Effects of Nitrogen on Mesophyll Cell Division and Epidermal Cell Elongation in Tall Fescue Leaf Blades[1]

Jennifer W. MacAdam, Jeffrey J. Volenec[2], and Curtis J. Nelson*

Department of Agronomy, University of Missouri, Columbia, Missouri 65211

ABSTRACT

Leaf elongation rate (LER) in grasses is dependent on epidermal cell supply (number) and on rate and duration of epidermal cell elongation. Nitrogen (N) fertilization increases LER. Longitudinal sections from two genotypes of tall fescue (*Festuca arundinacea* Schreb.), which differ by 50% in LER, were used to quantify the effects of N on the components of epidermal cell elongation and on mesophyll cell division. Rate and duration of epidermal cell elongation were determined by using a relationship between cell length and displacement velocity derived from the continuity equation. Rate of epidermal cell elongation was exponential. Relative rates of epidermal cell elongation increased by 9% with high N, even though high N increased LER by 89%. Duration of cell elongation was approximately 20 h longer in the high- than in the low-LER genotype regardless of N treatment. The percentage of mesophyll cells in division was greater in the high- than in the low-LER genotype. This increased with high N in both genotypes, indicating that LER increased with cell supply. Division of mesophyll cells adjacent to abaxial epidermal cells continued after epidermal cell division stopped, until epidermal cells had elongated to a mean length of 40 micrometers in the high-LER and a mean length of 50 micrometers in the low-LER genotype. The cell cycle length for mesophyll cells was calculated to be 12 to 13 hours. Nitrogen increased mesophyll cell number more than epidermal cell number: in both genotypes, the final number of mesophyll cells adjacent to each abaxial epidermal cell was 10 with low N and 14 with high N. A spatial model is used to describe three cell development processes relevant to leaf growth. It illustrates the overlap of mesophyll cell division and epidermal cell elongation, and the transition from epidermal cell elongation to secondary cell wall deposition.

Leaf growth in grasses is predominantly unidirectional, parallel with the longitudinal axis of the leaf. A gradient of development exists near the base of the leaf blade and consists successively of cell division, elongation, and structural SLW[3] accumulation. The LER of grasses is a function of the cell supply (the number of cells produced per file) and the rate and duration of cell elongation in the epidermal system. Nitrogen increases leaf growth rate of grasses (27); however, previous studies have not separated the effects of N on cell division and cell elongation.

Volenec and Nelson (29) used high and low rates of N fertilization to further alter LER of two genotypes of tall fescue selected for contrasting leaf growth rates. Mean LER of these genotypes was 89% higher with high N, but length of fully elongated epidermal cells was unaffected by N treatment. The number of epidermal cells produced per file per day increased 90% with high N, suggesting that much of the increase in LER following N fertilization was due to increased cell division.

Our long-term goal is to understand carbohydrate metabolism during the growth of grass leaves. Our objectives in the present study were to evaluate the effect of N on mesophyll cell division and rate and duration of epidermal cell elongation in leaf blades of two genotypes of tall fescue. Data for epidermal cell lengths utilized here have been reported previously (28, 29).

Mesophyll cell division data reported here, and data for leaf elongation rate and structural SLW accumulation reported previously (17, 28) are integrated in this paper into a spatial model of cell development in the grass leaf blade. This model will facilitate the evaluation of concomitant physiological processes, such as carbohydrate metabolism (21).

MATERIALS AND METHODS

Plant Culture

Details of plant culture were reported previously (28, 29). Briefly, five vegetative tillers of two tall fescue (*Festuca arundinacea* Schreb.) genotypes (14) which display high and low LER were transplanted into a low-organic-matter sandy loam soil contained in 11-cm-diameter by 15-cm-deep plastic pots. After 16 weeks regrowth in a greenhouse where diurnal temperature ranged between 20 and 30°C and day length was 14 h (extended with fluorescent lamps) to deplete soil N, plants were transferred to a controlled-environment chamber. A 14-h photoperiod of 500 μmol s^{-1} m^{-2} PPFD was provided by cool-white fluorescent and incandescent lamps. Temperature was maintained at a constant 24°C and RH varied between 50 and 70% of saturation. Fifty mL of Hoagland No. 2 nutrient solution (8) modified to exclude N, *i.e.* KNO$_3$, Ca(NO$_3$)$_2$·4H$_2$O, and NH$_4$H$_2$PO$_4$ were replaced by KH$_2$PO$_4$, and CaSO$_4$, was applied weekly. After 4 weeks, groups of six pots of each genotype received the equivalent of 22 (low N) or 336 (high N) kg ha^{-1} N as NH$_4$NO$_3$. Leaf blades were sampled 8 weeks after N application.

In a second study, carried out entirely in a greenhouse, tillers were transplanted into 57- by 42- by 20-cm-deep wooden boxes containing a mixture of silt loam, peat, and sand (2:1:1). Plants were clipped to leave a 5-cm stubble and

---

[1] Contribution from the Missouri Agricultural Experiment Station, Journal Series No. 10529.

[2] Present address: Agronomy Department, Purdue University, West Lafayette, IN 47907.

[3] Abbreviations: SLW, specific leaf weight; LER, leaf elongation rate; PPFD, photosynthetic photon flux density.

fertilized with the equivalent of 20, 24, and 23 kg ha^{-1} N, P, and K every 6 weeks. After 24 weeks, leaves which had developed under diurnal temperatures which ranged between 25 and 35°C and full sunlight were sampled.

Calculation of LER

Distance from the ligule of the youngest collared leaf to the tip of the elongating leaf blade was recorded for three tillers per pot on 4 consecutive d, or nine tillers per box for 3 d prior to sampling. LER was calculated as the slope of a linear regression of leaf blade length *versus* time.

Microscopy

Tillers were sampled approximately 3 h into the photoperiod. The lower 40 mm of elongating leaf blades was exposed by removing and discarding older enveloping leaf sheaths. Blade tissue was excised at the point of its attachment to the terminal meristem, fixed in formalin:glacial acetic acid:70% ethanol (1:1:18, v/v) for 48 h, dehydrated in a tertiary butanol series, infiltrated with paraffin (Paraplast Plus, Fisher Scientific, St. Louis, MO; melting point 56°C), and chromosomes of cells in 10- or 12-μm-thick longitudinal sections were stained using safranin-fast green (13).

Mesophyll Cell Division

Percentages of mesophyll cell nuclei in mitotic stages from prophase through telophase were determined using light microscopy analysis of longitudinal sections. Counts were made at 1-mm intervals beginning 1 mm above the point of attachment of the leaf blade to the terminal meristem. This is the approximate location of the ligule in leaf blades at this stage of development, before the sheath meristem becomes active. The leaf blade meristem is located above the ligule, and the sheath meristem below it.

Epidermal Cell Displacement Velocity

The rate at which an epidermal cell elongated as it was displaced through the elongation zone was determined by the use of the relationship between cell length and distance from the base of the leaf blade (Fig. 1), and the velocity of cell displacement from the leaf base at successive positions along the leaf. A relationship derived from the continuity equation ($d = \delta P/\delta t + V \delta P/\delta x + P \delta V/\delta x$; 10, 23, 24) was used to determine the velocity of cell displacement at successive positions along the elongating leaf blade. In this application, cell supply (division rate) at any position (d) is equal to change in cell density (P) with change in time (t), plus displacement velocity (V) multiplied by change in cell density with change in distance from the leaf base (x), plus cell density multiplied by the change in displacement velocity with change in distance (WK Silk, personal communication).

Lengths of epidermal cells used in this study were reported previously as a function of distance (28, 29). Only lengths of abaxial epidermal cells which were elongating, but no longer dividing, were used. Epidermal cell elongation begins approximately 1 mm above the ligule or 2 mm above the leaf base. No mitotic events were seen above this position in the epi-
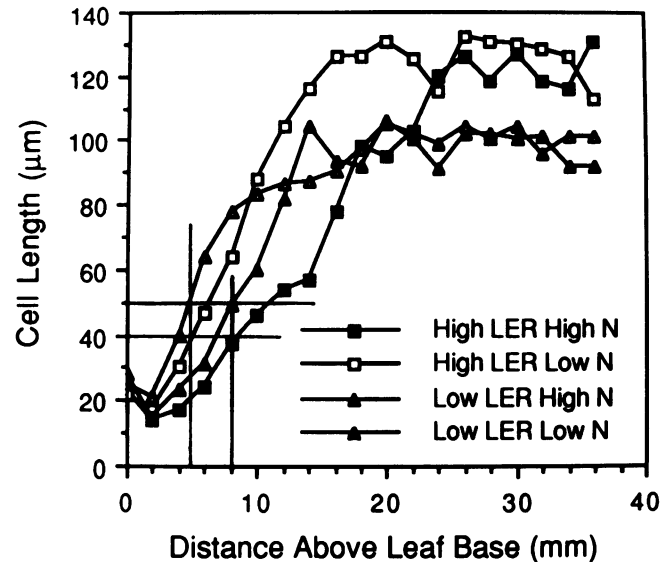
Figure 1. Mean lengths of abaxial epidermal cells in 10-μm-thick longitudinal sections from the basal 40 mm of elongating leaf blades. Perpendicular intersecting lines indicate positions where cells of the high-LER genotype were 40 μm long and cells of the low-LER genotype were 50 μm long. Plants were grown under high (336 kg ha^{-1}) or low (22 kg ha^{-1}) N fertilization, at 24°C and 500 μmol s^{-1} m^{-2}. Data are means of 90 observations. Adapted from Volenec and Nelson (29).

dermis. Since only nondividing cells were measured, the d term of the continuity equation, or epidermal cell division rate at a given distance from the leaf base, was set equal to zero.

The $\delta P/\delta t$ term of the continuity equation can also be set equal to zero if cell density at a given distance from the leaf base is constant with time. Plants used in this study were grown under a 14-h photoperiod. The daily increase in leaf length is constant from the time the tip of the leaf blade emerges from the whorl of older leaf sheaths until shortly before leaf elongation stops. Although LER varies within the diurnal cycle, growth trajectory data presented in the "Results and Discussion" section indicate that cell density is effectively constant with time at a given distance from the leaf base.

In the absence of cell division or change in cell density, the continuity equation can be rewritten as $-V(\delta P/\delta x) = P(\delta V/\delta x)$. This relationship implies a relationship between displacement velocity and cell length: $V_A = (V_F/L_F) L_A$, where V_A and L_A are velocity and cell length, respectively, at a point A from the leaf base, and V_F and L_F are final velocity (LER) and final cell length (WK Silk, personal communication). This relationship was used by Scott *et al.* (22) in studies of bean (*Vicia faba*) root development, and by Carmona and Cuadrado (3) in studies of onion (*Allium cepa*) root growth. Using this relationship, the velocity of cell displacement from the leaf base can be determined at each distance from the leaf base for which cell length is known.

Epidermal Cell Elongation Rate

Velocity is equal to change in distance divided by change in time. Therefore, the length of time required for a cell to move the 2 mm surrounding each position at which cell

length was measured (*e.g.* 1–3, 3–5 mm) was determined by rearranging the equation for velocity, and multiplying the reciprocal of velocity at each position by 2 mm. Movement from 0 to 1 mm from the position where epidermal cell elongation began was assumed to require one-half the time that was determined from cell lengths where elongation began. The time value corresponding to each distance from the leaf base was the sum of that and all preceding time intervals. For convenience, a curve of the reciprocal of velocity, time as a function of distance, then allowed us to determine cumulative time for any distance within the elongation zone from the point where epidermal cell elongation began.

Finally, the natural logarithm of cell length at each distance was plotted against cumulative time at the same distance for every position where epidermal cell length was increasing. We also attempted to fit both logistic and Gompertz equations to these data, but only exponential equations were appropriate (9). A linear regression of the logarithm of cell length data *versus* time was used to determine a formula for the line. Relative cell elongation rate was calculated as the slope of the line, and duration of cell elongation was the number of hours the cell required to attain its final length.

## Spatial Model of Cell Development

The model of cell development processes presented in this paper utilizes data for segmental elongation rate and structural SLW data published previously (17, 28). Segmental elongation rate was determined by exposing elongating leaf blades briefly and marking them with India ink at intervals of 1 mm (28). Distance between marks was determined 24 h later. Increase in distance between marks with change in time was plotted as segmental elongation rate.

Structural SLW is dry weight per unit leaf area, less water-soluble carbohydrates (17). Area of the leaf blade was determined by measuring the width of 10-mm-long segments of elongating leaf blades. Water-soluble carbohydrates were extracted from these segments by grinding dried samples with water and sand. After acid hydrolysis, the reducing power of the filtrate was determined by the copper-iodometric method of Smith (26). Mass of water-soluble carbohydrates per unit leaf area was subtracted from SLW to give structural SLW.

## RESULTS AND DISCUSSION

Epidermal Cell Elongation

Data for cell length as a function of distance above the leaf base (Fig. 1) were published earlier (29), and are presented again for convenience. When these cell length data were recalculated, the increase in cell length was exponential with time (*i.e.* cell length = initial cell length · e^{kt}) (Figs. 2 and 3). The rate constant (k) or relative growth rate as described by Erickson (9), representing length gained per unit length present (12), was 6% higher for the low- than the high-LER genotype for both N treatments (Fig. 2). High N caused a 9% increase in the rate constant for cell elongation in both genotypes. In an earlier study, using only initial and final cell lengths (29), mean epidermal cell elongation rate in tall fescue leaf blades was calculated to be 22% higher with high than with low N.
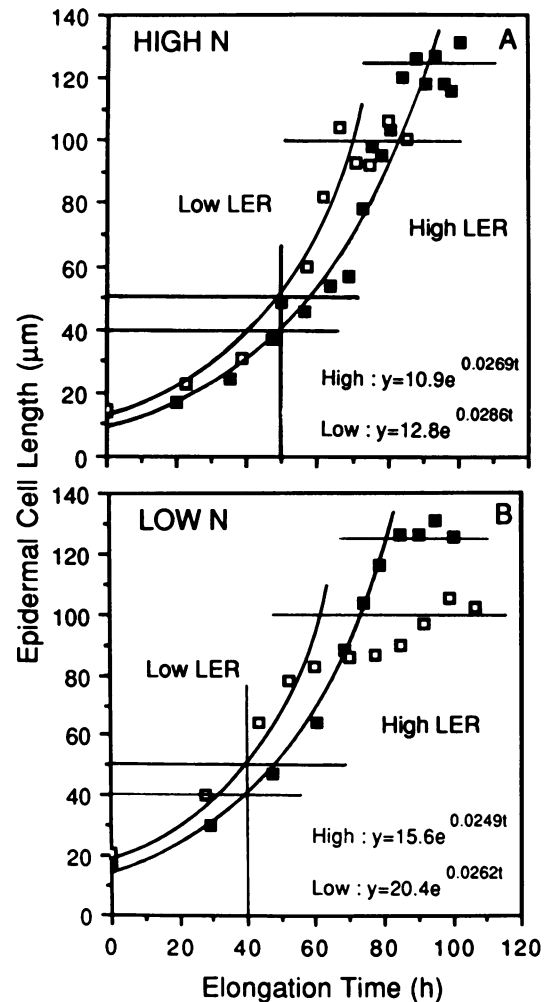
Figure 2. Mean lengths of abaxial epidermal cells in 10-μm-thick longitudinal sections from the basal 40 mm of elongating leaf blades. Intersecting perpendicular lines indicate that epidermal cells were 40 μm long for the high-LER genotype and 50 μm long for the low-LER genotype after 40 h at low N and after 50 h at high N. Plants were grown at 24°C and 500 μmol s^{-1} m^{-2} with high (336 kg ha^{-1}) or low (22 kg ha^{-1}) N fertilization. Data are means of 90 observations (29). Derivation of the relationship between cell length and time is described in "Materials and Methods."

Final epidermal cell length averaged 125 μm for the high- and 100 μm for the low-LER genotype, regardless of N treatment (Fig. 1). Using these final cell lengths in the exponential equations to calculate duration of cell elongation, epidermal cells of the high-LER genotype elongated for 82 h at low N and 90 h at high N, and those of the low-LER genotype elongated for 61 h at low N and 72 h at high N. The rate of cell elongation for the low-LER, low-N treatment was calculated on the basis of the first 50 h; final mean cell length was 100 μm after 90 h.

In the earlier report by Volenec and Nelson (28), duration of epidermal cell elongation was found to be 56% greater for the high-LER genotype compared with the low-LER genotype. Using the calculated exponential rate of cell elongation, duration of epidermal cell elongation for both the high- and low-N treatment is approximately 20 h greater in the high- than in the low-LER genotype. The 25 μm or 25% difference
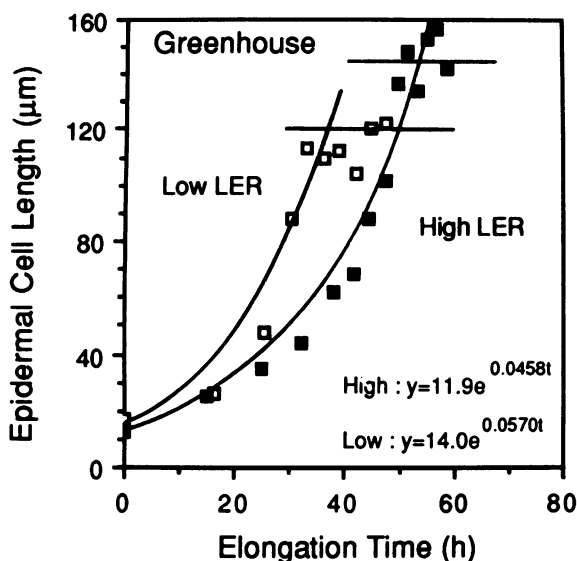
Figure 3. Mean lengths of abaxial epidermal cells in 12-μm-thick longitudinal sections from the basal 40 mm of elongating leaf blades. Leaf blades developed in a greenhouse at a mean temperature of 30°C. Data are means of 45 observations (28). Derivation of the relationship of cell length with time is described in "Materials and Methods."

in final cell length between the genotypes results primarily from this 20 h difference in duration of cell elongation (Fig. 2).

We also determined relative rates of epidermal cell elongation for both genotypes under high N fertilization and full sunlight during a summer greenhouse study where diurnal temperatures ranged between 25 and 35°C. Initial cell lengths were similar to the earlier study, but rate constants were nearly twofold higher for both genotypes (Fig. 3). Epidermal cells elongated for 55 h to 145 μm in the high-LER genotype and for 38 h to 120 μm in the low-LER genotype. This 17 h difference in duration of elongation is similar to that reported in the earlier experiment, despite a 50% reduction in duration of elongation in both genotypes. The genotypic difference of 25 μm in final cell length was also maintained. The differences in rate and duration between cell elongation under greenhouse and controlled-environment-chamber conditions indicate that, along with genotype, either temperature or light may affect epidermal cell elongation in grass leaf blades.

With the exception of the low-LER, low N treatment, epidermal cells continued to elongate exponentially until approximately the time they reached their final lengths (Figs. 2 and 3). Cessation of cell elongation may occur as a result of spatial or temporal controls. The spatial distribution of one or more growth regulators along the leaf blade may influence growth rate. In tall fescue, 100 ppm GA$_3$ applied to roots for 24 h increases LER of both genotypes by 90% (19). Exogenously applied GA$_3$ increases growth rate of lettuce (*Lactuca sativa* L.) hypocotyls, but is not thought to increase duration of cell expansion or rate of cell division (15). Therefore, the higher rate of cell elongation in the low-LER genotype (Figs. 2 and 3) may result from a higher endogenous GA concentration in leaf blades of this genotype.

Auxins move basipetally through growing leaf tissue with highest accumulation at the leaf base (11). The number of

isozymes of peroxidase with IAA-oxidase activity increase with maturity of root tissue (6), and would therefore tend to decrease tissue concentration of IAA with cell differentiation. Cytokinins may also play a role in leaf elongation through either cell division or cell elongation. The increase in LER following N fertilization may be due to an increase in the availability of cytokinins to the shoot, which could occur through proliferation of root tips, the major source of cytokinins (11).

Presented as a function of time, the cessation of increase in cell length occurs rapidly. We are currently investigating both the physical and biochemical changes that coincide with cessation of cell elongation. The exponential elongation rate of epidermal cells and their relatively abrupt cessation of elongation have also been demonstrated in leaf blades of perennial ryegrass (*Lolium perenne*) using other methods (H Schnyder, personal communication).

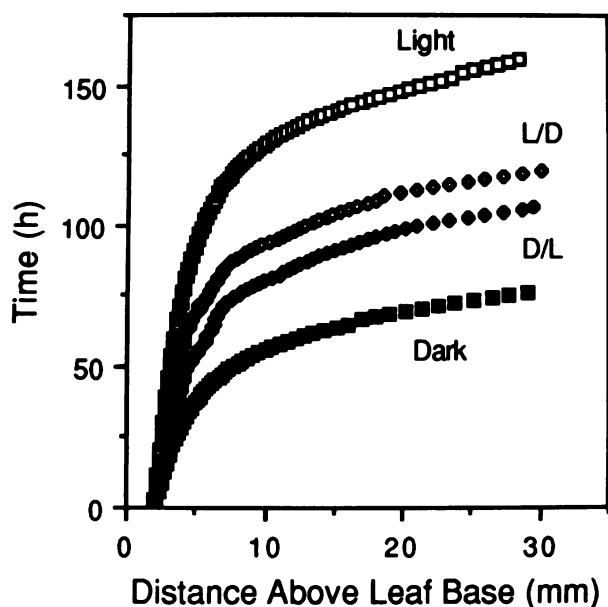## Epidermal Cell Displacement Velocity

We investigated the effect of a diurnal light/dark regimen on cell density. At constant temperature, LER is 60 to 65% greater during darkness than during the light period (20). Local displacement velocities of the elongating leaf blade are higher throughout the elongation zone during the dark period (*e.g.* Fig. 2 from Ref. 20). It normally takes several days for an epidermal cell to become displaced through the elongation zone, so that under a diurnal light/dark regimen, each cell experiences a combination of several high and low displacement velocities.

Movement of an elongating cell away from the leaf base (its growth trajectory) under different diurnal regimens was calculated by alternately applying light and dark local displacement velocities (Fig. 4). Percentage of cells in mitosis in the shoot apex of *Silene coeli-rosa* L. was approximately 4.5 in darkness and 5 during the photoperiod (18), indicating that the rate of cell division in shoots is relatively constant during the light/dark cycle. Therefore, a cell is equally likely to begin elongation at any time.

We found that if a cell begins elongation at the start of the 9-h dark period (designated D/L), it attained its final length 12.5 h earlier than a cell that begins elongation at the start of the 15-h photoperiod (L/D) (Fig. 4). However, this difference in time to final cell length is established within 5 mm of the base of the leaf blade, where cells are small and cell density is high for all treatments. From 5 to 30 mm above the leaf base, growth trajectories are similar, but displaced from each other by a constant difference in time. Growth trajectories for most cells under a diurnal regimen would be intermediary to the extremes represented by the D/L and L/D curves. No periodicity of cell lengths reflecting the length of the photoperiod was evident upon examination of single columns of epidermal cells (JW MacAdam, CJ Nelson, unpublished data).

Mesophyll Cell Division

Mesophyll cells comprise approximately 42% of the cross-sectional area of fully expanded tall fescue leaf blades, while epidermal tissue represents less than 19% (4). Mesophyll cells have a significant impact on photosynthetic rate, but they represent a major sink for photosynthate in the leaf blade
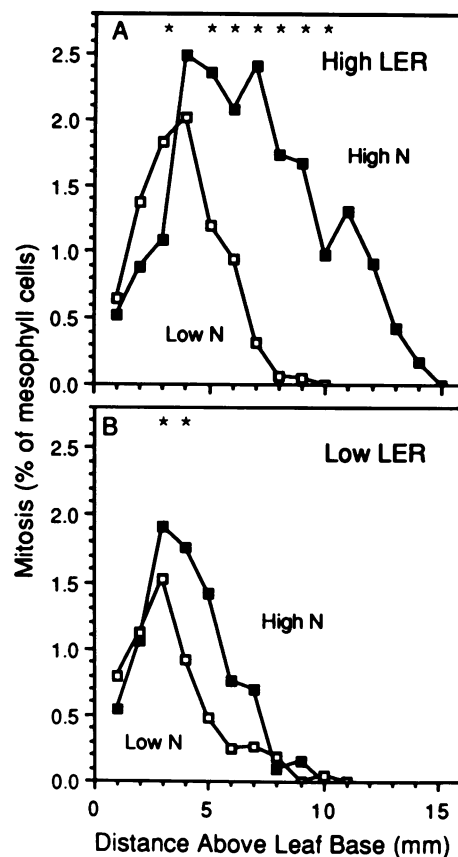
Figure 4. Growth trajectories, or movement of a cell away from the leaf base, for a high-LER genotype of tall fescue. Calculations were made using local displacement velocities measured during either the light or dark phases of the diurnal cycle, at 21°C and 500 μmol s^{-1} m^{-2}. A combination of light and dark displacement velocities beginning with the 9-h dark period (D/L) or the 15-h photoperiod (L/D) were used to simulate growth trajectories for an elongating cell under a diurnal regimen. Displacement velocity data were means of 16 observations. Calculated from Figure 2 of Schnyder and Nelson (20).

Figure 5. Percentages of mesophyll cells in mitosis observed in 10-μm-thick longitudinal sections from the basal 40 mm of elongating leaf blades. Plants were grown under high (336 kg ha^{-1}) or low (22 kg ha^{-1}) N fertilization at 24°C and 500 μmol s^{-1} m^{-2}. Data are means of 24 observations. Significant difference (P < 0.05) between treatments at a given position is indicated by *.

meristem. Therefore, we determined percentage of mesophyll cells in division with high and low N fertilization.

Percentage of mesophyll cells in division was higher for the high- than for the low-LER genotype at every position more than 1 mm above the leaf base (compare the two graphs in Fig. 5). Meristematic activity peaked at 3 or 4 mm above the leaf base, and distal to these positions, cell division was increased with high N. High N also increased the distance from the leaf base over which mesophyll cell division occurred for the high-LER genotype.

We determined the number of mesophyll cells adjacent to the inner wall of a single abaxial epidermal cell at 1-mm intervals (Fig. 6). At 1 mm above the leaf base the ratio was 1.0 and lengths of both cell types were similar. As individual epidermal cells elongated and were displaced farther from the leaf base, the number of adjacent mesophyll cells along their lengths increased. Nitrogen increased the final ratio of mesophyll to epidermal cells similarly for both genotypes, even though epidermal cells were 25% longer for the high-LER genotype. The final mean ratio was 10 mesophyll cells per epidermal cell with low N compared with 14 in leaves of plants receiving high N (Fig. 6).

Volenec and Nelson (29) inferred that increase in the number of epidermal cells undergoing elongation was the major factor which resulted in increased LER with high N. The data presented here confirm that N fertilization of tall fescue also increases cell division in mesophyll cells. High N also increased the ratio of mesophyll to epidermal cells (Fig. 6), which should increase the photosynthetic capacity of N-fertilized leaf blades. Wilson and Cooper (30) found that

photosynthetic capacity of eight populations of perennial ryegrass is negatively correlated with mean cross-sectional area of mesophyll cells. Grasses such as tall fescue respond to an increased supply of N with higher photosynthetic rates (2).

Coordination of Mesophyll Division and Epidermal Elongation

The number of mesophyll cells adjacent to elongating epidermal cells increased over the basal 5 mm of the leaf blade with low N and 8 mm with high N (Fig. 6). At these positions, epidermal cell length was 40 μm for the high-LER genotype and 50 μm for the low-LER genotype, regardless of N treatment (perpendicular intersecting lines, Fig. 1). This indicates a mutual dependence of mesophyll cell division and epidermal cell elongation, in that division of mesophyll cells occurs until epidermal cells reach a given length. These epidermal cell lengths for both genotypes are reached after 50 h of elongation with high N and after 40 h of elongation with low N (perpendicular intersecting lines, Fig. 2). The difference in time required for epidermal cells to reach a length of 40 or 50 μm, which is partially offset by higher rate constants, is due to the 30 to 37% smaller mean epidermal cell length with high N when epidermal cell elongation began (Fig. 2). The higher ratio of mesophyll to epidermal cells with high N could thus
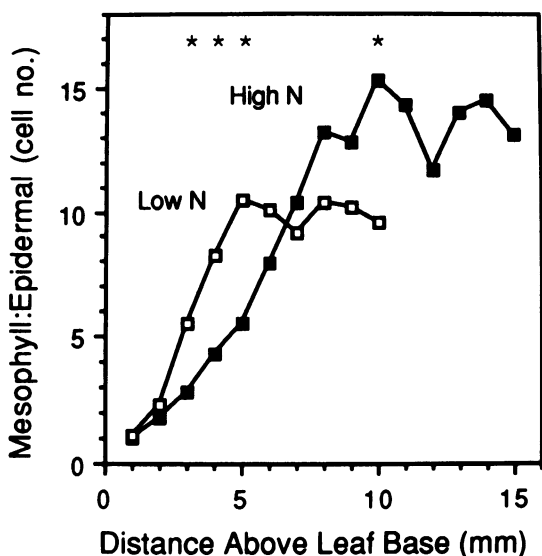
Figure 6. Number of mesophyll cells adjacent to single abaxial epidermal cells in 10-μm-thick longitudinal sections from the basal 40 mm of elongating leaf blades. Plants were grown at 24°C and 500 μmol s^{-1} m^{-2} with high (336 kg ha^{-1}) or low (22 kg ha^{-1}) N fertilization. Data are means of 10 observations, 5 per genotype. Significant difference (P < 0.05) between treatments at a given position is indicated by *.

be associated with the amount of time required for epidermal cells to reach a genetically predetermined length.

We commonly observed a 1:1 mesophyll to epidermal cell ratio near the leaf base, where epidermal cell elongation began (Fig. 6). We assume that up to this position a 1:1 ratio is maintained, and that N increases the number of both cell types similarly. Columns of epidermal and mesophyll cells likely originate from initials at the bases of their respective files. In contrast with mesophyll cells, which continue to divide, epidermal cells elongate as they are displaced from this position. If each mesophyll cell adjacent to an epidermal cell remains meristematic, it takes 3.8 cell cycles to produce 13 additional cells from the division of an initial in the high-N treatment during 50 h, or about 13 h per cycle. Similarly, it takes 3.3 cell cycles to produce 9 additional mesophyll cells in the low-N treatment in 40 h, or about 12 h per cycle. These calculated cell cycles for the two treatments are surprisingly similar and within a commonly reported range for leaf meristem cells (5). The similarity of these calculated cell cycles indicates that increase in mesophyll cell number with high N is more likely due to additional cycles of division than to a shortening of the cell cycle length. The additional cycles would extend mitosis over a greater distance from the leaf base (Fig. 5).
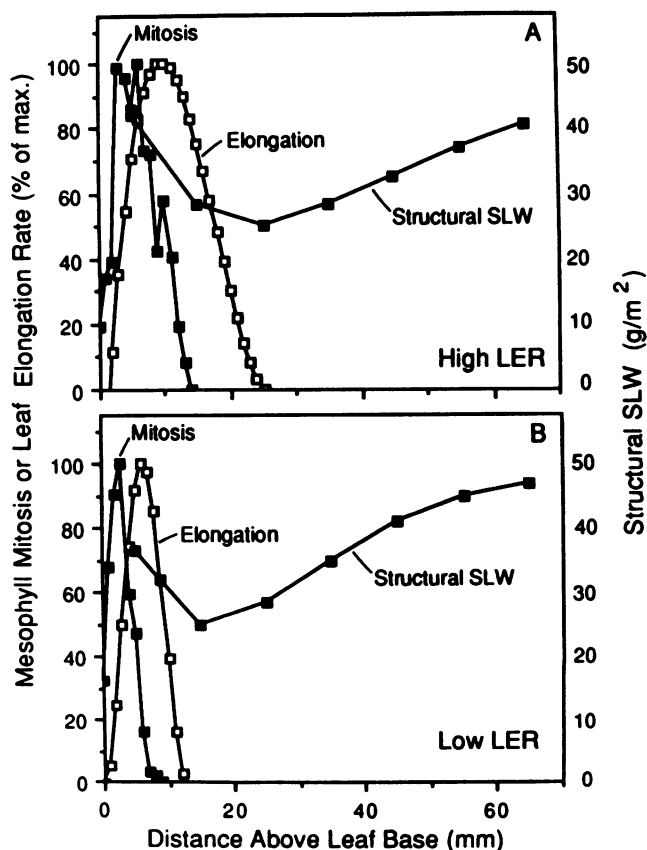
Spatial Model of Cell Development

A longitudinal gradient of development exists in an elongating grass leaf blade, and successive segments of the leaf blade represent the developmental course of cells produced in the leaf blade intercalary meristem. Such a gradient has been used to study the development of photosynthetic capacity in wheat (*Triticum aestivum*) (7) and other gramineous crops. In tall fescue, this system has been used to study leaf elonga-

tion (20, 28, 29), carbohydrate metabolism (21), and secondary cell wall deposition (17).

Our understanding of the physiological relationships within the developmental gradient at the base of the tall fescue leaf blade is aided by a spatial model in which data on cell division, segmental elongation rate (28), and deposition of structural material (17) are presented together (Fig. 7). We appreciate the histological complexity of the leaf blade and are fully in agreement with Allan and Trewavas (1) concerning problems inherent in reducing a complex system such as that by which leaf growth occurs to a simple model. In practice, however, we find this model of cell development useful as a conceptual framework.

In this model, based on high N treatments for both genotypes, mesophyll cell division occurs at the base of the leaf blade. While epidermal cell elongation begins within 2 mm of the leaf base, mesophyll cell division continues over 10 (low LER) or 14 mm (high LER). Structural SLW is highest in the cell division region and decreases during epidermal cell elongation because water influx exceeds deposition of dry matter (20). Structural SLW is minimal where cells become fully expanded, and increases linearly with deposition of secondary cell wall material in structural tissue (16). Increase

Figure 7. Spatial distribution of cell development in leaf blades of genotypes of tall fescue differing in LER. Data are (a) percent of mesophyll cells in mitosis for leaf blades at high (336 kg ha^{-1}) N fertilization (this paper), (b) leaf elongation rate (mm mm^{-1} d^{-1}) at 24°C and 500 μmol s^{-1} m^{-2} (28) (a and b presented as percent of maximum), and (c) structural SLW (dry weight minus water-soluble carbohydrate) as weight per unit area for leaf blades at 25°C and 490 μmol s^{-1} m^{-2} (17).

in structural SLW occurs entirely within the whorl of older leaf sheaths (17).

In general, cells occur in ordered columns oriented along the longitudinal axis of the leaf blade. The most protracted and extensive meristematic activity occurs in the mesophyll tissue, eventually resulting in 15 to 25 times more mesophyll than epidermal cells per unit area of mature leaf blade (JW MacAdam, CJ Nelson, unpublished data). It is evident that epidermal cell elongation is coordinated with mesophyll cell division, as shown here, and with elongation of cells comprising the vascular bundles, because radial columns of mesophyll cells are in contact with cells of both the epidermis and those of the bundle sheath throughout leaf elongation and maturation (JW MacAdam, CJ Nelson, unpublished data).

Only certain tissues (epidermal, vascular, and fiber) elongate longitudinally. The mesophyll is longitudinally continuous only within the lower 3 to 7 mm of the leaf blade, after which radial columns of mesophyll cells begin to separate, and intercellular air spaces are formed by continued epidermal and bundle sheath elongation. Mesophyll cells do, however, remain radially continuous after air spaces form, maintaining symplastic (plasmodesmatal) connections from the bundle sheath to either the upper or lower epidermis.

Leaf elongation occurs within the basal 15 mm in the low-LER genotype and 25 mm in the high-LER genotype (Fig. 7). The additional 10 mm in the high-LER genotype results from both a greater number of epidermal cells (28) and an increased duration of cell elongation (Figs. 2 and 3). This elongation region can be delimited by measuring epidermal cell lengths (Fig. 1) or by marking an intact growing leaf blade to determine the original length of the region in which growth subsequently occurs (28). The shape of the segmental elongation rate curves in Figure 7 (A and B) might suggest that cell elongation is also a process which increases and then decreases in rate. However, as epidermal cells are displaced through the elongation zone, increase in their length causes the number of cells per unit leaf length to decrease with distance from the leaf base. This increase in cell length occurs at a constant exponential rate, as indicated in Figs. 2 and 3. Leaf segmental elongation rate therefore declines from the maximal rate with distance because cell number per unit leaf length decreases rapidly at the distal end of the elongation region.

Since the cell is the unit of metabolic activity, any metabolic process which occurs during elongation growth should be considered in light of the fact that the number of cells mm^{-1} decreases with distance from the leaf base. In the case of tall fescue leaf blades, epidermal cell density declines linearly. Silk et al. (25) discuss this caveat for K$^+$ deposition in maize (Zea mays) root tips. They point out that although K$^+$ deposition rate falls in the distal half of the growth zone, absorption of K$^+$ per cell increases throughout cell expansion.

The deposition of structural material (dry weight less water-soluble carbohydrate), which occurs during cell elongation, utilizes about half the carbohydrate imported into the elongation region (20) and serves to maintain growth of the cell wall and supply other metabolic requirements during increase in cell volume. Content of structural material calculated on a unit area basis (structural SLW) is high at the base of the leaf blade, where cells are small, dense, and mesophyll cells are

actively dividing, and decreases coincidentally with epidermal cell elongation and formation of air spaces in the mesophyll. Near the position of cessation of epidermal cell elongation, structural SLW begins to increase. This increase corresponds with secondary cell wall deposition in the inner bundle sheath, fiber cells, and lower epidermal cells of the leaf blade (16). Increase in structural SLW occurs over 2 to 3 d, and rates of accumulation have been estimated as 5.2 and 8.5 g d^{-1} m^{-2} for leaf blades of the low-LER and high-LER genotypes, respectively (17). The total amount of carbohydrate required for secondary cell wall deposition has not been quantified, but would be substantial. Development of photosynthetic enzymes also occurs in this region (7).

In conclusion, high N fertilization increased the percentage of mesophyll cells in division. The ratio of mesophyll to epidermal cells also increased with N fertilization, indicating that N had an even greater influence on mesophyll than epidermal cell division. Nitrogen increased the time required for epidermal cells to reach a predetermined length by 25%, and the number of subtending mesophyll cells was increased by 40%, while final epidermal cell length remained unchanged. Rates of epidermal cell elongation were found to be exponential and genotypes maintained an approximate 20-h difference in duration of elongation under environmentally altered elongation rates. The rapid transition from exponential increase in cell length to constant final cell length implies an abrupt cessation of cell growth.

ACKNOWLEDGMENTS

LITERATURE CITED

1. **Allan EF, Trewavas A** (1986) Tissue-dependent heterogeneity of cell growth in the root apex of Pisum sativum. Bot Gaz **147**: 258–269
2. **Bolton JK, Brown RH** (1980) Photosynthesis of grass species differing in carbon dioxide fixation pathways. V. Response of Panicum maximum, Panicum milioides, and tall fescue (Festuca arundinacea) to nitrogen nutrition. Plant Physiol **66**: 97–100
3. **Carmona MJ, Cuadrado A** (1986) Analysis of growth components in Allium roots. Planta **168**: 183–189
4. **Cohen CJ, Chilcote DO, Frakes RV** (1982) Leaf anatomy and stomatal characteristics of four tall fescue selections differing in forage yield. Crop Sci **22**: 704–708
5. **Dale JE** (1976) Cell divisions in leaves. In MM Yeoman, ed, Cell Division in Higher Plants. Academic Press, London, pp 315–345
6. **Dencheva A, Klisurska D** (1982) Interaction between peroxidase and IAA-oxidase in the course of growth and differentiation of plant cells. Physiol Veg **20**: 385–394
7. **Ellis JR, Jellings AJ, Leech RM** (1983) Nuclear DNA content and the control of chloroplast replication in wheat leaves. Planta **157**: 376–380
8. **Epstein E** (1972) Mineral Nutrition of Plants: Principles and Perspectives. Wiley, New York
9. **Erickson RO** (1976) Modeling of plant growth. Annu Rev Plant Physiol **27**: 407–434
10. **Gandar PW** (1980) The analysis of growth and cell production in root apices. Bot Gaz **141**: 131–138
11. **Goodwin PB, Erwee MG** (1983) Hormonal influences on leaf growth. In JE Dale, FL Milthorpe, eds, The Growth and Functioning of Leaves. Cambridge University Press, New York, pp 207–232

12. **Green PG** (1976) Growth and cell pattern formation on an axis: critique of concepts, terminology, and modes of study. Bot Gaz **137**: 187–202

13. **Jensen WA** (1962) Botanical Histochemistry. Freeman, San Francisco

14. **Jones RJ, Nelson CJ, Sleper DA** (1979) Seedling selection for morphological characters associated with yield of tall fescue. Crop Sci **19**: 631–634

15. **Jones RL** (1983) The role of gibberellins in plant cell elongation. CRC Crit Rev Plant Sci **1**: 23–47

16. **MacAdam JW, Nelson CJ** (1986) Differentiation of fiber cells in tall fescue leaf blades. Agron Abstr **1987** 98–99

17. **MacAdam JW, Nelson CJ** (1987) Specific leaf weight in zones of cell division, elongation and maturation in tall fescue leaf blades. Ann Bot **59**: 369–376

18. **Ormrod JC, Francis D** (1985) Effects of light on the cell cycle in the shoot apex of *Silene coeli-rosa* L. on the first day of floral induction. Protoplasma **124**: 96–105

19. **Poskuta JW, Nelson CJ, Coutts JH, Vassey TL** (1984) Effect of gibberellic acid on photosynthesis and leaf characteristics of tall fescue. Curr Topics Plant Biochem Physiol **3**: 174

20. **Schnyder H, Nelson CJ** (1988) Diurnal growth of tall fescue leaf blades. I. Spatial distribution of growth, deposition of water and assimilate import in the elongation zone. Plant Physiol **86**: 1070–1076

21. **Schnyder H, Nelson CJ, Spollen WG** (1988) Diurnal growth of

tall fescue leaf blades. II. Dry matter partitioning and carbohydrate metabolism in the elongation zone and adjacent expanded tissue. Plant Physiol **86**: 1077–1083

22. **Scott BIH, Gulline H, Pallaghy CK** (1968) The electrochemical state of cells of broad bean roots I. Investigations of elongating roots of young seedlings. Aust J Biol Sci **21**: 185–200

23. **Silk WK** (1984) Quantitative descriptions of development. Annu Rev Plant Physiol **35**: 479–518

24. **Silk WK, Erikson RO** (1979) Kinematics of plant growth. J Theor Biol **76**: 481–501

25. **Silk WK, Hsiao TC, Diedenhofen U, Matson C** (1986) Spatial distributions of potassium solutes, and their deposition rates in the growth zones of the primary corn root. Plant Physiol **82**: 853–858

26. **Smith D** (1981) Removing and analyzing total nonstructural carbohydrates from plant tissue. Wis Agric Exp Sta Res Rep R2107

27. **Thomas H** (1983) Analysis of the nitrogen response of leaf extension in *Lolium temulentum* seedlings. Ann Bot **51**: 363–371

28. **Volenec JJ, Nelson CJ** (1981) Cell dynamics in leaf meristems of contrasting tall fescue genotypes. Crop Sci **21**: 381–385

29. **Volenec JJ, Nelson CJ** (1983) Responses of tall fescue leaf meristems to N fertilization and harvest frequency. Crop Sci **23**: 720–724

30. **Wilson D, Cooper JP** (1967) Assimilation of *Lolium* in relation to leaf mesophyll. Nature **214**: 989–992