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Analysis of Spatial Variation in CO<latex>$_2$</latex> Uptake Within the Intact Leaf and Its Significance in Interpreting the Effects of Environmental Stress on Photosynthesis [and Discussion]

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Analysis of spatial variation in CO_2 uptake within the intact leaf and its significance in interpreting the effects of environmental stress on photosynthesis

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The responses of CO_2 uptake to environmental variables and to stress effects *in vivo* have been largely limited to measurements of whole leaves or 'representative' portions of the leaf. These responses therefore reflect an average for the photosynthetic cells within a leaf, which may differ in environmental pre-history, stage of development and ontogeny. These differences may also result in variation in responses to stress. Improvements in the resolution and accuracy with which gaseous exchanges can be measured in open and closed systems allow the use of small chambers that facilitate a separation of the contributions made by different parts of the leaf to total CO_2 uptake. In amphistomatous leaves with large internal resistances simultaneous measurements of CO_2, N_2O and H_2O vapour allow separation of the contributions made by the upper and lower mesophyll. These techniques are applied to *Zea mays* leaves to examine (1) heterogeneity in the responses of CO_2 uptake to light and to internal air-space CO_2 pressure fraction, and (2) heterogeneity in the susceptibility to photoinhibition during chilling.

1. Introduction

Most previous studies of leaf gas exchange have concerned measurements of either the whole leaf or a part considered representative of the whole, e.g. the mid-region of a grass leaf (reviewed by Körner *et al.* 1971; Long 1985). Generalized patterns for the responses of photosynthetic CO_2 uptake to environmental variables are largely based on such data (reviewed by Long 1985). Implicit in previous studies has been the idea that intra-leaf variability is low relative to variability between leaves or that induced by environmental variation.

In grass leaves, marked variation in activities of various enzymes of carbon metabolism has been shown along the length of the leaf, a product at least in part of the linear developmental gradient (Leech *et al.* 1973; Miranda *et al.* 1981*a*). Recently, Terashima *et al.* (1988) have shown that within sunflower leaves patches can be identified where no starch is formed and the overlying stomata remain closed. Other regions of these leaves have open stomata and can assimilate CO_2. Not only is there significant variation across the surface of the leaf, but with depth into the leaf (Terashima & Saeki 1985; Terashima 1986). Many leaves will transmit no more than about 10% of incident light (Ross 1975). Thus even without variation in the photosynthetic apparatus very different rates of photosynthesis would be expected of the cells near the upper surface, which might receive full sunlight, compared with those near the lower surface in marked shade (Long *et al.* 1988).

Separation of the photosynthetic contributions of the different parts of the leaf has significance for a number of different problems.

(*a*) If there is considerable intra-leaf heterogeneity, properties of the responses of the whole leaf will reflect this. As a hypothetical example, if one half of the leaf shows a response curve of photosynthesis to light with a sharp transition between the light-limited and light-independent phases and the other half similarly shows a sharp transition, but at a very different photon flux, when combined they would produce a very different curve with a broad transition. This decreased convexity will therefore be an artefact of the heterogeneity and not a result of any biochemical or other subcellular features of the photosynthetic cells (Terashima & Saeki 1985; Kirschbaum 1987*a*).

(*b*) There has been much interest in simultaneous measurement of chlorophyll fluorescence and photosynthetic CO_2 uptake (Walker *et al.* 1983; Ireland *et al.* 1984, 1988). The fluorescence signal will largely emanate from the cells just below the epidermis; however, the rate of photosynthetic CO_2 uptake that is measured will reflect the activity of the whole tissue. Separate estimation of CO_2 uptake by the upper cells would provide a more meaningful basis for comparisons with fluorescence emission.

(*c*) Apparent stress inhibition to whole-leaf photosynthetic CO_2 uptake might reflect inhibition to one part of the leaf, but not the whole leaf. Identifying the most and least susceptible parts of the leaf would aid understanding of the bases of susceptibility and resistance. For example, in *Xanthium* leaves treated with abscisic acid the development of patches with closed stoma, rather than a uniform deterioration of the photosynthetic apparatus across the leaf, appears to explain the decline in photosynthesis (Farquhar 1988; Terashima *et al.* 1988).

(*d*) Homogeneity of photosynthetic capacity within the leaf would be a disadvantage in a heterogeneous light microclimate. If we assume that the leaf has finite resources of energy and nutrients to invest in proteins then whole leaf photosynthesis would be maximized by investing resources disproportionately in favour of those areas receiving most light. For example, in leaves in a planophile canopy we might expect a higher investment in photosynthetic apparatus on the upper rather than lower surface (Kirschbaum 1987*a*). Similarly, in a grass leaf the base of the lamina adjacent to the stem or pseudostem will be more frequently in shade than the tip and thus greater investment towards the tip might be expected (Miranda *et al.* 1981*a*, *b*).

Formerly, measurement of CO_2 uptake by a whole leaf or large portion of the leaf may have been necessary to attain sufficient change in CO_2 concentration for the resolution of the infra-red gas analysers employed. To determine the rate of CO_2 uptake by 1 cm^2 of leaf in a stream of air flowing at 200 $cm^3\ min^{-1}$ to a precision of 0.1 $\mu mol\ m^{-2}\ s^{-1}$, a differential infrared gas analyser capable of resolving a difference in the CO_2 pressure fraction in air of 0.1 $\mu Pa\ Pa^{-1}$ (0.1 p.p.m.) is required. A number of commercial instruments now meet this requirement (Bingham & Long 1985) making measurement of small areas (*ca.* 1 cm^2) of a leaf practical. Previously, we have shown that a diffusive barrier of significant resistance separates the upper and lower mesophyll of *Zea mays* leaves, making separate estimation of CO_2 uptake by these two cell populations possible (Long *et al.* 1989).

The objective of this study was to define the extent of variability of capacity for CO_2 uptake along the length of the *Z. mays* leaf lamina and between the two surfaces, and to examine the variability of the effects of chilling-dependent photoinhibition between these different parts

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of the leaf. Variation in photosynthetic capacity across the width of the young *Z. mays* leaf was estimated with a microscope chlorophyll fluorimeter, capable of measuring the chlorophyll fluorescence from three or four mesophyll cells.

2. Materials and methods

Plant material

Plants of *Zea mays* L. *cv.* LG11 (Nickersons Seed Specialists Ltd, U.K.) were grown in controlled environments at 20–25 °C, in a minimum photosynthetically active photon flux density (PPFD) of 400 μmol quanta m^{-2} s^{-1} and a water vapour pressure deficit of less than 1.0 kPa. Mature seventh leaves were used to examine the distribution of photosynthesis between the upper and lower halves of the mesophyll. To determine spatial variation along the length and across the width of the leaf, the lamina of the second leaf on emergence of the ligule was used; at this stage of development the lamina is *ca.* 12 cm from ligule to tip.

Leaf gas exchange

The influx of CO_2 and efflux of water vapour were measured with infrared gas analysers and capacitance hygrometers in an open gas-exchange system, as described previously (Bongi & Long 1987; Long *et al.* 1989). To determine the separate rates of CO_2 uptake by the upper and lower surfaces of the leaf, a leaf-disc chamber adapted from the design of Harris *et al.* (1983), to allow temperature control, was used. Leaf discs were positioned in this chamber to form a diaphragm between the upper and lower cavities of the chamber. This chamber was also used to estimate the leaf internal conductance to CO_2 transfer between the upper and lower substomatal cavities. Total leaf conductance to gaseous diffusion across the leaf was determined from the rate of diffusion to N_2O, measured with a differential infrared N_2O analyser. Both empirical measurements and theoretical calculations suggest that the binary diffusion coefficient of CO_2 in air equals that of N_2O in air (Reid *et al.* 1977; Pritchard & Currie 1982). Conductance to CO_2 was therefore considered to equal that measured for N_2O. The internal conductance (g_i) was then calculated by subtracting the reciprocals of the upper and lower stomatal conductance (g_s, calculated from water vapour efflux) from the reciprocal of total conductance (Long *et al.* 1989). Rates of CO_2 uptake, stomatal conductance and intercellular CO_2 concentration for both surfaces were calculated by the equations of von Caemmerer & Farquhar (1981). The net rate of diffusion from the upper to lower air space systems was taken as the product of the difference in intercellular CO_2 pressure fractions (p_{CO_2}) and g_i. This flux was used to correct the apparent rate of CO_2 uptake by each surface for any internal diffusion of CO_2 (Long *et al.* 1989).

Variation in photosynthesis along the length of the second leaf was determined in a stirred Parkinson leaf chamber (Long 1986), modified so that gas exchange could be monitored on 1 cm or 2 cm lengths of attached leaf. The rate of CO_2 uptake (A), g_s and p_{CO_2} were calculated by the equations of Long & Hällgren (1985). Apparent quantum yields (ϕ_{app}) were determined as the slope of the response of CO_2 uptake to incident PPFD in the range of 50–150 μmol m^{-2} s^{-1}. Leaf absorbance was measured with an integrating sphere, as described previously (Bongi & Long 1987). Responses of leaf segments to variation in incident PPFD and p_{CO_2} were described by fitted non-rectangular hyperbolae, following the procedure of Marshall & Biscoe (1980).

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Chlorophyll fluorescence

To assess variability in photosynthetic capacity across the width of the leaf the kinetics of chlorophyll fluorescence from points on the leaf were determined. Second leaves of *Z. mays* seedlings were placed on the stage of a microscope fluorimeter, described previously by Bolhár-Nordenkampf & Lechner (1988). Between 10 and 30 measurements were made in a line across the leaf at 5 cm below the tip. The leaf was positioned so that the image of the rectangular measuring diaphragm (125 μm × 30 μm) was focused on a row of *ca.* four mesophyll cells either between or overlying the bundle sheaths. The excitation light gave a PPFD of 630 μmol quanta m^{-2} s^{-1} (435 nm). After a period of dark adaptation, the excitation beam was turned on and the kinetics of fluorescence emission were recorded for 695 nm. This wavelength was chosen to minimize reabsorption losses. Fluorescence parameters were automatically computed following the equations of Hipkins & Baker (1986).

Chilling-dependent photoinhibition

Leaves were chilled at 5 °C and exposed to a PPFD of *ca.* 1000 μmol quanta m^{-2} s^{-1} on the upper surface for 6 h (Long *et al.* 1983), unless stated otherwise. Care was taken to ensure even illumination across the leaf, which was held in a horizontal position. Photoinhibition of CO_2 uptake was assessed by comparing leaves after chilling both in darkness (control) and in the presence of high light. To assess the effect of environmental perturbation on the pattern of development of photsynthesis along the length of the second leaf, *Z. mays* plants were chilled to 5 °C in a PPFD of 1000 μmol quanta m^{-2} s^{-1} for 14 h, 3 days before emergence of the second leaf ligule. Photosynthetic gas exchange along the length of the lamina was then measured 2 days after ligule emergence.

3. Results and discussion

Variation between the upper and lower mesophyll

The conductance for CO_2 in air between the upper and lower air space system of the *Z. mays* leaf was 17.2 ± 0.5 mmol m^{-2} s^{-1}, i.e. about one twentieth of the stomatal conductance of an illuminated leaf. Farquhar & Rashke (1978) similarly noted a markedly higher resistance to the diffusion of helium across the leaf of *Z. mays*, in comparison with a range of leaves of dicotyledons. Conceptually, then, the *Z. mays* leaf may be envisaged as two more or less separate air-space systems supplying CO_2 to the cells of the upper mesophyll via the stomata of the upper epidermis and the cells of the lower mesophyll via the stomata of the lower epidermis (Long *et al.* 1989). The calculated p_{CO_2} differed at most by 30 μPa Pa^{-1} and this, coupled with the low intercellular conductance, would mean little net diffusion of CO_2 between the upper and lower mesophyll. Differences in the rates of CO_2 uptake can be almost entirely attributed to the difference in mean light level received by the two tissues, i.e. there is no evidence of any adaptation of the upper mesophyll to high light or the lower mesophyll to shade, as might be expected in an erect-leaved canopy, where direct sunlight might commonly strike either leaf surface. In a light level approaching full sunlight (PPFD = 1500 μmol quanta m^{-2} s^{-1}), calculated rates of CO_2 uptake into the upper and lower mesophyll are similar. On chilling in the light, CO_2 uptake declines in both the upper and lower mesophyll; however, as the duration of the chill progresses CO_2 uptake declines rapidly in the upper mesophyll,

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becoming negative after 3 h. In contrast, CO_2 uptake by the lower mesophyll remains more or less constant (figure 1). On return to 20 °C, CO_2 uptake by the lower surface returned within one hour to a value not significantly different ($t, p < 0.05$) from its pre-chill value. The upper surface showed a rate of CO_2 uptake of just one quarter of its pre-chill rate, clearly showing that photoinhibition of CO_2 uptake is localized to the exposed cells of the upper mesophyll, which appear to protect the underlying lower mesophyll (figure 1). A similar distribution of photoinhibition has been suggested for *Eucalyptus pauciflora* leaves by Kirschbaum (1987*b*).

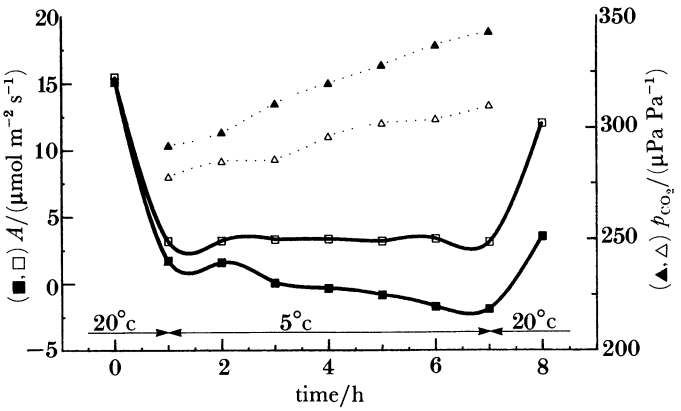

FIGURE 1. Change in calculated CO_2 uptake rate (A) of the upper (■) and lower (□) mesophyll with a photon flux density of 1500 μmol quanta m^{-2} s^{-1} incident on the upper surface of a disc cut from the seventh leaf of *Z. mays*. After 1 h at 20 °C, leaf temperature was reduced to 5 °C for 6 h and then returned to 20 °C. Parallel estimates of the intercellular air space p_{CO_2} are given for the upper (▲) and lower (△) mesophyll: ambient CO_2 pressure fraction was 345 μPa Pa^{-1}. Each point is the mean of three replicate measurements.

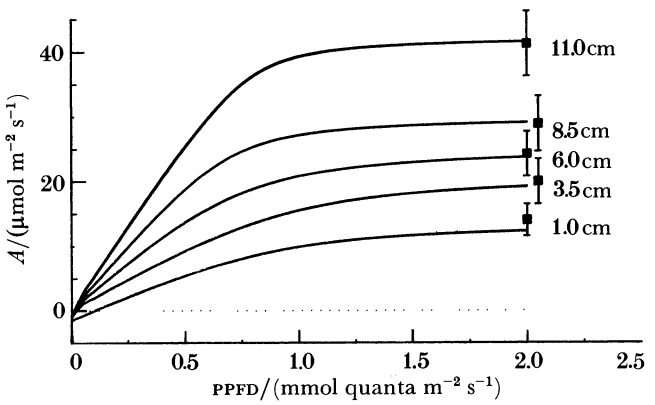

FIGURE 2. The response of CO_2 uptake rate (A) by 2 cm segments of the lamina of the second leaf of *Z. mays* to incident PPFD. Curves are fitted to measurements for five leaves. The final point indicates the actual mean $A \pm$ s.e. at PPFD = 2 mmol m^{-2} s^{-1}. Distances of the mid-point of each segment from the ligule are indicated; the distance from ligule to leaf tip was 12 cm.

Variation along the length of the lamina

On emergence of the ligule there is a marked gradient along the leaf in the rates of CO_2 uptake at a near-saturating PPFD of 2000 μmol quanta m^{-2} s^{-1} (A_{sat}). Rates vary from 41 μmol m^{-2} s^{-1} at the tip to just 14 μmol m^{-2} s^{-1} in the basal 2 cm (figure 2). A decline in the initial slope of the response of CO_2 uptake to light (ϕ_{app}) is also indicated down the length of the leaf (figure 2). The decline in ϕ_{app} is most marked in the basal 2 cm. There is also a change in the

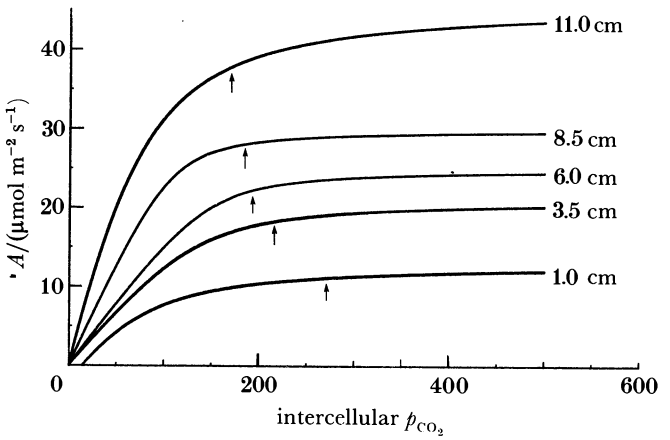

FIGURE 3. The response of CO_2 uptake rate (A) by 2 cm segments of the lamina of the second leaf of *Z. mays* to intercellular air space p_{CO_2}. Measurements were made at 20 °C and a photon flux density incident on the leaf of 2 mmol m^{-2} s^{-1}. Curves are fitted to measurements for five leaves. Distances of the mid-point of each segment from the ligule are indicated; the distance from ligule to leaf tip was 12 cm. The operating intercellular p_{CO_2} is indicated for each curve by an arrow.

convexity of the fitted curve, which is greatest in the segment closest to the tip (figure 2). The magnitude of variation in A_{sat} reported here for different positions on the same leaf when measured in the same microclimatic environment is just as large as the variation in A_{sat} of whole laminae induced by a change in leaf temperature from 25 °C to 5 °C (Long *et al.* 1983). The decline in ϕ_{app} towards the ligule cannot be fully explained by a decrease in light absorptivity, which only declines by *ca.* 10% from 11 to 3.5 cm above the ligule. The decline does, however, correspond to the decrease in the relative quantity of variable chlorophyll fluorescence reported previously (Miranda *et al.* 1981*b*). The increase in A_{sat} along the leaf is reflected in an increase in the carboxylation efficiency, i.e. the initial slope of the response of A to P_{CO_2} and in the CO_2-saturated value of A (A_{max}). The change in carboxylation efficiency, which approximately doubles between the mid-point and tip of the leaf, corresponds to the approximate doubling in phosphoenolpyruvate (PEP) carboxylase activity (Miranda *et al.* 1981) and ribulose bisphosphate carboxylase–oxygenase (Rubisco) protein (W. Masih, unpublished data) shown to occur between these two leaf positions. The operating point is the intercellular p_{CO_2} obtained at the ambient CO_2 pressure fraction of 345 μPa Pa^{-1}. This point can be seen to increase towards the ligule (figure 3). In most cases A at the operating point is on the upper part of the curve, suggesting a low stomatal limitation, as defined by Farquhar & Sharkey (1982). Only in the tip segment does stomatal limitation exceed 10%. In the lower 10 cm, the operating point is close to the upper linear portion of the A/p_{CO_2} response (figure 3).

The photoinhibitory treatment reduces the apparent quantum yield of the tip segment by 60% (t, $p < 0.01$) (figure 4). Although all segments were subjected to the same photon flux during the chilling treatment, a gradient of photoinhibition may be seen with no apparent damage at the ligule and maximal damage at the tip. A similar pattern is apparent in the photoinhibitory reduction of A_{sat} (figure 5).

Figure 6 illustrates the variation in A_{sat} in 1 cm segments along the second leaf two days after ligule emergence. By this stage the position of the highest A_{sat} has moved back about 3 cm from the tip (cf. figure 3). The photoinhibitory treatment similarly produces the maximal proportionate decrease near the tip and least at the base, presumably reflecting greater

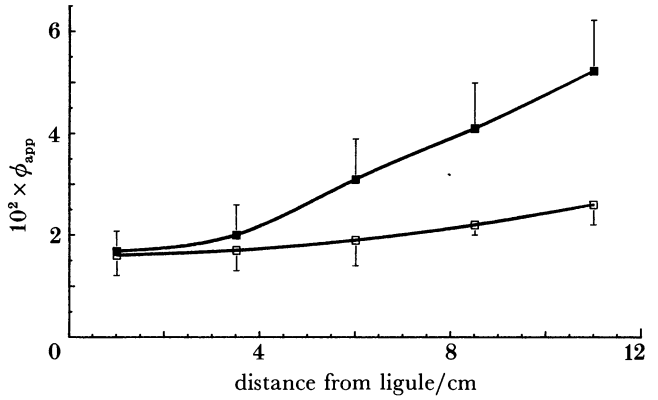

FIGURE 4. The apparent quantum yield (ϕ_{app}) of CO_2 uptake in relation to position along the lamina of the second leaf of *Z. mays*; for controls (■) and for leaves previously at 5 °C in a photon flux density of 1000 μmol quanta m^{-2} s^{-1} and returned to 20 °C 1 h before measurement (□).

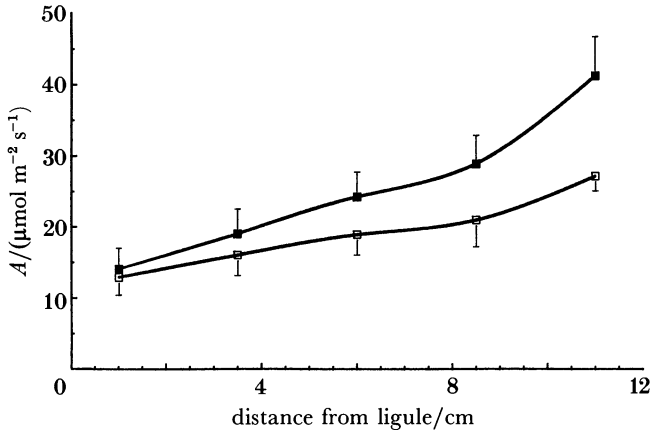

FIGURE 5. The rate of CO_2 uptake (A), at a photon flux density of 2000 μmol quanta m^{-2} s^{-1}, in relation to position along the lamina of the second leaf of *Z. mays*; for controls (■) and for leaves previously chilled at 5 °C in a photon flux density of 1000 μmol quanta m^{-2} s^{-1} and returned to 20 °C 1 h before measurement (□).

capacity for resynthesis of damaged components of the photosynthetic apparatus in the younger tissue. Chilling of plants in high light before emergence of the ligule produces a chlorotic band or restriction in the lamina that formed subsequently. The formation of chlorotic bands following chilling in tropical grasses is well known and was first described by Faris (1926) for *Saccharum officinarum*. Miedema *et al.* (1987) describe the conditions producing such banding in *Z. mays* cultivars. Besides the well-known visual symptoms, significant changes in the distribution of photosynthetic capacity throughout the leaf are apparent. The chlorotic band occurred 6 cm from the ligule, and an obvious depression of A_{sat} relative to control leaves may be seen at this point (figure 6). Above the band all leaf segments show a depression of 10–20 % in A_{sat} relative to controls. Below the band, there is a significant increase in A_{sat} relative to the control, which would in part compensate for the loss of photosynthetic capacity in the older parts of the lamina.

Variation across the width of the leaf

Heterogeneity or patchiness in photosynthesis observed in *Helianthus annuus* and *Phaseolus vulgaris* leaves suggests that variation occurs around areas of the air space system isolated by

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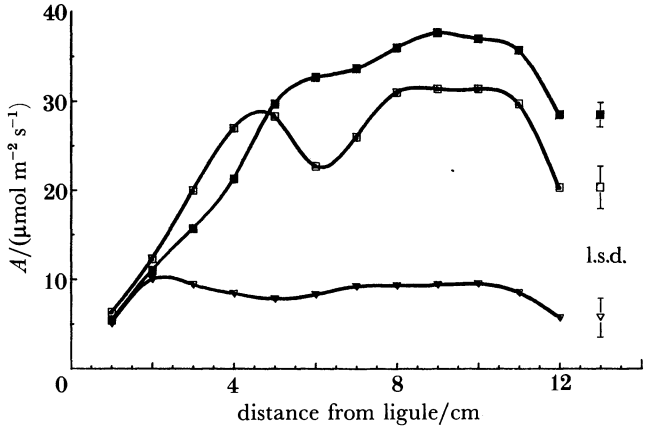

FIGURE 6. CO_2 uptake rate (A) at 2 cm intervals along the lamina of the second leaf of *Z. mays* *ca.* 2 days after emergence of the ligule; for controls (■), leaves chilled at 5 °C for 8 h in a photon flux density (PPFD) of 1000 μmol quanta m^{-2} s^{-1} and returned to 20 °C 1 h before measurement (▽), and leaves from plants which had been chilled at 5 °C with a PPFD of 1000 μmol m^{-2} s^{-1} for 14 h, 3 days before the emergence of the ligule (□). The measured leaves showed a narrow chlorotic band 6 cm from the ligule. l.s.d. indicates the least significant difference between points at the 95% confidence interval of Student's t-test.

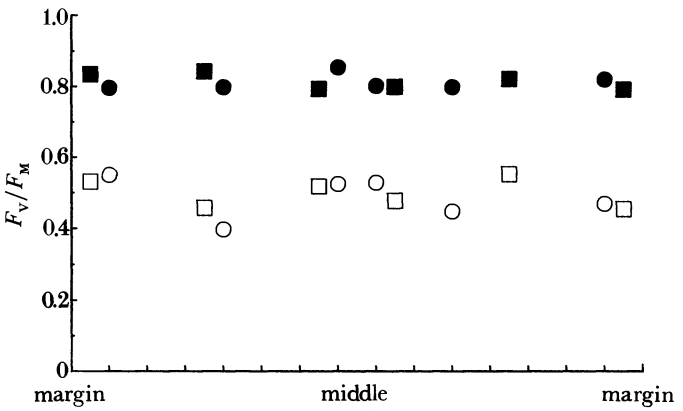

FIGURE 7. The ratio of variable chlorophyll fluorescence to maximum fluorescence (F_V/F_M) measured from 125 μm × 30 μm areas across the width of the lamina of the second leaf of *Z. mays*. Measurements were made on the mesophyll cells between vascular bundles (■, □) and on the mesophyll cells overlying the vascular bundles (●, ○). Closed symbols are for controls; open symbols for leaves chilled at 5 °C in a photon flux density of 1000 μmol quanta m^{-2} s^{-1} for 6 h, and returned to 20 °C about 1 h before measuring.

veins. In the *Z. mays* leaf, air spaces form longitudinal channels along the length of the leaf, between the parallel lines of the vascular bundles and each below a row of stomata. Paradermal and longitudinal sections show that these channels are not continuous, but that the mesophyll appears partly to plug the channels at frequent intervals, forming a restriction to longitudinal diffusion (Long *et al.* 1989). Thus isolated air spaces can be found in both the upper and lower halves of the mesophyll. Heterogeneity across the width of the leaf might therefore be expected to occur on the scale of the interveinal distance, i.e. the width of three or four mesophyll cells. Clearly gas exchange techniques, with the possible exception of radioactive CO_2 uptake, do not allow identification of variability over such small distances. A close correlation between quantum yield and the ratio of the variable component of chlorophyll fluorescence to the maximal fluorescence signal has been widely demonstrated (F_V/F_M) (see, for example,

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Björkman & Demmig 1987; Baker *et al.*, this symposium). Use of a rectangular excitation beam (125 μm × 30 μm) allowed measurement of F_V/F_M within the gap between veins of the *Z. mays* leaf. Figure 7 illustrates the typical results of these point measurements across one *Z. mays* leaf. Measurements at 15–30 points across six leaves failed to reveal any marked heterogeneity either before or after photoinhibitory treatment. The reduction in F_V/F_M, which corresponds to a decline in the apparent quantum yield of CO_2 by the whole leaf (Baker *et al.*, this symposium) appears to result from a more or less uniform decline in F_V/F_M across the leaf (figure 7).

Some implications of the observed spatial variability

The most marked variability in capacity for photosynthetic CO_2 uptake in *Z. mays* leaves is that which occurs along the length of the lamina. At emergence, the fifth of the leaf closest to the tip would account for 35 % of the total CO_2 uptake in high light. However, this pattern may be substantially modified by the environment. Photoinhibition is most damaging at the tip, decreasing the proportion of total photosynthesis contributed by the tip region. The environment during development of the leaf can also have a marked effect on the distribution along the leaf. The variability observed along the lamina of the second leaf equals that produced by major environmental differences and considerably exceeds variation between leaves. The marked gradients also illustrate the practical problem of selecting a 'representative' area for gas-exchange measurements. A shift in position of 2 cm between leaves could produce a 30 % change (figure 2). This problem is worsened when examining the effects of a chilling pre-treatment. Selection of an area at 4 cm from the ligule as 'representative' would yield the result that chilling pre-treatment enhances subsequent CO_2 uptake, whereas selection of an area at 6 cm would provide the opposite result (figure 6).

Figure 3 illustrates that the A/p_{CO_2} response varies markedly between different parts of the leaf. Each section of the leaf shows a different carboxylation efficiency, operating point, and A_{max}. The response curve that would be obtained for the whole lamina would be the average of the five curves illustrated. The shape of this response would be different, because by averaging responses for the different positions, the inflexion of the response would be greatly extended; this would, however, be purely a result of the variability and not a reflection of any special property within the mesophyll.

The high A_{sat}, A_{max}, ϕ_{app} and carboxylation efficiency of the tip region suggests a disproportionate investment of resources for protein and pigment synthesis in this part of the leaf. This may, however, be a strategy for maximizing total leaf photosynthesis. As the second leaf emerges the unfolding third and fourth leaves rise vertically through the sheath and above the ligule of the second leaf. As the orientation to the sun changes through the day the portion of the second lamina closest to the ligule will be shaded for the longest period. Similarly, reduction in diffuse radiation through interception by the developing third and fourth leaves will be greatest at the lamina base. In this situation and uneven distribution of photosynthetic capacity towards the tip provides a higher light-saturated rate of photosynthesis to the region that would receive the highest photon fluxes for the longest periods, possibly optimizing the distribution of resources for CO_2 uptake by the leaf as a whole.

Properties attributed in earlier studies to the whole leaf may in fact be the result of changes in one part. Thus the well-documented chilling-dependent photoinhibition of whole *Z. mays* seedling leaves in fact reflects photoinhibition of the region closest to the tip, while the lamina base remains largely unaffected. Similarly, across the leaf the 50 % photoinhibition observed

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following chilling in high light is predominantly the result of damage to the upper mesophyll, the lower mesophyll showing little loss of photosynthetic capacity.

In conclusion, marked heterogeneity in capacity for photosynthetic CO_2 uptake exists within the leaf, necessitating (1) careful consideration of its significance in selecting 'representative' areas for determination of environmental effects on photosynthesis or in genotype comparisons; and (2) consideration in the interpretation of responses of CO_2 uptake to light and intercellular CO_2 levels.

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References

Bingham, M. J. & Long, S. P. 1985 Equipment for field and laboratory studies of whole plant and crop photosynthesis and productivity research. In *Techniques in bioproductivity and photosynthesis*, 2nd edn. (ed. J. Coombs, D. O. Hall, S. P. Long & J. M. O. Scurlock), pp. 229–271. Oxford: Pergamon Press.

Björkman, O. & Demmig, B. 1987 Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origin. *Planta* **170**, 489–504.

Bolhár-Nordenkampf, H. R. & Lechner, E. G. 1988 Temperature and light dependent modifications of chlorophyll fluorescence kinetics in spruce needles during winter. *Photosynth. Res.* **18**, 287–298.

Bongi, G. & Long, S. P. 1987 Light-dependent damage to photosynthesis in olive leaves during chilling and high temperature stress. *Pl. Cell Environ.* **10**, 241–249.

Faris, J. A. 1926 Cold chlorosis of sugar cane. *Phytopathology* **16**, 885–891.

Farquhar, G. D. 1989 Models relating subcellular effects of temperature to whole plant responses. In *Plants and temperature* (ed. S. P. Long & F. I. Woodward), pp. 395–415. Cambridge University Press.

Farquhar, G. D. & Raschke, K. 1978 On the resistance to transpiration of the sites of evaporation within the leaf. *Pl. Physiol.* **61**, 1000–1005.

Farquhar, G. D. & Sharkey, T. D. 1982 Stomatal conductance and photosynthesis. *A. Rev. Pl. Physiol.* **33**, 317–376.

Harris, G. C., Cheesbrough, J. K. & Walker, D. A. 1983 Measurement of CO_2 and H_2O vapour exchange in spinach leaf discs. *Pl. Physiol.* **71**, 102–107.

Hipkins, M. F. & Baker, N. R. 1986 *Photosynthesis energy transduction: a practical approach.* Oxford: IRL Press.

Ireland, C. R., Long, S. P. & Baker, N. R. 1984 The relationship between carbon dioxide fixation and chlorophyll *a* fluorescence during induction of photosynthesis in maize leaves at different temperatures and carbon dioxide concentrations. *Planta* **160**, 550–558.

Ireland, C. R., Telfer, A., Covello, P. S., Baker, N. R. & Barber, J. 1988 Studies on the limitations to photosynthesis in leaves of the atrazine-resistant mutant of *Senecio vulgaris* L. *Planta* **173**, 459–463.

Kirschbaum, M. U. F. 1987*a* A model of photosynthesis for isobilateral leaves incorporating biochemical constraints and the light gradient within the leaf. In *Progress in photosynthesis research* (ed. J. Biggins), vol. 4, pp. 261–264. Dordrecht: Martinus Nijhoff.

Kirschbaum, M. U. F. 1987*b* Water stress in *Eucalyptus pauciflora*: comparison of effects on stomatal conductance with effects on the mesophyll capacity for photosynthesis, and investigation of a possible involvement of photoinhibition. *Planta* **171**, 466–473.

Körner, C., Scheel, J. A. & Bauer, H. 1971 Maximum leaf diffusion conductance in vascular plants. *Photosynthetica* **13**, 45–82.

Leech, R. M., Rumsby, M. G. & Thomson, W. W. 1973 Plastid differentiation, acyl lipid and fatty acid changes in developing green maize leaves. *Pl. Physiol.* **52**, 240–245.

Long, S. P. 1985 Leaf gas exchange. In *Photosynthetic mechanisms and the environment* (ed. J. Barber & N. R. Baker), pp. 453–499. Amsterdam: Elsevier.

Long, S. P. 1986 Instrumentation for the measurement of CO_2 assimilation by crop leaves. In *Advanced agricultural instrumentation. Design and uses* (ed. W. G. Gensler), pp. 39–91. Dordrecht: Martinus Nijhoff.

Long, S. P., East, T. M. & Baker, N. R. 1983 Chilling damage to photosynthesis in young *Zea mays*. I. Effects of light and temperature variation on photosynthetic CO_2 assimilation. *J. exp. Bot.* **34**, 177–188.

Long, S. P., Farage, P. K., Bolhár-Nordenkampf, H. R. & Rohrhofer, U. 1989 Separating the contribution of the upper and lower mesophyll to photosynthesis in *Zea mays* leaves. *Planta.* (In the press.)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Long, S. P. & Hällgren, J.-E. 1985 Measurement of CO_2 assimilation by plants in the field and the laboratory. In *Techniques in bioproductivity and photosynthesis*, 2nd edn. (ed. J. Coombs, D. O. Hall, S. P. Long & J. M. O. Scurlock), pp. 62–94. Oxford: Pergamon Press.

Marshall, B. & Biscoe, P. V. 1980 A model for C_3 leaves describing the dependence of net photosynthesis on irradiance. I. Derivation. *J. exp. Bot.* **31**, 29–39.

Miranda, V., Baker, N. R. & Long, S. P. 1981*a* Anatomical variation along the length of the *Zea mays* leaf in relation to photosynthesis. *New Phytol.* **88**, 595–605.

Miranda, V., Baker, N. R. & Long, S. P. 1981*b* Limitations of photosynthesis in different regions of the *Zea mays* leaf. *New Phytol.* **89**, 179–190.

Miedema, P., Post, J. & Groot, P. J. 1987 *The effects of low temperature on seedling growth of maize genotypes*. Wageningen: Pudoc.

Pritchard, D. T. & Currie, J. A. 1982 Diffusion coefficients of carbon dioxide, nitrous oxide, ethylene and ethane in air and their measurement. *J. Soil Sci.* **33**, 175–184.

Reid, R. C., Prauznitz, J. M. & Sherwood, T. K. 1977 *The properties of gases and liquids*. New York: McGraw Hill.

Ross, J. 1975 Radiative transfer in plant communities. In *Vegetation and the atmosphere* (ed. J. L. Monteith), vol. 1, pp. 13–55. London: Academic Press.

Terashima, I. 1986 Dorsiventrality in photosynthetic light response curves of leaf. *J. exp. Bot.* **37**, 399–405.

Terashima, I. & Saeki, T. 1985 A new model for leaf photosynthesis incorporating the gradients of light environment and of photosynthetic properties of chloroplasts within a leaf. *Ann. Bot.* **56**, 489–499.

Terashima, I., Wong, S.-C., Osmond, C. B. & Farquhar, G. D. 1988 Characterisation of non-uniform photosynthesis induced by abscisic acid in leaves having different mesophyll anatomies. *Pl. Cell Physiol.* **29**, 385–394.

von Caemmerer, S. & Farquhar, G. D. 1981 Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **153**, 376–387.

Walker, D. A., Sivak, M. N., Prinsley, R. T. & Cheesbrough, J. K. 1983 Simultaneous measurement of oscillations in oxygen evolution and chlorophyll *a* fluorescence in leaf pieces. *Pl. Physiol.* **73**, 542–549.

Discussion

R. Hill (*Department of Biochemistry, University of Cambridge, U.K.*). Wilstätter & Stohl's classic book (1918) was concerned with the activity of chlorophyll in relation to its natural function. In a young leaf the chlorophyll had its greatest specific activity. In modern terms the specific activity of the chlorophyll is inversely related to the amount of chlorophyll concerned with light harvesting. In the leaves used by Wilstätter the formation of the chlorophylls belonging to the antenna was spread over many days.

Reference

Willstätter, R. & Stoll, A. 1918 *Untersuchungen über die Assimilation der Kohlensäure*. Berlin: Springer.

S. P. Long. Modern techniques largely support Wilstätter and Stohl's view. Previous work (Baker & Leech 1977; Miranda 1980; Miranda 1981) indeed shows that the light-saturated rates of oxygen and CO_2 evolution per chlorophyll molecule decrease along the second leaf of 7-day-old maize from about 1 cm above the leaf base, i.e. the specific activity is indeed highest in the younger tissue. Similarly, PSI and PSII activities per unit chlorophyll show a parallel decline, reflecting the higher levels of antenna pigment formed in the older segments. However, total PSI and PSII activity does increase along the length of the leaf, suggesting that the decline in specific activity reflects a gradual shift in favour of antenna pigment formation relative to formation of active reaction centres.

References

Baker, N. R. & Leech, R. M. 1977 Development of photosystem I and photosystem II activities in leaves of light-grown maize (*Zea mays*). *Pl. Physiol.* **60**, 640–644.

Miranda, V. 1980 *Changes in anatomy and photosynthetic activity of the Zea mays leaf during biogenesis*. Ph.D. thesis, University of Essex.

Miranda, V. 1981 Limitations of photosynthesis in different regions of the *Zea mays* leaf. *New Phytol.* **89**, 179–190.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES