Growth Rates and Carbohydrate Fluxes within the Elongation Zone of Tall Fescue Leaf Blades

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ABSTRACT

Investigations were performed to better understand the carbon economy in the elongation zone of tall fescue leaf blades. Plants were grown at constant 21°C and continuous 300 micromoles per square meter per second photosynthetic photon flux density where leaf elongation was steady for several days. Elongation occurred in the basal 20 mm of the blade (0–20 millimeters above the ligule) and was maximum at 9 to 12 millimeters. Eight 3-millimeter long segments were sampled along the length of the elongation zone and analyzed for water-soluble carbohydrates. Sucrose concentration was high in the zone of cell division (0–6 millimeters) whereas monosaccharide concentration was high at and distal to the location where cell elongation terminated (20 millimeters). Fructan concentration increased in the basal part, then remained constant at about 85% of the total mass of water-soluble carbohydrates through the remainder of the elongation zone. Data on spatial distribution of growth velocities and substance contents (e.g., microgram fructan per millimeter leaf length) were used to calculate local net rates of substance deposition (i.e., excess rates of substance synthesis and/or import over substance degradation and/or export) and local rates of sucrose import. Rates of sucrose import and net deposition of fructan were positively associated with local elongation rate, whereas net rates of sucrose deposition were high in the zone of cell division and those of monosaccharide were high near the termination of elongation. At the location of most active elongation imported sucrose (29.5 milligrams per square decimeter per hour) was used largely for synthesis of structural components (52%) and fructan (41%).

Elongation of the leaf blade in grasses is confined to the basal region of the blade which is enclosed within a whorl of encircling leaf sheaths (2, 14, 20). With elongating leaf blades of tall fescue (Festuca arundinacea Schreb.) the zone of cell division is located at the base of the elongation zone, just above the ligule, which is about 1 to 2 mm above the point of attachment of the leaf to the tiller base (7). Like leaf growth, cell division is predominately unidirectional, the meristematic tissue producing parallel files of cells. A cell within a file is displaced away from the site of division as a result of production and longitudinal growth of younger cells. Simultaneous to being displaced from the base, each cell also grows and differentiates. Thus, the distance between a cell and its ontogenetic origin is a function of both its age and developmental stage.

Typically, a tall fescue leaf elongates at 15 to 30 mm·d⁻¹ and has a 15- to 30-mm-long elongation zone (14, 20, 22). Thus, an elongation zone essentially reproduces its own length approximately once a day suggesting that the elongation zone is an active sink for carbohydrates. Carbohydrates can be used for (a) carbon skeletons and respiration used in biosynthetic processes; (b) maintaining carbohydrate concentrations which are being diluted when water is deposited into expanding cells; and (c) respiration associated with protein turnover and maintenance of cellular structures, ion gradients, and metabolite gradients (i.e., maintenance respiration; 12).

In spite of seemingly high rates of carbohydrate use, however, carbohydrate concentrations are often high in the growth zones of grass leaf blades (4, 5, 9, 10, 23, 24). Few experimental data have been reported on rates of carbohydrate utilization in elongation zones of grass leaves. Our long-term objective is to understand carbon use in leaf growth processes. In this study, spatial distributions of carbohydrate concentrations and growth velocities were measured in elongation zones of tall fescue leaf blades growing at a steady rate. From these data local net rates of carbohydrate deposition were calculated using the continuity equation as described by Silk (15). Local rates of carbohydrate import were estimated from net deposition rates of WSC³ and WSC-free DM throughout the elongation zone.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Tall fescue plants were propagated vegetatively, established, and fertilized as previously described (23). All plants were free of the endophytic fungus (Acremonium coenophialum). After establishment 20 pots, each containing three plants, were transferred to a growth chamber, and allocated into three replicates of 7, 7, and 6 pots each. Plants were clipped to leave a 6-cm stubble. Regrowth occurred at continuous 300 μmol·m⁻²·s⁻¹ PPFD at stubble height, with radiation being provided by cool-white fluorescent and incandescent lamps. RH was controlled near 70%. A temperature of 21°C was maintained at the base of tillers where the leaf meristem is located (20). After 5 weeks in continuous light plants were again clipped to a 6-cm stubble, and regrown in continuous light for 4 weeks prior to experimentation. Plants had about 10 tillers each at the time of data collection.

Leaf Elongation Measurement. Distances between the tip of an elongating leaf and the top of the sheath of the second-youngest fully developed leaf were measured with a ruler on 4 consecutive d. Distances were regressed against time and slopes used to estimate LER. Leaves elongating at similar rates were selected for further experimentation. To verify that selected

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³ Abbreviations: WSC, water-soluble carbohydrate; WSC-free DM, dry matter less water-soluble carbohydrate; PPFD, photosynthetic photon flux density; LER, leaf elongation rate; SER segmental elongation rate; VD, velocity of displacement; DP, degree of polymerization.
leaves elongated at a steady rate on a shorter time scale, LERs of four leaves were measured continuously for two separate time periods by means of a radial position transducer (21).

Spatial Distribution of Growth. SERs of undisturbed leaves were calculated from their LERs and data on short-term displacement of holes in the growth zone of leaves matched for LER (14). VD was calculated from SER data as follows:

\[ VD = L \cdot (SER_1 + SER_2 + \cdots + SER_n) + 0.5 \cdot L \cdot SER_i \]

where \( L \) is the length of segments (i.e. 3 mm). The first term on the right side represents displacement due to elongation growth of all segments basal to the location of segment \( i \). The second term describes displacement of the midpoint of segment \( i \) relative to its basal limit. VD was thus calculated for midpoints of segments.

Tissue Sampling. Undamaged tillers that had been selected for uniformity in size and LER were removed from the pots. The elongation zone of blades was carefully freed from surrounding leaf sheaths and cut from the tiller base at the ligule which was about 1 mm from the point of attachment to the unelongated stem. Younger leaf blades that had started to develop within the blade of interest were removed. A cutting device holding 13 parallel razor blades spaced 3-mm apart was used to cut the elongation zone into 12 segments. Segments of 8 to 10 leaves per replicate were combined by position and held in aluminum trays on ice. After determination of fresh weight the samples were dried at 70°C for 40 h and dry weights determined. Water content was calculated.

Carbohydrate Analysis. Dried samples were stored at -20°C and analyzed for carbohydrates within 60 d. The WSC were extracted with water during grinding with a mortar and pestle. Extracts were filtered through Whatman 40 paper. Total WSC in an aliquot was determined by hydrolyzing with 0.1 N \( \text{H}_2\text{SO}_4 \) at 100°C for 15 min, then measuring the reducing power using a copper reduction-iodate titration technique (17). The remaining filtrate was dried at 35°C under reduced pressure and redissolved in 500 \( \mu \)l water. An aliquot was applied to a silica gel TLC plate, Fisher Redi-plate 06-600A, and developed three times with 1-butanol/glacial acetic acid:water (2:1:1, v/v). This system allowed a clear-cut separation of mono- and oligosaccharides up to a DP of 9 hexose units. Another study confirmed that the oligosaccharides in the leaf growth zone were a homologous series of low mol wt fructans (18).

Sugars on TLC plates were made visible by spraying with urea-phosphoric acid which stains mainly ketoses (27). The silica gel bands containing individual sugar fractions were scraped from the plate, eluted with water, and centrifuged. Carbohydrates in the supernatant were measured using the anthrone procedure (1). Carbohydrates separated by TLC were also determined after acid hydrolysis using the copper reduction-iodate titration technique (17) with no difference found between methods. Data reported are from the anthrone procedure.

A preliminary investigation was conducted to determine if drying at 70°C for 40 h caused alteration in carbohydrate content or composition of samples. Elongation zones of leaf blades were boiled in 80% ethanol immediately after removal from the tiller. Carbohydrates were extracted in 80% ethanol and subsequently with water. Combined extracts from this procedure were compared with water extracts obtained from tissue previously dried at 70°C for 40 h. Reducing power of extracts before and after acid hydrolysis was measured (17). Carbohydrate contents of 97.5 and 98.3 mg·g⁻¹ fresh weight were obtained from fresh and dried tissue, respectively. Reducing sugars accounted for 29.5 and 28.5% of the WSC, respectively. Separation of carbohydrates by TLC also indicated no difference between fresh and dried tissue.

Growth Analysis. Local net rates of substance deposition (\( D \), \( \mu g \cdot mm^{-1} \cdot leaf \ length \cdot h^{-1} \)) were calculated from data on the spatial distributions of growth velocities and tissue substance content (\( P \), e.g. \( \mu g \) fructan·mm⁻¹ leaf length) using the one-dimensional version of the continuity equation as discussed by Silk (15, 16):

\[ D = (\partial P/\partial t) + (VD \cdot \partial P/\partial x) + (SER \cdot P) \]

where \( t \) is time (h) and \( x \) is distance (mm) from the origin (ligule) of the leaf blade. In Silk’s terms SER corresponds to the strain rate or relative elemental growth rate and VD to displacement or growth velocity (15, 16).

The first term \( (\partial P/\partial t) \) represents the rate change in substance content at a fixed location from the ligule. The second term \( (VD \cdot \partial P/\partial x) \) is the product of the VD and the spatial gradient in substance content. The latter was calculated as

\[ \frac{\partial P}{\partial x} = 0.5 \left( \frac{P_i - P_{i-1}}{x_i - x_{i-1}} + \frac{P_{i+1} - P_i}{x_{i+1} - x_i} \right) \]

where \( P_i \) is the substance content in segment \( i \), and \( x_i \) is the distance (mm) from the origin to the midpoint of segment \( i \). For segment \( i = 1 \), which is the segment nearest the base, \( \partial P/\partial x \) was calculated to be \( (P_{i+1} - P_i) \cdot (x_{i+1} - x_i)^{-1} \). The error associated with this is relatively small since VD is low near the origin. The third term of the continuity equation \( (SER \cdot P) \) reflects deposition due to elongation of segments.

RESULTS AND DISCUSSION

Leaf Elongation. Leaf lengths were measured during 4 consecutive days on plants that had grown in continuous 300 \( \mu \)mol·m⁻²·s⁻¹ PPFD for 4 weeks after clipping. The three daily (incremental) LERs were similar, namely 0.88, 0.95, and 0.86 mm·h⁻¹ (\( n = 39 \)). Measurements over a shorter time period also revealed no major variation in LER suggesting that cell division and cell elongation occurred at near steady rates.

Spatial Distribution of Growth Velocities. Elongation occurred within a region extending from 0 to 20 mm above the ligule, a reference point which is at the base of the leaf blade (Fig. 1). SER was slow near the ligule, where most cell division occurs, and increased with distance to reach a maximum about 10 mm from the ligule. Cessation of elongation occurred at approximately 20 mm from the ligule. No difference in LER or spatial distribution of SER in the elongation zones was found among leaves varying in blade length (data not shown). Thus, leaf growth...
appeared to be uniform among selected leaves during these investigations.

Segments were displaced from the ligule, due partly to their own elongation, but mostly due to elongation of more basal tissues. Integration of SER along the elongation zone yields the VD function which is a sigmoidal curve (Fig. 1). VD was nearly zero at the origin and equal to LER at the distal end of the growth zone. Thus, the time required for a cell to be displaced by 1 mm was much longer in the basal than in the distal part of the growth zone, approximately 7.2 h mm\(^{-1}\) between 4 and 5 mm, and 1.2 h mm\(^{-1}\) between 19 and 20 mm from the ligule. Thus, cells remain close to the origin for a relatively extended time period, but rapidly leave the growth zone. The time required for a tissue element to be displaced from 1 to 20 mm from the ligule was calculated to be 3.8 d. This value compares closely with the data of Volenec and Nelson (20).

**Carbohydrate Concentrations.** Carbohydrate contents of segments in the growth zone are shown in Figure 2. Variation in tissue carbohydrate contents was minimal among replicates, as indicated by the low SE, even though replicates were sampled sequentially over a 6-h period. This suggests that carbohydrate content remained relatively constant during the steady leaf growth in continuous light.

Monosaccharide content was low near the ligule and in the zone of most active elongation, but increased 2.8-fold within the zone where SER decreased rapidly, i.e. between 12 and 21 mm from the ligule (Fig. 1). The large increase in monosaccharide concentration above the zone of most active elongation (Fig. 2) was also observed in barley leaves grown in a natural environment (9). Volenec and Nelson (23) found that fructose (57-67%) and glucose (16-27%) comprised most of the mass of monosaccharides in the elongation zone of tall fescue leaf blades, although myoinositol and small amounts of arabinose, mannose, and galactose were also present.

Tissue sucrose content decreased by 35% between the ligule and the region of highest SER (Fig. 2). Distal to this location tissue sucrose content was maintained nearly constant. In an earlier investigation (23) sucrose was the only disaccharide found in elongation zones of tall fescue leaf blades.

Throughout the elongation zone the fructan fraction comprised most of the WSC (Fig. 2). There is much evidence that fructan metabolism is confined to the vacuoles of cells (25, 26) which occupy more than 50% of the cell volume in the zone of cell division of tall fescue leaf blades (19). The fructan content of tissue increased strongly in the basal part of the elongation zone, (0-6 mm from the ligule) and was maintained distal to this location (Fig. 2). A high rate of growth-related water uptake into expandable cells was expected at the location of highest SER yet no dilution of fructans was observed, indicating that fructan synthesis occurred at a high rate at this location.

In contrast with the mature leaf blade where fructans are mainly high mol wt (17), the majority of fructans in the elongation zone were low mol wt, i.e. 70 to 85% of the mass of fructans had a DP of less than 10 hexose units (Fig. 2). Significant changes in the molecular size distribution of fructans occurred between the zone of cell division and the zone of most active elongation (Fig. 3). The trisaccharide was the most abundant fructan species in the cell division zone, whereas DPs 4 and 5 were most abundant in more distal parts of the elongation zone.

The findings that sucrose concentrations were highest in the zone of cell division, and then decreased, and fructan concentrations were lowest in the zone of cell division, and then increased, may be explained by induction of fructan-synthesizing enzymes in this zone. Induction may be causally related to the high sucrose concentration in this tissue (26).

**Osmotic Potential of WSC.** Molar concentrations of total WSC (excluding polyfructans) decreased from 164 mm near the ligule to 126 mm at the site of highest SER, and increased to 155 mm at 18 to 21 mm from the ligule (Fig. 4). Thus, local WSC concentrations were inversely related to SER. Nevertheless, concentration of total WSC was high throughout the elongation zone, and was calculated to contribute 0.32 to 0.41 MPa to the osmotic potential. King and Bush (6) reported a total osmotic potential of about 1.2 MPa for the elongation zone of tall fescue leaves.
The decrease in WSC solute concentration with distance just above the ligule was due mostly to a decrease in sucrose. The increase in WSC solute concentration in the distal part of the elongation zone was due mostly to an increase in monosaccharides. In contrast, the molar concentration of oligofructans was nearly constant throughout most of the elongation zone (3–21 mm from the ligule).

In the region of most active elongation (6–15 mm from the ligule) oligofructans contributed more to the osmotic potential of the tissue than sucrose and monosaccharides combined (0.18 versus 0.14 MPa). Vacuoles comprised a mean 63% of the total cell volume in the most actively elongating tissue of tall fescue leaf blades (19). Assuming that fructans are exclusively vacuolar (26) and that 90% of the tissue water was intracellular, fructan concentration in the vacuole was calculated to be about 130 mM. Thus, fructans would have contributed 0.32 MPa to the osmotic potential of vacuoles in the most actively elongating tissue. Some tissue sucrose and monosaccharide was probably also vacuolar. Vacuoles isolated from barley leaf blade protoplasts contained most of the cellular glucose, fructose, and sucrose (25). It appears, therefore, that carbohydrates contributed more to the osmotic potential of the vacuoles than to the rest of the cell.

Net Rates of Carbohydrate Deposition. Local net rates of monosaccharide and sucrose deposition within the elongation zone were calculated using the continuity equation (Fig. 5). These values indicate the rate at which carbohydrates are deposited at each location along the elongation zone in order to maintain the observed spatial distribution of tissue carbohydrate contents while the tissue is growing. For comparison, local net rates of water deposition are also indicated. As most of the tissue water is cellular and water is essentially noncompressible, local net rates of water deposition reflect local rates of tissue volumetric growth, neglecting formation of intercellular air spaces.

Rates of monosaccharide deposition were not in phase with rates of growth-related water deposition (Fig. 5). The net rate of monosaccharide deposition increased exponentially from the base through the region of most active elongation to a maximum at 15 to 18 mm from the ligule. At this location, elongation rates (Fig. 1) and associated net rates of water deposition (Fig. 5) were rapidly decreasing. This caused the increase in tissue monosaccharide content shown in Figure 4.

Maximum monosaccharide deposition rate at 15 to 18 mm may have resulted from a real increase in rate of monosaccharide production at this location. Alternatively, since the net rate of deposition describes the excess rate of import and synthesis over utilization, an increase in net rate of monosaccharide deposition could also have resulted from a decrease in monosaccharide utilization. Actually, the net rate of WSC-free DM deposition (which reflects biosynthetic activity) decreased strongly distal to 12 mm from the ligule (data not shown). A decrease in monosaccharide consumption in biosynthetic processes may result in an increased net rate of monosaccharide deposition.

The net rate of sucrose deposition was relatively high at the leaf base, whereas little growth-related water deposition occurred at this location (Fig. 5). As a consequence the sucrose concentration of tissue was high in this region (Fig. 4). The net rate of water deposition increased 5-fold between the base and the region of most active elongation (9–12 mm from the ligule), whereas the net rate of sucrose deposition increased only 2-fold. As a consequence, the tissue sucrose content decreased significantly. Distal to 12 mm from the ligule net rates of sucrose and water deposition both decreased in concert (Fig. 5), such that the tissue sucrose concentration was maintained (Fig. 4).

Local net rates of fructan deposition were closely associated with local net rates of water deposition (Fig. 6). For example, maximum rate of fructan accumulation occurred at the location of most active elongation, i.e. highest net rates of water deposition. As a consequence, the tissue fructan content was maintained relatively constant throughout the region of most active elongation (Figs. 2 and 4).

Sucrose Import and Use. In a steady state, where local growth rates, respiration rates, and concentrations of carbohydrates and other substances do not vary with time, the rate of carbohydrate use equals the rate of carbohydrate import. Assuming that carbohydrates are exclusively translocated in the form of sucrose, rate of sucrose import can be estimated from rates of sucrose use in all processes consuming sucrose, namely (a) biosynthetic processes and associated respiration; (b) WSC deposition; and (c) respiration associated with protein turnover, maintenance of cellular structures, and maintenance of gradients of ions and metabolites (maintenance respiration).

Sucrose use in (b) WSC deposition was calculated from the data in Figures 3, 5, and 6 assuming that no respiratory costs were involved. Sucrose use in (a) biosynthetic processes and associated respiration was derived from local net rates of WSC-free DM deposition (not shown) assuming that 1.36 g of a mixed organic substrate containing 1.055 g sucrose is required for biosynthesis of 1 g leaf dry matter (11). In addition, local rates of sucrose use in both processes (a) and (b) were calculated from local net rates of DM deposition using 1.055 g sucrose required per g leaf DM synthesized as given by Penning de Vries (11). Estimates of sucrose use in (a) and (b) were about 2% higher with the latter method (Fig. 7).

Data on maintenance respiration of the leaf elongation zone...
In the most actively elongating region of the blade 52% of the imported sucrose was utilized in biosynthetic processes and 48% was used in WSC deposition, which was 85% fructan (Fig. 7). Only 5% of the imported sucrose was deposited as sucrose, i.e. was used to maintain the tissue sucrose concentration. Thus, nonmetabolized sucrose appears to be quickly removed from the cytoplasm and sequestered as fructans. The mode of assimilate unloading in the elongation zone of tall fescue leaf blades is unknown but symplastic unloading in young leaves has been suggested (28). Symplastic unloading of sucrose was observed in growing primary roots of corn (3). Symplastic unloading may be facilitated by fructan synthesis causing removal of unmetabolized sucrose from the cytoplasm.

In summary, we have shown large changes in carbohydrate metabolism occurred together with growth and development of tissue in tall fescue leaf blades. WSC contributed significantly to the osmotic potential of the elongating zone. More than half of the osmotic partial pressure of carbohydrates in the most actively growing part of the growth zone was due to low mol wt fructans. However, total osmotic partial pressure due to carbohydrates was inversely related to SER. Nevertheless, a strong relationship was found between growth and carbohydrate import and use. Most carbohydrate imported into the rapidly growing part of the elongation zone was either rapidly metabolized, or removed from the cytosol and used for fructan synthesis.

**LITERATURE CITED**

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CARBOHYDRATE METABOLISM AND LEAF GROWTH