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Leaf Development in *Lolium temulentum*: Photosynthesis and Photosynthetic Proteins in Leaves Senescing under Different Irradiances

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Changes in carbon fixation rate and the levels of photosynthetic proteins were measured in fourth leaves of *Lolium temulentum* grown until full expansion at 360 μmol quanta $m^{-2} s^{-1}$ and subsequently at the same irradiance or shaded to 90 μmol $m^{-2} s^{-1}$. Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), light-harvesting chlorophyll *a/b* protein of photosystem II (LHCII), 65 kDa protein of photosystem I (PSI), cytochrome *f* (Cyt *f*) and coupling factor 1 (CF_1) declined steadily in amount throughout senescence in unshaded leaves. In shaded leaves, however, the decrease in LHCII and the 65 kDa protein was delayed until later in senescence whereas the amount of Cyt *f* protein decreased rapidly following transfer to shade and was lower than that of unshaded leaves at the early and middle stages of senescence. Decreases in the Rubisco and CF_1 of shaded leaves occurred at slightly reduced rates compared with unshaded leaves. These results indicate that chloroplast proteins in fully-expanded leaves are controlled individually, in a direction appropriate to acclimate photosynthesis to a given irradiance during senescence.

Key words: *Lolium temulentum* — Leaf senescence — Photosynthesis — Protein metabolism — Shading.

Photosynthetic capacity and nitrogen content diminish during leaf senescence. Nitrogen and photosynthesis are directly related because 70–80% of the nitrogen of mature leaves is located in the chloroplasts (Morita and Kono 1974, Makino and Osmond 1991). Furthermore, roughly 90% of the nitrogen released from senescent leaves can be accounted for by a loss of chloroplast nitrogen (Morita 1980). Most of this nitrogen is invested in carbon reduction cycle enzymes and thylakoid proteins (Evans and Seeman 1989).

There have been relatively few studies of changes in chloroplast components in relation to photosynthesis during natural senescence of leaves attached to the intact plant. The relationship between Rubisco and photosynthesis is the best understood (Makino et al. 1984, Evans 1987, Crafts-Brandner et al. 1990); the relatively great abundance of Rubisco in leaves where it represents 20 to 30% of total leaf nitrogen (Makino et al. 1984, Evans 1989) makes it experimentally convenient. Similarly there have been observations on the most abundant thylakoid protein, LHCII (Thornber 1975) and other components of the light harvesting system (Thornber 1975, Thomas 1988, Hidema et al. 1992) but these have been less directly related to photosynthesis. Other studies include the relationship of PSI and PSII to electron transport capacity (Jenkins and Woolhouse 1981a, b) and also observations on the loss of electron transport capacity during senescence associated with a loss of the Cyt b_6/f complex in a number of species (Ben-David et al. 1983, Holloway et al. 1983, Roberts et al. 1987). In order to understand how these changes in chloroplast components interact during senescence to give rise to the photosynthetic rates observed, there is a need for an integrated study relating the major photosynthetic components to photosynthetic rates.

Abbreviations: CF_1, coupling factor 1; LHCII, light-harvesting chlorophyll *a/b* protein complex of PSII; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase.

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We present here a quantitative study of changes in *Lolium temulentum* leaf proteins representative of the major photosynthetic processes: Rubisco (carbon reduction); LHCII (light harvesting); Cyt *f* (electron transport); CF_1 (phosphorylation); and the 65 kDa protein of PSI (photosystem reaction centre). In previous studies, it was shown that photosynthesis and photosynthetic components in rice leaves are adaptively sensitive to the light environment during senescence (Hidema et al. 1991, 1992). The acclimation capability of the mature photosynthetic apparatus was also investigated in *L. temulentum* fourth leaves exposed to two different irradiances from full expansion through senescence.

Materials and Methods

Plant materials—Plants of *Lolium temulentum* Ba3081 were grown hydroponically from seed under controlled environment conditions as described by Pollock (1982). The temperature was 20°C, water vapour pressure deficit 0.6 kPa, photoperiod 8 h and photosynthetic photon flux density approximately 360 μmol quanta m^{-2} s^{-1} (white fluorescent tubes) at the surface of the plant at the time of full expansion of the fourth leaf. Plants were divided into two groups, one unshaded, the other shaded to 90 μmol quanta m^{-2} s^{-1}. The latter treatment used shade net (75% cutoff) with neutral light transmission characteristics, applied continuously from full expansion of the fourth leaf on the main tiller, which occurred 30 days after sowing. All measurements were made on the fourth leaf. Assays of photosynthesis, nitrogen and Rubisco were expressed per unit leaf area, and relative assays of other proteins were carried out on a fresh weight basis. The ratio of fresh weight to leaf area was 196 ($\pm$1.4 sem) g m^{-2} overall, allowing expression of protein and pigment on the same leaf area basis as photosynthesis and nitrogen.

Sample preparation and measurement of chlorophyll and nitrogen—After measuring photosynthesis and leaf area, tissue was weighed and homogenised in a chilled mortar and pestle with 9 ml (g fresh weight)$^{-1}$ of ice-cold 50 mM lithium phosphate buffer pH 7.2 containing 120 mM 2-mercaptoethanol, 1 mM sodium monoiodoacetate, 1 mM phenylmethylsulfonylfluoride and 5% (v/v) glycerol. Duplicate 100 μl portions of the homogenate were used for Chl determination, measured spectrophotometrically after extraction in 80% acetone with pigment content calculated using the coefficients quoted by Hill et al. (1985). Total leaf nitrogen content was determined with Nessler's on 100 μl aliquots of homogenate after Kjeldahl digestion and addition of sodium potassium tartrate. A 0.36 ml portion of the remaining homogenate was treated with 20% (w/v) lithium dodecyl sulfate (LDS) solution, added to a final concentration of 2%. The mixture was heated immediately at 100°C for 45 s and then centrifuged at 10,000 $\times$ g_{ave} for 5 min. The supernatant was stored at −20°C until used for sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).

Determination of Rubisco—The amount of Rubisco was determined spectrophotometrically following SDS-PAGE separation on 12.5% gels (Laemmli 1970) and formamide extraction of Coomassie brilliant blue R-250 from the band of the large subunit as described by Makino et al. (1986). Calibration curves were made with purified rice Rubisco.

Antisera—Antibodies against CF_1 (α and β subunits) and Cyt *f* were raised separately in rabbits as previously described (Hidema et al. 1991). Chloroplast CF_1 was purified from spinach leaves according to Lien and Racker (1975) and Nelson et al. (1983) and then prepared by SDS-PAGE. Spinach Cyt *f* was purchased from Sigma Chemical Co. and additionally purified by SDS-PAGE. Antiserum against *Festuca pratensis* LHCII was prepared as described by Hilditch (1986) and antiserum against the reaction center complex of PSI was the kind gift of Professor G M Giacometti, University of Padova, Italy.

Quantitative western blotting—Blotting from SDS-polyacrylamide gel onto nitrocellulose and immunodetection were carried out according to the instruction manual of the Bio-Rad Immuno Blot Assay Kit. Horseradish peroxidase-conjugated swine-anti-rabbit secondary antibodies (DAKO, High Wycombe, U.K.) were used for immunodetection. The intensity of developed colour on the filter was quantified by reflectance scanning at 520 nm using a Helena Quick-scan densitometer. Relative amounts of proteins in leaves at different stages of senescence under the two light regimes were estimated by adopting the following routine procedure. The two outer tracks of the ten on each gel were not used. Tracks 2 and 9 were always loaded with protein extract from the fourth leaf at full expansion, i.e. day 0. Tracks 3 to 8 inclusive were loaded with proteins from leaves at −4, 4, 7, 11, 14 and 18 days (unshaded) or 4, 7, 11, 14, 18 and 24 days (shaded) after full expansion. Linearity of the densitometer response was ensured by scanning tracks on which different amounts of protein from 0 days leaves were loaded. The amount of antigen in each track was determined as peak area and expressed as a percentage of the 0 days sample run on the same gel. Analyses were replicated at least twice and only results from blots in which the two reference tracks gave identical intensities were accepted.

Measurement of photosynthesis—Photosynthetic rates were determined on attached leaves sealed into cuvettes which were part of an open gas exchange system. CO_2 exchange was measured by infrared gas analyser (225 Mk 3, ADC Ltd., Hoddesdon, U.K.) calibrated in absolute mode with gas mixing pumps (Bate et al. 1969) and in the differential mode by the divided tube method (Parkinson and Legg 1978). Calculations of photosynthesis were made ac-

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cording to Farquhar and Sharkey (1982) after allowances for the dilution of the gas stream by transpiration (Penning de Vries et al. 1984) and the self-broadening of CO_2 absorption bands in the presence of water vapour (Kirschbaum and Farquhar 1984). Water vapour content was measured with a dew point hygrometer (EG & G, Waltham, Mass., U.S.A.) and the dewpoint of the air supplied to the cuvettes was maintained at 1°C. The air supplied to the cuvettes was maintained at over 780 μbar CO_2, giving a minimum internal CO_2 concentration of 650 μbar, saturating for this material. Leaf temperature was 20.5±0.5°C and irradiance was 800 μmol quanta $m^{-2} s^{-1}$, above light saturation for this tissue.

Statistical treatment—Analysis of the degree of coordination between amounts of photosynthetic components used functional relationships which assume that both components are measured subject to experimental errors. The comparisons between shaded and unshaded plants used parallel curve analysis assuming a straight line relationship. If there were significant differences (at $P=0.05$) then separate lines were fitted to shaded and unshaded plants; otherwise data were pooled and a single line was fitted. Correlation coefficients were calculated for shaded and unshaded plants separately and combined, and all statistical analyses were performed using the Maximum Likelihood Program (Ross 1987).

Results

Fourth leaves of *Lolium temulentum* plants grown as described were fully expanded 30 days after sowing. Measurements were made on leaves aged under unshaded conditions compared with leaves developing from full expansion to senescence at approximately 25% of the previous irradiance level. In unshaded plants, self-shading by foliage above the fourth leaf only occurred to a small extent at the last stage of senescence. In the shaded treatment the light-acclimated leaves developed after full expansion of the fourth leaf were longer than those of unshaded plants and so tended to exert a somewhat greater shading influence. The experiment was conducted three times independently with some variations in the particular analyses carried out during each repeat. In the first, preliminary, experiment, leaf growth, photosynthetic rate and contents of total N, Rubisco and Chl were examined. In experiment 2 we additionally measured LHCII, Cyt *f*, CF_1 and PSI 65 kDa protein. In experiment 3 photosynthetic rate, LHCII, Cyt *f*, CF_1 and PSI 65 kDa protein were determined. Essentially reproducible results were obtained from the three experiments; data from experiment 2 are presented here.

Nitrogen content in both shaded and unshaded leaves decreased rapidly after full expansion (Fig. 1a). For the next 7 days in the unshaded and 14 days in the shaded plants, nitrogen was relatively stable, before falling to lower levels during the last 6–7 days of measurement. Assessed in terms of leaf appearance and function, complete senescence of unshaded tissue occurred earlier than shaded. Rubisco content decreased rapidly in the early stages of senescence at both irradiances (Fig. 1b). Over the entire senescence period, Rubisco was lost slightly faster from unshaded than shaded leaves. For about 12 days after full expansion CO_2-saturated photosynthesis declined in a similar fashion in the two treatments (Fig. 1c). Thereafter the rate of photosynthesis fell more quickly in unshaded leaves.

The pattern of Chl followed a similar trend to that of

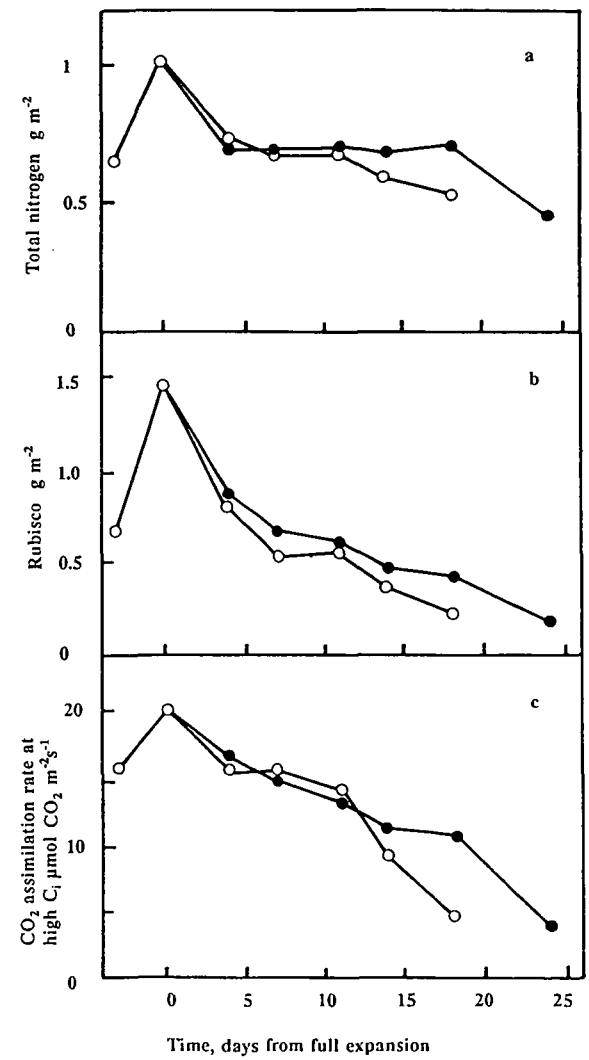

Fig. 1 Changes in the amounts of: (a) total leaf nitrogen; (b) Rubisco; (c) rate of CO_2 assimilation at high intercellular CO_2 in *Lolium temulentum* 4th leaves aged under two different irradiances from full expansion through senescence. Irradiance at plant height: 360 μmol quanta $m^{-2} s^{-1}$ (unshaded treatment ○) and 90 μmol quanta $m^{-2} s^{-1}$ (shaded ●).

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nitrogen content during senescence, with greater differences between shaded and unshaded plants and a more rapid decline in pigment content at the end of the measurements (Fig. 2a). Chlorophyll *a/b* ratio gradually decreased from 10 days after full expansion in both treatments (Fig. 2b).

Changes in LHCII, the major Chl-binding protein of chloroplasts, closely followed those of the pigment in both treatments. Fig. 3 shows that Chl and LHCII were highly correlated in both shaded and unshaded tissue. Amounts of LHCII gradually decreased in the leaves of unshaded

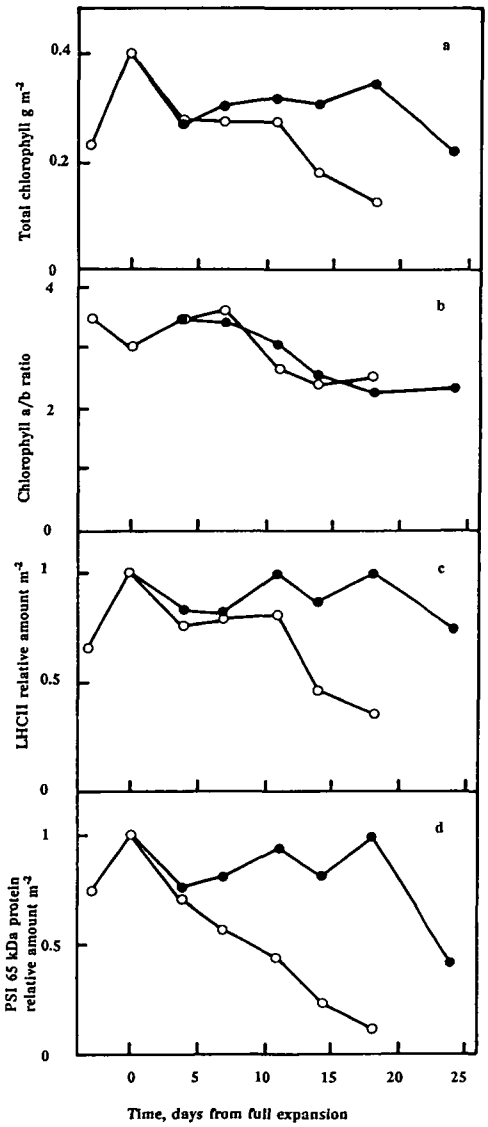

Fig. 2 Changes in: (a) amount of total chlorophyll; (b) ratio of chlorophyll *a/b*; (c) relative amount of light harvesting chlorophyll protein complex II; (d) relative amount of Photosystem I 65 kDa protein in *L. temulentum* 4th leaves senescing under two different irradiances. Unshaded (○), shaded (●).

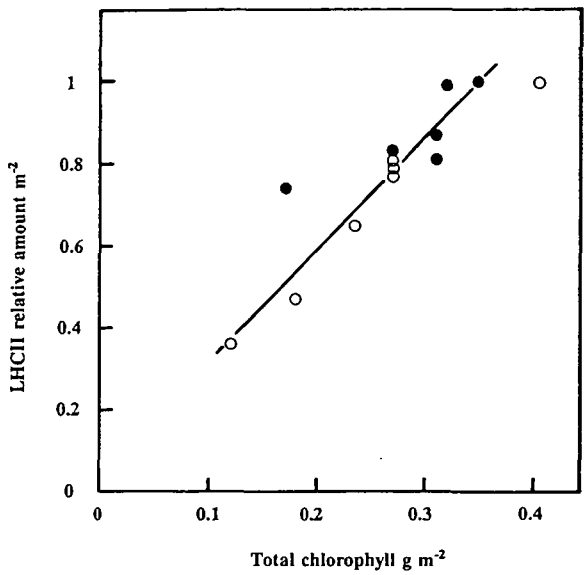

Fig. 3 Relation between amounts of LHCII and chlorophyll in *L. temulentum* 4th leaves under shaded (●) and unshaded (○) conditions. The line shown is a functional relationship fitted to the combined shaded and unshaded data, as there were no significant differences between the data sets.

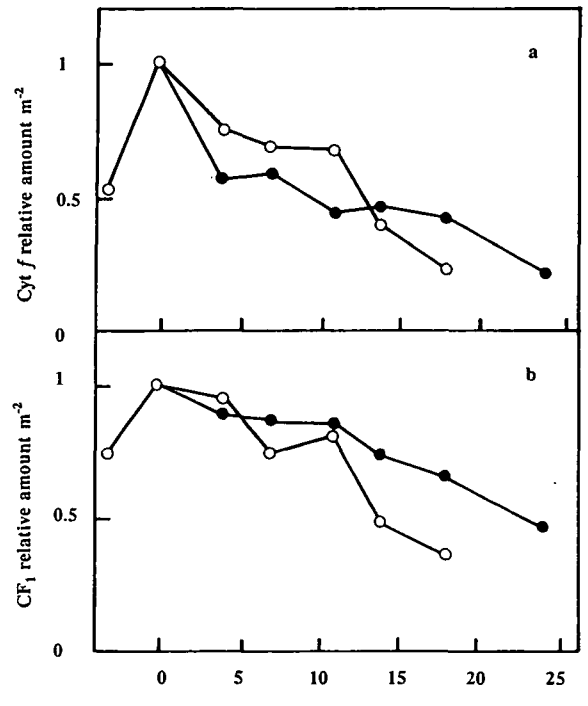

Fig. 4 Changes in the amounts of: (a) Cyt *f*; (b) coupling factor 1 during senescence of the 4th leaf of *L. temulentum* under shaded (●) and unshaded (○) conditions.

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plants but hardly changed at all in tissue exposed to reduced irradiance (Fig. 2c). The 65 kDa protein of PSI, representing the reaction centre subunit 1A/B, is also chl-binding and like LHCII it was much more stable in shaded than unshaded leaves (Fig. 2d). Attempts were made to determine the level of the D_1 protein of PSII by western blotting but the abundance of this component was too low for accurate measurement using the antibody available to us.

Cyt *f* content declined in both shaded and unshaded leaves throughout senescence (Fig. 4a). The decrease was more rapid in shaded than unshaded tissue immediately after full expansion and shaded leaves retained lower levels of Cyt *f* until 14 days after full expansion. Subsequently the Cyt *f* of shaded leaves was more stable than that of unshaded, possibly as a consequence of the delaying effect of shading on senescence. CF_1 content gradually decreased in both treatments, slightly slower in shaded than unshaded tissue (Fig. 4b).

To quantify the extent to which changes in different photosynthetic components are coordinated during senescence, an orthogonal correlation analysis was carried out. Table 1 summarises the result in the form of a correlation coefficient matrix for the shaded and unshaded treatments. It is immediately clear that all components in unshaded leaves moved in step throughout senescence, resulting in uniformly high, significant ($P=0.05$) correlation coefficients. In contrast, shading differentially altered the syndrome so that trends in some components became detached from coordinate control, leading to low coefficients which are not significant at $P=0.05$.

According to the biochemical model of C_3 photosynthesis by Farquhar and von Caemmerer (1982), the rate of CO_2 assimilation at high C_i is limited by RuBP regeneration mediated by electron transport capacity. Thus, photosynthesis at high C_i is expected to correlate with the amounts or activities of the components of electron transport. Fig. 5a presents the relationship between Cyt *f* content and rate of CO_2-saturated photosynthesis. Parallel curve analysis determined that the lines for shaded and unshaded leaves were not significantly different and that the data for the two treatments may be fitted to a single curve (Table 1). Thus Cyt *f* content was well correlated with the rate of photosynthesis irrespective of the irradiance during senescence. CF_1 behaved similarly.

The relationship between the 65 kDa protein of PSI and CO_2-saturated photosynthesis was also analysed

Table 1 Correlation coefficients and parallel line analysis for amounts of photosynthetic components during senescence

	Chl	Psyn	Rubisco	Cyt *f*	LHCII	PSI	CF_1	
Chlorophyll content		0.89*	0.94*	0.98*	0.97*	0.90*	0.91*	U
g m^{-2} (Chl)		0.66	0.44	0.61	0.81*	0.97*	0.58	S
CO_2 Assimilation rate at high	0.71*		0.82*	0.88*	0.89*	0.96*	0.90*	U
C_i μmol m^{-2} s^{-1} (Psyn)	+		0.96*	0.96*	0.33	0.64	0.97*	S
Rubisco content	0.72*	0.85*		0.90*	0.85*	0.91*	0.85*	U
g m^{-2} (Rubisco)				0.90*	0.16	0.45	0.95*	S
Cytochrome *f* relative amount	0.71*	0.88*	0.89*		0.98*	0.88*	0.95*	U
m^{-2} (Cyt *f*)					0.15	0.53	0.92*	S
LHCII relative amount	0.90*	0.51	0.52	0.51		0.86*	0.94*	U
m^{-2} (LHCII)		+	+	+		0.91*	0.27	S
PSI 65 kDa protein relative amount	0.91*	0.64*	0.62*	0.50	0.89*		0.90*	U
m^{-2} (PSI)		+	+	+			0.56	S
CF_1 relative amount	0.80*	0.89*	0.85*	0.85*	0.71*	0.74*		U
m^{-2} (CF_1)				+				S

Right-hand side of the diagonal: coefficients for unshaded (U) and shaded (S) treatments analysed separately. *Left-hand side of the diagonal* presents correlation coefficients for combined U and S data. A significant difference (at $P=0.05$) between lines fitted to shaded and unshaded plants is indicated by +. Significant correlation coefficients (at $P=0.05$) are shown by *.

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(Fig. 5b). The points from the shaded and unshaded leaves were not on the same line. Photosynthesis per unit of the protein was clearly higher in unshaded leaves. This indicates that PSI content is not correlated with the rate of CO_2-saturated photosynthesis in leaves aged under low irradiance. The pattern for LHCII was similar, an indication of preferential retention of this protein in shaded plants in spite of a decline in photosynthesis.

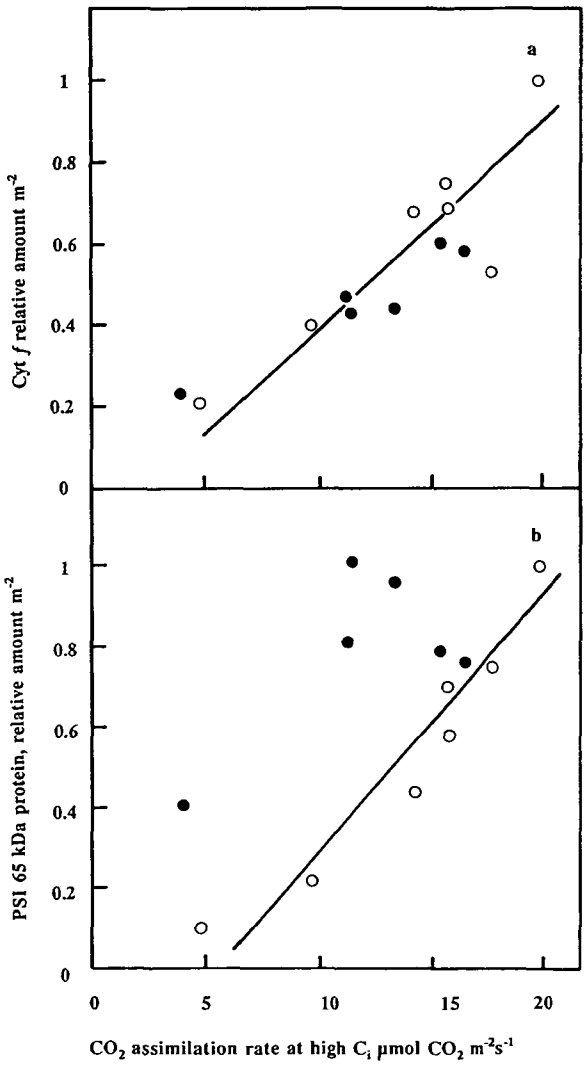

Fig. 5 Amounts of: (a) Cyt *f*; (b) the 65 kDa protein of PSI in relation to rates of CO_2 assimilation at high intercellular CO_2 pressure in shaded (●) and unshaded (○) leaves of *L. temulentum*. The line shown in (a) is a functional relationship fitted to the combined shaded and unshaded data, as there were no significant differences between the data sets. In (b) the line shown is the functional relationship fitted to the data for the unshaded plants only. Since there were significant differences between data sets and as the correlation coefficient for the shaded data was not significant, no line is shown.

Discussion

Control of the protein levels of photosynthetic components during senescence—Changes in the amounts of photosynthetic components were compared during senescence of *L. temulentum* leaves under shaded and unshaded conditions (Fig. 1, 2, and 4). Clear differences in pattern were observed. In unshaded leaves, Rubisco decreased most rapidly, followed by the 65 kDa protein of the PSI reaction centre, CF_1, Cyt *f*, and LHCII. In shaded leaves there was greater heterogeneity in the behaviour of the different proteins (Table 1). Rubisco decreased almost as rapidly as it did in unshaded leaves, whereas the decline in LHCII, 65 kDa protein and Chl was delayed until late senescence. Consequently, the ratio of Rubisco to LHCII, to the 65 kDa protein or to Chl greatly changed through senescence in the shaded leaves. It is concluded that although senescence is carefully controlled, the stoichiometries of photosynthetic components are not constant and are responsive to environmental changes.

Changes in the amounts of components may reflect differences in the rates of synthesis and degradation of the respective proteins during senescence. Protein synthesis in developing leaves has been extensively studied in many plants but less attention has been paid to protein synthesis during senescence. Mae et al. (1983) showed that 90% of the total Rubisco was synthesised by maturity, and that only 10% was synthesised during senescence. Thus for Rubisco, the biosynthetic side of turnover is dominant in developing leaves whereas catabolism is emphasised during senescence. Trends in the balance between synthesis and breakdown of LHCII are similar to those of Rubisco (Hidema et al. 1992). It seems to be generally true that degradation rather than synthesis plays a central role in controlling the levels of photosynthetic components in senescing leaves. One exception appears to be the D1 protein of PSII reaction centre, which turns over rapidly throughout the life of the leaf, probably as a direct consequence of the unstable photochemical environment in which it performs its photosynthetic function (Barber and Andersson 1992). But here too there is evidence of a separate underlying development-related turnover system in which catabolism predominates during senescence (Hilditch et al. 1986).

Neither Chl nor the proteins with which it is associated in the thylakoid change much throughout senescence in the shaded leaves. Hidema et al. (1992) also found that LHCII protein and Chl pigments were comparatively stable in rice leaves senescing under shaded conditions. A pattern of senescence in which pigment-proteins are retained while Rubisco, phosphorylation and overall CO_2-fixation capacity decline in a normal fashion is characteristic of certain "stay-green" senescence mutants, such as those observed in *Festuca* (Thomas 1987), *Phaseolus* (Ronning et al. 1991) and *Glycine* (Guiamet et al. 1990). The exceptional lability

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of the chlorophyll-protein degradation pathway is also evident in the failure of bananas to turn yellow at elevated temperatures, which Seymour et al. (1987) showed is the result of a specific lesion in pigment breakdown. This evidence and many other observations (see Thomas and Smart 1993) strongly suggest that one or a small number of activities controlling chlorophyll-protein breakdown are particularly sensitive to genetic or environmental (e.g. irradiance level) perturbation. It is known that both biosynthesis and catabolism of thylakoid apoproteins are directly and indirectly regulated by the availability and metabolism of associated pigments (Thomas et al. 1989). Using ^{15}N labelling, Hidema et al. (1992) showed that the high level of LHCII retained by shaded rice leaves during senescence is a result of suppression of protein breakdown rather than accelerated synthesis. It would be interesting to correlate the pattern of turnover of Chl proteins with that of the associated pigments.

The proteins of the photosynthetic apparatus are localised in chloroplasts. They may be selectively degraded in situ by imported proteolytic enzymes, by formerly latent enzymes already present in the organelle or by active proteases which target proteins with structural or conformational modifications. Alternatively there may be selective secretion of proteins from chloroplasts followed by rapid proteolysis in the cytosol or vacuole. The heterogeneous nature of chloroplast disassembly described here, as well as in studies of mutants (Thomas 1984), argues strongly against elimination of whole chloroplasts as a mechanism of breakdown, at least in the early and middle stages of senescence. Simultaneous decreases in all the components were observed only towards the end of senescence in the leaves from both treatments. These conclusions are consistent with previous observations that Rubisco and some other components decreased rapidly, but chloroplast number in leaves did not change very much until late senescence (Martinoia et al. 1983, Thomas 1983, Mae et al. 1984, Wardley et al. 1984). There are, however, contrary reports claiming that reduction of photosynthesis is mainly caused by the decrease in chloroplast numbers during senescence (Lamppa et al. 1980, Wittenbach et al. 1982, Camp et al. 1982, Kura-Hotta et al. 1990). The reason for this discrepancy is not known. We previously showed that nitrogen nutrition during leaf development greatly influences lifespan and rate of senescence of leaves (Makino et al. 1984). The present study shows that growth irradiance also affects leaf lifespan and senescence rate. If destruction of whole plastids is a normal feature of the terminal stages of senescence, separate studies of leaves undergoing senescence at very different rates because of variations in growth conditions may encounter diversity in the degree to which the process is expressed. Moreover, as senescence does not occur at the same time in all parts of a leaf, degradation of individual components within chloroplasts and eradication of whole chloroplasts may be happening simultaneously in the same tissue. Thus caution is required when attempting to reconcile the results of experiments in which species, growth conditions and tissue uniformity are not comparable.

Acclimation of the photosynthetic system to low irradiance during senescence—Acclimation of the photosynthetic apparatus to low irradiance during leaf growth has been studied in detail (Anderson 1986, Anderson et al. 1988, Evans 1988); less attention has been paid to the acclimatory capacities of post-maturation and senescing leaves. *L. temulentum* leaves exposed to low irradiance at full expansion showed some characteristics of light acclimation during subsequent senescence. The treatment markedly retarded loss of chl, LHCII and 65 kDa protein of PSI. In contrast, Cyt *f* content declined specifically after the transfer of the plants to low irradiance. Reduction of Cyt *f* protein content per unit Chl and per unit leaf area was also shown in senescing leaves of bean and rice under low irradiance (Ben-David et al. 1983, Roberts et al. 1987, Hidema et al. 1991). It has been reported that Cyt *f* content could be a rate-determining step of electron transport capacity (Evans 1987, Terashima and Evans 1988). The correlation between Cyt *f* and the rate of photosynthesis irrespective of the irradiance during senescence (Fig. 5a) indicates that the level of the protein in thylakoid membranes sensitively responds to the change of irradiance during senescence, and is consistent with a rate-limiting function in CO_2-saturated photosynthesis.

A similar analysis (Fig. 5b) suggests that the PSI reaction centre is not related to CO_2-fixation at the two irradiances in a rate-limiting fashion. No significant change was found in the amount of PSI reaction centre during senescence of oat and bean leaves although a sharp decline in the Cyt b_6/f complex was detected (Roberts et al. 1987, Gepstein 1988). PSI and PSII activities of chloroplast membranes were less labile than non-cyclic electron transport during senescence of bean leaves (Jenkins and Woolhouse 1981a, b). The relationship between PSII and the rate of photosynthesis in *Lolium temulentum* remains to be studied.

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