RESEARCH

Seasonal Trends in Nonstructural Carbohydrates in Cool- and Warm-season Grasses

Kevin B. Jensen,* Phil Harrison, N. Jerry Chatterton, B. Shaun Bushman, and J. Earl Creech

ABSTRACT

While increases in nonstructural carbohydrate (NSC) content of forages are generally considered advantageous, there are times during the growing season when grasses with increased NSC levels may not be the desired forage, particularly for horses (*Equus caballus)*. Hence, there is a need to better understand carbohydrate trends across the growing season. This study evaluated 15 grass species from May to August (growth phase-1) and from September to November (growth phase-2) in northern Utah during 2004 and 2005 for sugars, fructans, water-soluble carbohydrates (WSC), starch, and total nonstructural carbohydrates (TNC). Sampling date and species had a significant effect on carbohydrate concentrations in cool-season grasses, with a lesser effect on warm-season grasses. Warm-season grasses had uniformly lesser sugar, fructan, WSC, and TNC concentrations than cool-season grasses; however, they had dry-matter yields (DMY) greater than cool-season grasses in August. If high TNC forage is desired, then, of the grasses examined, perennial ryegrass and timothy would be the species of choice for irrigated pastures. However, if forage lower in TNC is desired, then meadow bromegrass, which had the least overall sugar, fructan, WSC, starch, and TNC concentrations, would be best. On dryland pastures, Sandberg bluegrass (*Poa secunda* J. Presl.) and tall wheatgrass (*Thinopyrum ponticum* (Podp.) Z.-W. Liu and R.-C. Wang) had the least overall forage carbohydrates, while crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.) maintained its carbohydrate concentrations better on 3 August. Fructan concentrations contributed 54, 47, and 42% of the total WSC concentrations in perennial ryegrass, crested wheatgrass, and Kentucky bluegrass, respectively, compared to 26 and 29% in tall wheatgrass and creeping meadow foxtail, respectively. Water-soluble carbohydrates accounted for between 86 and 91% of the total TNC concentration in the cool-season grasses compared to 75 to 80% in the warm-season grasses.

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Abbreviations: Dry matter, DM; dry matter yield, DMY; nonstructural carbohydrates, NSC; total nonstructural carbohydrates, TNC, water-soluble carbohydrates, WSC.

Temperate cool-season forage grasses make a major contribution to the profitability of agriculture and have been the subject of plant-improvement programs for many years (Miller et al., 2001). In an attempt to increase forage intake by animals, some grass breeders have recently selected in grasses for their ability to accumulate carbohydrates, which include soluble sugars (referred to hereafter as sugars, including glucose, fructose, and sucrose), fructans, and starch, which are collectively referred to as total non-structural carbohydrates (TNC) (Humphreys et al., 2006). Water-soluble carbohydrates (WSC) are defined as the sum of water-soluble sugars, including glucose, fructose, sucrose, and fructans. The role of TNC in grasses includes intermediary metabolism, energy transport, and energy storage (Moore and Hatfield, 1994). Greater TNC concentrations lead to increased animal preference (Mayland et al., 2000), intake (Burns et al. (2007), and increased animal gains (Gregorini et al., 2006). While increases in TNC content of forages are generally considered advantageous, there are times during the growing season when increased TNC levels, particularly fructans, have been associated with the increased incidence of equine laminitis (Pollitt et

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al., 2003; Van Eps and Pollitt, 2006). Forage nutritional quality is defined here in relation to the supply of readily available carbohydrate encompassed by TNC, thus potentially providing more energy to the animal. Cool-season grasses grown under cool temperatures accumulate sugars and fructans, whereas warm-season grasses accumulate sugars and starch with low fructan concentrations. Chatterton et al. (1989) concluded that these supposed fructan derivatives in warm-season grasses are either of the raffinose series or other non-fructan sucrososyloligosaccharides rather than actual fructans.

In cool-season grasses, fructans are a major component of TNC (Miller et al., 2001). Fructans are branched or linear poly-fructose molecules synthesized *de novo* from sucrose that act as storage polymers of carbon, performing similar functions as starch in temperate grasses and other plants (Gallagher et al., 2007). Fructans in grasses are stored mainly in the cell vacuoles (Wagner et al., 1983), and have been reported in roots, rhizomes, stem bases, and lower leaves (Moore and Hatfield, 1994). In contrast, starch accumulates in amyloplasts of cells in these storage organs in warm-season grasses (Moore and Hatfield, 1994). Storage carbohydrate levels in plants are in a constant state of flux due to accumulation from photosynthetic activity and utilization for growth and development (Bowden et al., 1968; Holt and Hilst 1969). Generally, any environmental condition that restricts plant growth (TNC utilization) to a greater extent than photosynthesis (TNC synthesis) results in increased amounts of TNC in forage (Housley and Pollock, 1985; Chatterton et al., 1988).

Carbohydrate content of forages can vary widely as a result of the interactions between plants and their environments (Buxton and Fales, 1994). Environmental factors affecting carbohydrate content include temperature, light intensity, and availability of water and nutrients (Watts and Chatterton, 2004). Environmental conditions or agents which tend to reduce growth, such as exposure to high levels of heavy metals or to non-lethal levels of herbicides, may increase fructan accumulation (Frossard, 1989). In Kentucky bluegrass, the WSC concentration of leaves increased during heat stress and was 21 and 44% greater in drought-preconditioned versus non-preconditioned plants at 14 and 21 d, respectively (Jiang and Huang, 2001). In orchardgrass, WSC increased by 40% of dry matter as drought stress increased (Volaire and Lelievre, 1997). Increases in available nitrogen allow for a more rapid growth rate and thus tend to result in lower levels of fructan accumulation (Jones, 1970).

Pollock and Cairns (1991) suggested that the ability to produce and store fructans was a physiological advantage to plants under environmental stress that require reserve carbohydrates to sustain growth. Spollen and Nelson (1994) suggested that fructans serve as an osmoticum to facilitate water uptake of expanding cells. Suzuki (1993) reported an increase in sugars instead of fructans or the conversion of fructans to sugars to lower the freezing temperature in cells of cool-season grasses exposed to cold-hardening conditions. Nelson (1994) suggested that changes in the general relationship between concentrations of fructans and other sugars may be a general stress response associated with slow growth.

A better understanding of carbohydrate trends in stockpiled forage from May to August (growth phase-1) and from September to November (growth phase-2) is critical to better manage forage for desired carbohydrate levels. The objectives of this study were to evaluate the effect of sampling date (May to November) on 13 cool- and two warm-season grass species in northern Utah as it relates to sugar (glucose, fructose, and sucrose), fructan, WSC, starch, and TNC levels.

METHODS AND MATERIALS

A field study was conducted near Logan, UT (41° 48′ N- 111° 49′ W; 1390 m elevation) to evaluate sugar, fructan, WSC, starch, and TNC concentrations of cool-season and warm-season grasses managed as stockpiled forages from May to August (growth phase-1), followed by a forage harvest of the entire plot, and forage regrowth from September to November (Growth Phase 2) in 2004 and 2005. Species and cultivars within species and their Latin names are listed in Table 1. Soil at the study site was a Nibley silty clay loam. Annual mean minimum air temperatures were 3°C during 2004 and 2005 with mean maximum annual temperatures of 15°C and 14°C in 2004 and 2005, respectively. Total precipitation was 50 cm and 66 cm in 2004 and 2005, respectively. Temperature variations during the growing season are shown in Fig. 1.

Seeding was accomplished on mechanically prepared, weed-free seedbeds with a drill equipped with double-disk furrow openers and depth band regulators. Seeds were placed 1.25- 2.0 cm below the soil surface at a rate of approximately one seed cm^{-1} in May 2003. Plots of 1.0 m × 8.2 m in dimension were arranged in a randomized complete block design with four replications. Throughout the study, plots were uniformly irrigated to field capacity (40 mm $irrigation^{-1}$) every 2 wk (to eliminate confounding with drought) and fertilized with 50 units of nitrogen (N) ha^{-1} after sampling dates 18 May and 15 September 2004 and 16 May and 14 September 2005. Forage samples for the determination of carbohydrates across the growing season was initiated on 3 May with subsequent samples taken 18 May, 2 June, 8 July, 3 August (growth phase-1). Forage DMY was estimated by harvesting the whole plot to an 8-cm stubble height using a Swift Current sickle bar harvester (Swift Machining and Welding LTD, Swift Current, SK, Canada) on 20 August 2004. Forage regrowth samples for carbohydrate determination were taken 15 September, 5 October, and 2 November (growth phase-2). After the 2 November sampling, the whole plot was mowed to a stubble height of 8-cm. In 2005, using the same protocol, sampling was initiated on 2 May 2005 with subsequent sampling on 16 May, 8 June, 6 July, 1 August (growth phase-1), followed by a whole plot DMY harvest on August 22. Regrowth sampling was initiated

Table 1. Cool- and warm-season forage grasses used to evaluate sugars, fructans, water-soluble carbohydrates, starch, total nonstructural carbohydrates, and dry-matter yield (DMY) in northern Utah during 2004 and 2005.

Species	Entry	Material Status	Mean DMY † t ha^{-1}
Cool-season grasses			
Creeping meadow foxtail (*Alopecurus arundinaceus* Poir.)	Garrision	Cultivar	3.83
Meadow bromegrass (*Bromus riparius* Rehm.)	Cache	Cultivar	6.59
	Regar	Cultivar	6.88
Kentucky bluegrass (*Poa pratensis* L.)	Ginger	Cultivar	2.03
Orchardgrass (*Dactylis glomerata* L.)	Potomac	Cultivar	6.07
	OG-2010	Breeding line	5.38
Perennial ryegrass (*Lolium perenne* L.)	VNS	Unknown	3.32
Tall fescue (*Schedonorus arundinacea* (Schreb.) Dumort	Fawn (E$^-$)	Cultivar	6.24
Timothy (*Phleum pratense* L.)	Climax	Cultivar	6.39
Sandberg bluegrass (*Poa secunda* J. Presl.)	Sherman	Cultivar	5.37
Crested wheatgrass (*Agropyron cristatu*m (L.) Gaertn.)	CD-II	Cultivar	4.68
	Hycrest	Cultivar	4.33
	Cold-Germ	Breeding line	3.78
Intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth and D.R. Dewey)	Greenar	Cultivar	5.11
	Rush	Cultivar	5.99
RS wheatgrass hybrid (*Elymus hoffmannii* K.B. Jensen and K.H. Asay)	NewHy	Cultivar	7.35
	RS-salt	Breeding line	7.42
Smooth bromegrass (*Bromus inermis* Leyss.)	Manchar	Cultivar	6.37
Tall wheatgrass (*Thinopyrum ponticum* (Podp.) Z.-W. Liu and R.-C. Wang)	Alkar	Cultivar	9.82
Warm-season grasses			
Blue grama (*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths)	Bad River	Cultivar	7.42
Sideoats grama (*Bouteloua curtipendula* (Michx.) Torr.)	El Reno	Cultivar	14.48

† LSD at ($P < 0.05$) for DMY = 1322.

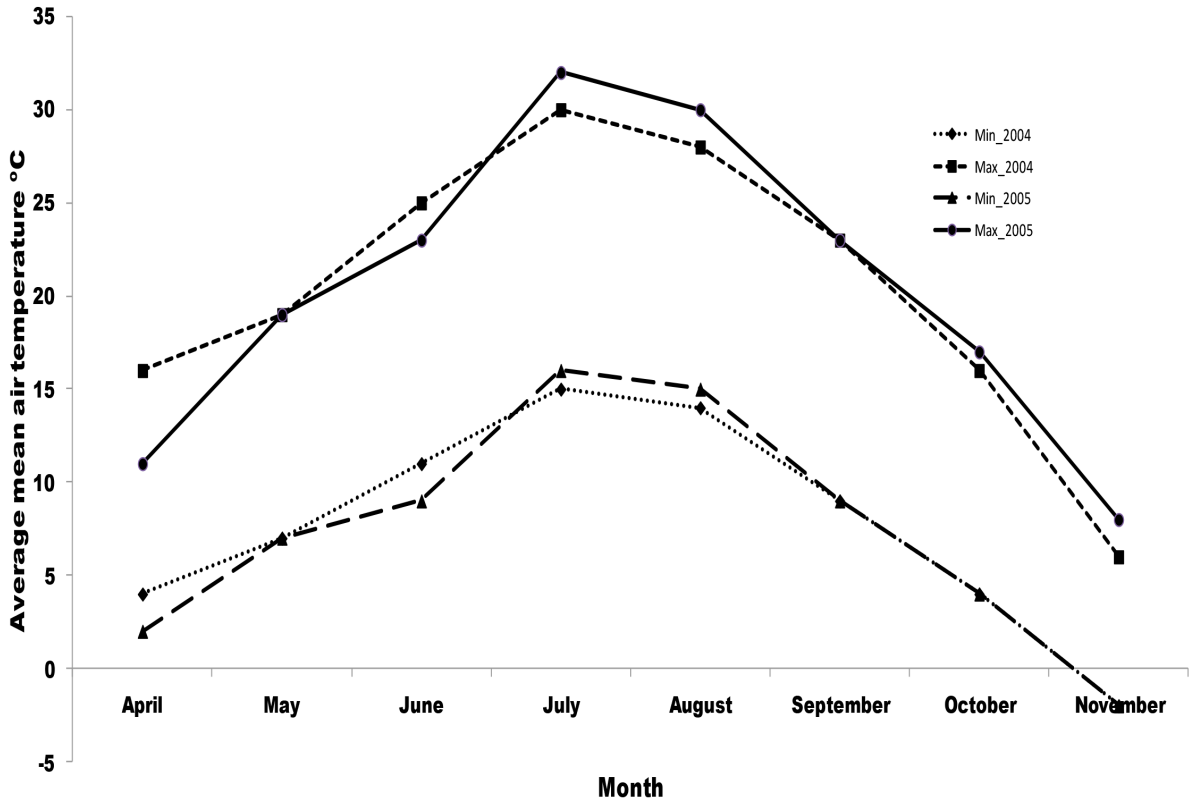

Figure 1. Mean annual minimum and maximum temperatures at Hyde Park, UT between April and November of 2004 and 2005.

on 14 September, 6 October, and 10 November (growth phase-2), for a total of eight sampling dates each year. Data is reported combined over years; hence within the tables the 2004 sampling dates are used as headings, but represent the mean of both years. Forage sampling consisted of hand cutting three random samples approximately 0.5 m × 0.5 m to a stubble height of 8 cm from stockpiled forage within the whole plot. The three samples were then bulked in the field.

Specific plant maturity varied by species at each harvest; however, in general cool-season grass plots were in the vegetative stage during May sampling dates, boot stage at the June sampling date, and at seed set at July and August sampling dates in northern Utah (Moore et al., 1991). After the August forage harvest, all cool-season grasses remained in the vegetative stage through November. The warm-season grasses did not initiate growth until after May sampling dates, remained vegetative through July, were beginning to flower by the August forage harvest, and remained vegetative thereafter.

Forage samples were taken between 1500 h and 1700 h to facilitate collection of maximum diurnal sugar, fructan, and starch concentration (Mayland et al., 2000) and were used for the determination of sugar, fructan, and starch concentrations at each sampling date. Samples were immediately placed in coolers with dry ice, then within 1 h of sampling placed in liquid nitrogen and kept at −20°C until freeze dried. After freeze-drying, forage samples were double ground, first with a Wiley mill and then with a Tecator- Cyclotec tissue grinder to pass through a 0.5-mm screen. Water-soluble carbohydrates were computed by summing sugars (glucose, fructose, and sucrose) and fructan concentrations, and TNC was calculated by summing WSC and starch concentrations (Chatterton et al., 1987).

Water-Soluble Carbohydrate Analysis

Water-soluble carbohydrates were extracted by placing 20-mg samples in 15-mL centrifuge tubes with 5 mL of deionized water. Samples were boiled for 10 min, and then centrifuged. The supernatant was decanted and the pellet re-extracted with an additional 10 mL of boiling distilled water. Following centrifugation, the supernatants from both extractions were combined. Water-soluble carbohydrates were quantified (4 replicated measurements sample^{-1}) according to a modified AOAC Method 999.03. Briefly, 50 μL of the water extract was combined with 50 μL of sucrase/β-amylase/pullulanase/maltase enzymes (Megazyme, Bray, Ireland) and incubated at 40°C for 30 min. One 25-μL aliquot was set aside for determination of sucrose and reducing sugars, and another 25-μL aliquot was set aside for determination of fructans. For determination of sugars, 25 μL of enzyme digest was added to 300 μL of 4-hydroxybenzoic acid hydrazide (PAHBAH) reagent, following the manufacturer's instructions (Megazyme). Samples were then incubated for 6 min at 95°C and cooled at room temperature for 10 min. A volume of 200 μL was read in a 96-well SpectraMax Plus spectrophotometer (Molecular Devices) plate reader at 410 nm.

For fructan determination, sucrose and reducing sugars were first precluded from quantification by conversion to sugar alcohols with sodium borohydride, after which fructans were hydrolyzed with HCl. The HCl was used in lieu of inulinase to better hydrolyze 2–6-linked fructan (phlein) as well as inulin. To the 25-μL volume of the original enzyme-digested material set aside earlier, 40 μL of 10 mg/ml sodium borohydride was added, and the mixture was incubated at 40°C for 30 min. Excess borohydride was liberated by the addition of 100 μL 0.2 N acetic acid, and samples were centrifuged at 5000 × *g* for 1 min. To hydrolyze the remaining fructans into reducing sugars, 100 μL of the reaction solution was added to 50 μL of 1.5 N HCl and incubated at 70°C for 60 min. After cooling, 25 μL was used for reducing sugar determination with PAHBAH reagent as noted above. Since oligosaccharides of the raffinose series (raffinose, stachyose, ajugose, etc.) are not hydrolyzed by the steps leading up to fructan hydrolysis, these carbohydrates would be measured as fructan in the fructan value (Chatterton et al., 1989).

Sucrose and reducing sugar products were compared to a standard curve of an equimolar mixture of glucose and fructose at concentrations ranging from 50 to 350 μM. Reducing sugars produced by HCl digestion of fructans were measured in the same way, except the standard contained only fructose in the same concentration range. As the presence of HCl in the PAHBAH reaction caused an increase in color production, 10 μL of 1.25 N HCl was added to the standards and 10 μL of deionized water was added to the samples before the PAHBAH reagent was added. Values obtained from measurements on the sucrose and fructan controls were used as correction factors.

Starch Digestion and Measurement

Ground forage samples (40–42 mg) were placed in 50-mL glass screw-cap tubes. Thermostable α-amylase was diluted from a stock solution at a ratio of 0.1 mL amylase to 2.0 mL 0.05 M MOPS, pH 7.0 with 5 mM calcium chloride. The amyloglucosidase solution was made up in the same proportions, with the enzyme being diluted in 0.2 M sodium acetate buffer, pH 4.5. Before starch digestion, 300 μL of 80% (v/v) ethanol were added to each tube to facilitate wetting of the tissue, followed by the addition of 2.0 mL of the α-amylase solution. Tubes were gently agitated and placed (uncapped) in a 95°C water bath for 6 min, followed by 10°C water bath for 10 min. Two milliliters of amyloglucosidase solution were added to each tube, gently mixed, and placed in a 50°C water bath for 30 min. Each sample was then diluted with deionized water up to 12 mL, capped, and mixed by inversion. Samples were allowed to settle for 30 min. Glucose hydrolyzed from starch was measured enzymatically with Megazyme's Chromogen reagent (GOPOD = glucose oxidase/peroxidase). Glucose standards ranging from 0 to 400 μM were used. A 25-μL aliquot each of sample and standards was added to 250 μL of the GOPOD reagent, incubated for 20 min in a 50°C water bath, and allowed to cool. Of this, 200 μL were pipetted into an optically clear 96-well plate and read in the plate reader at 510 nm (Chatterton et al., 1987).

Statistical Analysis

Data were analyzed within and across years and harvest dates using the GLM procedure of SAS with a random statement (SAS Institute, 1999). The main effects species, cultivars within species, sampling dates, and years were treated as fixed effects and replication as random (Table 2). Main effects and interactions were tested with their first-order interactions with replications as the error terms. Species mean separations were based on species averages in accordance with Fisher's protected least significant difference (LSD) at the $P < 0.05$ level of probability.

Table 2. Mean squares (based on type III sums of squares, non-additive) from the analysis of variance for sugar, fructan, water-soluble carbohydrates (WSC), starch, total nonstructural carbohydrates (TNC), and dry-matter yield (DMY) on 15 semi-irrigated and irrigated grasses at eight harvests in 2004–2005. Mean squares for replications and interactions with replications not shown.

Source of variation	df	Sugar	Fructan	WSC	Starch	TNC	DMY
Species (Sp)	14	19356**	46367**	105795**	875**	116375**	51**
Cult(Sp)	5	284**	1660*	1165ns†	79**	1120ns	0.7ns
Harvest (Harv)	7	39475**	62535**	153537**	4143**	166449**	
Harv x Sp	98	802**	4124**	6193**	86**	6391**	
Harv x Cult(Sp)	35	99ns	635**	897**	22ns	932**	
Year (Y)	1	1341**	31874**	3508ns	2007**	208ns	14ns
Yr x Sp	14	510**	1168**	932**	108**	1140**	5**
Yr x Cult(Sp)	5	309**	737ns	7682*	25ns	1565*	2ns
Yr x Harv	7	18999**	2740ns	21703**	1184**	30668**	
Yr x Harv x Sp	96	460**	714**	1130*	75**	1346**	
Yr x Harv x Cult(Sp)	35	108ns	232ns	359ns	18*	395ns	

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

†ns = not significant.

Intercharacter correlations were computed on entry by replication means using PROC CORR (SAS Institute, 1999). This study was conducted on stockpiled forage from growth phase-1 and forage regrowth from growth phase-2 (data not shown) to monitor trends in sugar, fructan, WSC, starch, and TNC concentrations across growth phases within species. Thus linear, quadratic, and cubic trends of sugar, fructan, WSC, starch, and TNC within growth phases and across sampling dates were determined for each species using orthogonal polynomials with unequal harvest-date intervals (Gomez and Gomez, 1984). Partial regression coefficients for sampling dates were computed as Julian days beginning 1 January.

RESULTS AND DISCUSSION

There was a significant ($P < 0.01$) species by sampling date interaction for sugar, fructan, WSC, starch, and TNC values, and hence the data are reported herein by sampling date (Table 2). A significant ($P < 0.01$) species by year interaction was also observed. However, positive correlations of $r > 0.83$ for fructan, WSC, and TNC concentrations between 2004 and 2005 suggests that the interactions were more of a shift in the magnitude of values rather than changes in species rank between years. Thus, for data summarization, data are combined over years and within sampling dates.

Sugars

Warm-season Grasses

Grasses cycle through periods when carbohydrates are used and stored between spring growth and maturity (Mayland et al., 2000). Although cool- and warm-season grasses have similar seasonal carbohydrate cycles, warm-season grasses generally store less carbohydrate than cool-season grasses (Nelson, 1995). This pattern was observed in the warm-season grasses, where blue and sideoats grama averaged 44.6 and 44.3 g of sugar kg^{-1} dry matter (DM), respectively, compared to an overall cool-season grass average of 83.6 g kg^{-1} DM (Table 3). Sugar concentration means across species ranged from 32.1 to 60.5 g kg^{-1} DM in the warm-season grasses on 18 May and 5 October, respectively, compared to 51.2 to 104.9 g kg^{-1} DM in the cool-season grasses on 3 August and 5 October sampling, respectively (Table 3). During growth phase-1, there was a significant ($P < 0.01$) linear trend towards increased concentrations of sugars in the warm-season grasses across sampling dates, which accounted for 51% of the explainable variation in sampling date (Table 3). Quadratic and cubic trends were small, yet significant ($P < 0.01$), and were caused by an increase in sugars between 18 May and 2 June. During growth phase-2, a 24% decrease in sugar concentrations from 3 October to 5 November resulted in a significant quadratic trend accounting for 84% of the sampling date sum of squares (data not shown).

Cool-season Grasses

Differences ($P < 0.05$) in sugar concentrations between species were evident regardless of sampling date (Table 3). Kentucky bluegrass sugar concentrations on 3 May ranked third at 115.1 g kg^{-1} DM and by 2 November sampling date it ranked last at 62.6 g kg^{-1}, conversely, tall wheatgrass ranked twelfth in sugar concentrations on 3 May and on the last two sampling dates ranked third (Table 3) both contributing to a significant species-by-sampling date interaction (Table 2). Timothy, on the other hand, had sugar concentrations that ranked first or second regardless of sampling date (Table 3). A 5% increase in sugar concentration across sampling dates in the crested wheatgrass cultivar Hycrest compared to the other crested wheatgrass lines resulted in a significant cultivar-within-species effect. (Table 2). General trends in sugar concentrations across the cool-season grasses during growth phase-1 were significant ($P < 0.01$) and described as linear, quadratic, and cubic accounting for 74, 4, and 13% of the sampling

Table 3. Means and trends in sugar (glucose, fructose, and sucrose) concentration of 15 grass species at eight sampling dates, combined across 2 yr in northern Utah.

Species	Overall mean	Sampling dates								Orthogonal trends†		
		3 May	18 May	2 June	8 July	3 Aug.	15 Sept.	5 Oct.	2 Nov.	Linear	Quadratic	Cubic
	g kg^{-1} DM									%		
Cool-season grasses												
Timothy	106.1	115.3	102.2	114.1	79.1	77.8	116.8	125.0	118.6	63**	8*	4ns
Creeping meadow foxtail	103.4	127.5	93.7	98.3	81.1	64.0	115.3	146.9	100.8	84**	0ns	14**
Perennial ryegrass	93.6	103.2	100.4	107.1	69.9	53.4	98.2	111.8	104.9	62**	26**	3*
Tall fescue	92.0	106.3	87.0	101.2	73.9	58.0	102.2	106.8	100.4	65**	9**	16**
Crested wheatgrass‡	87.7	99.9	75.0	95.2	72.7	53.5	98.6	108.5	99.2	53**	6*	33**
Intermediate wheatgrass‡	85.1	104.2	80.4	99.5	70.7	49.4	91.0	98.7	87.3	56**	9*	28**
Tall wheatgrass‡	84.4	86.6	72.0	95.0	58.0	56.6	90.5	113.3	102.7	37**	12**	12**
Smooth bromegrass‡	80.4	97.4	82.1	77.1	63.8	46.7	96.3	109.5	70.2	91**	3ns	5*
Orchardgrass	80.1	102.1	81.5	81.4	51.4	35.6	88.3	103.5	97.0	87**	5**	4**
Kentucky bluegrass	79.6	115.1	75.8	81.1	58.8	65.5	83.6	96.1	62.6	74**	9**	5**
RS wheatgrass hybrid‡	72.7	82.7	63.6	76.0	58.3	43.7	85.1	97.7	74.8	63**	5**	25**
Meadow bromegrass§	71.6	93.8	68.3	73.2	52.6	40.5	80.7	91.3	72.3	84**	1ns	10**
Sandberg bluegrass‡	66.4	85.5	66.6	73.9	53.2	51.5	66.8	75.6	87.8	76**	0ns	8**
Mean	**83.6**	**100.1**	**78.5**	**89.1**	**64.3**	**51.2**	**92.4**	**104.9**	**88.3**	**74****	**4****	**13****
Warm-season grasses												
Blue grama‡	44.6	35.2	30.7	47.5	45.9	43.7	37.9	61.3	49.8	44**	1ns	34**
Sideoats grama‡	44.3	31.9	33.5	50.2	45.0	44.2	41.6	59.7	41.7	55**	10**	16**
Mean	**44.4**	**33.5**	**32.1**	**48.9**	**45.4**	**44.0**	**39.7**	**60.5**	**45.8**	**51****	**4****	**24****
$LSD_{(0.05)\ SPECIES}$	3.3	12.3	9.5	9.9	5.1	5.3	11.2	13.1	12.2			
$LSD_{(0.05)\ GRASS\ TYPE}$	3.9	9.5	5.9	7.9	3.7	2.3	5.5	10.8	15.3			

† Orthogonal trends expressed as percent of sampling date sums of squares due to linear, quadratic, and cubic effects, based on orthogonal polynomials with unequal sampling date intervals from May to August (growth phase-1).

‡ Dryland range grasses.

§ Grasses used under irrigation and dryland.

*,** Significant at the 0.05 and 0.01 levels of probability, respectively.

date variation (Table 3). Within each species, there was a decline in sugar concentration from 3 May to 18 May; however, at the 2 June sampling data all grasses except orchardgrass and smooth brome experienced an increase is sugar concentration, resulting in a significant quadratic effect (Table 3). From 2 June to 3 August there was a linear decline in sugar concentrations within all the cool-season grasses. The sugar concentration on the first sampling date of the regrowth (15 September) was greater than that of the last sampling date of the previous growth cycle (Table 3). During growth phase-2 (regrowth), all cool-season grasses exhibited a significant increase in sugar concentration from 3 August to 15 September, which is likely associated with forage regrowth in the vegetative state compared to mature plant forage and the plant's attempt to cold harden by reducing growth rate and accumulating TNC in the crown. However, there was a subsequent decline in sugars thereafter contributing to a significant ($P < 0.01$) quadratic trend accounting for 99% of sample date variation during growth phase-2 (data not shown).

Western ranchers have expressed a need for early-spring, fall, and winter grazing with adequate nutritional quality to offset the costs of mechanically harvesting forage for winter feeding (Jensen et al., 2002). Spring (3 May) sugar levels were greatest in creeping meadow foxtail (127.5 g kg^{-1} DM) followed by timothy and Kentucky bluegrass (115.3 and 115.1 g kg^{-1} DM, respectively) (Table 3). Since creeping meadow foxtail is best used in wet meadows and is known for its early maturity (Jensen et al., 2001), it is not surprising that it produced the greatest sugar levels on 3 May. However, its sugar levels dropped rapidly by 18 May and remained comparatively low until 15 September. By comparison, sugar levels declined less in timothy and tall wheatgrass from 3 May to 3 August, while sugar levels in intermediate wheatgrass, orchardgrass, and smooth and meadow bromegrass declined by more than 50% during the same growth phase. Sugar levels in the fall (growth phase-2), critical to nutritional needs in the fall and winter, were above 100.0 g kg^{-1} DM in timothy, creeping meadow foxtail, and tall fescue (Table 3).

Dryland grasses such as crested wheatgrass, intermediate wheatgrass, and smooth bromegrass had sugar concentrations above 92.7 g kg^{-1} DM on 3 May (Table 3). By 2 June, crested and intermediate wheatgrass, as well as tall wheatgrass, maintained increased sugar concentrations (95.0 g kg^{-1} DM). Tall wheatgrass is a late-maturing wheatgrass, which possibly contributed to the increased sugar concentrations detected on 2 June. Sugar concentrations of crested

Table 4. Means and trends in fructan and raffinose (warm-season grasses) concentration of 15 grass species at eight sampling dates, combined across 2 yr in northern Utah.

Species	Overall mean	Sampling dates								Orthogonal trends†		
		3 May	18 May	2 June	8 July	3 Aug.	15 Sept.	5 Oct.	2 Nov.	Linear	Quadratic	Cubic
	g kg^{-1} DM									%		
Cool-season grasses												
Perennial ryegrass	110.5	94.1	183.6	189.9	51.0	43.6	49.7	131.3	140.5	19**	62**	4**
Crested wheatgrass‡	76.7	92.1	130.7	96.0	23.9	52.5	37.0	104.2	78.2	49**	16**	23**
Timothy	64.0	36.6	76.9	64.6	18.6	47.2	25.5	101.1	141.2	6ns	28**	43**
Kentucky bluegrass	57.7	106.1	101.1	101.8	24.5	32.2	15.1	49.5	30.1	68**	11**	0ns
Orchardgrass	52.6	45.6	78.9	69.6	24.0	14.9	19.4	68.3	100.1	35**	54**	5*
Tall fescue	50.6	37.2	60.1	51.6	35.6	42.3	29.6	68.6	80.1	3ns	47**	42**
RS wheatgrass hybrid‡	48.2	58.7	72.4	57.3	19.0	41.3	24.3	62.1	50.3	47**	7**	25**
Intermediate wheatgrass‡	45.1	59.2	62.3	48.1	15.6	24.0	21.2	56.6	74.1	85**	0ns	2ns
Smooth bromegrass‡	43.9	60.1	68.4	45.9	16.6	33.6	30.1	61.0	34.6	68**	2ns	21**
Creeping meadow foxtail	42.0	41.1	54.1	47.4	26.7	51.3	20.3	48.2	46.7	2ns	1ns	61*
Sandberg bluegrass‡	36.6	26.5	59.1	66.8	24.0	40.9	15.8	34.4	20.4	1ns	40**	21**
Meadow bromegrass§	36.5	35.6	43.0	32.5	15.1	20.1	25.4	67.1	53.6	65**	12*	16**
Tall wheatgrass‡	29.7	31.4	39.9	30.2	12.1	9.0	15.1	42.4	57.3	68**	24**	5ns
Mean	**54.2**	**58.3**	**74.9**	**63.2**	**21.3**	**32.3**	**24.2**	**65.6**	**64.8**	**43****	**25****	**15****
Warm-season grasses												
Blue grama‡	7.1	5.9	9.0	10.2	8.4	5.1	4.6	7.4	5.9	0ns	86**	3ns
Sideoats grama‡	12.4	8.2	14.6	14.9	10.3	9.8	10.5	15.3	13.5	0ns	82**	10ns
Mean	**9.8**	**7.1**	**11.8**	**12.6**	**9.4**	**7.5**	**7.6**	**11.4**	**9.7**	**0ns**	**91****	**1ns**
$LSD_{(0.05)\ SPECIES}$	8.4	19.5	20.3	21.8	9.0	12.1	6.9	15.2	15.2			
$LSD_{(0.05)\ GRASS\ TYPE}$	5.4	18.0	11.1	5.1	4.7	4.9	4.9	14.8	14.9			

† Orthogonal trends expressed as percent of sampling date sums of squares due to linear, quadratic, and cubic effects, based on orthogonal polynomials with unequal sampling date intervals from May to August (growth phase-1).

‡ Dryland range grasses.

§ Grasses used under irrigation and dryland.

*,** Significant at the 0.05 and 0.01 levels of probability, respectively.

and tall wheatgrass remained greater on 5 October and 2 November, while those of smooth bromegrass declined from 5 October to 2 November. Sandberg bluegrass had sugar concentrations less than the other grasses (Table 3).

Fructans and Raffinose Oligosaccharides

Warm-season Grasses

As noted above, fructans have not been documented in warm-season grasses (Chatterton et al., 1989). Chatterton et al. (1989) further suggested that putative fructans in some C-4 grasses may actually be of the raffinose series or possibly other non-fructan sucrosyloligosaccharides. Therefore, the positive values obtained when the sample was analyzed for fructans are assumed to be small amounts of raffinose series oligosaccharides and/or non-fructan sucrosyloligosaccharides, and they will be referred to as raffinose in the warm-season grass discussion (Chatterton et al., 1989). Within each harvest, raffinose concentrations in blue grama and sideoats grama were not significantly different; however, on average, sideoats grama had 3.9 g kg^{-1} DM more raffinose than blue grama (Table 4). Unlike the sugars, which expressed a strong negative linear trend across harvests (Table 3) during growth phase-1, raffinose concentrations were almost exclusively quadratic in nature, accounting for 91% of the explainable variation in sampling date (Table 4). This trend is characterized by an increase in raffinose from 3 May to 2 June followed a decline in 8 July and 3 August. During growth phase-2, raffinose concentrations peaked at 5 October and declined at 2 November (Table 4). In the warm-season grasses during growth phase-2, there was a positive correlation between raffinose and sugar concentrations ($r = 0.48$; $P < 0.01$); however, during growth phase-1 that correlation did not exist.

Cool-season Grasses

There was significant ($P < 0.05$) variation observed for fructan concentration in the different grass species within each sampling date (Table 4). A significant species-by-sampling date interaction suggested that species were not consistent in their fructan concentrations across sampling dates (Table 2). Most notable was the change in Kentucky bluegrass–with 106.1 g fructan kg^{-1} DM on 3 May, it ranked first, yet by 15 September it ranked twelfth at 15.1 g fructan kg^{-1} DM (Table 4). Perennial ryegrass consistently ranked high across all sampling dates. Conversely, tall wheatgrass consistently had less fructan concentrations across sampling dates (Table 4). A 15 and 20% increase in fructan concentration across sampling dates in 'Regar'

meadow brome (39.4 g kg^{-1} DM) and 'Greenar' intermediate wheatgrass (50.2 g kg^{-1} DM), respectively, over 'Cache' meadow brome and 'Rush' intermediate wheatgrass resulted in a significant ($P < 0.05$) cultivar-within-species effect. (Table 2)

Based on orthogonal polynomials, across all species, linear, quadratic, and cubic trends in fructans during growth phase-1 were significant ($P < 0.01$) (Table 4). The sampling date sum of squares was partitioned with 43% associated with linear effects and 25 and 15% with quadratic and cubic effects, respectively. Nonlinear effects in fructan concentrations from 3 May to 18 May were associated with an observed increase in all cool-season grasses, except Kentucky bluegrass, which was already high in fructan. This was subsequently followed by a reduction in fructan concentration from 18 May to 8 July with the exception of Kentucky bluegrass, perennial ryegrass, and Sandberg bluegrass, which exhibited an increase in fructan concentrations from 18 May to 2 June (Table 4). Pelletier et al. (2009, 2010) observed increased fructan concentrations in timothy and other grasses from early heading to anthesis and in summer regrowth forage compared to spring growth in grasses. In general, fructan concentration on 3 August continued to decline by the first sampling date of regrowth (15 September) except for perennial ryegrass, orchardgrass, meadow brome, and tall wheatgrass which experienced a 12, 23, 20, and 40% increase in fructan concentrations during that period (Table 4). Subsequently, all cool-season grasses exhibited an increase in fructan concentration from 15 September to 5 October, which resulted in significant linear (44%) and quadratic (56%) effects during growth phase-2 (data not shown).

Interestingly, fructan concentration increased in perennial ryegrass at 18 May (183.6 g kg^{-1} DM) and 2 June (189.9 g kg^{-1} DM) and then again at 5 October (131.3 g kg^{-1} DM) and 2 November (140.5 g kg^{-1} DM) sampling dates. At low temperatures, synthesis and breakdown of fructan occurs more readily than for starch (Pollock and Cairns 1991). This suggests that perennial ryegrass, which does not achieve the same level of fall dormancy seen in other temperate grasses, may accumulate fructans, allowing it to continue growth at lower temperatures, such as in the early spring and late fall (Chatterton et al., 1989).

Crested wheatgrass had comparatively greater fructan concentrations, particularly in the spring, and is one of the few grasses that competes effectively with cheatgrass (*Bromus tectorum* L), an invasive annual grass (Jensen et al., 2001). Pollock and Cairns (1991) concluded that low-temperature adaptation associated with fructan metabolism likely gives cool-season grasses a competitive growth advantage in grassland ecosystems at temperatures less than 10°C. Hence, crested wheatgrass' ability to produce fructans that can be metabolized under cold temperatures may contribute to its ability to compete with cheatgrass. Conversely, meadow bromegrass is one of the most winter-hardy grasses (Jensen et al., 2001), yet this species exhibited reduced concentrations of fructans (Table 4). Correlations during growth phase-1 between sugar and fructan concentrations were significant ($P < 0.01$) but relatively low ($r = 0.24$), suggesting that some grasses produce greater sugar levels yet relatively less fructan levels.

Water-soluble carbohydrates

Warm-season Grasses

Water-soluble carbohydrates in plants are defined as the sum of water-soluble sugars (glucose, fructose, and sucrose) and fructans (Zhao et al., 2008). Concentrations of WSC in warm-season sideoats and blue grama were 61% less than those detected in the cool-season grasses (Table 5). Sugars were on average 82% of the total WSC content in the warm-season grasses examined. Linear trends across sampling dates for WSC concentration in growth phase-1 accounted for 44% of the sampling date sum of squares (Table 5). Quadratic trends in WSC concentrations accounted for 80% of the variation in sampling dates during growth phase-2 (data not shown). On average, WSC concentrations were greatest at 5 October (Table 5), which corresponds to increased sugar concentrations (Table 3).

Cool-season Grasses

Significant ($P < 0.05$) variation was observed for WSC concentration in the different grass species within each sampling date (Table 5). Contributing to the species-by-sampling date interaction were rank changes in Kentucky bluegrass which went from having the greatest WSC concentrations on 3 May at 221.3 g kg^{-1} DM to nearly the least at 92.6 g kg^{-1} DM by 2 November; conversely orchardgrass had the least WSC concentrations on 3 August (50.5 g kg^{-1}) and by 2 November was ranked third with 197.6 g kg^{-1} (Table 5). With the exception of the 3 August sampling date, perennial ryegrass had the greatest WSC concentrations across the growing season. In general, Sandberg bluegrass and meadow bromegrass had the least WSC concentrations within sampling dates (Table 5). Cultivars within species did not differ in their WSC levels (Table 2). Combined across sampling dates, fructan concentrations accounted for 54, 47, and 42% of total WSC concentrations in perennial ryegrass, crested wheatgrass, and Kentucky bluegrass, respectively, compared to 26 and 29% in tall wheatgrass and creeping meadow foxtail, respectively.

During growth phase-1, linear and quadratic trends in WSC concentrations across sampling dates were significant ($P < 0.01$), with linear and quadratic accounting for 68 and 16% of the sampling date sum of squares, respectively (Table 5). Across species, WSC concentration ranged from 157.6 to 160.0 g kg^{-1} from 3 May to 2 June followed by a decline to 86.8 g kg^{-1} by 8 July and remaining at that level through 3 August. All grasses except Sandberg bluegrass (15% decline)

Table 5. Means and trends in water-soluble carbohydrate (WSC) concentration of 15 grass species at eight sampling dates, combined across 2 yr in northern Utah.

Species	Overall Mean	Sampling dates								Orthogonal trends†		
		3 May	18 May	2 June	8 July	3 Aug.	15 Sept.	5 Oct.	2 Nov.	Linear	Quadratic	Cubic
	g kg^{-1} DM									%		
Cool-season grasses												
Perennial ryegrass	204.1	197.3	283.9	297.0	120.9	97.1	147.8	243.1	245.4	29.0 **	55.0**	1.0ns
Timothy	170.1	151.9	179.2	178.8	97.7	124.9	142.2	226.1	259.8	32.0 **	24.0**	11.0**
Crested wheatgrass‡	164.4	192.0	205.7	191.2	96.6	106.0	135.6	212.7	177.3	66.0 **	16.0**	3.0ns
Creeping meadow foxtail	145.4	168.6	147.8	145.7	107.7	115.3	135.6	195.1	147.5	84.0 **	0.0ns	0.0ns
Tall fescue	142.6	143.6	147.1	152.8	109.4	100.3	131.7	175.4	180.5	56.0 **	30.0**	0.0ns
Kentucky bluegrass	137.2	221.3	176.8	182.9	82.3	97.7	98.7	145.4	92.6	79.0 **	2.0ns	0.0ns
Orchardgrass	132.8	147.7	160.5	151.0	75.4	50.5	107.7	171.8	197.6	67.0 **	27.0**	0.0ns
Intermediate wheatgrass‡	130.3	163.3	142.7	147.6	86.3	73.4	112.1	155.4	161.3	82.0 **	3.0ns	3.0ns
Smooth bromegrass‡	124.3	157.9	150.4	123.0	80.4	80.3	126.4	170.5	105.1	91.0 **	3.0**	2.0ns
RS wheatgrass hybrid‡	120.9	141.4	136.0	133.3	77.3	84.9	109.3	159.7	125.1	72.0 **	8.0**	1.0ns
Tall wheatgrass‡	114.0	118.1	112.0	125.3	70.1	65.7	105.6	155.7	160.3	57.0 **	19.0**	1.0ns
Meadow bromegrass§	108.1	129.4	111.4	105.7	67.6	60.6	106.7	158.3	125.9	89.0 **	4.0*	1.0ns
Sandberg bluegrass‡	102.9	111.9	125.7	140.7	77.2	97.4	82.6	109.9	78.1	19.0 **	27.0**	4.0ns
Mean	**137.8**	**158.4**	**160.0**	**157.6**	**86.8**	**86.1**	**118.3**	**176.3**	**158.9**	**68.0 ** **	**16.0****	**0.0ns**
Warm-season grasses												
Blue grama‡	51.7	41.1	39.8	57.7	54.3	48.8	42.5	68.7	55.7	38.0 **	10.0*	36.0*
Sideoats grama‡	56.7	40.1	48.1	65.1	55.3	54.1	52.1	75.0	55.3	46.0 **	35.0**	6.0*
Mean	**54.2**	**40.6**	**43.9**	**61.4**	**54.8**	**51.4**	**47.3**	**71.9**	**55.5**	**44.0 ** **	**22.0****	**18.0****
$LSD_{(0.05)\ SPECIES}$	9.8	27.3	23.7	22.2	9.9	12.8	13.1	18.5	20.1			
$LSD_{(0.05)\ GRASS\ TYPE}$	8.8	24.5	13.5	9.4	2.3	5.7	9.3	20.1	24.5			

† Orthogonal trends expressed as percent of sampling date sums of squares due to linear, quadratic, and cubic effects, based on orthogonal polynomials with unequal sampling date intervals from May to August (growth phase-1).

‡ Dryland range grasses.

§ Grasses used under irrigation and dryland.

*,** Significant at the 0.05 and 0.01 levels of probability, respectively.

exhibited an increase in WSC concentrations on the first sampling date of the regrowth (15 September) and that increase continued in all grasses through 5 October contributing to a significant linear effect (data not shown). A significant quadratic effect resulted from a decline in WSC concentrations in creeping meadow foxtail, Kentucky bluegrass, crested wheatgrass, smooth and meadow bromegrass, Sandberg bluegrass, and RS-hybrid from 5 October to 2 November. There was a stronger correlation between WSC and fructan concentrations ($r = 0.88$; $P < 0.01$) than WSC and sugar concentrations ($r = 0.67$; $P < 0.01$).

Dryland grasses are typically grazed beginning in late May through October on rangeland or used as winter forage from October through February. Early-spring WSC concentrations were greater in crested wheatgrass across all sampling dates compared to the other dryland grasses examined (Table 5). Meadow bromegrass and tall wheatgrass are multi-purpose grasses that can be grown under dryland conditions or where irrigation is limited. Both of these grasses had reduced WSC concentrations through 3 August when compared to the other species examined. Within species, the sugar portion of WSC ranged from 49 to 75% in perennial ryegrass and tall wheatgrass, respectively ($P < 0.05$).

Starch

Starch and fructan stored in leaves are hydrolyzed to form sucrose for transport when sucrose derived from photosynthesis is low. In general, the enzymes for fructan metabolism are functional at a lower temperature than those for starch metabolism (Pollock and Cairns, 1991). Hence, starch accumulation is relatively low in grasses of temperate origin (Chatterton et al., 1987).

Warm-season Grasses

Average starch concentrations on 3 May were greater ($P < 0.05$) in cool-season grasses (24.0 g kg^{-1} DM) compared to the warm-season grasses (15.4 g kg^{-1} DM). Conversely, warm-season grass forage at 3 August was significantly greater ($P < 0.05$) in starch concentration than the cool-season grasses. This result may be partially explained by differences in plant maturity, as cool-season grasses were fully mature and warm-season grasses were actively growing on 3 August. With the exception of 15 September and 5 October sampling dates, blue grama had greater starch concentrations than sideoats grama (Table 6). Despite having significant linear (14%) and quadratic (25%) effects, trends in starch concentrations in growth phase-1 were not well defined and differed between blue

Table 6. Means and trends in starch concentration of 15 grass species at eight sampling dates, combined across 2 yr in northern Utah.

Species	Overall mean	Sampling dates								Orthogonal trends†		
		3 May	18 May	2 June	8 July	3 Aug.	15 Sept.	5 Oct.	2 Nov.	Linear	Quadratic	Cubic
	g kg^{-1} DM									%		
Cool-season grasses												
Crested wheatgrass‡	22.1	27.8	21.6	29.8	28.1	17.6	20.5	17.9	13.7	10ns	15*	76**
Timothy	19.5	24.2	19.1	31.3	18.8	16.6	14.1	13.6	18.6	9ns	19ns	29*
Perennial ryegrass	19.4	19.6	19.6	27.2	18.0	16.8	18.6	17.9	17.7	2ns	43**	13ns
Orchardgrass	19.3	30.3	25.1	24.2	20.1	12.0	16.1	14.1	12.5	85**	6ns	9ns
Creeping meadow foxtail	18.6	28.3	22.7	33.1	18.5	9.8	12.6	11.1	12.8	39**	26**	21**
Intermediate wheatgrass‡	17.7	26.4	18.2	29.4	20.6	14.3	12.6	10.5	9.6	22ns	10ns	48**
Tall wheatgrass‡	17.5	19.5	17.6	28.9	17.2	18.0	13.4	12.7	12.6	0ns	24**	17*
Meadow bromegrass§	16.0	26.1	18.8	21.3	19.0	12.2	11.4	9.4	10.2	67**	1ns	32*
Tall fescue	15.9	18.7	16.1	26.4	15.9	9.5	12.5	13.8	13.9	13*	39**	30**
Smooth bromegrass‡	14.3	21.3	17.6	17.6	18.1	9.7	11.7	10.3	8.3	60**	8ns	26*
Kentucky bluegrass	13.7	22.8	12.0	22.1	12.1	11.7	10.9	10.2	7.2	31**	0ns	33**
RS wheatgrass hybrid‡	12.9	17.8	13.4	19.7	16.1	9.6	9.2	9.2	7.9	19**	19**	59**
Sandberg bluegrass‡	11.6	17.2	11.0	18.0	10.2	7.0	10.3	9.6	9.4	39*	8ns	32*
Mean	**17.3**	**24.0**	**18.5**	**25.4**	**19.2**	**13.1**	**13.9**	**12.6**	**11.7**	**35****	**16***	**41****
Warm-season grasses												
Blue grama‡	18.0	17.2	21.5	26.4	19.3	26.4	9.5	13.6	9.3	35*	6ns	11ns
Sideoats grama‡	14.5	13.6	13.4	25.1	15.1	13.0	12.3	14.3	8.4	2ns	33**	21**
Mean	**16.2**	**15.4**	**17.5**	**25.8**	**17.2**	**19.7**	**10.9**	**14.0**	**8.8**	**14***	**25****	**2ns**
$LSD_{(0.05)\ SPECIES}$	1.5	5.7	3.0	4.6	4.2	3.4	2.8	2.4	3.8			
$LSD_{(0.05)\ GRASS\ TYPE}$	1.1	3.6	4.0	4.8	2.5	1.8	3.8	2.7	3.4			

† Orthogonal trends expressed as percent of sampling date sums of squares due to linear, quadratic, and cubic effects, based on orthogonal polynomials with unequal sampling date intervals from May to August (growth phase-1).

‡ Dryland range grasses.

§ Grasses used under irrigation and dryland.

*, ** Significant at the 0.05 and 0.01 levels of probability, respectively.

and sideoats grama (Table 6). During growth phase-2, starch concentrations fit a quadratic relationship, accounting for 92% of the sampling date variation (data not shown). Contributing to this trend was a 22% increase in starch concentration from 15 September to 5 October followed by a 37% decline by 2 November (Table 6). Significant ($P < 0.01$) positive correlations during growth phase-1 were found between starch and sugars ($r = 0.56$), WSC (0.49), and TNC (0.82). A nonsignificant correlation between starch and fructans ($r = -0.04$; $P < 0.75$) was detected. Growth phase-2 correlations were similar to growth phase-1 except for the correlation between starch and fructans ($r = 0.33$; $P < 0.05$).

Cool-season Grasses

Differences ($P < 0.05$) in starch concentrations between species were evident regardless of sampling date (Table 6). Crested wheatgrass consistently ranked in the top four across all sampling dates and Sandberg bluegrass ranked in the bottom three across sampling dates (Table 6). However, the other grasses were inconsistent in starch concentrations at the different sampling dates contributing to a significant species-by-sampling date interaction. Significant differences in starch concentrations between crested wheatgrass cv. Hycrest (23.7 g kg^{-1}), cold-selected line (22.0), and CD-II (20.6) resulted in a significant cultivar-within-species effect. Linear trends as a percent of the sampling date sum of squares were larger in orchardgrass (85%), meadow bromegrass (67%), and smooth bromegrass (60%) during growth phase-1. Quadratic trends within species accounted for 43% or less of the sampling date sum of squares. Cubic trends explained 76, and 59% of the variation in sampling date sum of squares in crested wheatgrass and RS-hybrid, respectively (Table 6). In growth phase-1, a significant negative linear trend for starch concentration was observed from 15 September to 2 November with the exception of creeping meadow foxtail, timothy, and tall fescue, which showed an increased starch concentration by 2 November (data not shown). Positive correlations during growth phase-1 between starch and sugar ($r = 0.71$; $P < 0.01$), fructan ($r = 0.07$; $P < 0.05$), WSC ($r = 0.40$; $P < 0.01$), and TNC concentrations ($r = 0.53$; $P < 0.01$) were detected. Trait correlations during growth phase-2 were similar to those of growth phase-1, except that the correlation between starch and fructan increased to $r = 0.26$ ($P < 0.01$).

Table 7. Means and trends in total non-structural carbohydrate (TNC) concentration of 15 grass species at eight sampling dates, combined across 2 yr in northern Utah.

Species	Overall mean	Sampling dates								Orthogonal trends†		
		3 May	18 May	2 June	8 July	3 Aug.	15 Sept.	5 Oct.	2 Nov.	Linear	Quadratic	Cubic
	g kg^{-1} DM									%		
Cool-season grasses												
Perennial ryegrass	223.5	216.9	303.6	324.2	138.9	113.9	166.4	260.9	263.1	28.0**	55.0**	1.0ns
Timothy	189.6	176.1	198.3	210.1	116.5	141.6	156.4	239.7	278.5	31.0**	26.0**	5.0*
Crested wheatgrass‡	186.5	219.8	227.3	221.0	124.7	123.7	156.1	230.6	191.0	67.0**	18.0**	1.0ns
Creeping meadow foxtail	164.0	196.9	170.5	178.9	126.2	125.1	148.2	206.2	160.3	78.0**	3.0ns	2.0ns
Tall fescue	158.5	162.3	163.2	179.2	125.3	109.8	144.3	189.2	194.4	48.0**	34.0**	3.0ns
Orchardgrass	152.0	178.0	185.6	175.2	95.4	62.5	123.7	185.9	210.1	71.0**	24.0**	0.0ns
Kentucky bluegrass	150.9	244.1	188.9	204.9	95.4	109.4	109.7	155.6	99.8	77.0**	1.0*	1.0ns
Intermediate wheatgrass‡	147.9	189.6	160.8	177.0	106.9	87.7	124.7	165.9	170.9	77.0**	4.0ns	6.0ns
Smooth bromegrass‡	138.6	179.2	168.1	140.6	98.5	90.0	138.1	180.8	113.4	94.0**	4.0**	1.0ns
RS wheatgrass hybrid‡	133.7	159.2	149.3	153.0	93.4	94.4	118.5	169.0	133.0	72.0**	10.0**	0.0ns
Tall wheatgrass‡	131.5	137.6	129.6	154.2	87.3	83.6	119.0	168.8	172.6	46.0**	22.0**	3.0ns
Meadow bromegrass§	124.2	155.4	130.1	127.0	86.6	72.8	117.5	167.7	136.1	89.0**	3.0ns	2.0ns
Sandberg bluegrass‡	114.5	129.2	136.7	158.7	87.4	104.4	92.9	119.5	87.5	24.0**	25.0**	1.0ns
Mean	**155.1**	**182.4**	**178.5**	**183.0**	**106.0**	**99.1**	**132.2**	**188.8**	**170.7**	**67.0****	**17.0****	**0.0ns**
Warm-season grasses												
Blue grama	69.7	58.3	61.3	84.1	73.5	75.2	52.0	82.3	65.0	50.0**	12.0ns	11.0ns
Sideoats grama	71.1	53.7	61.6	90.2	70.3	67.1	64.4	89.4	63.6	23.0**	37.0**	12.0**
Mean	**70.4**	**56.0**	**61.4**	**87.2**	**71.9**	**71.1**	**58.2**	**85.8**	**64.3**	**35.0****	**25.0****	**12.0****
$LSD_{(0.05)\ SPECIES}$	10.1	29.6	24.1	22.5	11.1	14.1	15.6	19.9	22.4			
$LSD_{(0.05)\ GRASS\ TYPE}$	9.7	27.5	15.6	12.8	4.6	7.1	11.3	22.7	30.8			

† Orthogonal trends expressed as percent of sampling date sums of squares due to linear, quadratic, and cubic effects, based on orthogonal polynomials with unequal sampling date intervals from May to August (growth phase-1).

‡ Dryland range grasses.

§ Grasses used under irri gation and dryland.

*, ** Significant at the 0.05 and 0.01 levels of probability, respectively.

Total nonstructural carbohydrates (TNC)

The concentration of TNC in forage is an important determinant of its nutritive value (Miller et al., 2001). In fact, total TNC concentration in forages has been identified as one of the most important characteristics requiring the attention of forage breeders (Wheeler and Corbett, 1989) because of its close correlation with preference and production in ruminants (Shewmaker et al., 2006).

Warm-season Grasses

Within sampling dates, TNC concentrations in blue grama and sideoats grama were similar (Table 7). As might be expected, the warm-season grasses had, on average, 84.7 g kg^{-1} DM less TNC concentrations ($P < 0.05$) than the cool-season grasses. The difference in TNC concentrations between the warm- and cool-season grasses examined was less pronounced during 8 July and 3 August than in the spring and fall. In blue grama, there was a positive linear trend (50%) towards increased TNC during growth phase-1, suggesting that as the plants matured TNC increased (Table 7). However, in sideoats grama trends were less defined with linear, quadratic, and cubic components accounting 23, 37, and 12%, respectively (Table 7). Forage regrowth on 15 September exhibited an 18% decline in TNC compared to TNC concentrations on 3 August. Correlations between TNC concentrations and sugars ($r = 0.95$; $P < 0.01$), fructans ($r = 0.68$; $P < 0.01$), and WSC concentrations ($r = 0.72$; $P < 0.01$) were larger during growth phase-2 than growth phase-1, but still significant.

Cool-season Grasses

A significant species by sampling date ($P < 0.01$) resulted from species rank changes between sampling dates (Table 2). For example on 3 May, Kentucky bluegrass ranked first in TNC concentrations at 224.1 g kg^{-1} DM and by 2 November had one of the lesser TNC concentrations at 99.8 g kg^{-1} DM (Table 7). Cultivars within species did not differ in their TNC concentrations (Table 2). Within each sampling date, significant ($P < 0.05$) variation was observed for TNC concentrations among the species (Table 7). Within species and sampling dates, TNC concentrations ranged from 62.5 g kg^{-1} DM in orchardgrass on 3 August to 324.2 g TNC kg^{-1} DM in perennial ryegrass on 2 June (Table 7). Pelletier et al. (2010) reported afternoon TNC values ranging between 69 g kg^{-1} DM in spring growth of smooth bromegrass to 117.9 g kg^{-1} DM in tall fescue in summer regrowth forage. Perennial

ryegrass consistently ranked in the top four in TNC concentration across all sampling dates ranging from 113.9 to 324.2 g kg^{-1} compared to meadow bromegrass which was always in the bottom four in TNC concentrations ranging from 72.8 to 167.7 g kg^{-1} DM. Total non-structural carbohydrate concentrations exceeded 200 g kg^{-1} DM in Kentucky bluegrass, crested wheatgrass, and perennial ryegrass on 3 May (Table 7). Concentrations of TNC at 15 September and 5 October in perennial ryegrass, timothy, and crested wheatgrass were above 156 g kg^{-1} DM and 230 g kg^{-1} DM for their respective sampling dates. By 2 November, these grasses were joined by orchardgrass and tall fescue in having comparatively greater TNC concentration (mean exceeded 191 g kg^{-1} DM; Table 7). With the exception of Sandberg bluegrass, which had 11% less TNC concentrations in subsequent sampling dates following the August forage harvest, the other cool-season grasses averaged a 25% increase in TNC concentration after defoliation from 15 September to 3 October (Table 7). Supporting this premise, Richards and Caldwell (1985) estimated that under typical grazing situations at least 89 to 99% of the carbon in regrown forage of crested wheatgrass and bluebunch wheatgrass [*Pseudoroegneria spicata* (Pursh) Á. Löve] is derived from new photosynthate (i.e., regrowth forage) rather than stored carbohydrates. Water-soluble carbohydrates accounted for between 86 and 91% of the total TNC concentration in the cool-season grasses compared to 75 to 80% in the warm-season grasses.

The strongest positive correlations detected were between TNC and WSC concentrations ($r = 0.99$; $P < 0.01$) and TNC and fructan concentrations ($r = 0.87$; $P < 0.01$) regardless of growth phase. Burns and Smith (1980) reported significant positive correlations between digestibility and TNC concentrations of tall fescue, but in that study correlations between sugar and starch concentrations, although significant ($P < 0.01$) were moderate ($r = 0.68$). Hence, it is not surprising that trends in TNC concentrations were similar to trends observed in WSC concentrations (Table 5) with linear and quadratic accounting for 67 and 17% of the total variation among sampling dates during growth phase-1 (Table 7). Nonlinear effects in TNC concentrations were more apparent in growth phase-2 with 46% of the harvest date variation attributed to quadratic effects associated with a decline in TNC on 15 September followed by an increase on 5 October, with a subsequent decline by 2 November (Table 7). Chatterton et al. (1987) reported a linear decrease in TNC in crested wheatgrass when temperatures increased from 5°C to 20°C. Deviations from these trends were reported by Bertrand et al. (2008), where timothy grown under high day/night temperatures of 28/15°C had the greatest NSC concentrations compared to 17/5°C and 22/10°C day/night temperatures. They attributed that increase to a stress response associated with elevated temperatures.

Dry-Matter Yield

There was a significant ($P < 0.01$) species by year interaction. However, a significant positive correlation ($r = 0.81$; $P < 0.01$) between DMY in 2004 and 2005 suggests that instead of rank changes among the species the interaction is likely attributable to a magnitude increase in DMY within species from 2004 to 2005. Mean stockpiled forage taken on 20 August 2004 and 22 August 2005 in the warm-season grasses was significantly ($P < 0.01$) greater than the cool-season grasses examined by 5.33 t ha^{-1} (Table 1) This increase in DMY may be attributable to an increased day/night temperatures in July and August in northern Utah, which favors warm-season forage production when compared to cool-season grasses and the onset of plant senescence in the cool-season grasses.

Mean DMY varied from zero (perennial ryegrass) to 18.17 t ha^{-1} (sideoats grama cv. El Reno) among replications. Tall wheatgrass cv. Alkar had a significantly greater DMY (9.82 t ha^{-1}) followed by RS-hybrid (cv. NewHy) (7.35 t ha^{-1}) in the cool-season grasses. Of the irrigated grasses, meadow bromegrass averaged 6.70 t ha^{-1} compared to 6.39 t ha^{-1}, 6.24 t ha^{-1}, and 6.07 t ha^{-1} in timothy, tall fescue, and orchardgrass, respectively. Kentucky bluegrass (2.03 t ha^{-1}) and perennial ryegrass (3.32 t ha^{-1}) produced the least DMY.

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