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Interaction between External and Internal Conditions in the Development of Photosynthetic Features in a Grass Leaf

I. REGIONAL RESPONSES ALONG A LEAF DURING AND AFTER LOW-LIGHT OR HIGH-LIGHT ACCLIMATION

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ABSTRACT

Morphological and functional features were compared along a developing third leaf and fully expanded leaf from high-light- and low-light-acclimated seedlings of *Lolium multiflorum*.

The young leaf contains a gradient of differentiating tissue, ranging from meristematic cells at the leaf base to mature tissue at the tip; this gradient can be related to the maturation of a functional photosynthetic apparatus. Along the fully expanded leaf, a decreasing gradient from tip to base is maintained for functional characteristics (net maximum photosynthesis, chlorophyll content, and ribulose bisphosphate carboxylase activity) and for a number of structural parameters (number of mesophyll cells and their external surface area, number of chloroplasts and their envelope area), irrespective of the light regime. In contrast, a constancy in the absolute intrachloroplastic lamellar content per plastid was revealed whatever the position in the leaf or irradiance received. However, the relative membrane content was lower in high-light chloroplasts due to their larger volume compared to low-light plastids (dilution effect).

The longitudinal differences in functional and morphological characteristics are interpreted as the result of interaction between the internal gradient of differentiating tissue along a developing young leaf and the external light conditions during development.

Environmentally induced effects can yield overall phenotypes of quite different structural and physiological features; in particular the effect of irradiance is well-established for a variety of species (see review, ref. 2). Individual leaves can respond differently depending upon their exposed or shaded position on the plant (11). Since a particular response is the result of both external and internal (ontogenetic) factors (10), the question arises whether developmental differences can contribute to regional responses within one leaf.

Grass leaves provide a convenient material for evaluating this problem. First, the young leaf blade is composed of a gradient of tissues of different ages (3, 18). The meristematic activity is restricted to a zone at the base of the blade, and the leaf matures from the tip downward. Second, grass leaves have a distinctive developmental pattern: early leaf differentiation takes place within the precedent leaf sheath under extremely reduced irradiance and is dependent on assimilates from the older leaves. Cell elongation ceases in the exposed leaf region upon emergence from the sheath and this area then becomes photosynthetically active and directly receptive to the prevailing environment (see review, ref. 19). Thus, the duration of exposure to full-light conditions decreases progressively from leaf tip to base, in parallel with the developmental gradient.

In dicotyledons, photosynthetic capacity is affected by individual leaf age. In general, photosynthetic rate increases progressively during leaf expansion and reaches a maximum when leaf expansion is complete (4, 10). However, little attention has been given to photosynthetic capacity in relation to the processes of maturation unique to grass leaves (monocotyledons). We have sought to assess the consequences of an internal gradient on photosynthetic performance and the degree of interaction of biochemical and structural parameters in reaching this performance.

We have examined the following parameters: CO_2 transfer resistances, carboxylation activity, leaf anatomy, chloroplast ultrastructure, and Chl content. These characteristics were compared in apical, medial, and basal regions of developing and fully expanded leaves from plants grown under two contrasting light conditions. The reversibility of the localized adaptive responses is examined in the accompanying paper (16).

MATERIALS AND METHODS

Plant Material and Growth Condtions. *Lolium multiflorum* Lam. (var. Westerwold Barenza) seeds were germinated under shade conditions for 1 week on a nylon sieve cloth impregnated with deionized H_2O. Seedlings were selected and replanted in regular arrays in plastic boxes containing sand-gravel and then placed under the experimental culture conditions.

Plants were grown under controlled conditions: day-night regime, 17 C/13 C; RH, 70% either under an irradiance of 110 w m^{-2} ($\simeq$500 μE m^{-2} s^{-1}) or 16 w m^{-2} ($\simeq$75 μE m^{-2} s^{-1}). The higher irradiance is 25% of maximum solar irradiance and is nearly saturating for photosynthesis of these plants. For simplicity, the two light treatments will be designated hereafter as high and low light. Light was from fluorescent tubes (Philips 33 TL 140 W) and measured with a Kipp and Zonen thermopile or Quantum sensor (190S, Lambda Instruments). The cultures were irrigated automatically for 45 min each h with a modified Hoagland solution.

All structural and functional measurements were carried out on the third leaf of the main shoot at two developmental stages: (*a*) a 10- to 13-cm developing leaf, the emerged portion of which has attained 6 to 8 cm (about one-third of its final length) with 4 to 5 cm enclosed in the preceding leaf sheath; (*b*) a fully expanded leaf (21–26 cm) taken after growth had stopped (26 days under high light and 34 days under low light). Wherever possible, measurements were carried out at three positions along the blade.

Additional measurements of gas exchange were made at intermediate steps between the initial and final stage. Details of

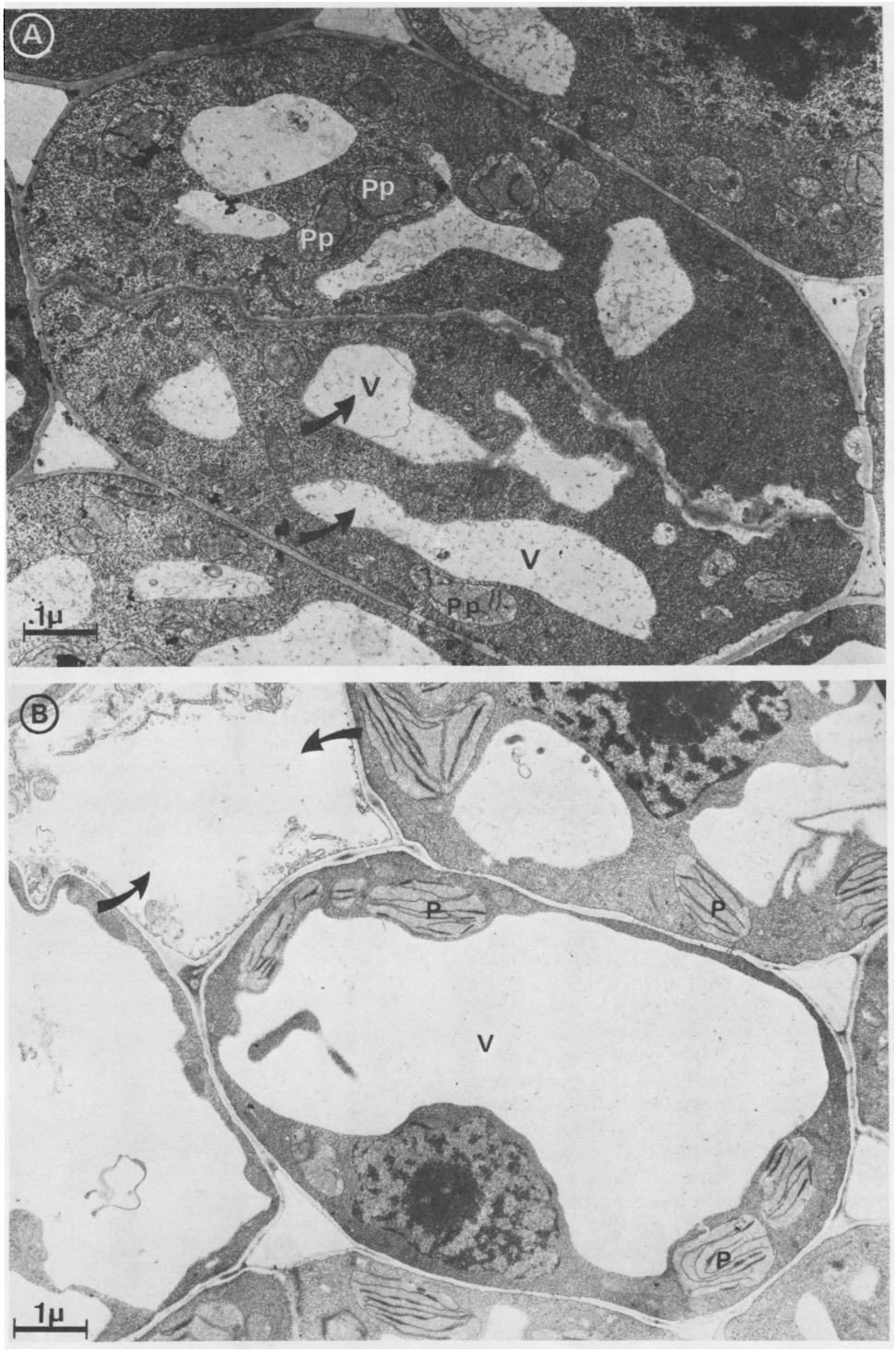

FIG. 1. Photomicrograph of meristematic cell containing numerous proplastids (Pp) at the base of emerging leaf (A) compared to vacuolated mesophyll cell containing juvenile plastids representative of middle region of young leaf (B). Arrows in A show beginning of vacuolation (V); arrows in B indicate degradative process in mesophyll cell.

samplings are described under each technique section.

Gas-Exchange Measurements. Gas-exchange measurements were carried out by conventional IR analysis in an open system previously described by Hariri and Prioul (8), except that a small rectangular assimilation chamber was used to measure leaf portions 2.5 cm long. The chamber was designed for simultaneous measurements of six to eight attached leaves. The small chamber volume (5 cm^3) provided good gas mixing as shown by the low boundary layer resistance to CO_2, estimated by blotting paper models to be 20 s m^{-1}. Air temperature was measured with a copper-constantan thermocouple. The whole chamber was inserted in a small Plexiglas cabinet surrounded by a 10-cm thick water jacket kept constant at 25 C. Light was provided by a high pressure mercury-vapor lamp (Philips HPL 2 kw). Irradiance incident on the leaves was continuously monitored with a silicon cell calibrated against the thermopile. Appropriate neutral filters

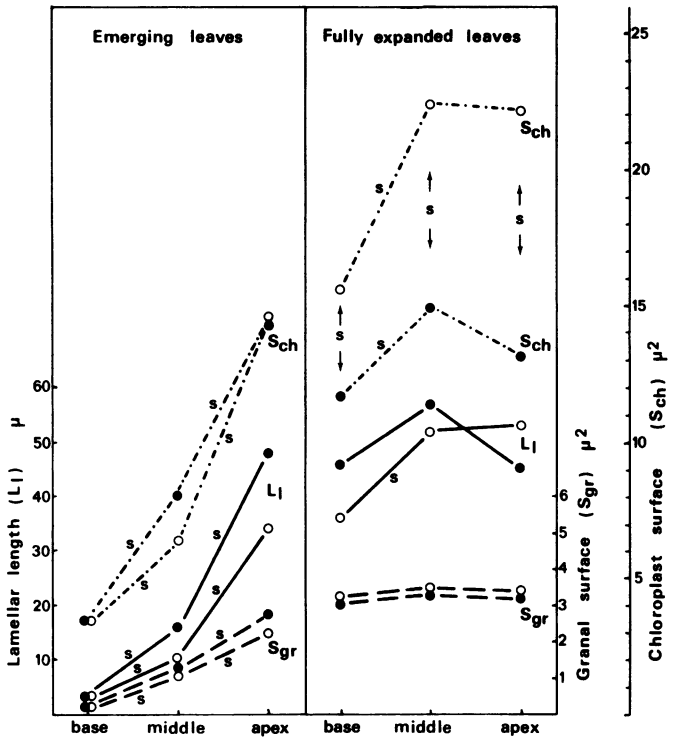

FIG. 2. Chloroplast ultrastructural parameters characteristic of base, middle, and apex of developing and fully expanded leaves grown in high (○) or low light (●). Chloroplast surface (S_{ch}), total lamellar length (L_l), and total granal surface (S_{gr}) were measured on transverse sections. s, significant at 5% level; no symbol when nonsignificant.

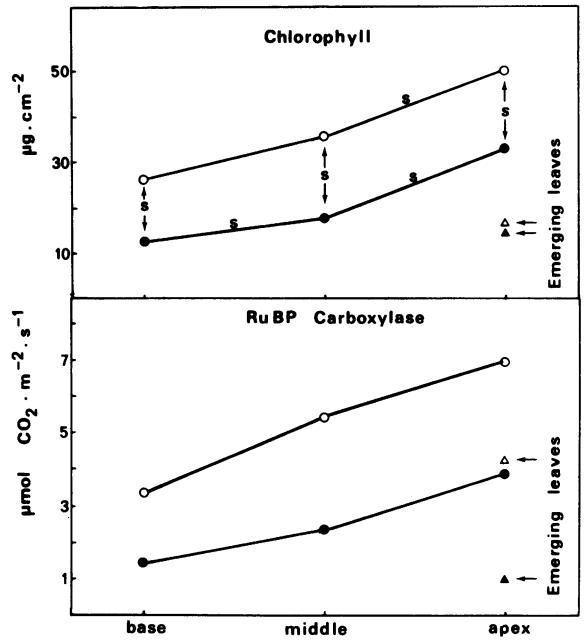

FIG. 3. Chlorophyll content and RuBP carboxylase activity on a leaf-area basis in emerging leaves (arrows) and in the apical, middle, and basal portions of fully expanded leaves grown in high (○, △) or low (●, ▲) light. Chl, four to five replicates; s, significant difference at 5% level; no symbol when nonsignificant; RuBP carboxylase; average from two replicates, experimental variability is less than the difference along the leaf or between light conditions.

were used to vary irradiance and to obtain light photosynthesis-response curves.

Net photosynthesis was measured at the initial stage (emerged length, 6–8 cm) and then every 2 or 3 days until full leaf expansion (the successive sample lengths were typically 6–8, 13–16, 18–20, and 21–26 cm). Experiments were repeated two to three times with three independent cultures.

Biochemical Measurements. RuBP carboxylase[1] was prepared and assayed as described by Reyss and Prioul (17) except for a modification in the enzyme assay: the enzyme extract was activated by a 5-min preincubation with $NaHCO_3$ (6) before starting the reaction with RuBP (final concentration of substrates: $NaHCO_3$, 60 mM; RuBP, 0.3 mM). Incubation times were 10, 20, and 30 min; only enzyme activities on the linear part of the curve were used for comparisons. Activity was determined for emerged leaves at the initial stage (6–8 cm) and for the apical, medial, and basal portions of fully expanded leaves. Fourteen leaves (approximate fresh weight, 0.5 g) were sampled for each determination and in duplicate sets of experiments. All results were expressed on a leaf-area basis.

Leaf Chl content was determined in 80% acetone (four to five replicates) as described by Arnon (1).

Leaf Anatomy and Choroplast Ultrastructure. Three ontogenetic steps in the developing leaf were tested by sampling (*a*) at the leaf base (within sheath); (*b*) in the medial zone (site of leaf emergence); and (*c*) 3 cm from the leaf tip. Samples from fully expanded leaves were taken at the leaf base, in the medial zone, and 3 cm from the tip. The apical zone is the same in developing and mature leaves because cell elongation ceases upon leaf emergence. Electron microscopy methods were as previously described (3).

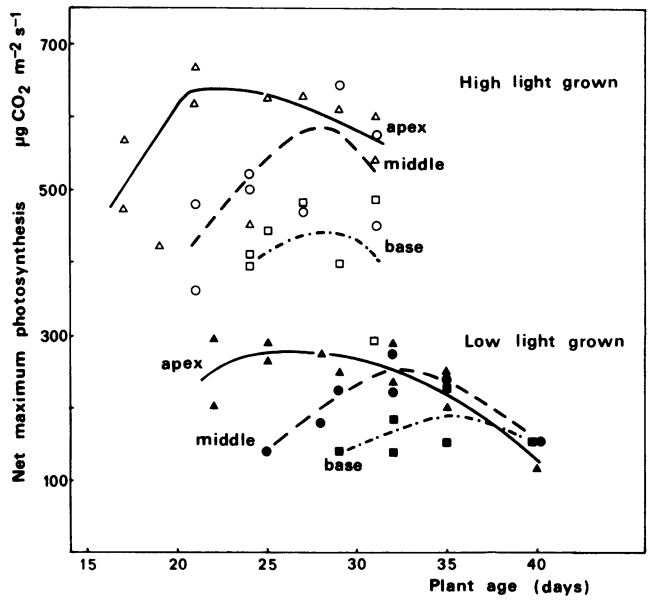

FIG. 4. Net maximum photosynthesis in apical (△, ▲), medial (○, ●), and basal (□, ■) leaf zones as a function of age for plants grown in high (△, ○, □) and low (▲, ●, ■) light. Each point corresponds to a measurement of a pooled sample from six leaves.

Chloroplast ultrastructural parameters were evaluated on photomicrographs by biometric techniques: perimeters of plastid profiles were measured with a curvimeter and surface areas with a planimeter (starch grain surfaces were excluded). Within each profile, the total intergranal thylakoid lengths were measured by

[1] Abbreviations: RuBP carboxylase, ribulose bisphosphate carboxylase oxygenase; RuBP, ribulose 1,5-bisphosphate.

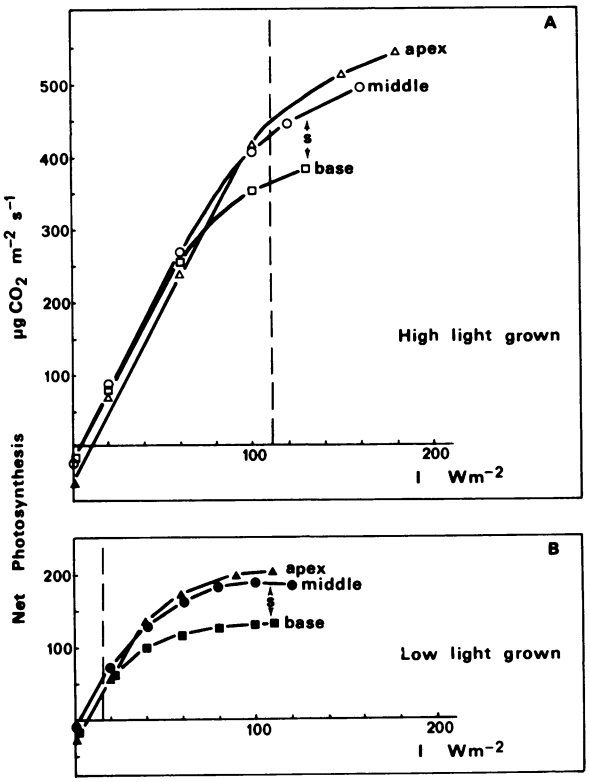

FIG. 5. Photosynthetic light-response curves from apical, medial, and basal zones of fully expanded leaves grown in high or low light. Measurements were carried out 0 to 3 days after full leaf expansion. Each curve is an average of five (A) or seven (B) independent measurements. s; significant difference at 5% level; no symbol when nonsignificant.

tracing with a curvimeter, and the total granal surfaces were measured with a planimeter. The analyzed chloroplasts were taken at random from a large number of micrographs. As shown previously (15), a sample size of 5 to 10 chloroplasts is sufficient for a significant evaluation. To take into account the variability between experiments, leaves from three separate cultures were used (125 chloroplasts measured).

Samples for optical microscopy were prepared from the three leaf regions indicated above from whole or half cross-section slices of five blades/experiment. Fixation and embedding procedures were those used for electron microscopy. Sections (about 1 μm) were cut on an LKB ultratome with a glass knife and stained with Paragon SL (Serlabo). Cell numbers, chloroplast numbers, and cell perimeters in contact with intercellular air spaces are expressed either per unit of cross-sectional area or per unit of abaxial epidermis length. As section thickness is very small, it may be assumed that the ratio of cell wall or chloroplast envelope profile to cross-section area is representative of the ratio of cell or envelope area to leaf volume. Similarly, these parameters, expressed per abaxial epidermis length, are representative of the ratio of cell or envelope area to leaf surface area.

As shown in paradermal sections, mesophyll cells are essentially spherical (diameter, 20–30 μm) and chloroplasts are ellipsoidal (diameter, 7–8 μm). Mean cell and chloroplast diameters must be considered when estimating total cell or chloroplast numbers per leaf volume or leaf area.

Granal surfaces and stroma lamellae lengths were plotted against chloroplast profile surfaces on a log-log scale and the resulting regression lines were compared. A measure of stroma lamellae or granal content relative to total chloroplast section area (lamellae or granal indexes) was derived from the distance between regression lines as previously described (15). Otherwise, conventional statistical methods were used (Student's t test and analysis of variance).

RESULTS AND DISCUSSION

Ontogenetic Features of the Developing Leaf. A gradient of differentiating tissue exists along the developing leaf. At the leaf base, closely packed, meristematic cells characterize the beginning of the ontogenetic process (Fig. 1A). Although some vacuolation has begun (arrows), most cells are filled with cytoplasm rich in juvenile organelles, particularly proplastids. A poorly developed membrane system, as quantified by biometry, characterizes proplastids (Fig. 2, left). In the leaf medial region (Fig. 1B), most cells have become fully vacuolated and the cytoplasm, containing organelles, has been pressed against the cell wall. Plastid differentiation has reached an intermediate stage in terms of lamellar and granal content (Fig. 2, left). Near the tip of the developing leaf, chloroplasts are comparable to those found in fully expanded leaves and take on a characteristic aspect of their high- or low-light control counterparts (as in Fig. 10). However, quantitative evaluation of granal or stoma lamellar content shows great variability between experiments with some values lower than in "mature" plastids, indicating that membrane synthesis was not always complete in this apical zone (Fig. 2, left, apex).

Chl content and RuBP carboxylase activity in the exposed part of developing leaves (Fig. 3, arrows) differ considerably from those of the adult leaf. However, at this young stage, a difference in the carboxylase activity between a plant grown in low or high irradiance is already apparent, whereas this is not yet so for the Chl content. This fact suggests that the rates of maturation differ for these two parameters, Chl synthesis being more light-dependent. It should be stressed that the emerged part of the leaf is not homogeneous. This is evident from the visible differences in color: green near the tip, light green or yellow-green near the leaf sheaths. The observed biochemical values are the average for the entire emerged part, whereas the ultrastructural features represent more localized zones.

Photosynthetic gas exchange measurements of the developing leaf confirm the differences in maturity along the leaf. For example, the photosynthetic rate at light saturation in the apical part (Fig. 4) reaches a maximum 2 or 3 days after the beginning of the measurement and then declines steadily. The medial and basal zones then, in turn, attain their maximum rates (Fig. 4). The maximum exchange rate for the whole leaf corresponds to the maximum capacity measured for the medial portion; the apex has begun its decline, whereas the basal part has not yet reached its maximum. The relation between age and photosynthesis of the whole leaf, as described elsewhere (4, 10), can be observed along a single grass leaf, each leaf zone maturing and senescing in turn. The decline in photosynthetic rate is accelerated by high light conditions as noted by Jurik (10).

Physiological Gradients along Fully Expanded Leaves. Light-response curves of the apical, medial, and basal regions of fully expanded leaves confirm differences between high- and low-light-grown plants (14). Also, a significant decrease in net maximum photosynthesis is shown from leaf tip to base (Fig. 5), irrespective of growth conditions.

Initial slopes of curves are not significantly different under any of the experimental conditions. However, a decreasing Chl gradient from tip to base is observed under both light conditions (Fig. 3).

RuBP carboxylase activity is strongly affected by light intensity and also shows a marked gradient along the leaf (Fig. 3).

Specific leaf fresh weight is rather constant for each light condition, but specific leaf dry weight decreases from tip to base and is lowered by low light (1.9 to 1.31 mg cm^{-2} for 16 w m^{-2} and 2.73 to 1.71 for 1110 w m^{-2}), indicating that the leaf water content increases from tip to base.

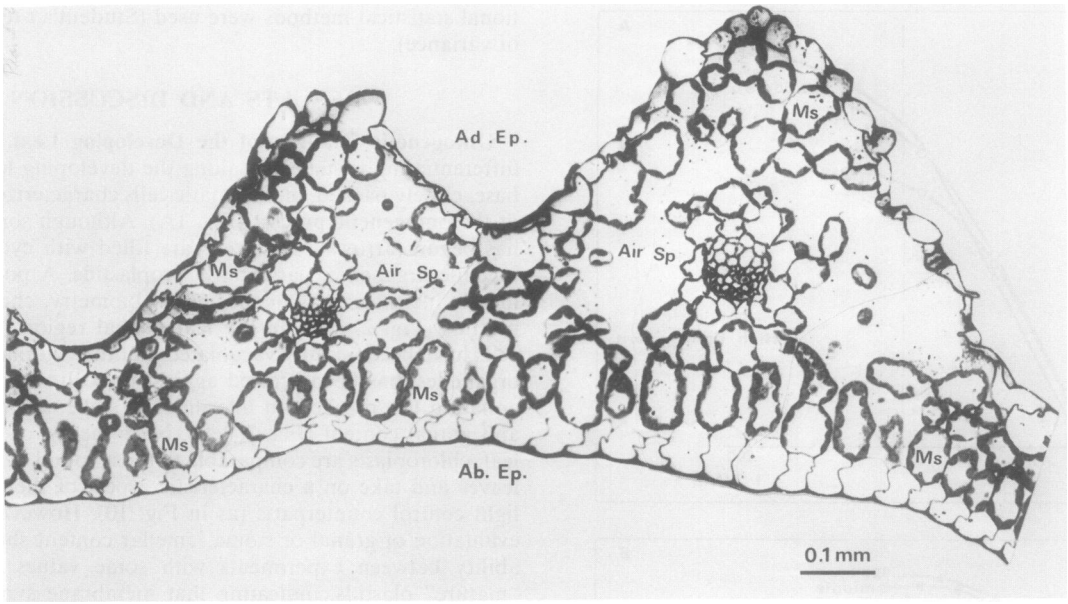

FIG. 6. Cross-sectional view in the medial zone of an adult high-light leaf illustrating foliar anatomy typical in *Lolium*. Loosely arranged mesophyll cells (Ms) are separated by large air spaces (Air Sp.). Ad.Ep, adaxial epidermis; Ab.Ep., abaxial epidermis.

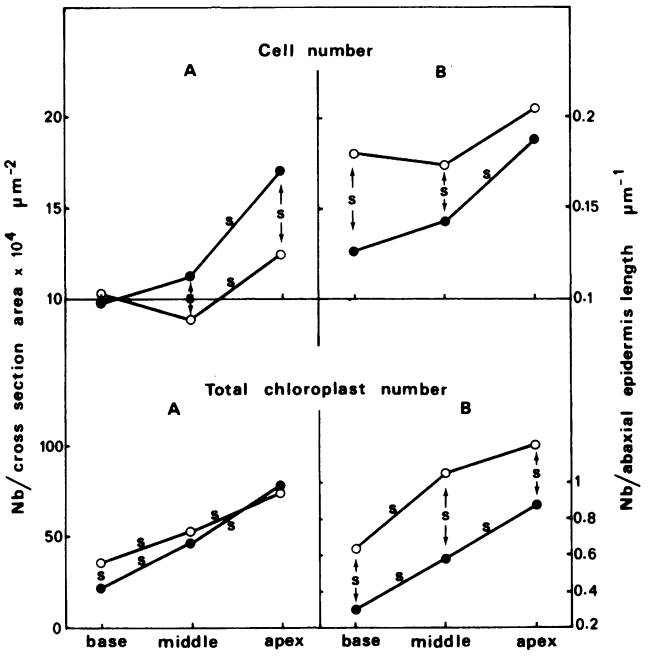

FIG. 7. Cell and chloroplast numbers expressed per leaf cross-section area (A) or per abaxial epidermis length (B) for the base, middle, and apex of high-light (○) and low-light (●) fully expanded leaves. s as in Fig. 5.

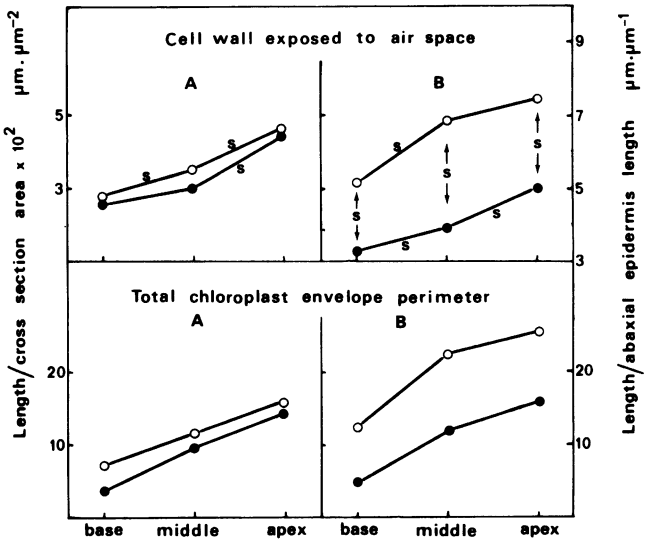

FIG. 8. Cell wall lengths exposed to internal air space and chloroplast envelope perimeters expressed per leaf cross-section area (A) or per abaxial epidermis length (B). Symbols as in Figure 7. Total chloroplast envelope perimeters are the products of mean chloroplast perimeter × mean chloroplast number in the same zone. Statistical comparison is not possible.

Structural Parameters along Fully Expanded Leaves. An example of leaf anatomy is shown in Figure 6, illustrating loosely arranged mesophyll cells separated by large air spaces in the inner leaf zone, whereas a more compact mesophyll cell layer is contiguous with the abaxial epidermis. Adaxial leaf margins with a high degree of ridging are characteristic of *Lolium*.

A decreasing apex to base gradient in structural features is shown in leaves from both light treatments (Figs. 7 and 8). However, differences in apparent responses for certain parameters are highly dependent upon the unit of comparison. Leaves grown in high or low light show similar gradients in total plastid numbers (Fig. 7A, lower), cell wall, and chloroplast envelope lengths (Fig. 8A), when expressed per cross-section (indicative of leaf volume). But when data are expressed on a leaf area basis, high-light leaves always contain more cells and more chloroplasts (Fig. 7B), which leads to extensive exchange surfaces as reflected by the internal cell wall and chloroplast envelope data (Fig. 8B). These data suggest the importance of leaf thickness in determining CO_2 exchange rates (5). However, Nobel and co-workers (12, 13) have pointed out that a more appropriate parameter for dealing with variations in leaf thickness is the internal cell wall area per unit leaf area. When the maximum photosynthetic rate is expressed as a function of this characteristic, the gradient along the leaf disappears, but the difference in photosynthetic rate between leaves grown in high and low light remains (Fig. 9, left). Similarly, when

Table I. *Quantification of Chloroplast Ultrastructural Characteristics as a Function of Position in Leaf and of Irradiance during Growth*

Granal and lamellar indexes represent the relative granal and lamellar content per unit area of chloroplast section. Indexes are derived from covariance analysis and are computed by reference to the mean value of the low-light leaf which is taken as 100. Values indicated by the same letter are not significantly different at 5% level (covariance analysis for indexes and variance analysis for other parameters).

Leaf Region	Irradiance									
	Granal Index		Lamellar Index		Plastid Profile Perimeter		Plastid Long Axis		Plastid Short Axis	
	Low	High	Low	High	Low	High	Low	High	Low	High
					μm		μm		μm	
Apex	104a	78c	95a	68b	18b	21a	7.5a	8.3a	3.1b	4.2a
Middle	98a	67c	102a	69b	20.2a	21.5a	8.8a	8.6a	2.8b	4.0a
Base	101a	86b	106a	64b	16.5b	19.5a	7.3b	8.1a	2.3c	3.0b

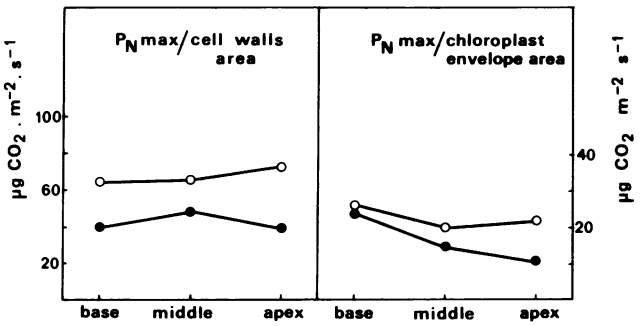

FIG. 9. Net maximum photosynthesis (P_N expressed per unit area of cell wall in contact with internal air spaces (left) or per unit area of chloroplast envelope (right) from apical, basal, and medial zones of leaves grown under high light (○) or low light (●). These values are the products of mean net photosynthesis per unit leaf area multiplied by the ratio of leaf area to internal surface determined on cross-sections. No statistical comparison is possible.

the photosynthetic rate is expressed in terms of the chloroplast envelope area, not only the leaf gradient but also the effect of light is reduced (Fig. 9, right). These data emphasize the potential importance of chloroplast ultrastructure as CO_2 barrier(s) and, more generally, the role of gas-exchange interfaces in accounting for adaptive responses.

Chloroplast Ultrastructure along Fully Expanded Leaves. A comparative biometric analysis on a large number of chloroplast profiles from high-light and low-light leaves reveals a constancy in the absolute intrachloroplast membrane parameters (Fig. 2, right). Both granal and stroma lamellar contents are identical, irrespective of the position in the leaf or the light treatment. This observation apparently contrasts with reports for certain sun and shade species (2, 7). However, chloroplast size differences distinguish plastids having developed under the two light regimes (Fig. 2, right). As a consequence, the density of the lamellar network per chloroplast unit area measured by the granal and lamellar indexes (Table I) differs significantly for the two irradiances. Because of an increased chloroplast size, a less dense intrachloroplastic system is observed under high light, in agreement with previous results on *Lolium* (15).

Flattened, lenticular forms of low-light plastids are readily distinguishable from the more spherical forms of their high-light counterparts (Fig. 10). In addition, it appears that granal configuration is affected by irradiance. Under high light, granal stacks are quite uniform in width (partition length) and in thickness and present rectangular arrays in cross-sectional views (Fig. 10A, inset), whereas the low-light plastids are characterized by more staggered interconnected stacks (Fig. 10B, inset).

Plastid perimeters and the long and short axes were measured to evaluate differences in plastid forms (Table I). Since perimeters vary only slightly between the two plastid populations, whereas the short plastid axis increases considerably under high light, it is suggested that the observed size differences are mainly due to changes in the chloroplast shape. This signifies that, although envelope size remain nearly identical, the change in plastid form results in significant volume increase.

The increase in plastid volume under high light is correlated with an increase in RuBP carboxylase activity, a relationship compatible with the idea that this enzyme is the principal constituent of the stroma (9).

CONCLUSION

A grass leaf starts its growth within the precedent leaf sheaths under extremely reduced irradiance before it emerges into the ambient light. Concerning this unique developmental pattern, it can be asked whether each individual leaf or parts of it respond to the light which it receives, or does the whole plant (older leaves) perceive and transmit the light signal to the young developing leaf?

We have obtained evidence for both phenomena operating. The cell number is determined early in leaf differentiation and, consequently, prior to direct exposure to light. Nonetheless, differences in cell densities on a surface area or volume basis exist between high- and low-light leaves, suggesting that other plant parts must have influenced cell division (final number) in the developing leaf. Other parameters, such as intrachloroplast membrane structure of Chl content, are found to be identical in developing leaves (just prior to exposure to full light), irrespective of the light regimes. As the developing leaf emerges and matures, it takes on characteristics typical of the prevailing light condition. This would indicate a certain independence from the rest of the plant and suggest that the leaf itself is the receptor.

The functional and morphological gradients that exist along the developing leaf are maintained and expressed in the fully expanded leaf, irrespective of the irradiance in which the leaves have developed. These apparently local adaptations seem to be in part related to internal (developmental) factors. However, irradiance measurements at different leaf positions in the course of development have revealed a natural decreasing gradient of light from leaf tip to base. The amount of light encountered by the leaf varies, depending upon its position and angle with incident light (young leaf upright, adult leaf in curved position). The basal zone receives up to 4 times less light than the apex. This may also be a reason for regional differences.

The existence of different functional and structural properties within one leaf raises the question as to the time course of the responses and their reversibility. An attempt has been made to approach this problem by changing the light conditions of young developing leaves, as reported in the accompanying article (16).

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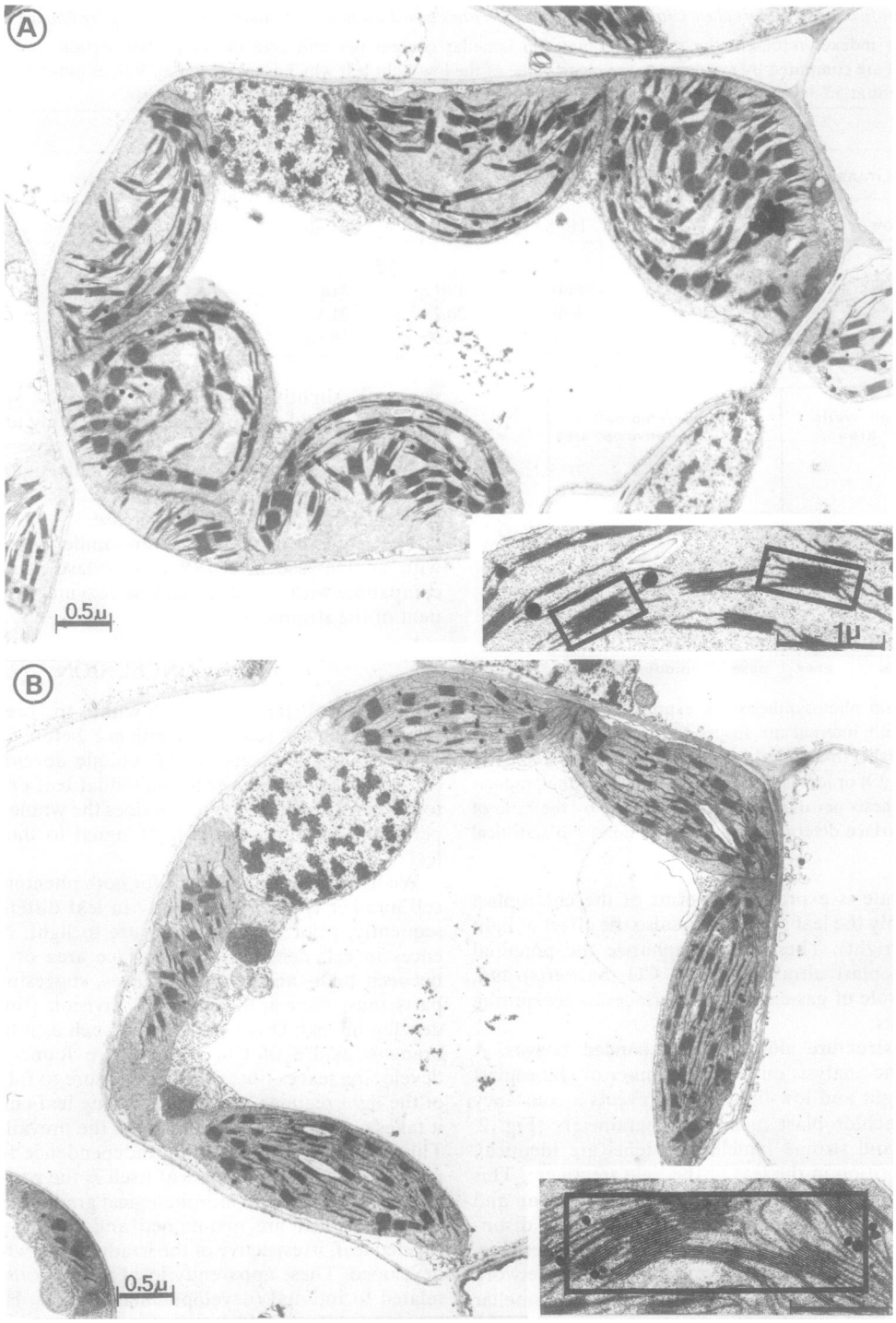

FIG. 10. Photomicrographs of fully differentiated mesophyll cells illustrating large, rounded high-light chloroplasts (A) *versus* the typical, flattened low-light plastids. Insets, enlarged view of individual granal stacks obtained in high light (A) compared to the irregular and connected stacks obtained in low light (B).

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LITERATURE CITED

1. ARNON DI 1949 Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24: 1–15
2. BOARDMAN NK 1977 Comparative photosynthesis of sun and shade plants. Annu Rev Plant Physiol 28: 355–377
3. BRANGEON J, L MUSTARDY 1979 The ontogenetic assembly of intrachloroplastic lamellae viewed in three dimension. Biol Cell 36: 71–80
4. ČATSKÝ J, I TCHA, J SOLAROVA 1976 Ontogenetic changes in internal limitations to bean-leaf photosynthesis. I. Carbon dioxide exchange and conductances for carbon dioxide transfer. Photosynthetica 10: 394–402
5. CHARLES-EDWARDS LA, LJ LUDWIG 1975 The basis of expression of leaf photosynthetic activities. *In* R Marcelle, ed, Environmental and Biological Control of Photosynthesis, Dr. W Junk, The Hague, pp 37–43
6. CHU DK, JA BASSHAM 1973 Activation and inhibition of ribulose-1,5-diphosphate carboxylase by 6-phosphogluconate. Plant Physiol 52: 373–379
7. HARIRI M, J BRANGEON 1977 Light-induced adaptive responses under greenhouse and controlled conditions in the fern *Pteris cretica* var. *ouvrardii*. I. Structural and infrastructural features. Physiol Plant 41: 280–288
8. HARIRI M, JL PRIOUL 1978 Light-induced adaptive responses under greenhouse and controlled conditions in the fern *Pteris cretica* var. *ouvrardii*. II. Photosyn-

thetic capacities. Physiol Plant 42: 97–102
9. JENSEN RG, JT BAHR 1977 Ribulose-1,5-bisphosphate carboxylase-oxygenase. Annu Rev Plant Physiol 28: 379–400
10. JURIK TW, JF CHABOT, BF CHABOT 1979 Ontogeny of photosynthetic performance in *Fragaria virginiana* under changing light regimes. Plant Physiol 63: 542–547
11. NOBEL PS 1976 Photosynthetic rates of sun *versus* shade leaves of *Hyptis emoryi* Torr. Plant Physiol 58: 218–223
12. NOBEL PS 1977 Internal leaf area and cellular CO_2 resistance: photosynthetic implications of variations with growth conditions and plant species. Physiol Plant 40: 137–144
13. NOBEL PS, LJ ZARAGOZA, WK SMITH 1975 Relation between mesophyll surface area, photosynthetic rate, and illumination level during development for leaves of *Plectranthus parviflorus* Henckel. Plant Physiol 55: 1067–70
14. PRIOUL JL 1971 Réactions de feuilles de *Lolium multiflorum* à l'éclairement pendant la croissance et variation des résistances aux échanges gazeux photosynthétiques. Photosynthetica 5: 364–375
15. PRIOUL JL 1973 Eclairement de croissance et infrastructure des chloroplastes de *Lolium multiflorum* Lam. Relation avec les résistances au transfert de CO_2. Photosynthetica 7: 373–381
16. PRIOUL JL, J BRANGEON, A REYSS 1980 Interaction between external and internal conditions in the development of photosynthetic features in a grass leaf. II. Reversibility of light induced responses as a function of developmental stages. Plant Physiol 66: 770–774
17. REYSS A, JL PRIOUL 1975 Carbonic anhydrase and carboxylase activities from plants (*Lolium multiflorum*) adapted to different light regimes. Plant Sci Lett 5: 189–195
18. SIGNOL M 1965 Modifications morphologiques, pigmentaries et cytologiques induites chez divers groupes de végétaux par quelques substances antichlorophylliennes. Rev Gen Bot 72: 417–575
19. SILSBURY JH 1970 Leaf growth in pasture grasses. Trop Grassl 4: 17–36