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Leaf development in *Lolium temulentum* L.: photosynthesis in relation to growth and senescence

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SUMMARY

Length, area, weight and concentrations of pigment were measured from just after emergence until senescence of the fourth leaf of *Lolium temulentum* L. seedlings grown in nutrient solution in a controlled environment. At the same time the progress of photosynthesis, respiration and carbon contents of the leaf were recorded. A new approach to calculating plastochron indices for grasses was developed, based on logistic curves fitted to leaf lengths. Dry weight and photosynthetic rates per unit area were greatest in young leaves. Subsequently photosynthetic capacity at light saturation declined, as did photosynthetic rates at the light incident on the leaf, although the decrease was less pronounced in the latter case. Dry weight per leaf remained fairly constant once full expansion was reached, with perhaps a slight rise in the oldest leaves. The photosynthesis and carbon content data were combined to calculate the carbon balance of leaf 4, with respect both to the atmosphere and to the rest of the plant. Net gas exchange became positive about 7 d before full leaf expansion. Leaf weight continued to increase so that the carbon balance with the rest of the plant did not become positive until just after final leaf size had been attained. These results are discussed in relation to the likely contribution of the various components of the balance to plant productivity.

Key words: Plastochron, leaf growth, photosynthesis, senescence, carbon balance.

INTRODUCTION

Lolium temulentum is a representative of the *Lolium–Festuca* complex of temperate grasses, within which there is an extremely wide range of interfertility crossing species and genus boundaries. *L. temulentum* appears to have originated as a weed of wheat and barley and to have evolved its distinctive inbreeding annual life-cycle by selective synchronization with that of companion species of cereal crops. *L. temulentum* therefore combines the physiological and adaptive characteristics of forage and grain monocots, which together are the dominant crops of temperate agriculture in general and the UK in particular. This makes *L. temulentum* an important experimental model, particularly for studies of vegetative development.

In a number of previous studies we have quantified the growth of *L. temulentum* leaves under controlled conditions and in response to environmental and genetic perturbations (Thomas 1983*a*, *b*; Thomas & Stoddart, 1984; Thomas & Potter, 1985; Stoddart *et al.*, 1986; Thomas *et al.*, 1989; Ougham *et al.*, 1992; Gay & Hauck, 1994). We have also made detailed studies of metabolic and cellular processes underlying the juvenile-emerging-mature-senescent sequence (Ougham, Thomas & Hilditch, 1987; Davies *et al.*, 1990; Ougham & Davies, 1990; Thomas, 1990). Although some observations have been reported of ontogenetic trends in photosynthetic capacity in this species, particularly in relation to low temperature responses (Mae *et al.*, 1993; Pollock *et al.*, 1983, 1984), there has been no detailed treatment of gas exchange and carbon relations under the standard controlled conditions employed for biochemical analyses. The objectives of the present study were to establish such a photosynthetic context for previous and continuing investigations of leaf growth, differentiation and senescence in this species, and to integrate developmental and functional observations so that a balance sheet can be drawn up and the contributions of a single leaf to the carbon economy of the whole plant be estimated.

MATERIALS AND METHODS

Plant material

Seeds of Ba3081, an inbred line of *Lolium*

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temulentum L., were grown under controlled conditions at 20 °C and lit by white fluorescent tubes for an 8 h/16 h light/dark photoperiod (short daylength to avoid production of flowering tillers) at a PPFD of 320 μmol m^{-2} s^{-1} (measured with a 190 SB quantum sensor, LI-COR Inc., Lincoln, NE, USA). Seeds were germinated on moist filter paper and, after 7 d, groups of 24 seedlings were transferred to boxes (14 × 21 × 7 cm) containing a nutrient solution with the following composition (Gay & Hauck, 1994): 0·5 mM NH_4NO_3; 1·75 mM NaH_2PO_4; 1·5 mM KCl; 2·0 mM $MgSO_4$; 0·2 mM FeNaEDTA; 1·5 mM $CaCl_2$; 4·5 μM $MnSO_4$; 0·7 μM $ZnSO_4$; 0·6 μM H_2MoO_4; 8·1 μM H_3BO_3; 0·18 μM KI; 0·4 μM $CuSO_4$; 0·34 μM $Co(NO_3)_2$. The nutrient medium was replaced every 7 d. All times are expressed as days after sowing.

Sampling and growth analyses

Photosynthesis, respiration, photorespiration, leaf area and tissue fresh weight were measured on attached fourth leaves beginning at 21 d, the earliest time at which leaves could be conveniently handled. Leaf area (either emerged portion or whole lamina) was estimated and tissue was divided into subsamples on a fresh weight basis for determination of chlorophyll (the central 2 cm of the sample) or dry weight, carbon and nitrogen content (the rest of the sample). Total leaf area (tip to ligule on fully expanded leaves or tip to top of the sheath tube on partly expanded leaves) was estimated from leaf dimensions using a single function to avoid discontinuities in the conversions. Because leaves change in shape from approximately triangular when young to rectangular when fully expanded there is no simple relationship between length and area. On the other hand a polynomial relating area to the square of leaf length accounted for 99·99 % of the total variation over all stages of development (1).

$$y = 2{\cdot}95x - 0{\cdot}3286x^2 + 0{\cdot}01805x^3, \quad (1)$$

where y is leaf area (cm^2) and x is the square of leaf length. To avoid numerical errors associated with very small values for the polynomial coefficients, leaf length was expressed as dm. Initially measurements were made daily on replicate groups of leaves; from 30 d observations were made at less frequent intervals until 45–50 d. Frequent measurements were also made of the lengths of leaves 3, 5, 6 and 7 up to full expansion.

The length of leaf n was measured from its tip to the ligule of leaf $n-2$ daily on 15 plants. Leaf growth curves were fitted with a logistic function:

$$L_t = a + \frac{c}{1 + e^{-b(t-m)}}, \quad (2)$$

with leaf length at time t, L_t, lower asymptote a, final leaf length at full expansion $a+c$, slope parameter b and point of inflection m. From the fitted curve the leaf length at m was also estimated. Because final leaf length varied slightly among leaves, m was used as an indicator of a similar physiological age, since it is the time of maximal leaf extension rate, and as the basis for a 'plastochron' versus time relationship.

Gas exchange measurements

Photosynthesis, photorespiration and respiration measurements were made using a ten-channel open gas-exchange system operated and calibrated as described by Gay & Hauck (1994) with an inlet air dewpoint of 4 °C. Three leaves were sealed in each cuvette and four or five cuvettes were used for each measurement date. When leaves were long enough to span the cuvettes the central portion of the leaf was measured. Photorespiration was estimated at 1000 μmol m^{-2} s^{-1} PPFD from the CO_2 efflux into CO_2-free (less than 0·4 μl l^{-1}) air. Photosynthesis was measured at a range of light treatments between 30 and 1250 μmol m^{-2} s^{-1} photosynthetically active radiation, and respiration was measured with the lights off. The background CO_2 concentration in each case was 360 μl l^{-1}. The photosynthetic light responses were fitted with an exponential curve:

$$P = P_{max} - kr^F \quad (3)$$

with photosynthetic rate P, maximal photosynthetic rate P_{max}, origin $P_{max}-k$, a parameter describing the curvature of the relationship r and photosynthetic photon flux density F.

Chlorophyll, carbon and nitrogen determination

The sub-sample used for chlorophyll determination was frozen in liquid nitrogen and stored at −20 °C. Pigments were extracted into 80 % aqueous acetone by grinding tissue in a pestle and mortar with a little acid-washed sand. After filtration the absorptance of the filtrate was measured at 470, 646 and 663 nm. Chlorophyll content was calculated using the coefficients of Hill *et al.* (1985) and carotenoid concentrations were determined according to Lichtenthaler & Wellburn (1983).

The sub-sample used for carbon and nitrogen determination was oven-dried and ground to a fine powder in a pestle and mortar. Weighed samples were analysed for total C and N using a Carlo Erba NA1500 nitrogen analyser (Erba Science (UK) Ltd).

Calculations from primary data

Parameters measured on the portion of the leaf in the cuvette, or on subsamples, were expressed on a unit area basis and multiplied by average area per leaf to give average values per leaf. Carbon balances were calculated using the rate of photosynthesis at 200 μmol m^{-2} s^{-1} (P_{200}), the average PPFD measured at the surface of leaf 4 in the environment used,

taking into account leaf angle and shading. P_{200} was estimated from light response curves fitted for each cuvette and average rate of respiration (R_r) was calculated for each cuvette. The hourly carbon gain or loss per cm^2 of leaf was calculated by multiplying P_{200} or R_r by 3600 (to convert from seconds to hours), dividing by 10000 (to convert from m^{-2} to cm^{-2}) and multiplying by 12/1000 (to convert μmol of CO_2 to mg of C). Daily carbon gains per leaf owing to photosynthesis and losses owing to respiration were then calculated by multiplying the hourly rates by 8 and 16 respectively (the day and night periods in h) and multiplying both by the average area per leaf. Net daily carbon exchange per leaf (C_D) was expressed as the difference between daily leaf photosynthesis and daily leaf respiration. Mean daily carbon export (C_e) from the leaf was then calculated as:

$$C_e = C_D - \frac{(C_2 - C_1)}{t} \quad (4)$$

where t is the time interval between successive measurements and C_1 and C_2 are the leaf carbon contents before and after the successive measurements. Accumulated carbon export was calculated as

$$C_f + \Sigma_s^x C_e t \quad (5)$$

where C_f is the carbon content of the leaf on the first day of measurements, s is the second day of measurements (up to which the first C_e value is calculated) and x is the day up to which the summation is made.

Statistical analyses

All gas exchange measurements were made using the cuvette containing three leaves as the basic unit, and where combined parameters are calculated (as shown above) the data from a single cuvette were used to estimate the combined parameter for the cuvette and the means and standard errors (SE) calculated from the replicate cuvettes with leaves of the same age. The nonlinear curves were fitted using MLP (Ross, 1987) which allows estimation of standard errors of fitted coefficients and the other derived values calculated, and determination of the slopes of the curves.

RESULTS AND DISCUSSION

Leaf extension and plastochron index

The logistic curves fitted to the lengths of leaves 3–7 (Fig. 1) were all of the same general shape, and the leaves attained asymptotic final lengths in excess of 200 mm. It has been noted previously that the primordia of the first three leaves of this species are already present in the embryo of the seed before germination; consequently leaf 4 represents the first to have initiated and developed independently of maternal influences and the effects of the environment during grain maturation (Evans, 1969). Leaves 4–6 were very similar in size and rate of development. Subsequent leaves, as exemplified by leaf 7, were significantly longer. Growing *L. temulentum* Ba3081 under short days greatly delays floral initiation, an event that profoundly alters vegetative development in grasses (Langer, 1972). When Ba3081 develops under a daylength in excess of 14 h, flowering occurs after only about six leaves have been produced; an 8 h photoperiod more than triples the number of leaves to flowering (Evans, 1969).

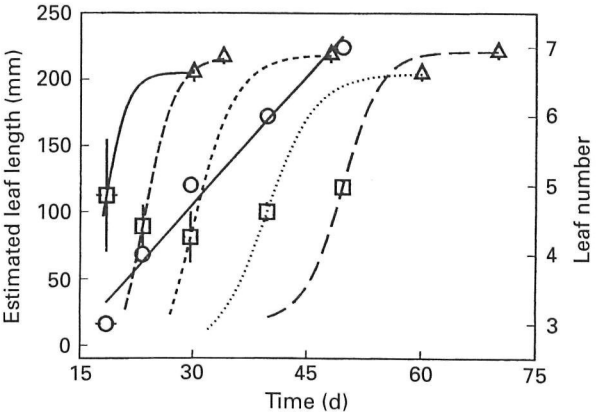

Figure 1. Fitted logistic growth curves and the corresponding plastochron index trend for leaves 3 to 7 of *Lolium temulentum*. Estimated asymptotic final length (△) and inflection point m (□) are indicated for each curve. The time at which m is reached is also plotted against leaf number (○), together with the linear regression line (equation $y = 0{\cdot}12677x + 0{\cdot}8917$; $r^2 = 0{\cdot}989436$). The bars are standard errors ($n = 15$).

There are inherent problems in applying the procedure for calculating plastochron index, as originally developed by Erikson & Michelini (1957) for *Xanthium italicum*, to monocot leaf growth. These authors recognized the particular difficulties in grasses, where leaves develop inside a sheath of older leaf bases making measurements near the reference length (typically 10 mm) impractical because of the likelihood of tissue damage. The main aim of a plastochron index is to identify corresponding stages of development of successive leaves. If leaves vary in their final length it seems more appropriate to define, as the basis of plastochron calculations, a robust physiological state which is independent of final size. Since the precise timing of each of the two most obvious choices, the initiation and cessation of leaf growth, is very difficult to determine, an alternative approach is needed. The use of m, the inflection point of the growth curve, which corresponds to the time of maximal extension rate, has been suggested previously (Thomas & Potter, 1985) and is adopted here. Since leaf lengths at m were between 80 and 119 mm in the present data and all laminae were emerged from the sheath they could be readily measured. A basic requirement for a successful plastochron index is that the reference point chosen is linearly related to time

(Erickson & Michelini, 1957), when averaged over a group of plants. This requirement is fulfilled here since the linear relation between m and time (illustrated in Fig. 1) was highly significant ($r^2 = 0.988$). It has a slope of 0.124 leaves d^{-1}, equivalent to a plastochron of 8·06 d. Thus the use of m is a simple solution to the problem of establishing a physiological time-base for comparative studies of grass leaf development.

Defining the plastochron index in terms of m raises the practical problem of how such indices can be estimated for individual plants. The whole number part of the plastochron index can be estimated from the number of leaves longer than 120 mm (or an appropriate length chosen from the values of m fitted to the material used). The length of the last leaf to emerge before the estimate of plastochron index is required is also recorded daily, to allow the timing of m for this leaf to be estimated by difference or interpolation between successive leaf length or extension measurements. Figure 2 presents an example: the time of maximal growth is very similar when a logistic function or a linear leaf extension approach is used. Once the timing of the last m is known, the fractional part of the plastochron index can be calculated as:

$$\mathrm{PI} = \frac{t - t_{\mathrm{m}}}{t_{\mathrm{p}}} \qquad (6)$$

where PI is the plastochron index, t is the current time, t_{m} is the time of m for the last leaf to reach this point, and t_{p} is the average plastochron. This method enables estimation of whole and fractional plastochron indices for grasses, a hitherto difficult group.

Growth and development of the fourth leaf

The changes in leaf length with time are well represented by the fitted logistic curve as the curve passes within the standard errors of the data points (Fig. 2). Also the curves for leaf extension rate (G) and relative leaf extension rate (R) are very similar to those calculated from the successive measurements. G rises initially then falls once the leaf is longer than 100 mm with maximal G (over 25 mm d^{-1}) only maintained for 3–4 d. Relative growth rates were originally devised to allow comparisons to be made between whole organisms differing in size. R for a single leaf, an organ of limited size that exports materials used for growth to other parts of the plant, is inherently more complex. R declined rapidly throughout leaf expansion, reaching zero when final size was achieved, although the leaf is still making a significant contribution to the plant at this stage and beyond.

The use of the curve fitting process described above simplifies comparison of the factors influencing growth. This is particularly useful in plants exhibiting considerable phenotypic plasticity. *L. temulentum* is such a species: for example G has been shown to be highly responsive to nitrogen supply (Thomas, 1983*a*), temperature (Thomas & Potter, 1985), genetic perturbation (Ougham *et al.*, 1992), PPFD or CO_2 concentration during development (Gay & Hauck, 1994). Moreover, experiments with high-resolution transducer instruments over a much shorter time scale have revealed large diel variations in the growth rate of *L. temulentum* leaf 4, with a different distribution between light and dark periods depending on temperature (Thomas & Stoddart, 1984). Such nuances of growth behaviour are themselves important indicators of processes underlying the macro-scale responses of leaf expansion to the environment, but they might introduce quite a lot of background noise into studies of trends over the time-span of whole leaf development. They suggest that it is important to measure leaves at the same time in the day/night cycle, and also show the advantage of curve fitting approaches in smoothing out and summarizing short-term fluctuations and accentuating longer-term patterns.

The pattern of fresh and dry weight increase during leaf expansion was similar to the trend for leaf area (Fig. 3*a*), reaching a plateau at about 27 d. Leaf dry weight did not continue to increase beyond full expansion. Leaves of tomato grown at normal CO_2 concentration are similar in this respect (Thornley, Hurd & Pooley, 1981), but in other Gramineae increases in leaf dry weight after full expansion have been observed (Williams & Rijven, 1965; Dale & Milthorpe, 1983). In experiments described in the latter report, samples were taken at relatively infrequent intervals and the exact point at which dry weight reaches its maximum is difficult to determine. The cases discussed by Dale & Milthorpe (1983) might be related to conditions under which the measurements were made. For example, increasing the CO_2 concentration causes a significant delay in reaching maximal dry weight after the completion of expansion in tomato leaves, and leaf area and dry weight maxima do not coincide (Thornley *et al.*, 1981). As well as environmental influences, species differences might also be important in the timing of maximal dry weight. Another significant factor in the degree of synchrony between weight and area during leaf development might be ontogenetic trends in assimilate export capacity. There was some indication in the present study of a further increase in weight at the very end of the observation period (Fig. 3*b*), possibly as result of the reduced efficiency of export in the oldest leaves.

Changes in pigments during leaf development

Chlorophyll per leaf increased over the first 10 d after emergence and declined thereafter (Fig. 3*b*).

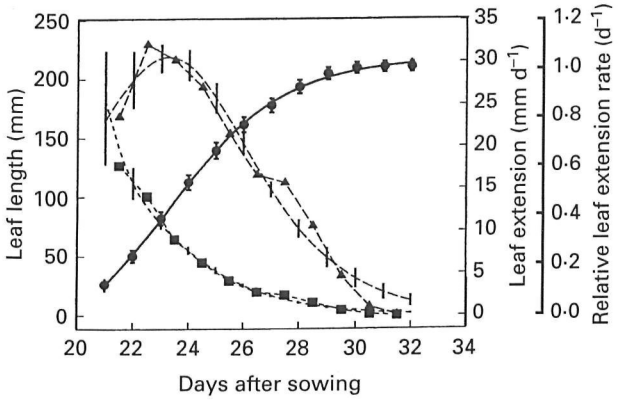

Figure 2. Time courses for growth and development of the fourth leaf of *Lolium temulentum*. Length (●; bars are single standard errors) and fitted curve (——), extension rate (——) calculated on differences between successive values (▲) and by calculation from length curve fitted (smooth curve with error bars) and relative extension rate (----) calculated for successive intervals (■) and derived from the fitted length curve (smooth curve with error bar).

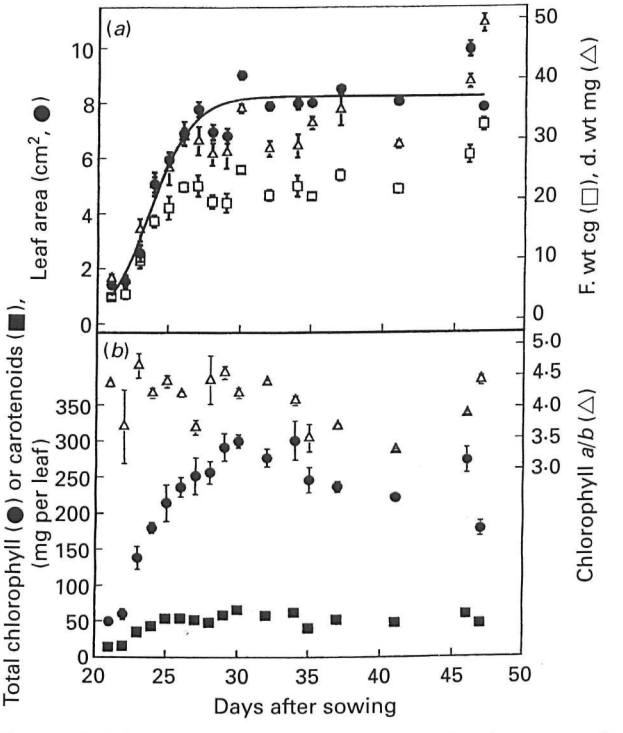

Figure 3. Time courses for growth and development of the fourth leaf of *Lolium temulentum*. (*a*) Area (●, with fitted curve), fresh weight (□) and dry weight (△). (*b*) Chlorophyll (●), chlorophyll *a*/*b* ratio (△) and carotenoids (■). Standard errors for data points (but not for parameters calculated over intervals in (*b*)) are shown as bars with ends where they are larger than symbols.

Carotenoids remained fairly constant after an initial rise in the first part of the expansion phase. The ratio of chlorophyll *a* to *b* decreased during the period of chlorophyll loss post-full expansion, though the oldest leaves did not follow the trend established earlier in senescence. These changes are similar to those observed in many herbaceous plants (Šesták, 1985). The reduction in the chlorophyll *a*/*b* ratio as

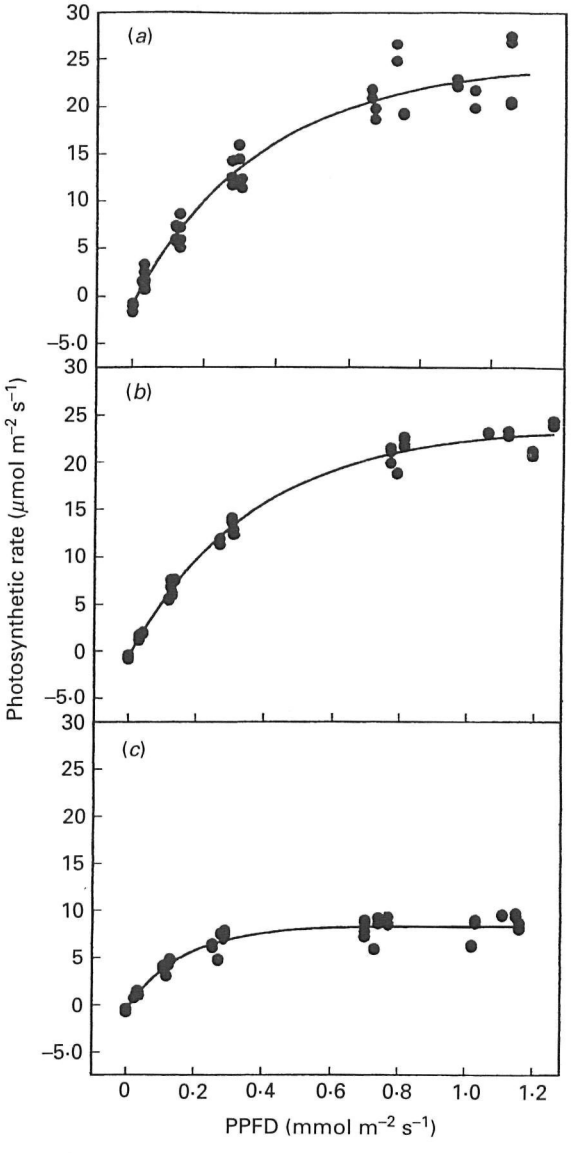

Figure 4. Photosynthetic light response curves for fourth leaves of *L. temulentum* (*a*) 22, (*b*) 29 and (*c*) 41 days after sowing.

the leaves aged suggests either a preferential breakdown of chlorophyll *a*, or a change in the relative rates of synthesis during replacement of existing chlorophyll. There is evidence that the principal route for physiological breakdown of chlorophyll *b* is via conversion to chlorophyll *a* (Hörtensteiner, Vicentini & Matile, 1995); the slow fall in the *a*/*b* ratio might reflect a lower activity of the *b* to *a* pathway than of the subsequent steps that dephytylate, dechelate and open the chlorophyll *a* macrocycle.

Ontogenetic trends in photosynthesis

Photosynthetic light response curves are shown for points representative of the main phases of leaf development, namely: early growth (Fig. 4*a*), near to full expansion (Fig. 4*b*) and when senescence was well advanced (Fig. 4*c*). The pattern for young tissue

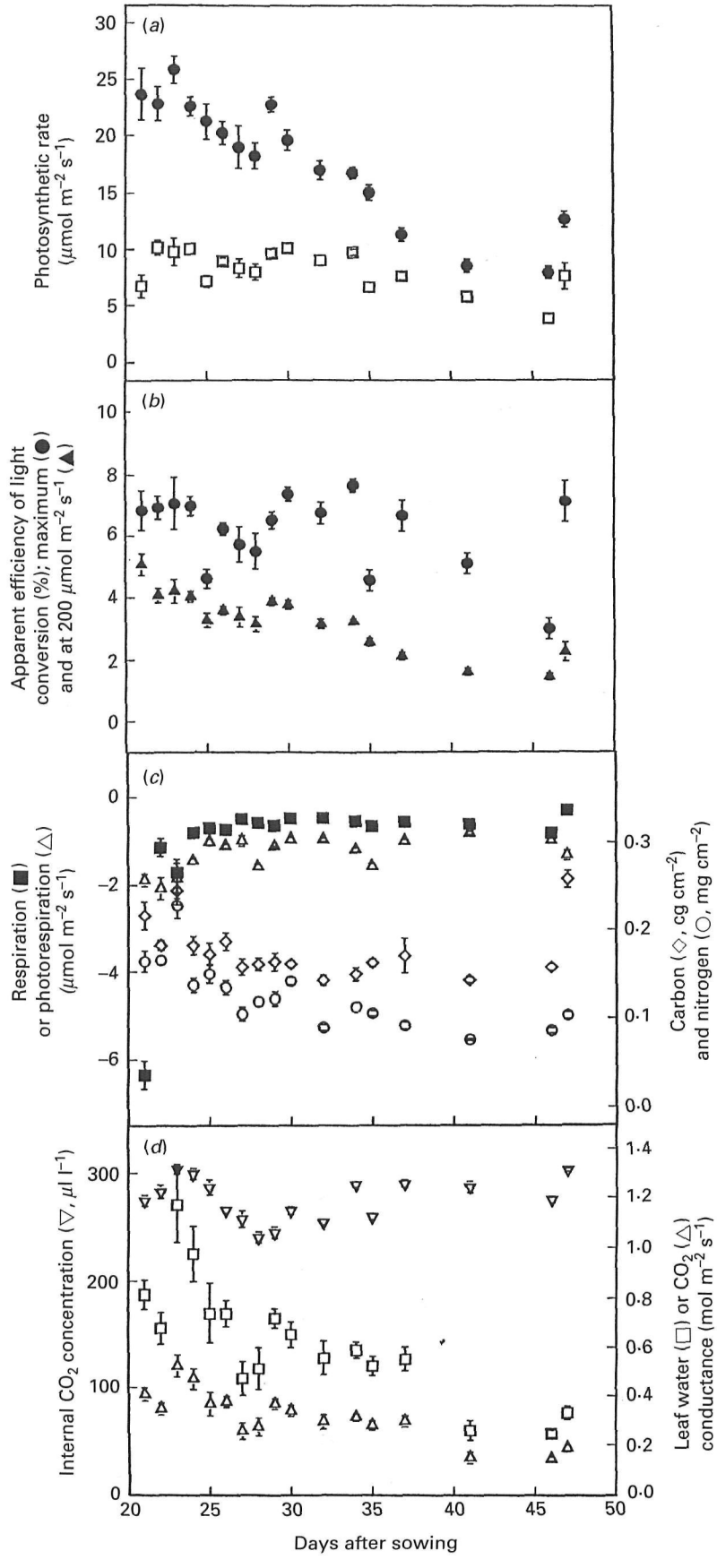

Figure 5. Ontogenetic changes in photosynthetic parameters per unit leaf area for fourth leaves of *L. temulentum*. (*a*) Rates of photosynthesis, light saturated (●) and at 200 μmol m^{-2} s^{-1} (□). (*b*) Efficiency of light conversion at 0 (●) and 200 (▲) μmol m^{-2} s^{-1}. (*c*) Dark respiration (■), photorespiration (△), carbon (◇) and nitrogen (○) content. (*d*) Parameters estimated at light saturation: internal [CO_2] (▽), and leaf water (□) or CO_2 (△) conductances. Standard errors of values shown as vertical bars where larger than symbols.

was very similar to that of fully expanded leaves, but in older leaves there was a significant reduction in photosynthesis in all light treatments, except at the two smallest irradiances used. Light-saturated photosynthetic rates continually declined in senescing leaves to about one third of the rates in younger tissue (Fig. 5*a*). This contrasts with the rates measured at 200 μmol m^{-2} s^{-1}, the light incident on the leaf surface during growth, where the reduction was smaller, to about half of the initial rate, and the onset of decline was delayed to about 36 d. The attainment of maximal photosynthetic rate before growth is complete contrasts with the data for the related grass *Festuca arundinacea*, in which the maximum is not reached until 10 d after full expansion (Jewiss & Woledge, 1967). On the other hand the behaviour of *L. temulentum* resembles that of many other plants (Tichá *et al.*, 1985) and might be related to the relatively high carbon content per unit area during the earlier stages of leaf expansion and the availability of adequate resources for early completion of the photosynthetic machinery. This suggestion is entirely consistent with the observation that cell expansion is confined to the basal 20 mm of the fourth leaf in *L. temulentum* (Davies *et al.*, 1989), a region enclosed in the sheaths and not accessible for the physiological measurements used in the present study.

The slope of the light response curve can estimate the apparent quantum efficiency of photosynthesis (Long & Hällgren, 1993). The maximal apparent quantum efficiency, estimated at the light compensation point (where the Kok effect should no longer influence the slope), was largely unaffected by leaf age in *L. temulentum* (Fig. 5*b*). Values towards the later stages of senescence tended to be much more variable, however. Similar conclusions are reached if slopes are calculated for other small irradiances (data not shown). By contrast, the quantum efficiency determined at the light flux experienced by the leaf during its development shows a continuous gradual decline from about 30 d, with relatively little variability (Fig. 5*b*). The decrease in apparent quantum efficiency with age is unlikely to be owing to changes in light transmission and reflection properties because, as Sheehy (1975) has shown, older grass leaves intercept more light than do younger leaves. Loss of chlorophyll (Fig. 3*b*) might partly explain the decrease, but a significant reduction in electron transport capacity is also likely, as indicated by falling rates of light saturated photosynthesis. The proposal that both components might contribute to diminishing photosynthetic efficiency in older tissue is supported by the data of Mae *et al.* (1993) showing a parallel decline in both light harvesting and electron transport components of *L. temulentum* leaves senescing under constant light. Apart from low activity on the first day of measurements, respiration and photorespiration maintained relatively constant rates during the life of the leaf (Fig. 5*c*). Leaf carbon content was initially large and also increased somewhat at the end of the measurement period, but otherwise was relatively constant. Nitrogen declined more or less continuously throughout the life of the leaf (Fig. 5*c*).

CO_2 exchange in relation to conductances

Another possible explanation for the decline in photosynthesis with age is that leaf (i.e. mostly stomatal) or mesophyll conductance, or both, change. Most studies of ontogenetic trends in photosynthesis have identified changing mesophyll resistance as the major factor in declining CO_2 fixation capacity in older leaves, but it has been suggested that stomatal limitations are significant too (Thimann & Satler, 1979). There were indications in the present study that leaf water conductance declined during senescence, over the period when photosynthetic capacity was decreasing (Fig. 5*d*). Nevertheless, neither CO_2 conductance nor substomatal CO_2 concentration underwent changes sufficient to account for a 60–70 % fall in light-saturated CO_2 exchange rate. We conclude that mesophyll resistance, probably at the level of rubisco, dominates ontogenetic trends in photosynthetic capacity in *L. temulentum*. Interestingly, the reduction in nitrogen content, to about half the value in younger leaves, was rather less pronounced than the decline in light-saturated photosynthesis (Fig. 5*c*). This suggests that there might be some delay in the complete breakdown and export of rubiso from the leaf since it is the major remobilizable source of nitrogen in the leaf. Rubisco might be inactivated without much proteolysis, or else the nitrogen is released from hydrolysed protein much faster than it is exported. The evidence favours the latter explanation. In experiments on *L. temulentum*, fourth leaves developed under the same conditions as those used in the present study, Mae *et al.* (1993) used quantitative immunoblotting to show considerable loss of rubisco protein during senescence.

Photosynthesis, respiration and leaf carbon balance

Rates of CO_2 exchange at 200 μmol m^{-2} s^{-1} (the average irradiance measured at the leaf surface), respiration and carbon content from emergence to senescence are shown on a per leaf basis in Fig. 6*a*. Except for a lower initial value, respiration remained fairly constant. Photosynthesis per leaf increased over a similar time course to leaf length, area and weight and then declined over the period of senescence from about 34 d, apart from one rather high value at 30 d. The latter outlier seems to result from the anomalously high leaf areas of the plants sampled on that day. The initial increase in photosynthesis per leaf was accounted for largely by the rise in leaf

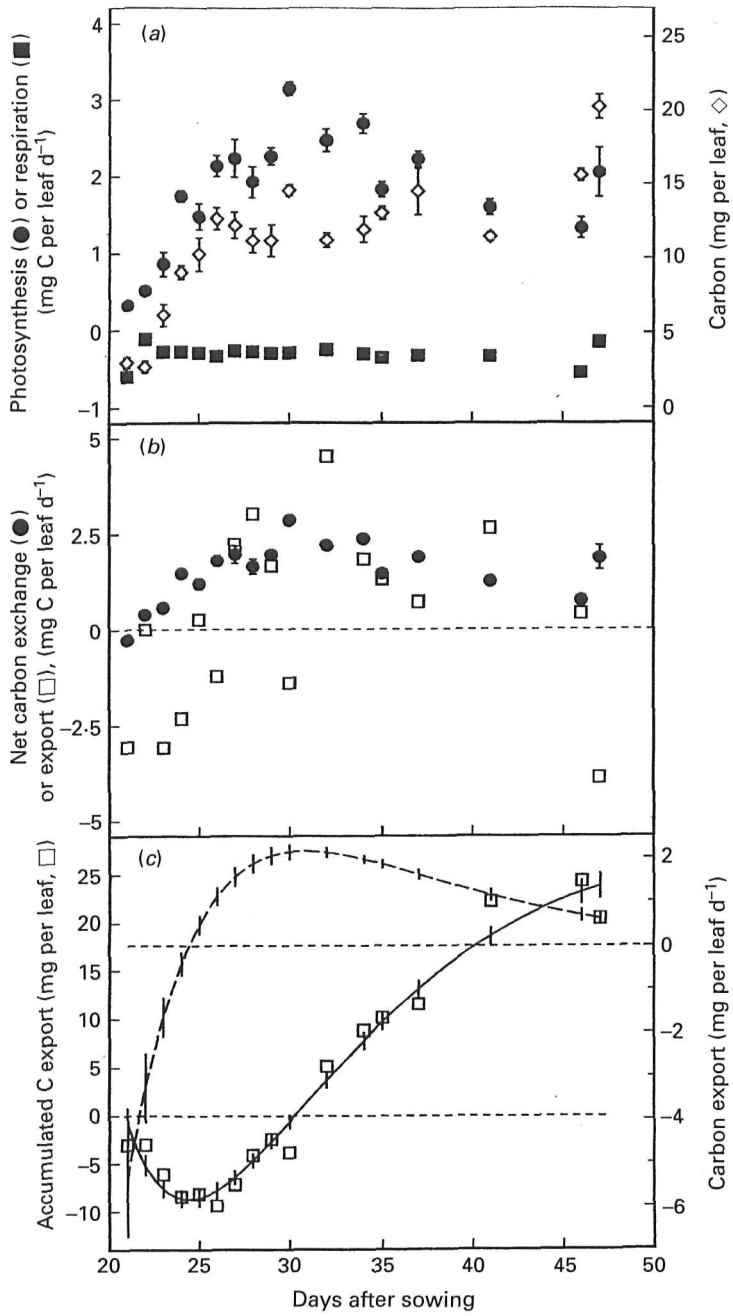

Figure 6. Ontogenetic changes in photosynthetic and carbon balance parameters per leaf for the fourth leaf of *L. temulentum*. (*a*) Photosynthesis (at 200 μmol m^{-2} s^{-1}, ●), respiration (■) and carbon content (◇). (*b*) Net carbon exchange with the atmosphere (photosynthesis *minus* respiration, ●) and net export of carbon (photosynthesis *minus* respiration *minus* increase in leaf weight, □). (*c*) Accumulated export of carbon (□, fitted curve: $y = 28{\cdot}8 + (4792 - 265x) * 0{\cdot}8555^{x}$) and the first derivative of the curve, (----) representing daily carbon export.

area, as the CO_2 fixation rate remained fairly constant when expressed on a unit area basis (Fig. 5*a*). Total carbon per leaf increased up to 26–30 d and remained relatively constant subsequently.

The data for net carbon exchange (photosynthesis minus respiration, allowing for daylength) show that, photosynthetically, leaf 4 was in positive carbon balance with the atmosphere from an early stage, 21 d (Fig. 6*b*). Taking into account leaf weight changes and calculating net carbon export, the passage of the leaf into positive carbon exchange with the rest of the plant was much later, at about 26 d; that is to say, up to this point the leaf was not fully autotrophic and there was a considerable demand on the rest of the plant for carbon to support growth and development. In these data the course of the net carbon export is likely to be distorted for the 30 and 31 d points by the particularly high leaf weight observed on 30 d, as previously noted.

By accumulating the export of carbon through the life of the leaf (Fig. 6*c*) we can quantify the maximal carbon demand on the rest of the plant during leaf

development, and the eventual carbon gain to the plant provided by the leaf. The carbon input to leaf 4 from the rest of the plant peaked at about 10 mg on day 25, about 1·5 d after maximal leaf extension rate was achieved (Fig. 2). The carbon balance with the rest of the plant then returned to zero at about 30 d, almost coincident with full leaf expansion (Fig. 2). The onset of the subsequent phase of net carbon contribution to the plant coincided with the expansion of leaf 5 which, if it follows the same pattern as leaf 4, will have attained the peak of its carbon demand at 31–32 d. If we assume that about the same amount of carbon is required to make each successive leaf, then leaf 4 will have produced enough carbon to support the establishment of leaf 5 plus, by the end of its life, an additional 22 mg of carbon, equivalent to in excess of two further leaves. These figures do not take into account the carbon requirements of root growth and respiration, and the relatively minor fraction diverted to stem growth in these vegetative plants, but probably more importantly any contribution to tillering. In a study of short-term allocation patterns Wardlaw (1976) found that a leaf of *L. temulentum*, just after full expansion, donated carbon in approximately equal proportions to shoot (presumably mainly leaf), tillering and roots. These data were obtained from plants grown at a greater irradiance than that employed in the present work. A similar experiment conducted with plants at a much smaller irradiance than used here revealed that about twice as much carbon is allocated to the shoot than to all other parts combined (Wardlaw, 1976). We suggest that the allocation pattern of carbon at the intermediate light treatment employed in the current experiment comes somewhere between the two extremes. Further data are required to decide the partition of carbon allocated to the shoot between the stored fraction and the fraction used for leaf growth, since it is well known that when carbon supply exceeds demand, *L. temulentum* and other grasses store large amounts of carbohydrate in leaf bases (Pollock & Eagles, 1988).

The first derivative of the accumulated C export curve (Fig. 6*c*) gives the *carbon credit contour* (Thomas, 1987) for leaf 4 of *L. temulentum*. The area under the curve on the positive side of zero defines the leaf's carbon contribution to the whole plant. We note that the curve achieved a maximum at about 30 d. This point seems to be highly significant in the life of the fourth leaf. At this time the leaf goes into positive carbon balance with the rest of the plant (Fig. 6*c*). It also coincides with the attainment of full size (Figs. 2, 3*a*). Functionally it represents the onset of senescence, making the interesting point that a large part of the productive life of the leaf coincides with a period conventionally considered to be one of deterioration.

From Figure 6*c*, it seems that within the brief span of 10 days, *L. temulentum* leaf tissue is formed, assembles the photosynthetic apparatus, emerges from the sheath into the light, maximizes its light-intercepting surface and initiates senescence. Successive stages of foliar development run into each other and each might be cueing the start, albeit at a low rate, of the following phase. This has a number of important implications, notably for studies of the regulation of leaf growth, differentiation and senescence in terms of gene expression. Ephemeral leaves like those of *L. temulentum* are not ideal for isolating genes concerned with initiation of senescence because of 'contamination' by overlapping phases of development; longer-lived leaves such as those of maize, where growth and attainment of maximal photosynthetic competence occur perhaps several weeks before senescence begins, offer better opportunities for studying the expression of senescence-related genes (Thomas, 1994*a*; Smart *et al.*, 1995).

Conclusions

The large world effort on understanding and increasing photosynthetic productivity is concerned with maximizing the size of the captured resource represented by the carbon credit contour. A great deal of attention has been paid to increasing the area of the contour by raising its upper limit – that is, by boosting photosynthetic capacity – without, it must be said, very much success. Some progress has been made towards minimizing losses through leaf respiration (Wilson & Jones, 1982), thereby reducing the height of the lower boundary and enlarging the contour. Thomas (1987) has argued that most agronomically significant increases in crop productivity have been achieved through pushing the right-hand side boundary back by delaying senescence, either as a result of breeding or by applying fertilizer, irrigation, pesticides and other ameliorating treatments.

Nevertheless, even the 'unimproved' productivity of a single leaf is considerable and we do not fully understand what the plant as a whole does with what might well be an excess of carbon income over capacity to turn it into biomass. Such excess resource might be an important feature of the survival strategies of herbage plants that have relatively small storage capacities and that suffer periodic defoliation. Alternatively it might be another demonstration of 'carbon dumping' in the life of a terrestrial plant (Thomas, 1994*b*). It will be necessary to understand the fate of all the carbon acquired by the plant if we want to address the question of increasing plant productivity in a more balanced manner.

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