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Carbohydrate Metabolism in Leaf Meristems of Tall Fescue[1]

II. RELATIONSHIP TO LEAF ELONGATION RATES MODIFIED BY NITROGEN FERTILIZATION

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ABSTRACT

Our objective was to examine alterations in carbohydrate status of leaf meristems that are associated with nitrogen-induced changes in leaf elongation rates of tall fescue (*Festuca arundinacea* Schreb.). Dark respiration rates, concentrations of nonstructural carbohydrates, and soluble proteins were measured in leaf intercalary meristems and adjacent segments of elongating leaves. The two genotypes used differed by 43% in leaf elongation rate. Application of high nitrogen (336 kilograms per hectare) resulted in 140% higher leaf elongation rate when compared to plants receiving low nitrogen (22 kilograms per hectare). Leaf meristems of plants receiving high and low nitrogen had dark respiration rates of 5.4 and 2.9 microliters O_2 consumed per milligram structural dry weight per hour, respectively. Concentrations of soluble proteins were lower while concentrations of fructan tended to be slightly higher in leaf meristems of low-nitrogen plants when compared to high-nitrogen plants. Concentrations of reducing sugars, nonreducing sugars, and takadiastase-soluble carbohydrate of leaf meristems were not affected by nitrogen treatment. Total nonstructural carbohydrates of leaf meristems averaged 44 and 39% of dry weight for low- and high-nitrogen plants, respectively. Within the leaf meristem, approximately 74 and 34% of the pool of total nonstructural carbohydrate could be consumed per day in high- and low-nitrogen plants, respectively, assuming no carbohydrate import to the meristem occurred. Plants were able to maintain high concentrations of nonstructural carbohydrates in leaf meristems despite a 3-fold range in leaf elongation rates, suggesting that carbohydrate synthesis and transport to leaf intercalary meristems may not limit leaf growth of these genotypes.

Herbage yield of tall fescue (*Festuca arundinacea* Schreb.) is positively associated with LER[3] (8), but the physiological basis for differences in LER is unclear. Cell division and elongation occur in a leaf intercalary meristem located in the basal 3 cm of elongating leaves (2, 5, 24). Genotypes of tall fescue that differed by 50% in LER had similar concentrations of nonstructural carbohydrates and R_D of leaf intercalary meristems (27). Hexose and protein concentrations within the base of elongating leaves of wheat (*Triticum aestivum* L.) were, likewise, not related to changes in LER (11, 12).

The LER of tall fescue is very responsive to applications of N (1, 19, 26), and occurs as a result of increased cell division within the leaf intercalary meristem with final or ultimate cell length remaining unchanged (26). It is unknown whether these cellular changes in response to N are accompanied by changes in carbohydrate metabolism within the leaf meristem. In addition, such information may provide valuable insight into the reasons for genetic differences in LER.

Objectives of this research were to: (a) confirm before-mentioned trends in R_D, nonstructural carbohydrates, and soluble proteins of meristems and segments of elongating leaves of genotypes selected for contrasting LER; (b) examine influence of N on R_D and concentrations of nonstructural carbohydrate and protein within leaf intercalary meristems and adjacent tissues; (c) estimate potential turnover rates of pools of nonstructural carbohydrates in leaf intercalary meristems; and (d) estimate leaf area per tiller needed to supply the leaf intercalary meristem with carbohydrate required for metabolism and biosynthesis.

MATERIALS AND METHODS

Plant Culture. Plastic pots, 11 cm wide by 15 cm deep, were filled with a 1:3 mixture of silt loam topsoil and coarse sand that was chosen in order to have a low organic matter content. Three vegetative tillers from each of two tall fescue genotypes described previously (27) were transplanted into individual pots. Plants were established in a greenhouse under natural daylength with air temperatures of 25 ± 5°C. During establishment plants received a single application of 50 ml of nutrient solution containing 950, 950, and 1178 mg/l N, P, and K, respectively.

Following establishment, 36 pots of each genotype were blocked into six replications. Three pots of each genotype in each replication received 22 kg/ha N as NH_4NO_3, while the three remaining pots of each genotype received 336 kg/ha N. These N rates were chosen to complement previous studies on leaf intercalary meristem morphology of these genotypes (26), and were equivalent to 21 and 319 mg N/pot, respectively. Plants were placed in a controlled environment chamber where a 14-h photoperiod of 550 μmol m^{-2} s^{-1} PAR was supplied by fluorescent and incandescent lamps. Canopy air temperature was 24/20°C (day/night), thus, maintaining temperature of the leaf intercalary meristem at a constant 20°C (24). RH was 50 to 70%. Plants were watered with distilled H_2O as needed and weekly with 50 ml of Hoagland solution modified to contain no N by substituting 6.4 mM KH_2PO_4 and 2 mM $CaSO_4$ for KNO_3, $Ca(NO_3)_2 \cdot 4H_2O$, and $NH_4H_2PO_4$.

Tissue Sampling and Analysis. After 5 weeks of regrowth, LER of four tillers per pot was estimated, and tissues sampled according to segment lengths in Table I using procedures described previously (27).

Concentrations of nonstructural carbohydrates and soluble proteins as well as R_D were estimated as outlined previously (27). Statistical analyses were as described previously (27) using a

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[3] Abbreviations: LER, leaf elongation rate; R_D, dark respiration rate; HYT, high yield per tiller; LYT, low yield per tiller; SDW, structural dry weight; TNC, total nonstructural carbohydrate; SLW, specific leaf weight.

randomized complete block design with six replications and three pots per replication.

RESULTS AND DISCUSSION

Leaf Growth. Effects of genotype and tissue age were similar to those reported previously (27) and, thus, will not be discussed. Emphasis will be placed upon the effect of N and relevant interactions. Averaged across genotypes, application of 22 (low) and 336 (high) kg/ha N resulted in significant differences in concentrations of Kjeldahl N in the herbage of 1.3 and 2.0% of dry weight, respectively. High N increased LER of both genotypes an average of 140% over LER of plants receiving low N (Table I).

Dark Respiration and Soluble Protein. Averaged across genotypes and segment ages, R_D of plants receiving high N was significantly greater than that of plants receiving low N, averaging 2.06 and 1.22 μl O_2 mg SDW^{-1} h^{-1}, respectively (Fig. 1). A positive relationship between R_D and tissue N concentration was reported previously (7, 14), and may be related to the high maintenance respiration costs associated with high tissue protein concentrations (Fig. 2) and turnover (9, 21). In a similar experiment (26), application of high N was found to increase LER of these genotypes by increasing cell division rates while ultimate cell length remained unchanged. Thus, the increased R_D and soluble protein of the leaf intercalary meristem of the high-N relative to the low-N plants probably reflected both the increased mitotic activity and the subsequent biosynthesis associated with expansion of the additional cells.

Photosynthate Requirements. Based on R_D and SDW of meristems (Fig. 1; Table II), the amount of leaf area per tiller needed to support growth within the leaf intercalary meristem can be estimated. For example, total R_D of the leaf intercalary meristem of the HYT genotype receiving 336 kg/ha N was 5.40 μl O_2 mg SDW^{-1} h^{-1}. About 25% is assumed to be due to maintenance R_D (16, 21), leaving 4.05 μl O_2 mg SDW^{-1} h^{-1} that is associated with growth. This is equivalent to 16.6 μl O_2 h^{-1} for the entire meristem. Assuming R_D of the leaf intercalary meristem was constant over a 24-h period and a respiratory quotient of 1.0 (1.83×10^{-3} mg CO_2/μl O_2), the resultant 0.727 mg CO_2 $meristem^{-1}$ d^{-1} is an estimate of the daily CO_2 equivalents associated with growth R_D. Growth R_D represents 25% of the CO_2 equivalents utilized in biosynthesis or, in other words, is equivalent to a 75% efficiency of conversion of substrate into dry matter (20, 29). This suggests 2.91 and 0.24 mg CO_2 equivalents were used daily in growth and maintenance processes, respectively, giving a total use of 3.15 mg CO_2 equivalents per $meristem^{-1}$ d^{-1}.

Using rates of net photosynthesis of these genotypes from Davidson (1) and a 14-h photoperiod, assimilation of needed CO_2 would require 109 mm^2 of leaf area for the HYT genotype receiving high N. With appropriate leaf width (Table II) and LER (Table I), growth of 109 mm^2 leaf area would require 1.3 d of regrowth following complete defoliation.

An alternative approach to determine the CO_2 equivalents and leaf area per tiller required to support growth within leaf intercalary meristems is shown in Table III. The SDW per area is calculated as meristem SLW minus the product of meristem SLW and proportion of TNC (MacAdam and Nelson, unpublished). Leaf area expansion rate (cm^2 d^{-1}) is the product of LER

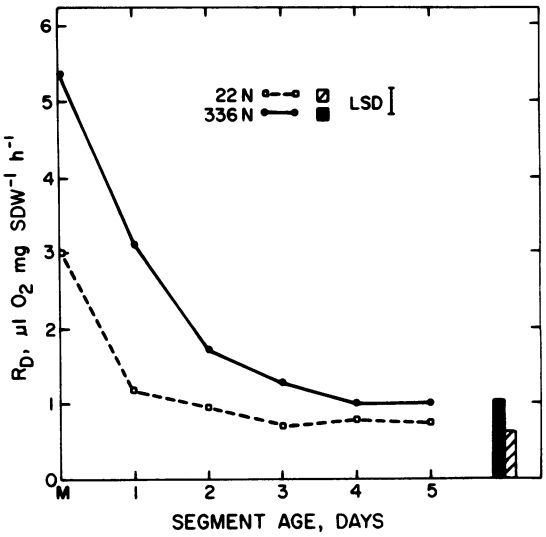

FIG. 1. Dark respiration rate (R_D) of leaf intercalary meristems (M) and segments of elongating leaves ranging from 1 to 5 d old. Vertical bars represent R_D of collared leaf blades. Data were averaged across genotypes selected for high (HYT) and low (LYT) yield per tiller and fertilized with 22 or 336 kg/ha nitrogen (N).

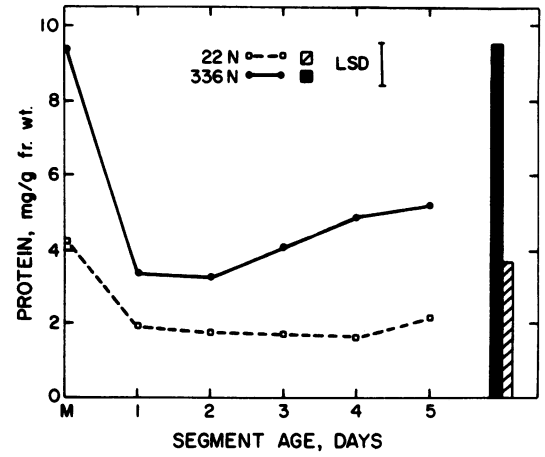

FIG. 2. Soluble protein concentrations in leaf intercalary meristems (M) and segments of elongating leaves ranging from 1 to 5 d old. Vertical bars represent soluble protein concentrations of collared leaf blades. Data were averaged across genotypes selected for high (HYT) and low (LYT) yield per tiller and fertilized with 22 or 336 kg/ha nitrogen (N).

Table I. *Position of Segments of Similar Age Within Elongating Leaves*

Relationship between segment age and cumulative distance of elongating leaves above the point of attachment of the previously collared leaf. Genotypes selected for high (HYT) and low (LYT) yield per tiller had received 22 or 336 kg ha^{-1} nitrogen. Segments of the elongating leaf above the leaf intercalary meristem were cut to lengths equal to the daily leaf elongation rate (LER).

Tissues	Cumulative Distance			
	HYT		LYT	
	22	336	22	336
	mm			
Meristem	16.0	24.0	16.0	14.0
Age (d)				
1	20.7	36.7	20.0	22.0
2	25.4	49.4	24.0	30.4
3	30.1	62.1	28.0	38.6
4	34.8	74.8	32.0	46.8
5	39.5	87.5	36.0	55.0
LER (mm d^{-1})	4.7[a]	12.7	4.0	8.2

[a] Least significant difference ($P < 0.05$) between LER means was 1.1 mm d^{-1}.

Table II. *Calculated Leaf Area per Tiller Needed for Assimilation of CO_2 Released via Meristem Growth Processes*

Dark respiration rate (R_D) and structural dry weight (SDW) per meristem, net photosynthetic rates (Pnet), and leaf area (LA) per tiller needed to supply carbohydrate to meet growth needs of leaf intercalary meristems. Days of regrowth per tiller needed to generate leaf area required were calculated from leaf width and leaf elongation rates (Table I). Genotypes were selected for high (HYT) and low (LYT) yield per tiller, and were fertilized with 22 or 336 kg ha^{-1} nitrogen.

N	Genotype	Total R_D	Growth R_D[a]	SDW Meristem^{-1}	Growth R_D[b]	Growth[c]	Pnet[d]	LA for Growth[e]	Leaf Width	Regrowth[f]
kg/ha		*$\mu l\ O_2\ mg^{-1}\ SDW\ h^{-1}$*		*mg*	*mg CO_2 meristem^{-1} d^{-1}*		*mg CO_2 dm^{-2} d^{-1}*	*mm^2 tiller^{-1}*	*mm*	*d*
336	HYT	5.40	4.05	4.09	0.727	2.91	266	109.2	6.5	1.32
	LYT	5.33	4.00	1.41	0.248	0.99	238	41.6	6.0	0.85
22	HYT	3.16	2.37	1.92	0.200	0.80	175	45.7	6.0	1.62
	LYT	2.72	2.04	1.37	0.123	0.49	154	31.9	5.5	1.45

[a] Total R_D − (Total R_D × 0.25); removes maintenance component of R_D from total (Moser *et al.*, 1982).
[b] (Growth R_D) × (SDW meristem^{-1}) × (24 h d^{-1}) × (1.83×10^{-3}); the last term converts μl O_2 to mg CO_2, assuming a respiratory quotient of 1.0.
[c] (Growth R_D per meristem)/0.25; assuming a 75% conversion efficiency in biosynthesis.
[d] From Davidson (1980), assuming a 14-h photoperiod.
[e] (Growth/Pnet) × 10^4; the last term converts dm^2 into mm^2.
[f] (LA for growth)/[(leaf width) × (left elongation rate)]; leaf elongation rate from Table I.

Table III. *Calculated Leaf Area per Tiller Needed for Assimilation of CO_2 Consumed in Meristem Growth Processes*

Specific leaf weight (SLW), total nonstructural carbohydrate (TNC), and structural dry weight (SDW) per unit area of leaf intercalary meristems. Data were used to calculate CO_2 used for daily growth, leaf area (LA) per tiller needed to assimilate CO_2 used in growth, and days of regrowth following defoliation needed to regenerate LA. Genotypes were selected for high (HYT) and low (LYT) yield per tiller, and were fertilized with 22 or 336 kg ha^{-1} nitrogen.

N	Genotype	Meristem SLW[a]	TNC	Meristem SDW Area^{-1}[b]	Growth SDW[c]	Growth[d]	LA for Growth[e]	Regrowth[f]
kg ha^{-1}		*mg cm^{-2}*	*% dry wt*	*mg cm^{-2}*	*mg meristem^{-1} d^{-1}*	*mg CO_2 meristem^{-1} d^{-1}*	*mm^2 tiller^{-1}*	*d*
336	HYT	3.65	40.0	2.19	1.81	3.54	133.2	1.61
	LYT	2.60	38.9	1.59	0.78	1.53	64.2	1.30
22	HYT	3.29	42.8	1.88	0.53	1.04	59.4	2.11
	LYT	2.24	44.7	1.24	0.27	0.53	34.4	1.56

[a] Unpublished (J. W. MacAdam and C. J. Nelson).
[b] Meristem SLW − [meristem SLW × (TNC/100)].
[c] (Meristem SDW area^{-1}) × (leaf width, cm) × (leaf elongation rate, cm d^{-1}).
[d] [(Growth SDW)/0.75] × 1.47; assuming 75% conversion efficiency; latter term converts carbohydrate to CO_2 equivalents.
[e] [(Growth)/Pnet (Table II)] × 10^4; latter term converts dm^2 to mm^2.
[f] (LA for growth)/[(leaf width, mm) × (leaf elongation rate, mm d^{-1})].

(Table I) and leaf width (Table II). Multiplication of SDW per area by leaf area expansion rate results in an estimate of SDW produced daily per meristem. The HYT genotype with high N actually produced 1.81 mg SDW d^{-1}. At 75% efficiency, 2.41 mg of carbohydrate meristem^{-1} d^{-1} are required for growth respiration and as substrate for biosynthesis. Multiplying by 1.47 converts this to a total of 3.54 mg CO_2 equivalents used in growth meristem^{-1} d^{-1}. These values can be equated with leaf area and days of regrowth knowing CO_2 assimilation rates, leaf widths in Table II, and LER in Table I.

Approximately 3.5, 1.0, 1.5, and 0.5 mg CO_2 meristem^{-1} d^{-1} were utilized for growth of the HYT high-N, HYT low-N, LYT high-N, and LYT low-N treatments, respectively (Table III). This quantity of CO_2 is equivalent to that assimilated by 133, 59, 64, and 34 mm^2 of leaf area, which represents 1.6, 2.1, 1.3, and 1.6 d of regrowth for the respective treatments. These values averaged 28% higher than those obtained in Table II, a difference that is acceptable considering the varied assumptions and methods used. Results were consistent in that, within N treatments, the HYT genotype required more CO_2 equivalents and more days of regrowth to generate the needed leaf area than the LYT genotype (Tables II and III). Similarly, plants receiving low N needed more days of regrowth to generate the required leaf area when compared to their high-N counterparts. Thus, although plants receiving high N require more CO_2 equivalents and, therefore, more leaf area to obtain growth needs through photosynthesis than do low-N plants, their 140% greater LER (Table I) more than compensates for the greater leaf area needed. Based upon this analysis, plants receiving low N may be more dependent than high-N plants upon stored carbohydrate reserves for regeneration of the canopy. White *et al.* (28) found depletion of TNC was greater and subsequent restoration was slower in green needlegrass (*Stipa viridula* Trin.) receiving no N when compared to plants receiving 70 or 140 kg/ha N. This demonstrates the interrelationships between leaf growth, metabolism of carbohydrate, and N.

Concentration of Reducing Sugars. Within elongating leaves, reducing sugar concentrations of high-N plants were increased significantly in 1-d-old segments, then decreased with age (Table IV). In contrast, reducing sugar concentration in elongating leaves of low-N plants generally decreased as segment age increased. The distribution of reducing sugars in elongating leaves of plants receiving high N was similar to that reported previously (4, 10, 17, 27). Reducing sugar concentration of the leaf inter-

Table IV. *Concentrations of Nonstructural Carbohydrates in Leaf Tissues of Tall Fescue Genotypes Receiving Two Rates of Nitrogen*

Tissues examined included leaf intercalary meristems and 1- to 5-d-old segments of elongating leaves, and center sections of recently collared leaf blades. Genotypes were selected for high (HYT) or low (LYT) yield per tiller, and exhibited rapid and slow leaf elongation, respectively. Plants received 22 or 336 kg ha^{-1} nitrogen.

Tissue	Genotype	Reducing sugars		Nonreducing sugars		Fructan		Starch		TNC	
		22	336	22	336	22	336	22	336	22	336
					% dry wt						
Meristem	HYT	1.81	2.33	5.62	6.34	30.4	26.0	4.97	5.34	42.8	40.0
	LYT	2.50	2.60	6.02	7.44	32.7	25.5	3.46	3.33	44.7	38.9
Age (d)											
1	HYT	1.73	3.41	3.05	6.20	17.7	12.3	1.04	5.37	23.5	27.3
	LYT	1.78	5.84	3.33	7.48	21.6	13.5	3.15	5.71	29.8	32.5
2	HYT	1.10	2.09	1.97	3.70	16.5	7.7	1.43	4.42	21.0	17.9
	LYT	1.65	4.58	2.60	5.32	18.5	8.4	1.01	4.33	23.8	22.6
3	HYT	1.02	1.34	1.52	2.49	15.1	5.7	0.41	2.77	18.1	12.3
	LYT	1.31	3.25	1.90	3.20	16.9	5.1	1.21	2.63	21.3	14.1
4	HYT	1.05	1.10	1.56	2.29	13.9	4.6	0.61	1.90	17.1	9.9
	LYT	1.18	2.57	1.65	2.59	15.5	3.5	1.17	1.34	19.5	10.0
5	HYT	0.98	0.97	1.68	2.18	13.6	4.8	0.45	1.51	16.7	9.5
	LYT	1.13	2.05	1.57	2.28	14.4	2.8	0.75	0.43	17.8	7.6
Collared blade	HYT	0.70	0.93	2.20	3.54	16.4	30.8	0.12	1.34	19.4	36.6
	LYT	0.53	1.64	2.64	6.39	6.6	8.3	0.13	0.19	9.9	16.6
LSD[a]		0.73		1.08		2.6		1.02		2.9	

[a] Least significant difference between means ($P \leq 0.05$).

Table V. *Potential Turnover Rates of Pools of Reducing Sugars and TNC in the Meristem*

Potential turnover rates of reducing sugars and total nonstructural carbohydrates (TNC) in leaf intercalary meristems of tall fescue. Turnover rates were calculated using growth dark respiration rate (R_D) and structural dry weight (SDW) per meristem. Genotypes were selected for high (HYT) and low (LYT) yield per tiller and were fertilized with 22 or 336 kg ha^{-1} nitrogen.

N	Genotype	Growth[a]	Reducing Sugar[b]	TNC[b]	SDW meristem^{-1}[a]	Reducing Sugars[c]	TNC[c]	Potential Turnover[d]	
								Reducing sugars	TNC
kg ha^{-1}		mg CO_2 meristem^{-1} d^{-1}	% meristem SDW		mg	mg CO_2 meristem^{-1}		d^{-1}	
336	HYT	2.91	3.88	66.7	4.09	0.233	3.99	12.5	0.73
	LYT	0.99	4.26	63.7	1.41	0.088	1.32	11.3	0.75
22	HYT	0.80	3.16	74.8	1.92	0.089	2.11	9.0	0.38
	LYT	0.49	4.52	80.8	1.37	0.091	1.62	5.4	0.30

[a] From Table II.
[b] (% Carbohydrate on dry wt basis)/(100 − % TNC on a dry wt basis).
[c] (% Carbohydrate on a SDW basis)/100 × meristem SDW × 1.47 (converts carbohydrates to CO_2 equivalents).
[d] (Growth)/(carbohydrate content in mg CO_2 meristem^{-1}).

calary meristem and LER appear to be largely independent, as a 3-fold range in LER (Table I) was accompanied by no change in concentration of reducing sugar in the meristem (Table IV). This is consistent with previous results (11, 13, 27).

Reducing Sugar Turnover. The flux of carbohydrate through the pool of reducing sugars may be related to LER more than reducing sugar concentration of tissues *per se*. These fluxes can be estimated as follows (Table V). For the HYT genotype receiving high N, 2.91 mg of CO_2 meristem^{-1} d^{-1} was consumed in growth processes (growth R_D and biosynthesis). The quantity of reducing sugar per meristem was calculated as the product of reducing sugar concentration and SDW per meristem. This value was multiplied by 1.47 to convert the carbohydrates to CO_2 equivalents. The 0.233 mg CO_2 equivalents of reducing sugars per meristem was divided into the required 2.91 mg CO_2 meristem^{-1} d^{-1} to obtain a potential turnover rate of the reducing sugar pool of 12.5 × d^{-1}. Potential turnover rates were similarly estimated for the HYT low-N, LYT high-N, and LYT low-N treatments at 9.0, 11.3, and 5.4 × d^{-1}, respectively (Table V). These estimates assume all biosynthesis and growth R_D occurred via reducing sugars and that the entire pool was active.

The high turnover rates of reducing sugars in meristems of the high-N plants reflect the high R_D and rapid LER of these treatments. The low turnover of the LYT genotype receiving low N

was due to the low growth R_D per meristem and large amount of reducing sugar per meristem (Table V). If R_D associated with maintenance processes represents about 25% of total O_2 consumption of the leaf intercalary meristem with no concomitant biosynthesis (16, 21), turnover rates of the pool of reducing sugars due to maintenance would be 8% of values reported for growth-associated process.

Monosaccharides, Sucrose, and Fructans. Monosaccharides of the reducing sugar fraction were identified using GLC (Table VI). Consistent with results on reducing sugar concentrations (Table IV), tissues from plants receiving high N had higher concentrations of each monosaccharide than did low-N plants. Lower fructose and glucose concentrations of collared leaves of *Agrostis palustris* Huds. and *Poa pratensis* L. have been reported in plants receiving no N when compared to plants receiving up to 584 kg/ha N (6). *Myo*-inositol concentrations were highest in leaf intercalary meristems and decreased rapidly with segment age whether plants received high or low N. The function of this cyclitol in the leaf intercalary meristem remains somewhat speculative and has been discussed previously (27).

Analysis using GLC revealed that sucrose was the only nonreducing disaccharide present in tissues examined. Segments of elongating leaves of plants receiving high N generally contained more nonreducing sugar than did segments of low-N plants (Table IV). Nonreducing sugar concentrations were high in leaf intercalary meristems of low-N plants and decreased rapidly with tissue age. High nonreducing sugar concentrations were maintained in 1-d-old segments of elongating leaves of high-N plants, after which concentrations declined rapidly with segment age. Similar distributions of nonreducing sugars in well-fertilized tall fescue have been reported previously (27).

Fructan is the major nonstructural polysaccharide present in tall fescue (22). Within elongating leaves, fructan concentrations of segments removed from plants receiving low N were always significantly higher than corresponding segments from plants receiving high N (Table IV). Higher fructan concentration in herbage of tall fescue receiving low N when compared to high-N has been reported previously (15). With both N treatments, leaf intercalary meristems contained more than 25% (w/w) fructan on a dry weight basis. The accumulation of fructan in the meristems of low-N plants suggests that these compounds may serve as a reserve pool of reduced carbon that is available for growth when LER is stimulated by high N. Alternatively, rapid utilization of sugars in high-N plants (Table V) may preclude synthesis of fructan.

Starch and TNC. Takadiatase was used to hydrolyze starch to glucose which was subsequently analyzed for reducing power. Concentrations of takadiastase-soluble carbohydrate were significantly greater in plants receiving high N when compared to low-N plants (Table IV). The largest difference between N rates in concentration of takadiastase-soluble carbohydrate occurred in 1-d-old segments. Clearly, starch or starch-like polymers accumulate in leaf intercalary meristems regardless of N treatment. The function of high concentrations of takadiastase-soluble carbohydrate in leaf intercalary meristems and day-old segments is unknown, but may be related to its reported function in organogenesis of callus cultures (23) as discussed previously (27).

The sum of the nonstructural carbohydrate fractions is termed TNC and represents the potential supply of energy and substrate available for cellular growth and metabolism. Generally, TNC concentrations of segments removed from low-N plants were greater than those from high-N plants (Table IV). TNC concentrations of leaf intercalary meristems averaged 39 and 44% (w/w) dry weight in the high- and low-N plants, respectively. Similar high TNC concentrations in this tissue were reported previously (27).

Relationship of LER to Carbohydrate Concentrations. Although LER, and presumably carbohydrate utilization were 1.4% times greater in high-N when compared to low-N plants (Table I), TNC concentrations were only slightly lower in leaf intercalary meristems of high-N plants (Table IV). With low N, LER was limited by N allowing TNC to accumulate, possibly in excess of concentrations needed for maintenance of maximum leaf growth rates. However, the similarity in TNC concentrations of meristems removed from high- and low-N plants suggests that TNC concentrations may not be a major factor influencing LER in these genotypes, regardless of N fertilization and resultant LER. Further support for this conclusion is apparent from the 50% genotypic difference in LER in this (Table I), and a previous study (27), that was independent of TNC concentrations in the leaf intercalary meristems (Table IV).

Concentrations of TNC are an estimate of the quantity of energy and biosynthetic precursors available for growth. However, the potential rate of turnover of the pool of TNC provides

Table VI. *Monosaccharides within Elongating Leaves of Tall Fescue*

Concentrations of monosaccharides in leaf intercalary meristems and segments of the elongating leaf ranging in age from 1 to 5 d. Values are averaged across genotypes selected for low (LYT) and high (HYT) yield per tiller that received 22 or 336 kg ha^{-1} nitrogen.

Tissue	Fructose		Glucose		*Myo*-inositol		Arabinose		Mannose		Galactose	
	22[a]	336	22	336	22	336	22	336	22	336	22	336
	mg g^{-1} dry wt											
Meristem	14.5	16.6	6.10	5.15	1.61	2.13	0.33	0.61	0.22	0.14	0.03	0.06
Age (d)												
1	14.9	33.4	4.67	8.21	0.63	1.15	0.24	0.54	0.18	0.43	0.13	0.11
2	13.5	29.6	3.71	6.04	0.27	0.61	0.29	1.03	0.15	0.43	0.18	0.15
3	11.1	19.5	3.18	3.84	0.18	0.43	0.26	0.73	0.13	0.32	0.15	0.25
4	10.0	16.3	3.47	3.94	0.15	0.29	0.26	0.56	0.13	0.27	0.15	0.44
5	9.9	11.5	3.77	4.08	0.14	0.28	0.33	0.63	0.09	0.19	0.23	0.93
Collared blade	4.9	9.6	4.23	6.87	0.13	0.21	0.28	0.55	0.03	0.11	0.03	0.15
LSD[b]	6.1		1.66		0.16		0.26		0.08		0.28	

[a] Kg ha^{-1} nitrogen applied.

[b] Least significant difference ($P < 0.05$).

a dynamic perspective of carbohydrate utilization of the leaf intercalary meristem. Assuming the entire pool of TNC of the leaf intercalary meristem is available for growth, turnover rates can be calculated in a fashion similar to turnover rates of reducing sugars. Given a total TNC pool size equal to 1.0, turnover rates of TNC in leaf intercalary meristems were higher in plants receiving high N when compared to low-N plants, averaging 0.73, 0.75, 0.38, and 0.30 of the pool d^{-1} for HYT high-N, LYT high-N, HYT low-N, and LYT low-N treatments, respectively (Table V). The high TNC turnover rates of high-N plants were similar to those found for reducing sugars, while the slow turnover of TNC within leaf intercalary meristems of low-N plants reflects their slow leaf growth rates (Table I) and reduced R_D (Fig. 1).

Enough TNC was present in leaf intercalary meristems of high-N treatments to support leaf growth of the tiller for at least 1 d without additional carbohydrate import, while meristems of low-N plants contained sufficient TNC to maintain leaf growth for nearly 3 d without additional import of photosynthesis. This supports a previous conclusion that daily photosynthate production was not necessary to maintain rapid LER in these genotypes of tall fescue (25). Soluble protein concentrations of tissue segments were significantly greater in plants receiving high N when compared to low-N plants (Fig. 2). Within the elongating leaves of plants receiving high N, soluble protein concentrations were high in leaf intercalary meristems, low in 1-d-old segments, and increased linearly with tissue age thereafter. Similar results have been reported previously (3, 10, 27). Soluble protein concentrations did not change with segment age beyond the leaf intercalary meristem in plants receiving low N.

CONCLUSION

It is apparent that changes in LER, whether genetically or environmentally induced, usually occur with little or no change in nonstructural carbohydrate concentrations and composition within the leaf intercalary meristem. Thus, the supply of energy and biosynthetic precursors appears sufficient for rapid LER. However, these data are static by nature and provide only preliminary estimates of the dynamics of nonstructural carbohydrate flux through the leaf intercalary meristem. Calculated turnover rates of nonstructural carbohydrate pools were similar for the genotypes when equal quantities of N were applied. Verification using labeled assimilate may provide insight into the specific functions and dynamics of fructan and starch pools within the leaf intercalary meristem.

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