod, M. N., and Minson, D. J. (1978). The accuracy of the pepsin–cellulase technique for estimating the dry matter digestibility *in vitro* of grasses and legumes. *Animal Feed Science and Technology* **3,** 277–87.

Michell, P. J. (1973). Relations between fibre and water soluble carbohydrate contents of pasture species and their digestibility and voluntary intake by sheep. *Australian Journal of Experimental Agriculture and Animal Husbandry* **13,** 165–70.

Munro, J. M. M., Davies, D. A., Evans, W. B., and Scurlock, R. V. (1992). Animal production evaluation of herbage varieties. 1. Comparison of Aurora with Frances, Talbot and Melle perennial ryegrasses when grown alone and with clover. *Grass and Forage Science* **47,** 259–73.

Payne, R. W. (1995). Procedure AREPMEASURES. *In* 'Genstat 5 Procedure Library Manual, Release 3[3]'. (Eds R. W. Payne and G. M. Arnold.) pp. 58–63. (Numerical Algorithms Group: Oxford, UK.)

Pell, A. N., and Schofield, P. (1993). Computerized monitoring of gas production to measure forage digestion *in vitro*. *Journal of Dairy Science* **76,** 1063–73.

Potter, L. R. (1987). Effect of crown rust on regrowth, competitive ability and nutritional quality of perennial and Italian ryegrasses. *Plant Pathology* **36,** 455–61.

Radojevic, I., Simpson, R. J., St John, J. A., and Humphreys, M. O. (1994). Chemical composition and *in vitro* digestibility of lines of *Lolium perenne* selected for high concentrations of water-soluble carbohydrate. *Australian Journal of Agricultural Research* **45,** 901–12.

Reed, K. F. M. (1978). The effect of season of growth on the feeding value of pasture: a review. *Journal of the British Grassland Society* **33,** 227–34.

Reed, K. F. M. (1996). Improving the adaptation of perennial ryegrass, tall fescue, phalaris and cocksfoot for Australia. *New Zealand Journal of Agricultural Research* **39,** 457–64.

Reed, K. F. M., Johnson, A. M., Goss, L. C., and Avery, A. L. (1995). Pasture grasses and legumes '95. Agriculture Victoria and Department of Primary Industries and Fisheries Tasmania Technical Report, May 1995, Melbourne. 98 pp.

Reheul, D., and Ghesquiere, A. (1996). Breeding perennial ryegrass with better crown rust resistance. *Plant Breeding* **115,** 465–9.

Ridout, M. S., and Payne, R. W. (1995). Procedure ANTORDER. *In* 'Genstat 5 Procedure Library Manual, Release 3[3]'. (Eds R. W. Payne and G. M. Arnold.) pp. 44–5. (Numerical Algorithms Group: Oxford, UK.)

Shenk, J. S., and Westerhaus, M. O. (1991*a*). Population structuring of near infrared spectra and modified partial least squares regression. *Crop Science* **31,** 1548–55.

Shenk, J. S., and Westerhaus, M. O. (1991*b*). Population definition, sample selection, and calibration procedures for near infrared reflectance spectroscopy. *Crop Science* **31,** 469–74.

Smith, K. F., and Flinn, P. C. (1991). Monitoring the performance of a broad based calibration for measuring the nutritive value of two independent populations of pasture using near infrared reflectance spectroscopy. *Australian Journal of Experimental Agriculture* **31,** 205–10.

Smith, K. F., Kearney, G. A., and Culvenor, R. A. (1998*a*). The use of repeated measurements analysis for the evaluation of seasonal variation in the dry matter yield and nutritive value of perennial ryegrass (*Lolium perenne* L.) cultivars. *Australian Journal of Experimental Agriculture* **38,** 145–54.

Smith, K. F., Simpson, R. J., and Armstrong, R. D. (1998*b*). Using near infrared reflectance (NIR) spectroscopy to estimate the nutritive value of senescing annual ryegrass (*Lolium rigidum*): a comparison of calibration methods. *Australian Journal of Experimental Agriculture* **38,** 45–54.

Smith, K. F., Simpson, R. J., Oram, R. N., Dove, H., Culvenor, R. A., and Humphreys, M. O. (1998*c*). Increasing the nutritive value of perennial ryegrass in dairy pastures of temperate Australia *In* 'Breeding for a Multi-functional Agriculture'. Proceedings of the 21st Eucarpia Fodder Crops and Amenity Grasses Section Meeting. (Eds B. Boller and F. J. Stadelmann.) pp. 16–19. (Swiss Federal Research Station for Agroecology and Agriculture: Zurich.)

Thomas, H. (1991). Accumulation and consumption of solutes in swards of *Lolium perenne* during drought and after rewatering. *New Phytologist* **118,** 35–48.

Thompson, A. J., and Rogers, H. H. (1971). The interrelationship of some components of forage quality. *Journal of Agricultural Science, Cambridge* **76,** 283–93.

Tyler, B. F., and Jones, E. L. (1982). Evaluation of forage genetic resources in relation to breeding objectives and varietal assessment. *In* 'The Utilisation of Genetic Resources in Fodder Crop Breeding'. Proceedings of the Eucarpia Fodder Crops Section Meeting, Aberystwyth. (Ed. M. D. Hayward.) pp. 148–62. (Institute for Grassland and Environmental Research: Aberystwyth, Wales.)

Van Soest, P. J., and Robertson, J. B. (1980). Systems of analysis for evaluating fibrous feeds. *In* 'Standardisation of Analytical Methodology for Feeds'. Proceedings of a workshop held in Ottawa, Canada, 12–14 March 1979. (Eds W. J. Pigden, C. C. Balch and M. Graham.) pp. 49–60. (International Development Research Centre: Ottawa.)

Volaire, F., and Lelièvre, F. (1997). Production, persistence, and water-soluble carbohydrate accumulation in 21 contrasting populations of *Dactylis glomerata* L. subjected to severe drought in the south of France. *Australian Journal of Agricultural Research* **48,** 933–44.

Waite, R., and Boyd, J. (1953). The water-soluble carbohydrates of grasses. I. Changes occurring during the normal life cycle. *Journal of the Science of Food and Agriculture* **4,** 197–204.

Walsh, G. L., and Birrell, H. A. (1987). Seasonal variations in the chemical composition and nutritive value of five pasture species in south-western Victoria. *Australian Journal of Experimental Agriculture* **27,** 807–16.

Wilkins, P. W. (1975). Inheritance of resistance to *Puccinia coronata* Corda and *Rhynchosporium orthosporum* Caldwell in Italian ryegrass. *Euphytica* **24,** 191–6.

Yemm, E. W., and Willis, A. J. (1954). The estimation of carbohydrates in plant extracts by anthrone. *Biochemistry Journal* **57,** 508–14.

Received 24 April 1998, accepted 16 September 1998