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Growth and carbon partitioning in perennial ryegrass (*Lolium perenne*) cultivars selected for high water-soluble carbohydrate concentrations

K. F. SMITH[1]*, R. A. CULVENOR[2], M. O. HUMPHREYS[3] AND R. J. SIMPSON[2]

[1] *Agriculture Victoria, Pastoral and Veterinary Institute, Private Bag 105, Hamilton, Victoria 3300, Australia*
[2] *CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia*
[3] *Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, SY23 3EB, UK*

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SUMMARY

Perennial ryegrass (*Lolium perenne* L.) cultivars with increased water-soluble carbohydrate (WSC) concentrations were evaluated under controlled environment conditions. The growth and carbon partitioning of these cultivars was compared with standard cultivars during vegetative growth. The high WSC cultivars had shoot growth rates that were not significantly different from the standard cultivars, confirming that the extra WSC in these cultivars was not made available through reductions in yield potential. The extra WSC stored in these cultivars coincided with lower concentrations of neutral detergent fibre in the dry matter. When the cultivars were grown in hydroponic solution the high WSC cultivars Aurora and Ba10727 were found to also have less root mass and a lower root : shoot ratio than the standard cultivars. However, this trait was not consistent across all high WSC cultivars with Cariad having the same root : shoot ratio as the standard cultivars at the end of the experiment. The reduction in the root mass of the cultivars Aurora and Ba10727 was far greater than necessary to provide the extra carbon stored as WSC in these cultivars. The implications of these results for the breeding of cultivars of perennial ryegrass with increased WSC concentrations are discussed.

INTRODUCTION

Perennial ryegrass (*Lolium perennel*) cultivars that accumulate high water-soluble carbohydrate (WSC) concentrations in their shoots have been developed in the UK (Humphreys 1989*a*, *b*, *c*). These cultivars have consistently expressed this trait in a range of UK (Davies *et al*. 1989*a*, *b*, 1991, 1992; Jones & Roberts 1991; Munro *et al*. 1992) and Australian environments (Radojevic *et al*. 1994; Smith *et al*. 1998*b*). The use of these cultivars in grazing systems in the UK has led to increased animal production (Davies *et al*. 1989*a*, *b*, 1991, 1992; Jones & Roberts 1991; Munro *et al*. 1992) and their use in Australia has been predicted to significantly increase milk production over summer (Smith *et al*. 1998*a*).

The mechanism(s) by which the high-WSC phenotype is achieved in these cultivars are unknown. However several alternatives may be postulated:

(i) Reduced growth rates.
(ii) Improved photosynthesis or respiration efficiency (i.e. reduced energy costs for maintenance or synthesis).
(iii) Altered carbon partitioning between structural and non-structural compounds.
(iv) Altered carbon partitioning between roots and shoot.

There is limited evidence from field experiments to support certain of the hypothesized mechanisms by which the high-WSC phenotype is achieved. When grown in a range of Australian environments, the total herbage yield of the high-WSC cultivars has often been lower than that of cultivars better adapted to Australian conditions (Smith *et al*. 1998*b*). However, during certain periods of the growing season, herbage production by the high-WSC cultivars was equivalent to the local cultivars and their high-WSC phenotype was expressed consistently. From this it was concluded that increased WSC concentrations were not due to lower growth rates and a consequent excess of photosynthate. The field trials showed that

* To whom all correspondence should be addressed. Email: kevin.f.smith@nre.vic.gov.au

the high-WSC cultivars had equivalent crude protein (CP) concentrations but reduced neutral detergent fibre (NDF) concentrations in their shoots. This supported the possibility that higher WSC concentrations may be a consequence of altered partitioning of carbon between structural and non-structural compounds.

There is also evidence that cv. Aurora (high WSC) had fewer roots at depth than cv. Melle (low WSC) when these cultivars were grown under drought conditions in southern France (Volaire *et al.* 1998). If so, storage of WSC in the shoots of high WSC ryegrass may be a result of less carbon partitioning to roots.

This paper describes the results of two experiments where perennial ryegrass genotypes with increased WSC concentrations were grown in controlled environments to examine their growth, chemical composition and aspects of dry matter partitioning between the structural and soluble components of their herbage.

The cultivars were also grown in hydroponic culture to allow the accurate measurement of root mass and composition, to test whether differences in root : shoot ratios exist between high- and low-WSC cultivars. Specific leaf weight was measured to determine whether other differences in allocation of carbon to plant structure had occurred.

MATERIALS AND METHODS

Germplasm

Seeds of perennial ryegrass cvv. Aurora, Cariad and Ba10727 (all high-WSC genotypes from the UK), Melle (low WSC, Belgium), and Ellett (low WSC, New Zealand) were germinated on blotting paper in Petri dishes.

Growth conditions

Experiment 1

Four seedlings of cvv. Ellett, Ba10727, Aurora or Cariad were transplanted into 13-cm diameter pots filled with coarse river sand. Seedlings of cv. Melle were not transplanted until 3 days later due to slow germination.

Twenty pots (5 harvests × 4 replicates) of each cultivar were placed in a controlled-environment growth cabinet in a randomized complete block design. Day temperature was 24 °C, night temperature 14 °C with a 16-h photoperiod. Light was provided by fluorescent tubes supplemented with incandescent bulbs. The photon flux density (400–700 nm) was 520 μmol/m²/s at pot height at the beginning of the experiment. Pots were watered daily with a complete nutrient solution (Smith 1999).

Experiment 2

Hydroponic tanks were placed in a similar controlled environment cabinet with temperatures of 24 °C/14 °C and a 16-h photoperiod. The photon flux density was 470 μmol/m²/s at plant height. Each tank (30 litres) was filled with a complete nutrient solution (Smith 1999). De-ionized water was added daily to the tanks to replace losses due to evaporation and transpiration. The nutrient solutions were renewed every 4 days. To avoid any possibility of allelopathic effects from growing varieties in common solution culture (Kraus *et al.* 1994), only one cultivar was grown in each tank. The solution in each tank was stirred and aerated continuously using diffuser stones. The tanks were arranged in the growth cabinet in a randomized complete block design with four replicates of each cultivar. The blocks were rotated around the growth cabinet when solutions were renewed to eliminate any potential impact of uneven light distribution. Seeds of each cultivar were germinated in coarse sand watered with a nutrient solution. Steps were taken to ensure that germination of seeds of all cultivars occurred at the same time. Four seedlings of a cultivar were transplanted into slits in a 100-mm diameter polystyrene disk, four disks were used per cultivar to give a total of 16 seedlings per cultivar in the experiment. Transplanting occurred 2–3 days after germination. Eight disks were inserted into holes cut in a black plastic lid of each tank.

Harvesting

Experiment 1

Four pots each of Ellett, Aurora, Cariad and Ba107272 were harvested at 19, 26, 35 and 41 days after germination. Because of the slow germination of Melle, no Melle plants were harvested at the first harvest then the harvests corresponded to 23, 30, 38 and 45 days after germination. All plants were in the vegetative growth stage during the experiment and were harvested 8 h into the light period. At each harvest the shoots were cut at the base of the plant. The roots were then washed to remove sand. The leaf blades were separated from the pseudostem by cutting through the lamina at its junction with the ligule. The roots, leaf blades and pseudostem were then frozen in liquid nitrogen and stored at −20 °C. Frozen plant material was freeze dried, weighed and ground to pass through a 1 mm screen in a Cyclotech mill.

Experiment 2

Plants were harvested at 21, 28, 35 and 42 days after germination. Two disks (8 plants) were harvested from each tank, 8 h into the light period. The plants were immediately dissected into leaf blade, pseudostem and root tissues and were then dried overnight at 100 °C and weighed (harvests 1 and 2) for estimation of dry matter yield, or were freeze dried (harvests 3 and 4) prior to being weighed for estimation of dry

matter yield and nutritive value components. All plant material from harvests 3 and 4 was ground to pass through a 1-mm sieve in a Cyclotech mill.

At the second harvest, the youngest fully emerged leaf blade was dissected from each plant, placed on white paper and photocopied before being dried. The photocopied images were then scanned into a computer and the leaf areas of the plants were calculated using image analysis software (ImageQuant TM). The leaf areas and dry weights were used to calculate the specific leaf weight of the youngest fully emerged leaves of each of the perennial ryegrass cultivars.

Chemical analyses

WSC were extracted for 60 min in 80% ethanol (80 °C) and twice in water (100 °C) at a dry tissue to volume ratio of 100 mg to 8 ml. WSC concentrations were then measured using the anthrone method (Yemm & Willis 1954). Total nitrogen (N) was determined using the Kjeldahl method, and crude protein (CP) estimated as $N \times 6{\cdot}25$.

Starch was extracted from the residue pellet remaining after WSC extraction (Expt 1, harvest 3) and was determined using the 'Megazyme' amyloglucosidase/α-amylase assay kit (McCleary *et al.* 1994). NDF was determined by digesting a sample of herbage in neutral detergent solution (Goering & Van Soest 1970) in an autoclave at 105 °C for 1 h (Pell & Schofield 1993). The NDF was then further digested in acid detergent solution (Goering & Van Soest 1970) with the residue weighed to give ADF, or in sulphuric acid according to the Klason lignin procedure. ADF and Klason lignin were expressed as a percentage of the NDF. The (organic matter) concentration of the samples was measured by weighing the inorganic residue present after ashing samples at 550 °C for 3 h.

Statistical analysis

Data from both experiments were analysed using analysis of variance procedures with the statistical program Genstat 5.3 for Windows.

RESULTS

Experiment 1

Shoot growth

Rates of OM increase in leaf blade (Fig. 1*a*) and pseudostem (Fig. 1*b*) for the cultivars, Aurora, Cariad, Ellett and Ba10727 were not significantly different ($P > 0{\cdot}05$) during the experiment. Although data from cv. Melle was excluded from the analysis of

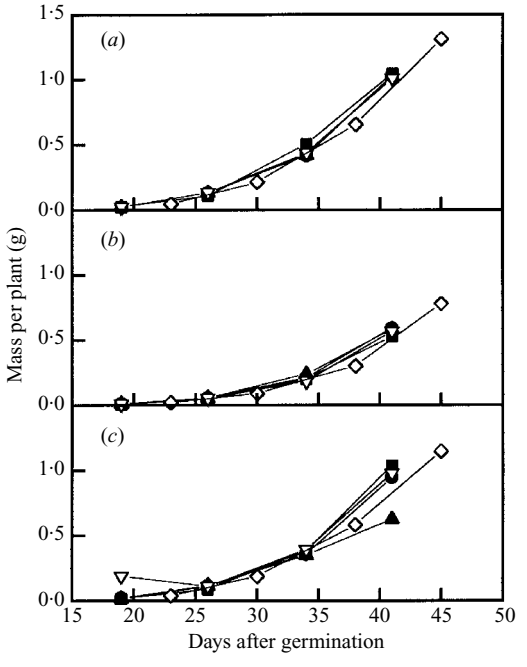

Fig. 1. Biomass accumulation (g OM/plant) in perennial ryegrass cultivars grown in sand culture: (*a*) leaf blades; (*b*) pseudostem; (*c*) roots. Aurora (●), Cariad (■), Ba10727 (▲), Ellett (▽) and Melle (◇). Differences between cultivars at individual harvests were not significant ($P > 0{\cdot}05$).

Table 1. *Composition of the neutral detergent soluble component of the organic matter, 35 days after germination, in contrasting perennial ryegrass cultivars grown in sand culture* (*g/kg OM*)

Cultivar	WSC	Starch	CP	Others
Leaf blade				
Aurora	128	34	356	160
Cariad	123	40	353	157
Ba10727	127	32	353	154
Ellett	108	34	329	224
S.E. (D.F. = 9)	3·7	2·2	10·4	8·8
Pseudostem				
Aurora	329	6	236	0
Cariad	265	5	225	37
Ba10727	278	6	221	0
Ellett	201	5	264	17
S.E. (D.F. = 9)	6·9	0·3	8·6	5·7
Shoot				
Aurora	184	31	323	106
Cariad	157	29	311	104
Ba10727	172	23	307	107
Ellett	135	26	310	154
S.E. (D.F. = 9)	2·5	2·0	8·9	10·4
Root				
Aurora	53	2	163	39
Cariad	29	2	191	200
Ba10727	62	2	170	90
Ellett	29	2	199	91
S.E. (D.F. = 9)	4·6	0·2	20·3	9·4

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Table 2. *Composition of the neutral detergent fibre component of the organic matter, 35 days after germination, in contrasting perennial ryegrass cultivars grown in sand culture (g/kg OM)*

Cultivar	NDF	ADF	ADF (% NDF)	Lignin	Cell wall protein
Leaf blade					
Aurora	320	164	51·1	32	65
Cariad	327	156	47·8	30	61
Ba10727	324	147	45·4	31	59
Ellett	345	158	45·8	29	62
S.E. (D.F. = 9)	31·4	10·3	1·2	2·1	3·2
Pseudostem					
Aurora	431	183	42·6	69	47
Cariad	468	185	39·5	57	24
Ba10727	485	190	39·3	65	30
Ellett	514	198	38·5	60	47
S.E. (D.F. = 9)	10·7	5·7	1·1	3·2	2·5
Shoot					
Aurora	336	168	49·9	42	61
Cariad	339	169	47·8	37	56
Ba10727	343	158	46·0	53	53
Ellett	376	155	41·1	49	56
S.E. (D.F. = 9)	8·3	5·6	0·8	3·1	3·0
Roots					
Aurora	743	Not measured	Not measured	Not measured	Not measured
Cariad	584				
Ba10727	676				
Ellett	680				
S.E. (D.F. = 9)	24·9				

variance due to the later germination of that cultivar, the graphs (Fig. 1*a*, *b*) demonstrate that the shoot growth rate of Melle was similar to those of the other cultivars. Leaf blades comprised approximately 60% of the shoot OM of all cultivars, at the end of the experiment (42 days after germination).

No significant differences were detected ($P > 0{\cdot}05$) in the root mass (OM) of the cultivars. However, problems with sand contaminating the root samples led to large variations in their ash content (data not shown) which could have obscured differences in the root mass of the cultivars. For instance, there appeared to be a large difference between the root mass of Ba10727 and the cultivars Aurora, Cariad and Ellett at 42 days after germination (Fig. 1*c*) although this difference was not statistically significant.

Chemical composition

Water-soluble carbohydrate concentrations in the leaf blades of Aurora, Cariad and Ba10727 were 20% greater than Ellett at 35 days after germination (Table 1). The WSC accumulation of Melle followed a similar pattern to that of Ellett. Concentrations of starch or CP in the leaf blades were not significantly different between cultivars ($P > 0{\cdot}05$). The sum of WSC, CP and starch concentrations explained a larger proportion of the neutral detergent soluble (NDS) component (100-NDF) of the leaf blades of the high-WSC phenotypes than in Ellett. This unexplained component of the NDS is likely to have consisted of a mixture of lipids, organic acids, pectins and other carbohydrate compounds (Van Soest 1982).

A similar situation to that measured in the leaf blades was observed in the pseudostem (Table 1), with Aurora, Cariad and Ba10727 having higher ($P < 0{\cdot}05$) WSC concentrations than Ellett. However, WSC concentrations were higher in the pseudostem than the leaf blade in all cultivars and there were differences in the WSC concentration of the pseudostems of the high-WSC cultivars. The WSC concentration in the pseudostems of Aurora were significantly higher ($P < 0{\cdot}05$) than those in Cariad or Ba10727. There were also differences ($P < 0{\cdot}05$) measured in the CP concentrations of the pseudostems of the cultivars, with Ellett having a 30–40 g/kg higher CP concentration in the pseudostem than any of the other cultivars.

No differences ($P > 0{\cdot}05$) wre observed in the concentration of NDS components in the roots of the perennial ryegrass cultivars.

No differences ($P > 0{\cdot}05$) were measured in the concentration of NDF, or of the NDF components measured in the leaf blades of any of the cultivars (Table 2). However, total NDF concentrations were greater ($P < 0{\cdot}05$) in the pseudostem and consequently

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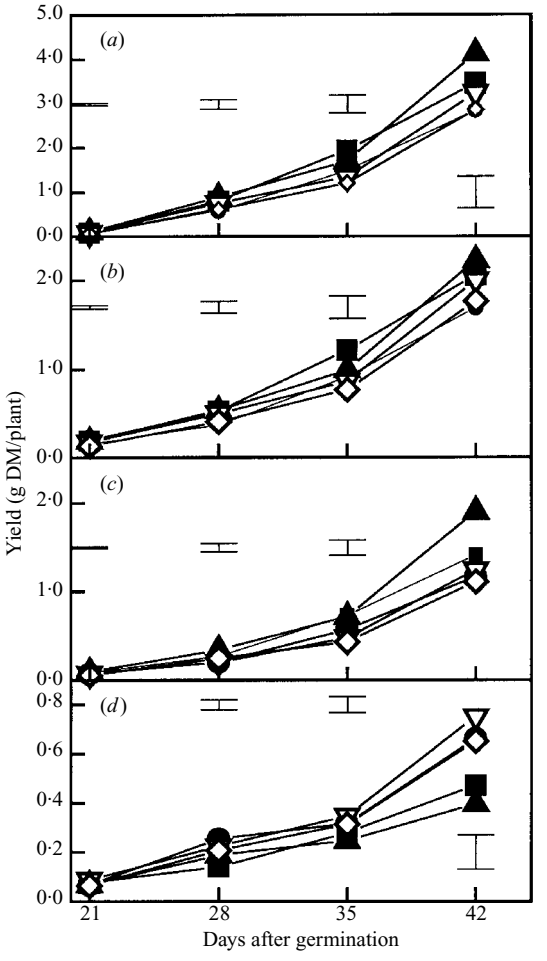

Fig. 2. Dry matter yields of (*a*) shoots; (*b*) leaf blades; (*c*) pseudostem and (*d*) roots of perennial ryegrass cultivars grown in hydroponic culture: Aurora (■), Cariad (●), Ba10727 (▲), Ellett (▽) and Melle (◇) (Bars represent least significant difference, $P < 0{\cdot}05$).

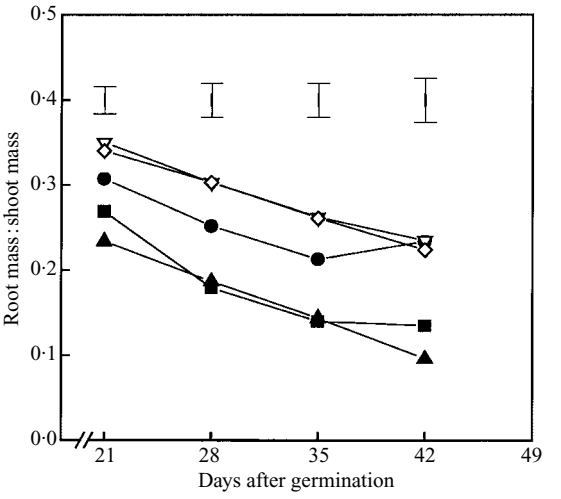

Fig. 3. Root : shoot ratio of perennial ryegrass cultivars grown in hydroponic culture: Aurora (■), Cariad (●), Ba10727 (▲), Ellett (▽) and Melle (◇) (Bars represent least significant difference, $P < 0{\cdot}05$).

the whole shoot of cv. Ellett than for any of the other cultivars. There were no differences ($P > 0{\cdot}05$) in the ADF, lignin or cell-wall protein concentrations of the pseudostems or shoots of the cultivars, despite the difference in total NDF concentration. However, the NDF composition of the perennial ryegrass genotypes was altered, such that the high-WSC genotypes had an increase in the ADF concentration of the NDF fraction.

Experiment 2

Shoot

At 42 days after germination Ba10727 had accumulated more shoot dry matter (DM) ($P < 0{\cdot}05$) than either Melle or Cariad (Fig. 2*a*). No other differences were observed in the final shoot dry matter of the perennial ryegrass cultivars. The majority of the differences in shoot dry matter between Ba10727 and Melle or Cariad were associated with pseudostem mass, although no significant ($P > 0{\cdot}05$) differences between cultivars were detected when the final leaf blade (Fig. 2*b*) or pseudostem (Fig. 2*c*) mass of the cultivars were analysed separately.

Root

Root dry matter accumulation was significantly greater ($P < 0{\cdot}05$) in the cvv. Melle, Ellett and Cariad than either Ba10727 or Aurora (Fig. 2*d*). For example, at 42 days after germination, Ellett plants had a mean root mass of approximately 0·75 g DM whereas the mean root mass of Ba10727 was only 0·4 g DM. Consistent differences in the root : shoot mass ratio of the perennial ryegrass plants were observed (Fig. 3). The cvv. Aurora and Ba10727 had significantly lower root : shoot ratios than either Ellett or Melle throughout the experiment. By the final harvest the root : shoot ratio of Ba10727 and Aurora was 0·10–0·14, whereas the root : shoot ratio of Ellett and Melle was 0·25. The root : shoot ratio of Cariad was intermediate between the two groups of cultivars at the early harvests but was not significantly lower than Ellett or Melle ($P > 0{\cdot}05$), and was equal to Melle and Ellett by the end of the experiment.

Leaf area and specific leaf weight (SLW)

At 28 days after germination the youngest fully emerged leaves of the perennial ryegrass plants weighed 28·5 mg on average, had a surface area of 414 mm² and hence a SLW of 0·07 mg/mm². There

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Table 3. *Water-soluble carbohydrate (WSC), crude protein (CP) and neutral detergent fibre (NDF) concentrations (g/kg DM), 35 and 42 days after germination, in perennial ryegrass cultivars grown in hydroponic culture*

	WSC		CP		NDF	
Cultivar	35 d	42 d	35 d	42 d	35 d	42 d
Leaf						
Aurora	135	161	340	320	300	279
Cariad	122	120	343	335	300	271
Ba10727	145	206	324	290	289	224
Ellett	94	102	334	333	311	291
Melle	99	99	348	336	309	292
S.E. (D.F. = 9)	10·7	11·2	10·4	11·5	15·7	12·2
Pseudostem						
Aurora	250	312	236	207	331	329
Cariad	247	254	242	217	340	332
Ba10727	249	315	230	217	331	283
Ellett	193	195	244	225	356	341
Melle	178	170	250	229	351	347
S.E. (D.F. = 9)	7·6	13·9	8·5	9·5	9·6	10·3
Root						
Aurora	80	74	207	190	628	581
Cariad	58	59	221	212	601	534
Ba10727	66	81	222	210	629	538
Ellett	48	47	204	216	642	503
Melle	56	51	199	216	602	534
S.E. (D.F. = 9)	3·3	6·7	7·6	12·3	21·6	13·4

were no significant differences ($P > 0{\cdot}05$) between cultivars for leaf mass, leaf area or SLW (data not presented).

Water-soluble carbohydrates

Water-soluble carbohydrate concentrations were highest in the pseudostem (mean 223 g/kg DM, 35 days; 249 g/kg DM, 42 days) and lowest in the roots (62 g/kg DM at both harvests), with WSC concentrations in the leaf blades intermediate (119 g/kg DM, 35 days; 137 g/kg DM, 42 days). However, significant differences ($P < 0{\cdot}05$) were observed between cultivars for the mean WSC concentration of leaf blades, pseudostems and roots (Table 3). Concentrations of WSC in all tissues of Aurora and Ba10727 were consistently higher than those of Ellett and Melle, the differences being greatest in the leaf blades and pseudostems at 42 days. WSC concentration in the pseudostems of Cariad was higher than concentrations in either Ellett or Melle pseudostems at both harvests, but unlike Aurora and Ba10727, the WSC concentrations in the leaf blades and roots of Cariad were intermediate between the highest- and lowest-WSC cultivars and not consistently different ($P = 0{\cdot}05$) to either grouping (Table 3).

Amount of WSC in plant tissues

The mass of WSC in the pseudostem of the high-WSC cultivars Aurora and Ba10727 was greater than the mass of WSC in their leaf blades. By contrast, approximately equal amounts of WSC were present in the leaf and pseudostem in each of Melle and Ellett. The total amounts of WSC accumulated in roots were: 34·8, 31·8, 38·8, 33·0 and 35·4 mg/plant in Aurora, Ba10727, Cariad, Melle and Ellett, respectively. Increases in plant size between 35 days and 42 days led to large increases in the amount of WSC per plant because WSC concentrations either increased or remained constant over the same period.

Crude protein

Crude protein concentrations were highest in the leaf blades (mean 330 g/kg DM) and lowest in the roots (209 g/kg DM) (Table 3), with pseudostems having similar protein concentrations as the roots (230 g/kg). Significant differences ($P < 0{\cdot}05$) between cultivars were only detected in the leaf blades at the final harvest, when CP concentrations were lower in Ba10727 than in Ellett, Melle and Cariad. No significant differences ($P > 0{\cdot}05$) between cultivars were detected for pseudostem and root CP concentrations at either harvest.

Neutral detergent fibre

Neutral detergent fibre concentrations were highest in the roots (mean 620 g/kg, 35 days; 538 g/kg, 42 days) with lower concentrations of NDF in the pseudostem (335 g/kg averaged over both harvests) and leaf

blades (287 g/kg averaged over both harvests) (Table 3). No significant differences in NDF concentration were detected between cultivars at 35 days, whereas cultivar effects were significant ($P < 0{\cdot}05$) at the final harvest. Ba10727 had significantly ($P < 0{\cdot}05$) lower NDF concentrations in the leaf blades and pseudostems than either Ellett or Melle.

DISCUSSION

The present experiments have provided further insight into how high WSC concentrations are achieved in these perennial ryegrass cultivars selected for this trait. It was hypothesized that there were four potential mechanisms by which higher WSC concentrations could be maintained.

Reduced growth rates: It is possible to alter the partitioning of C between structural and non-structural fractions by providing conditions that enable photosynthesis but are less conducive for growth. For instance, perennial ryegrass swards grown with long days (20 h) and low temperatures (5 °C) contained WSC concentrations of 235 mg/g, whereas those grown with 4-h photoperiods at 25 °C contained only 25 mg/g WSC (Robson 1981). During both experiments reported in this paper, the high-WSC cultivars had herbage growth rates equal to or better than the standard ryegrass cultivars. However, WSC concentrations in the shoot tissues were higher in the cvv. Aurora, Cariad and Ba10727. This result confirmed conclusions from earlier field trials (Smith *et al*. 1998*b*) that it was possible for these high-WSC genotypes to accumulate higher WSC concentrations while growing at equivalent rates.

Improved respiratory efficiency or more photosynthesis: Despite the apparent attractiveness of this hypothesis, photosynthetic rates and respiration losses were not measured in the present experiment because it can be calculated that the magnitude of changes in loss or gain of carbon need only be small to achieve the differences in WSC concentration that were observed between the high- and low-WSC cultivars of perennial ryegrass. For instance, when perennial ryegrass cv. Melle was fed with radioactive carbon, only 4% of the carbon was stored as WSC with the majority being used for respiration (40%) or growth (56%; Danckwerts & Gordon 1987). Only 5% of the carbon lost in respiration would need to be diverted to WSC storage to achieve a 50% increase in the WSC concentration of shoot DM, assuming that growth rates were unaffected. Clearly, if differences in respiration rate exist between the high- and low-WSC ryegrass cultivars, they would be difficult, or impossible, to detect with any certainty.

Other experiments provide indirect evidence that altered respiratory efficiency is probably not involved in the accumulation of high WSC concentrations in ryegrass. Dark respiration is often envisaged as having two components: one is conceptually associated with growth and the other with maintenance (McCree 1970). As the growth component is thought to operate at maximum efficiency (Penning de Vries 1974) it is likely that any change in respiration that leads to increased WSC accumulation would be due to a reduction in maintenance respiration. Divergent selection of perennial ryegrass for slow and fast mature-leaf (maintenance) respiration has been used to develop populations with significantly different respiration rates (Wilson 1982). This improved the growth rate of the slow-respiring line relative to the fast-respiring line (Robson 1982*a*, *b*; Wilson 1982), but did not alter WSC concentrations (Kraus *et al*. 1993).

Altered carbon partitioning between compounds within the shoot: Extra carbon may potentially be diverted for storage as WSC if synthesis of other compounds is reduced and particularly if there is less synthesis of complex compounds (e.g. lignins etc.), which require more energy to synthesize (Penning de Vries *et al*. 1974). The faster growing, slow-respiration selection of perennial ryegrass (Wilson & Jones 1982) provides an example of altered chemistry in this species. The slow-respiration line accumulated higher concentrations of cellulose and less hemicellulose than the slower growing, fast-respiration line. However, in this case the balance between the soluble and structural carbohydrate concentrations of dry matter was not altered.

It appeared likely from field experiments, that WSC was accumulated by the high-WSC cultivars at the expense of NDF (Smith *et al*. 1998*b*). The present experiments also support the notion that higher WSC concentrations were accumulated at the expense of other components of the dry matter. In Experiment 1, WSC accumulation also appeared to coincide with less NDF. For example, Aurora stored 50 g/kg OM more than Ellett in its pseudostems (Table 1). The amount of ADF accumulated by Aurora and Ellett did not differ (Table 2). However, the NDF concentration of Aurora was 40 g/kg OM lower than Ellett, due to there being less hemicellulose (NDF-ADF) in the cell walls of the pseudostems. Given that 1 g less hemicellulose synthesis would release enough glucose to produce 1·2 g of fructan (Penning de Vries *et al*. 1974), the differences in dry matter composition (50 g more WSC/kg OM and 40 g less hemicellulose/kg OM; Tables 1 and 2) were energetically equivalent and would have no impact on the potential growth rate of the cultivars.

However, in Experiment 2, higher WSC concentrations coincided with either less NDF (e.g. Ba10727; Table 3) or less of mainly the 'other' component of the NDS (e.g. Aurora and Cariad). This can be deduced from the data in Table 3, but is illustrated definitively by considering the differences in the concentrations of components when expressed per

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Table 4. *Percentage composition of the organic matter of the pseudostem of perennial ryegrass cultivars grown in hydroponic culture* (*standard error of the mean* (*S.E.M.*) *for each cultivar is in parentheses*)

	NDS			
Cultivar	WSC	CP	Other*	NDF
Aurora	36·9 (0·1)	24·4 (0·01)	−0·2	38·9 (0·5)
Cariad	30·0 (0·1)	25·6 (0·02)	5·3	39·1 (0·5)
Ba10727	37·2 (0·2)	25·6 (0·03)	3·8	33·4 (0·6)
Ellett	23·0 (0·3)	26·6 (0·02)	9·9	40·2 (1·0)
Melle	20·0 (0·2)	27·0 (0·04)	12·0	41·0 (1·2)

* Other, 100 − (CP + WSC + NDF); NDS, neutral detergent solubles; NDF, neutral detergent fibre; WSC, water-soluble carbohydrate; CP, crude protein.

unit of OM (e.g. Table 4). The 'other' component of NDS, is material soluble in neutral detergent, but not accounted for by crude protein, or the carbohydrates that may be extracted in ethanol and water. It may include starch, pectins, lipids and some other carbohydrate compounds (Van Soest 1982).

Altered carbon partitioning as a result of changed morphology: Partitioning of dry matter between roots and shoots of the cultivars was examined to ascertain whether partitioning of extra carbon to WSC storage might be a consequence of reduced root growth. Only small changes in carbon partitioning would be necessary to gain the enhanced WSC concentrations in high-WSC shoots (Smith *et al.* 2001). In Experiment 1, the WSC concentrations of roots did not differ significantly between the cultivars and there was no evidence of any significant differences in root dry matter. However, root weights were very variable due partly to sand contamination (e.g. as indicated by their high ash contents) and presumably to damage caused by washing to remove the contaminating sand particles. Hydroponic culture was therefore used to ensure a more accurate sampling of roots for determination of WSC concentrations and root dry matter.

The WSC concentrations of roots of plants grown in hydroponics were similar to the 50–100 g/kg DM measured in field experiments in Ireland (McGrath 1988) and were generally higher and less variable than the concentrations recorded for roots grown in sand culture (Table 1). Higher concentrations of WSC were measured in Ba10727 and Aurora, but the WSC concentration of roots of Cariad, which consistently expressed high pseudostem and total shoot WSC concentrations, was intermediate between the highest- and lowest-WSC cultivars and not consistently different ($P = 0{\cdot}05$) from either grouping. Neither the root WSC concentrations, nor the total amounts of WSC accumulated in roots, gave much credence to the possibility that high shoot WSC concentrations might result from altered accumulation of WSC in the roots. Although two of the high-WSC cultivars, Aurora and Ba10727, accumulated the lowest amounts of WSC in their roots, Cariad, the remaining high-WSC cultivar, accumulated the largest amount of root WSC.

However, root growth by Aurora and Ba10727 was significantly less than that of Melle and Ellett and resulted in consistently lower root : shoot ratios for these high-WSC cultivars (Figs 2*d* and 3). This result could be indicating that WSC accumulation in shoots might occur at the expense of root growth. However, these differences in root mass between Aurora and Ba10727, and the low-WSC cultivars were far in excess of what would be needed to account for extra WSC accumulated in their shoots. Cariad again indicated that altered carbon partitioning between roots and shoots was not necessary for expression of the high shoot-WSC phenotype. Root mass of Cariad was equivalent to that of the low-WSC cultivars, and by 42 days after germination, its root : shoot ratio was also equivalent.

Changes in specific leaf area of grasses have been hypothesized to depend on the relative amount of carbon storage that is available for growth (Johnson & Thornley 1983). When the diploid perennial ryegrass cultivar Wendy and the tetraploid Condesa were grown in hydroponic solution at two water potentials (van Loo 1992), increases in WSC concentration were associated with decreased specific leaf area (the inverse of SLW). However, there were no differences in the SLW, leaf area or leaf mass between the cultivars in the present experiment, indicating that differences in WSC concentrations did not result from altered leaf morphology.

Implications of these results for breeding high-WSC cultivars

On balance, we propose that the results of the present and previous experiments (Smith *et al.* 1998*b*) favour the hypothesis that high-WSC concentrations are associated with lower NDF concentrations in the shoot. However, in some cultivars, carbon may be available for storage in the shoot because root growth is poorer. The implications of these results for the likely field performance of Aurora and Ba10727 and cultivars related to them, therefore, remain equivocal.

High WSC concentrations may confer a number of advantages to a perennial ryegrass cultivar. Nutritive value is likely to be higher (Radojevic *et al.* 1994; Smith *et al.* 1998*b*) and they are likely to be preferred by grazing animals (Ciavarella *et al.* 2000). This would contribute to improved animal production, but might also lead to selective grazing. Accumulation of

WSC in the form of high molecular weight fructans in tiller bases has been positively correlated with drought survival, and regrowth after drought in both cocksfoot and perennial ryegrass in Mediterranean environments (Volaire & Lelièvre 1997; Volaire *et al*. 1998). However, because the high-WSC genotype may be linked to reduced root growth in some cultivars, they may be less competitive and less persistent. Reductions in root : shoot ratio have been associated with reduced competitive ability of perennial ryegrass genotypes (Hofman & Ennik 1980; Ennik & Hofman 1983) and decreased root mass is associated with reduced persistence during drought (Arcioni *et al*. 1980). Volaire *et al*. (1998) reported that Aurora had fewer roots than Melle at soil depths of 70–110 cm in a Mediterranean environment. Nevertheless, they also found that tiller death in the two cultivars during an 80-day drought, was equivalent.

We are concerned about the possibility of a link between expression of high shoot-WSC concentrations and a smaller root system. For instance, ranking of grain sorghum genotypes on the basis of their root : shoot ratios in hydroponics is claimed to be indicative of drought tolerance in the field (Sullivan & Ross 1979). However, our observations indicate that root mass may vary between the present high-WSC cultivars. Whilst Aurora and Ba10727 grew small root systems in hydroponic culture, Cariad, a high-WSC cultivar derived from the progeny of a cross between Aurora and Melle, expressed both a high shoot-WSC concentration and a large root mass. It remains possible that low root mass in Aurora and Ba10727 is a characteristic of these particular cultivars and is unrelated to their expression of high shoot-WSC, or that the low root mass was an adaptation to hydroponics culture because the cultivars did not appear to have less root mass when grown in sand.

We conclude that further investigations of root growth by high-WSC cultivars under field conditions are desirable to determine whether poor root growth and high shoot-WSC concentrations are genetically linked traits. We know that selection for a larger root system in ryegrass can be successful in perennial ryegrass. Veronesi (1990) has demonstrated that a 25% increase in root length, 40% increase in root volume and 67% increase in root mass was possible after only two cycles of recurrent restricted phenotypic selection for increased root mass within the perennial ryegrass cultivar, Vejo, and similar results have been obtained with annual ryegrass (Bullitta 1996). At this stage, our results indicate that selection for high-WSC concentrations in perennial ryegrass should be accompanied by evaluation of root production and, if necessary, concurrent selection for adequate or improved root development. In particular, Cariad indicates that high-WSC cultivars can be developed without adverse consequences for root growth.

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