

# Nitrogen Use within the Growing Leaf Blade of Tall Fescue<sup>1</sup>

Francois Gastal<sup>2\*</sup> and Curtis J. Nelson

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

Leaf elongation rate (*LER*) of grasses depends on N supply and is expressed mostly through cell production, whereas most N in mature leaf tissues is chloroplastic. Our objective was to evaluate a possible competition for N between cell production and chloroplast development processes, utilizing the gradient of cell development along the leaf growth zone of tall fescue (*Festuca arundinacea* Schreb.). Under the two contrasting N regimes, total N content was highest in the cell production zone, declined sharply as cells elongated, and remained relatively constant in more distal positions, at values close to those measured in mature tissues. A similar pattern was found for N in proteins and nucleic acids that were not soluble in 80% ethanol. Content of N compounds soluble in 80% ethanol was higher in the cell production and elongation zones than in mature parts of the leaf.  $\text{NO}_3^-$  N content was low in the cell production zone and increased in the cell elongation zone for high-N plants. The deposition rate of total N in the growth zone was much higher with plants in high N than in those shifted to no N. For both N regimes, most N was deposited during cell production and early cell elongation. Little N was deposited during cell maturation where ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) was being actively synthesized. This suggests that synthesis of Rubisco, and probably other chloroplastic proteins, occurs largely from recycling of N that was previously incorporated into proteins during cell production. Thus, Rubisco content in mature tissues is more closely associated with N deposited during cell production than with N deposited during its biosynthesis.

*LER* of grasses is very responsive to N application, often increasing 3- to 4-fold in field studies (Gastal et al., 1992). N also stimulates photosynthetic rate per unit leaf area, but to a much lesser extent (Chapin et al., 1988; Just et al., 1989; Nelson et al., 1992). These relationships suggest that enhanced leaf growth rate is the primary response to N. In more detailed experiments (Volenc and Nelson, 1984), *LER* was stimulated more efficiently with high-N than with low-N nutrition, but more water-soluble carbohydrates accumulated under low-N conditions in both the leaf growth zone and mature leaf tissues. These results strongly suggest that carbohydrate availability does not limit leaf elongation under low-N conditions, leading us to focus in more detail on the role of N in the leaf growth zone.

<sup>1</sup> Supported partially by a North Atlantic Treaty Organization grant to F.G., the Missouri Agricultural Experiment Station, and a U.S. Department of Agriculture-National Research Initiative grant to C.J.N.

<sup>2</sup> Permanent address: Institut National de la Recherche Agronomique-Station d'Ecophysiologie des Plantes Fourragères, 86600 Lusignan, France.

\* Corresponding author; fax 33-49-55-60-68.

The leaf growth zone of grasses exhibits sequential developmental patterns of cell production, cell enlargement, and cell maturation with distance from the leaf base (MacAdam et al., 1989). This longitudinal gradient of cell development was used to show that stimulation of *LER* by N is due mostly to an increase in cell production (Volenc and Nelson, 1984), with little effect on final cell size or rate of epidermal cell elongation (MacAdam et al., 1989). Subsequently, most N in mature leaf tissue is associated with chloroplastic components (Chapin et al., 1987), and chloroplast development in grass leaves occurs mainly in the cell maturation zone (Dean and Leech, 1982).

These considerations raised the question of possible competition for N imported in the growing zone between requirements for cell production and for chloroplast development. Therefore, we investigated N metabolism in the leaf growth zone of tall fescue (*Festuca arundinacea* Schreb.), and compared N accumulation patterns for various leaf developmental processes in response to altered N supply.

## MATERIALS AND METHODS

### Plant Culture

Three experiments were conducted (experiments I, II, and III) using similar protocols. Individual tillers of a tall fescue (*Festuca arundinacea* Schreb.) genotype with high *LER* (MacAdam et al., 1989) were vegetatively propagated and planted in 11-cm diameter × 15-cm deep pots containing perlite. After approximately 2 months in a greenhouse, top-growth was clipped to leave a 5-cm stubble, and plants were transferred to a growth chamber. Plants were grown under constant air temperature ( $20 \pm 1^\circ\text{C}$ ) and RH ( $70 \pm 5\%$ ), and under continuous light ( $450 \mu\text{m}^{-2} \text{s}^{-1}$  PPFD). Continuous light allowed leaves to elongate at a constant rate (Schnyder and Nelson, 1989), avoiding day/night transitions and simplifying calculation of net deposition rates. The *LER*s in our high-N conditions were similar to those reported earlier for plants grown under day/night cycles (MacAdam et al., 1989; Schnyder and Nelson, 1989).

Plants received nutrient solution twice a day [macroelements: 6 mM  $\text{KNO}_3$ , 2 mM  $\text{Ca}(\text{NO}_3)_2$ , 0.2 mM  $\text{NH}_4\text{H}_2\text{PO}_4$ , 1 mM  $\text{MgSO}_4$ , 2.9 mM  $\text{NH}_4\text{NO}_3$ ; microelements: 50  $\mu\text{M}$  KCl, 25  $\mu\text{M}$   $\text{H}_3\text{BO}_3$ , 2  $\mu\text{M}$   $\text{MnSO}_4$ , 2  $\mu\text{M}$   $\text{ZnSO}_4$ , 0.5  $\mu\text{M}$   $\text{CuSO}_4$ , 0.5  $\mu\text{M}$   $\text{H}_2\text{MoO}_4$ , 40  $\mu\text{M}$  Fe-citrate]. After about 4 weeks the plants were clipped a second time and separated into two sets. One

Abbreviations: 0N, no-N nutrient solution; HN, high-N nutrient solution; *LER*, leaf elongation rate; *REGR*, relative elemental growth rate.

set (HN) continued on the same nutrient solution. The second set (0N) received a solution modified to be without N (macroelements: 1 mM MgSO<sub>4</sub>, 0.2 mM K<sub>2</sub>HPO<sub>4</sub>, 2 mM CaSO<sub>4</sub>, 1 mM K<sub>2</sub>SO<sub>4</sub>; microelements: same as previously described). All plants were sampled 5 weeks after the second clipping.

### Sampling Procedure

Tillers that had an elongating blade less than half the length of the previous mature blade were sampled. The elongating leaf blade was removed from surrounding sheaths, cut into segments, and immediately placed on ice. In experiments I and II, 12 sequential segments of 3 mm (0–36 mm) were obtained, starting from the leaf base, followed by 12 sequential segments of 5 mm (36–96 mm). Two segments of 10 mm were taken from the exposed part of the growing blade (115–125 and 145–155 mm above the base), and two segments were taken from the blade of the previous mature leaf (one segment 10 mm above the ligule and the other in the middle of the blade). In experiment III, the same sampling procedure was followed except the basal 6 mm of the elongating leaf was cut into 1-mm segments to gain more detail. Segments obtained from different tillers on plants in the same N regime were combined according to position. Fresh weight was recorded and segments were oven-dried at 70°C unless specified differently and weighed.

### Leaf Elongation and Distribution of Growth

Leaf length was measured daily during the 4 d preceding sampling and used to calculate *LER*. Longitudinal distribution of growth was assessed on 15 tillers by making fine holes in the growing part of the expanding blade (experiments I and II) and measuring the displacement of these holes within a 6- to 8-h period (Schnyder et al., 1987). The *REGR* (mm mm<sup>-1</sup> h<sup>-1</sup>) was computed according to Erickson and Sax (1956):

$$REGR = \Delta d/d \times 1/\Delta t$$

where *d* is initial distance between holes,  $\Delta d$  is the increase in distance between holes during the period, and  $\Delta t$  is the time period. Since *LER* was decreased by making holes, *REGR* of each segment was corrected by the ratio of *LER* for leaves with holes to *LER* for a matched population of undisturbed leaves (Schnyder et al., 1987).

As an alternative to making holes to assess spatial distribution of growth, 15 additional tillers were selected (experiments II and III) for analysis of epidermal cell length. The bases of the growing leaves were exposed as above, and imprints of epidermal cells were taken as described by Schnyder et al. (1990). Lengths of epidermal cells adjacent to the row with stomata were measured on the imprints along the leaf growth zone. *REGR* was derived from cell lengths according to Schnyder et al. (1990).

### N Analysis

For experiments I and II, segment samples of 5 to 8 mg dry weight (consisting of 10 and 15 segments for HN and 0N plants, respectively) were extracted with hot 80% ethanol for 15 min and filtered three times in succession. The ethanol-

insoluble fraction contained large polypeptides and nucleic acids, whereas the soluble fraction included mainly small peptides, mineral N, and free amino acids. The N in each fraction was quantified with a micro-Kjeldahl procedure using K<sub>2</sub>SO<sub>4</sub>/HgO (15 and 0.7 g, respectively) as a catalyst. Ammonium generated from digestion was microdiffused and quantified by the indophenol method (Chaney and Marbach, 1962). Digestion of the soluble N fraction was preceded by a reduction step to fully include NO<sub>3</sub><sup>-</sup> in the N determination (Guiraud and Fardeau, 1977).

The NO<sub>3</sub><sup>-</sup> content of the ethanol-soluble fraction was evaluated by the sulfanilamide method after reduction to NO<sub>2</sub><sup>-</sup> (Technicon TRACCS 800 Autoanalyzer, Buffalo Grove, IL). Total N content was calculated as the sum of N contents in ethanol-soluble and insoluble fractions. Reduced soluble N content was calculated as the difference between ethanol-soluble N and NO<sub>3</sub><sup>-</sup> N contents. For experiment III, total N only was assessed by utilizing an elemental analyzer (Carlo-Erba, Milan, Italy, model NA1500). The limited amount of tissue available allowed us to repeat the whole-N analyses only twice for each experiment.

### Nitrate Reductase Assay

In vivo nitrate reductase activity was assessed according to Blevins et al. (1976). Briefly, 15 intact segments, 3 or 5 mm long, were incubated in sealed tubes at 30°C for 15 min in a potassium-phosphate buffer, pH 7.5, with exogenous NO<sub>3</sub><sup>-</sup> (0.05 mM KNO<sub>3</sub>). N gas was bubbled through the medium for 2 min at the beginning of the incubation period. Nitrite was assayed in the medium at the end of the incubation period.

### Rubisco Determination

Rubisco was assayed by rocket immunoelectrophoresis (Joseph and Randall, 1981) in experiments II and III. Ten to 15 segments, 3 or 5 mm long, were homogenized in a buffer (0.1 mM Tricine, 20 mM MgCl<sub>2</sub>, 10 mM NaHCO<sub>3</sub>, 1 mM DTT, pH 8.2) containing 0.5% Triton X-100. The homogenate was centrifuged. The protein content of the supernatant was determined according to Bradford (1976). A diluted aliquot of the homogenate was deposited on the agarose IgG gel. Rubisco was quantified by comparing peak height of the unknown leaf samples with a Rubisco standard purified from tall fescue.

### Net Deposition Rate of N

Net deposition rate (*D*, μg mm<sup>-1</sup> h<sup>-1</sup>) of N or dry matter was calculated according to Silk (1984) as:

$$D = (\Delta P/\Delta t) + VD \times (\Delta P/\Delta x) + REGR \times (P)$$

where *P* is density of N on a leaf length basis (μg mm<sup>-1</sup>), *t* is time (h), *VD* is velocity of displacement of a segment (mm h<sup>-1</sup>) calculated as the sum of *REGRs* from the leaf base to the considered segment, and *x* is distance (mm). The term  $\Delta P/\Delta t$  was neglected because growth was in a steady state due to continuous lighting, and  $\Delta P/\Delta x$  represented the gradient of density of N along distance. Unless specified differently,

**Table I.** *LER, REGR, and growth parameters of the leaf elongation zone of tall fescue receiving two N regimes*

	N Regime	LER	Final Cell Length	Zone Length	Maximum REGR	Elongation Time
		$mm\ h^{-1} \pm SD\ (n)$	$\mu m \pm SD\ (n)$	mm	$mm\ mm^{-1}\ h^{-1} \pm SD\ (n)$	h
Experiment I	High N	$1.79 \pm 0.24\ (30)$	$323 \pm 29\ (10)$	30	$0.086 \pm 0.024\ (10)$	70
	No N	$0.44 \pm 0.12\ (30)$	$312 \pm 35\ (10)$	15	$0.078 \pm 0.053\ (08)$	82
Experiment II	High N	$1.21 \pm 0.30\ (30)$	$319 \pm 29\ (08)$	46	$0.051 \pm 0.009\ (07)$	135
	No N	$0.78 \pm 0.26\ (30)$	$282 \pm 27\ (06)$	33	$0.049 \pm 0.011\ (10)$	143
Experiment III	High N	$1.21 \pm 0.25\ (30)$	$320 \pm 24\ (12)$	35	$0.062 \pm 0.015\ (10)$	92
	No N	$0.26 \pm 0.12\ (30)$	$250 \pm 21\ (08)$	18	$0.025 \pm 0.011\ (10)$	140

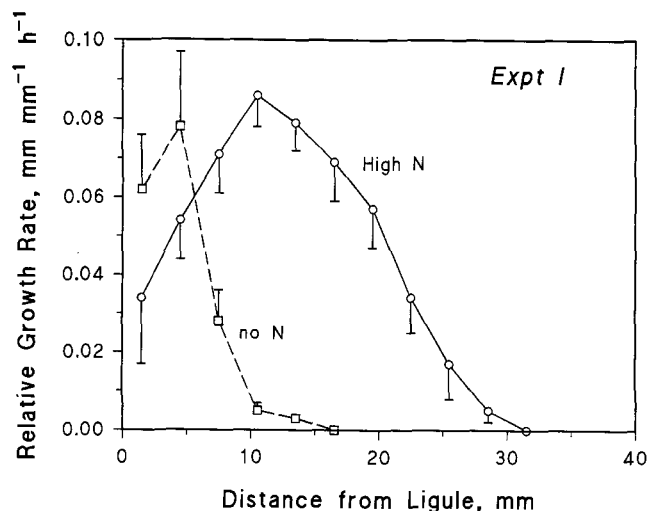
conclusions from all three experiments were similar. Thus, data from all experiments are not presented.

## RESULTS AND DISCUSSION

### Leaf Elongation and Distribution of Growth

LER with HN was 4- to 5-fold higher than with ON in experiments I and III, and 1.6 times higher in experiment II (Table I). Plants were larger in experiment II, and likely recycled more N from older tissue under the ON regime. Patterns of growth distribution for HN plants, which were similar to those reported by Schnyder et al. (1987), showed maximal REGR in the middle part of the growth zone (Fig. 1). In all cases, the growth zone was shorter for ON than HN plants (Table I). The N regime had relatively little effect on maximum REGR except for ON plants in experiment III, which had very low LER.

The final length of epidermal cells was unaffected (experiment I) or slightly increased (experiments II and III) by high N nutrition. The time necessary for a cell to grow from its initial to its final length was decreased by high N, but to a relatively small extent compared with the effect of N on LER (Table I). Since N had only a small effect on cell size and cellular growth rates, the major effect of N on leaf elongation occurred through altered cell production. The leaf growth

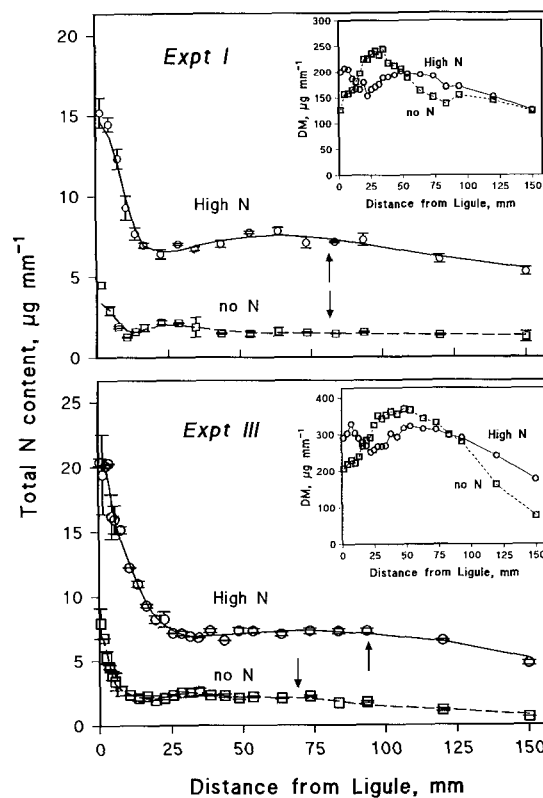


**Figure 1.** Distribution of REGRs in the elongation zone of leaf blades for plants receiving two contrasting N regimes. Error bars represent SE ( $n = 10$ ).

zone, therefore, is longer with HN than ON mainly because a larger number of epidermal cells are undergoing a similar rate of expansion at the same time. These data confirm those from MacAdam et al. (1989).

### N Composition

Total N content, expressed on a segment length basis, was high near the ligule, then declined up to 25 mm from the ligule for HN plants and to 10 mm for ON plants, i.e. throughout most of the elongation zone for both N regimes (Fig. 2). Distal to the elongation zone, N content increased



**Figure 2.** Content of total N (on a segment length basis) along the growing leaf blade of plants receiving two contrasting N regimes. Vertical arrow indicates location where leaf blade emerges to light (same in the following figures) and error bars represent SE ( $n = 4$ ). Dry matter (DM) content on a segment length basis is shown in the insets.

slightly (HN) or remained constant (0N), then declined beyond 60 to 80 mm from the ligule. Data beyond 60 mm have to be interpreted with respect to decreases in both width and dry matter per segment length.

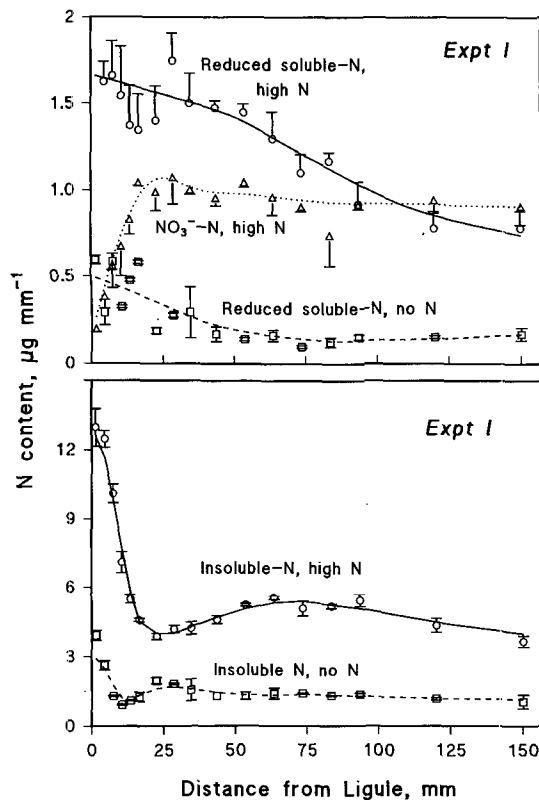
Within the elongation zone, width of the segments increased by 50 to 80% depending on the experiment and N regime (data not shown), whereas dry matter per segment length at high N decreased to a limited extent (see insets in Fig. 2) due to expansive dilution and vacuolation (MacAdam and Nelson, 1987). Therefore, the large decline in N content per segment length observed in the elongation zone (Fig. 2) is explained partly by a decrease in total dry matter (HN plants), but principally reflects a higher N content, on a dry matter basis, of immature cells compared with expanded cells. For example, total N concentrations of HN and 0N plants were 7.5 and 3% of the dry matter, respectively, at the leaf base, and 3.5 and 1%, respectively, at the end of the elongation zone.

In the 25- to 30-mm distal to the end of the elongation zone, leaf width was approximately constant and dry matter per segment length increased regularly due to secondary cell wall deposition (MacAdam and Nelson, 1987). In the 50- to 80-mm distal to the ligule, both leaf width and dry matter per segment length decreased, indicating that the decline in N content observed on a segment length basis in this part of the leaf was due primarily to blade narrowing. The 0N plants had lower total N content than HN plants along the entire blade length, due in part to a 20% narrower leaf width under the 0N regime, but due mostly to lower N concentration on a dry matter basis for the 0N segments.

For both N rates, content of reduced soluble N was higher throughout the zone of cell expansion compared with more distal segments (Fig. 3). The reduced soluble N fraction included free amino acids, nucleic acids, and small peptides. Its higher content within the elongation zone probably reflects intensive N metabolism or accumulation of one or several N solutes.

No  $\text{NO}_3^-$  was detected in 0N plants throughout the entire growing leaf (not shown). This was expected, since the  $\text{NO}_3^-$  source had been withdrawn. In contrast with reduced soluble N,  $\text{NO}_3^-$  N content in HN plants was low in the first segments (Fig. 3), then increased in the zone of rapid cell elongation to a content that was approximately stable in more mature parts of the leaf. Nitrate accumulates mainly in vacuoles, where its concentration has been estimated to be up to 100 times higher than in cytoplasm (Granstedt and Huffaker, 1982; Robin et al., 1983). Cells located at the base of the leaf are in early stages of development and probably have relatively small vacuoles that limit  $\text{NO}_3^-$  accumulation.

In contrast to the soluble N fraction, patterns of insoluble N content were similar to those observed for total N, i.e. a steep decline along the growth zone with concentration slightly increasing (HN) or remaining stable (0N) in more distal segments (Fig. 3). This insoluble N fraction contains both polypeptides and nucleic acids. In mature tissues, nucleic acids constitute only a small part of leaf N. Because of the gradient of cell differentiation along the growth zone, however, the small cells at the base presumably contain a larger proportion of total N in nucleic acids than do more distal cells. The N content by weight is near 16% for both proteins



**Figure 3.** Contents of reduced soluble N,  $\text{NO}_3^-$  N, and insoluble N along the growing leaf blade of plants receiving two contrasting N regimes. Error bars represent SE ( $n = 4$ ).

and nucleic acids, and since there was very little  $\text{NO}_3^-$  in the first segment of the growth zone, the total N concentration of 7.5% on a dry matter basis in HN plants means that nearly half the dry matter in the first segment is protein and nucleic acid material.

#### Nitrate Reductase Activity

No  $\text{NO}_2^-$  was produced under anoxia in either N rate at leaf positions below 100 mm from the ligule, and none was produced at higher positions with 0N (data not shown). With HN, however, the  $\text{NO}_2^-$  production rate increased linearly from 0 at 100 mm to about  $2.0 \mu\text{M g}^{-1}$  fresh weight  $\text{h}^{-1}$  at 150 mm, a rate similar to that for the previous fully developed leaf (not shown).

Induction of nitrate reductase activity in leaves is dependent on both  $\text{NO}_3^-$  and light, the effect probably being phytochrome mediated (Beevers and Hageman, 1980). In our study,  $\text{NO}_3^-$  was present several hours before segments emerged above the sheath in HN plants, yet nitrate reductase activity did not appear until segments were exposed to light, which suggests a very localized control of induction by light. The absence of  $\text{NO}_3^-$  reduction in the leaf growth zone implies that all N incorporated into these enclosed tissues is imported in a reduced form from mature tissues. This is consistent with the hypothesis drawn by Pearen (1991) based

on tissue construction costs within the leaf growth zone of the same genotype of tall fescue.

### Net Deposition Rates of N

Irrespective of N regime, there was a large import and deposition of total N in the leaf base, where cell production and cell elongation occur (Fig. 4). In contrast, net deposition rate of N was minimal in the maturation zone, i.e. above 30 mm from the base. Deposition rates for 0N plants were much lower than for HN plants, probably leading to both a lower rate of leaf elongation and a lower total N content.

Deposition of  $\text{NO}_3^-$  occurred only in the HN treatment (0N plants did not receive any  $\text{NO}_3^-$ ), and the rate was highest in the zone of maximal REGR (Fig. 5), i.e. approximately 10 to 15 mm from the base, and was near zero in the cell maturation zone. Deposition of reduced soluble N occurred at similar rates throughout the leaf elongation zone and was decreased to near zero when cell elongation ceased. In contrast, deposition of the insoluble N fraction was greatest in the basal 20 mm of the leaf, i.e. the basal 60% of the growth zone. Very low, but significant, positive deposition rates of insoluble N were recorded in the maturation zone, up to the location where segments were exposed to light. Integration of deposition rates on a distance basis for the HN treatment indicated that 70% of the insoluble N was deposited within the basal 30 mm, the remainder being deposited between 30 and 110 mm (data not shown). Nearly 100% of the insoluble N was deposited within the basal 15 mm for the 0N treatment.

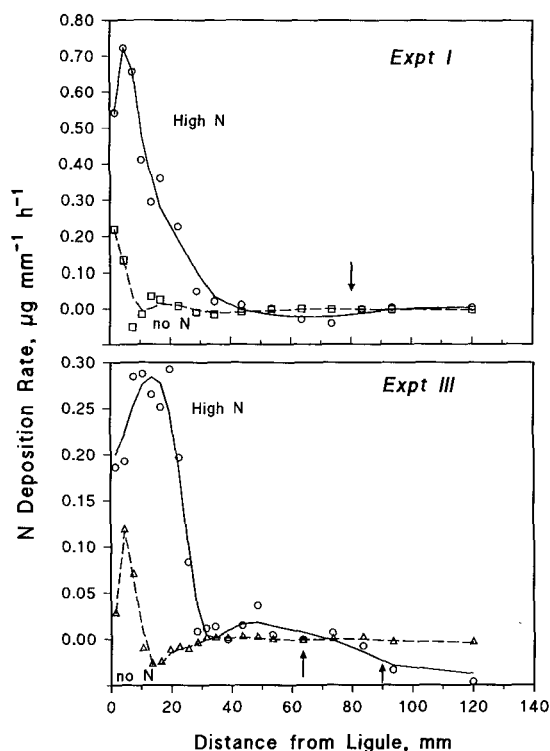


Figure 4. Net deposition of total N in the growing leaf blade of plants receiving two contrasting N regimes.

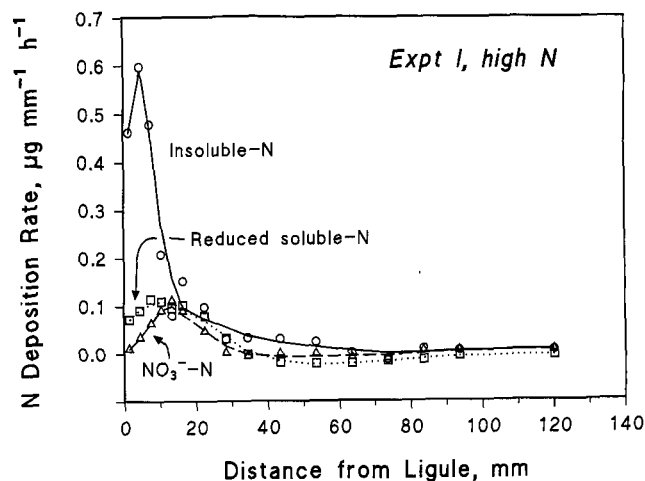


Figure 5. Net deposition of reduced soluble N,  $\text{NO}_3^-$  N, and insoluble N in the growing blade of plants receiving high N.

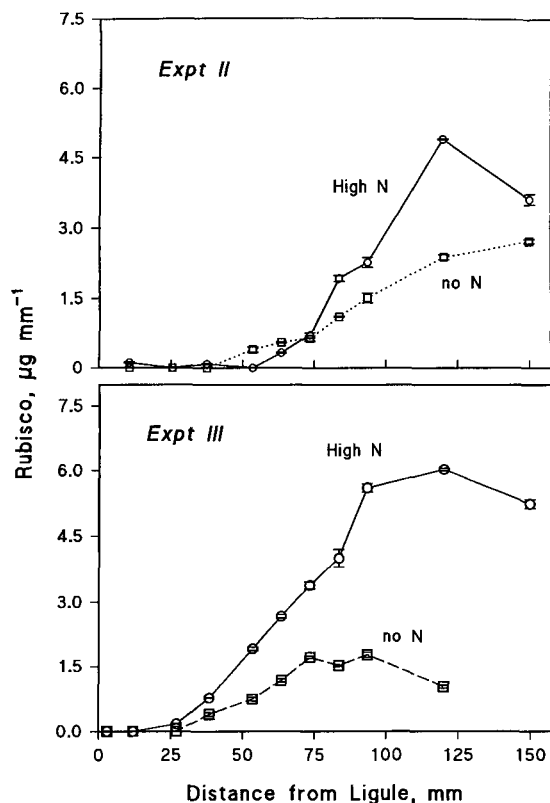
Net deposition rates need to be interpreted in conjunction with metabolic aspects of each form of N. Since  $\text{NO}_3^-$  is not reduced in the growth zone, the net deposition rate probably represents the rate of  $\text{NO}_3^-$  import into the various segments. Conversely, the net deposition of the soluble N fraction represents the net pool resulting from differences in rate between import and incorporation into nucleic acids and proteins. Our broad assessment of the soluble N fraction does not indicate accumulation of particular amino acids, such as Pro, as has been reported for *Lolium perenne* (Thomas, 1991). Further investigation of the form of imported soluble N is needed.

Net deposition of the insoluble N fraction, which represents the net quantity of N incorporated into nucleic acids and polypeptides, was of particular interest. Deposition occurred largely at the very base of the leaf, and the maximum was observed nearer the base than were maxima for other N fractions. Cell production occurs largely in the basal 10 mm of the tall fescue leaf blade (MacAdam et al., 1989). A part of this deposition is necessarily linked to synthesis of nucleic acids and polypeptides during mitosis. Only a limited deposition occurred in older segments of mature cells, however, where N associated with nuclear components is small compared with N incorporated into polypeptides. Thus, a large part of the deposition of insoluble N between 0 and 30 mm was likely associated with polypeptide synthesis.

### Rubisco Synthesis

The preponderance of early N deposition raised the question of localization of chloroplastic protein synthesis in the growth zone. Rubisco was quantitatively assayed as a major chloroplastic polypeptide. Very low amounts of Rubisco were detected near the leaf base (Fig. 6). Rapid Rubisco synthesis began after cells stopped elongating, and was mostly completed when segments emerged above the whorl. As expected, Rubisco represented 30 to 40% of total proteins in mature tissues in both N regimes (not shown).

The localization of Rubisco synthesis described here agrees



**Figure 6.** Content of Rubisco on a segment length basis in the growing leaf blade of plants receiving two contrasting N regimes. Error bars represent SE ( $n = 2$ ).

with data on wheat seedlings from Dean and Leech (1982), who also reported that Rubisco appeared after cells slowed in elongation and before tissue emerged above the whorl. Also, chloroplast maturation of *Avena* leaves, based on chloroplast volume, number of thylakoids, and acquisition of photosynthesis capability, occurred in this same distal portion (Nakamura and Hashimoto, 1988). According to greening experiments, synthesis of the main chloroplastic proteins is at least partly induced by light. In our study, Rubisco synthesis started before tissues were exposed to direct light.

### N Economy in the Leaf Growth Zone

The appearances of several major photosynthetic proteins (small and large subunits of Rubisco, light-harvesting Chl proteins) seem to be coordinated (Viro and Kloppstech, 1980), which indicates that in tall fescue leaf segments, synthesis of Rubisco represents positional synthesis of several polypeptides. In turn, this suggests a large requirement for amino acids in the leaf maturation zone before the tissue is exposed to light. However, our study indicates that most N is imported at the base of the leaf and is present in tissues before they reach the maturation zone. There is no apparent large N import associated with chloroplastic protein synthesis, nor is there any large net utilization of the ethanol-soluble N fraction (mineral N, free amino acids, and small peptides), which otherwise would have led to negative net deposition rates.

Therefore, N requirements for synthesis of chloroplastic polypeptides were probably met through internal N recycling.

Large changes in polypeptide profiles have been described along successive segments of the *L. perenne* leaf growth zone (Ougham et al., 1987a, 1987b), with simultaneous appearances of a number of polypeptides and disappearances of others. This experimental evidence strongly supports our hypothesis of N recycling during cell development and differentiation. It is also supported by data showing that early amino acid incorporation, assayed by feeding [<sup>35</sup>S]Met and measuring radioactivity of total proteins in various segments, occurred in the base (0–10 mm) of growing leaves of *L. perenne* (Ougham, 1987). The 0- to 10-mm zone corresponded closely with the zone of cell production, whereas the small and large subunits of Rubisco appeared mainly later, starting from 30 mm (i.e. after the zone of cell elongation), where [<sup>35</sup>S]Met incorporation was much more limited.

In summary, due to temporal and spatial separation, leaf growth processes, especially cell production, compete strongly for N that is newly imported into the growth zone, whereas development of chloroplastic polypeptides competes strongly for N recycled within segments of the growth zone. Thus, incorporation of N should be considered more as sequential use than as sink competition between various synthetic processes within nonmature segments of a developing grass leaf. This model for N is similar to that for carbohydrate import and utilization between zones of cell expansion and secondary cell-wall deposition in the same species (Allard and Nelson, 1991).

It is unclear how N enhances cell production rate, but *LER* in this study was closely associated with N concentration of the basal 3-mm segment ( $R^2 = 0.80$ ,  $n = 7$ ). MacAdam et al. (1989) suggested that N tended to increase the number of division cycles from an original cell with little effect on duration of the cell cycle. The question remains whether the N effect on cell production is direct and local or is mediated through hormonal control, perhaps originating from the roots. Experiments are being conducted to investigate these possibilities.

### ACKNOWLEDGMENTS

The authors would like to thank John Coutts for technical assistance and Professor Douglas D. Randall for help in assessment of Rubisco.

Received November 19, 1993; accepted January 19, 1994.  
Copyright Clearance Center: 0032-0889/94/105/0191/07.

### LITERATURE CITED

- Allard G, Nelson CJ (1991) Photosynthate partitioning in basal zones of tall fescue leaf blades. *Plant Physiol* 95: 663–668
- Beever L, Hageman RH (1980) Nitrate and nitrite reduction. In *The Biochemistry of Plants*, Vol 5. Academic Press, New York, pp 115–168
- Blevins DG, Lowe RH, Staples L (1976) Nitrate reductase in barley roots under sterile, low oxygen conditions. *Plant Physiol* 57: 458–459
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248–254
- Chaney AL, Marbach EP (1962) Modified reagents for determination of urea and ammonia. *Clin Chem* 8: 130–132

- Chapin FS, Bloom AJ, Field CB, Waring RH** (1987) Plant responses to multiple environmental factors. *BioScience* **37**(1): 49–57
- Chapin FS, Walter CHS, Clarkson DT** (1988) Growth response of barley and tomato to nitrogen stress and its control by abscisic acid, water relations and photosynthesis. *Planta* **173**: 352–366
- Dean C, Leech RM** (1982) Genome expression during normal leaf development. I. Cellular and chloroplast numbers and DNA, RNA and protein levels in tissues of different ages within a seven-day-old wheat leaf. *Plant Physiol* **69**: 904–910
- Erickson RO, Sax KB** (1956) Elemental growth rate of the primary root of *Zea mays*. *Proc Am Philos Soc* **100**: 487–498
- Gastal F, Belanger G, Lemaire G** (1992) A model of the leaf extension rate of tall fescue in response to nitrogen and temperature. *Ann Bot* **70**: 437–442
- Granstedt RC, Huffaker RC** (1982) Identification of the leaf vacuole as a major nitrate storage pool. *Plant Physiol* **70**: 410–413
- Guiraud G, Fardeau JC** (1977) Dosage par la méthode Kjeldahl des nitrates contenus dans les sols et les végétaux. *Ann Agron* **28**: 329–333
- Joseph MC, Randall DD** (1981) Photosynthesis in polyploid tall fescue. II. Photosynthesis and ribulose-1,5-bisphosphate carboxylase of polyploid tall fescue. *Plant Physiol* **68**: 894–898
- Just D, Saux C, Richaud C, André M** (1989) Effect of nitrogen stress on sunflower gas exchange. I. Photorespiration and carbon partitioning. *Plant Physiol Biochem* **27**: 669–677
- 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
- MacAdam JW, Volenec JJ, Nelson CJ** (1989) Effects of nitrogen on mesophyll cell division and epidermal cell elongation in tall fescue leaf blades. *Plant Physiol* **89**: 549–556
- Nakamura Y, Hashimoto H** (1988) Characteristics of photosynthate partitioning during chloroplast development in *Avena* leaves. *Plant Physiol* **87**: 458–462
- Nelson CJ, Choi SY, Gastal F, Coutts JH** (1992) Nitrogen effects on relationships between leaf growth and leaf photosynthesis. In N Murata, ed, *Research in Photosynthesis IV*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 789–792
- Ougham HJ** (1987) Gene expression during leaf development in *Lolium temulentum*: patterns of protein synthesis in response to heat-shock and cold-shock. *Physiol Plant* **70**: 479–484
- Ougham HJ, Jones TWA, Evans MLL** (1987a) Leaf development in *Lolium temulentum* L: progressive changes in soluble polypeptides complement and isoenzymes. *J Exp Bot* **38**: 1689–1696
- Ougham HJ, Thomas H, Hilditch P** (1987b) Leaf development in *Lolium temulentum* L: gradients of expression of growth and photosynthesis-related polypeptides revealed by immunoblotting. *J Plant Physiol* **129**: 181–186
- Pearen JR** (1991) The relationship of growth and maintenance respiration to leaf growth in tall fescue. PhD thesis, University of Missouri-Columbia
- Robin P, Conejero G, Passama L, Salsac L** (1983) Evaluation de la fraction métabolisable du nitrate par la mesure in situ de sa réduction. *Physiol Veg* **21**: 115–122
- Schnyder H, Nelson CJ** (1989) Growth rate and assimilate partitioning in the elongation zone of tall fescue leaf blades at high and low irradiance. *Plant Physiol* **90**: 1201–1206
- Schnyder H, Nelson CJ, Coutts JH** (1987) Assessment of spatial distribution of growth in the elongation zone of grass leaf blades. *Plant Physiol* **85**: 290–293
- Schnyder H, Seo S, Rademacher IF, Kübauch W** (1990) Spatial distribution of growth rates and of epidermal cell lengths in the elongation zone during leaf development in *Lolium perenne* L. *Planta* **181**: 423–431
- Silk WK** (1984) Quantitative descriptions of development. *Annu Rev Plant Physiol* **35**: 479–518
- Thomas H** (1991) Accumulation and consumption of solutes in swards of *Lolium perenne* during drought and after rewatering. *New Phytol* **118**: 35–48
- Viro M, Klopstech K** (1980) Differential expression of the genes for ribulose-1,5-bisphosphate carboxylase and light-harvesting chlorophyll a/b protein in the developing barley leaf. *Planta* **150**: 41–45
- Volenec JJ, Nelson CJ** (1984) Carbohydrate metabolism in leaf meristems of tall fescue. II. Relationship to leaf elongation modified by nitrogen fertilization. *Plant Physiol* **74**: 595–600