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Does assimilate supply limit leaf expansion in wheat grown in the field under low phosphorus availability?

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Abstract

Under conditions of phosphorus deficiency, reductions in plant leaf area have been attributed to both direct effects of phosphorus (P) on the individual leaf expansion rate and a reduced availability of assimilates for leaf growth. Simulation techniques have been used to test the hypothesis of a non-trophic limitation to leaf expansion in wheat grown on P-deficient soils; this study provides further experimental evidence supporting this hypothesis. In a field experiment, we studied the effects of soil P additions (0–200 kg P_2O_5 ha^{-1}) and assimilate availability (non-shaded and shaded treatments) on the expansion of individual leaves of wheat (*Triticum aestivum* L. cv. Oasis), light interception and radiation use efficiency. The soil was low in P (5.5 μg P g^{-1}), the crop was drip irrigated and nitrogen was applied at non-limiting rates during the experiment. At 61 days after emergence (DAE), phosphorus deficiency reduced above ground biomass 70 and 61%, leaf area index 73 and 69%, the percent intercepted radiation 63 and 46%, and radiation use efficiency 21 and 31%, in non-shaded and shaded plots, respectively. Leaves of shaded plants had lower %WSC and lower accumulation of structural dry weight than non-shaded ones. In shaded leaves, there was a more important restriction to the accumulation of structural material in high-P than in low-P treatments. This and the fact that leaf expansion rate of individual leaves was negatively related to %WSC led us to conclude that leaf expansion in low P plants is unlikely to be limited by the availability of assimilates. © 2000 Published by Elsevier Science B.V.

Keywords: Leaf expansion; Phosphorus; Water-soluble carbohydrates; Wheat

Abbreviations: DAE, days after emergence; LER, leaf expansion rate; LER_{rel}, relative LER; RUE, radiation use efficiency; RUE_{rel}, relative RUE; P, phosphorus; %WSC, % water-soluble carbohydrates

1. Introduction

Phosphorus (P) availability in soil is seldom adequate for optimal plant growth, and in many regions of the world P is the main environmental factor controlling crop growth and yield (Constant and Sheldrick, 1991). During early vegetative development, growth of wheat is directly related to the amount of intercepted radiation and therefore to the increase in leaf area. Particularly, during these early stages, deficiencies of P can affect wheat growth and productivity by

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reducing the amount of intercepted radiation (Terry and Rao, 1991), while the efficiency with which intercepted radiation is used for growth remains relatively constant (Lawlor, 1993; Rodríguez et al., 1998a). Leaf growth is the result of highly complex biochemical events, and several attempts have been made to simulate its growth (Charles-Edwards, 1979; Lainson and Thornley, 1981). When phosphorus is limited, growth is generally more reduced than the rate of photosynthesis per unit of leaf area in recently expanded leaves (Jacob and Lawlor, 1991; Rodríguez et al., 1998a). This would suggest that under P-limiting conditions, the availability of assimilates at leaf level might not be the principal factor in the reduction in leaf area expansion and plant growth. Direct effects of P on plant leaf area expansion and tillering have also been proposed in wheat (Sato et al., 1996; Rodríguez et al., 1999) and rice (Hanada, 1995). Though various authors have suggested direct effects of P deficiency on individual leaf expansion, experimental evidence of this in field-grown plants is limited. This paper considers whether the restriction to individual leaf expansion in wheat grown on P-deficient soils is caused by a reduced availability of assimilates or through other unaccounted mechanisms.

2. Materials and methods

A field experiment was conducted during the 1997 growing season at the INTA Research Station, Balcarce, Buenos Aires, Argentina (37°45′S, 58°18′W; 130 m above sea level) on a typic Argiudol. The soil was low in P, 5.5 mg P kg^{-1} soil (Bray and Kurtz, 1945), and had a minimum effective soil depth of 1.5 m. Treatments consisted of the combination of three levels of soil P fertilisation 0 (P1), 60 (P2) and 200 (P3) kg P_2O_5 ha^{-1}, and two levels of assimilate availability, a control (non-shaded) and 65% reduction in incident radiation from emergence to end of tillering (shaded). Commercial super phosphate was used as fertiliser, and shades were made of a neutral shading cloth stretched over the plots on cane and wire structures. Treatments were arranged in a split-plot design with P fertilisation treatments as the main plot and shading treatments the subplot. The main plots were arranged in a randomised complete block design with four replications. On 20 July, seeds of wheat (*Triticum aestivum* L. cv. Pro-INTA Oasis) were sown at a density of 320 seeds m^{-2}. The crop was kept free of weeds, and insect pests were adequately controlled. Soil water content was maintained above 60% field capacity in the first meter of soil depth by drip irrigation. The subplots consisted of four rows, 6 m long and 0.17 m apart with two border rows on each side. Urea was hand-applied in four equal amounts of 59.5 kg N ha^{-1} at sowing, emergence, beginning of tillering, and mid-tillering. Rainfall and daily total radiation were obtained from a meteorological station located 500 m from the experimental site. Daily air and soil maximum and minimum temperatures were recorded in the shaded and non-shaded treatments using a data-logger (LI 1100, Li-Cor, NE) and thermocouples. Photosynthetic photon flux density (PPFD, $\mu\mathrm{mol\ m^{-2}\ s^{-1}}$) intercepted by the shade cloth and the crop were measured by placing a line quantum sensor (Delta-T Sun-Scan type SS1, Cambridge, UK) 0.15 m above and below the shading cloth. Percent radiation interception by the crop (Q) was estimated from PPFD measurements as $100[1-(I_t/I_o)]$, where I_t is the incident PPFD at ground level and I_o is the incident PPFD at the top of the canopy. Data on Q versus days after emergence (DAE) were studied using Eq. (1), that was fitted by means of a curve-fitting software, Genstat 3.1 (Genstat 5 Committee, 1987),

$$Q = \frac{Q_{\max}}{1 + \exp\{-r(\mathrm{DAE} - \mathrm{DAE}_{50\%})\}} \tag{1}$$

where $Q_{\max}$ is the maximum percentage of radiation interception, r a relative rate with units per day, and $\mathrm{DAE}_{50\%}$ is the duration until 50% of $Q_{\max}$ is reached (days). The maximum rate of percentage radiation interception increase (Q_{rate}, % per day) was calculated from the maximum value of the first derivative of Eq. (1).

The length and width of the main stem leaf blades L1, L2, L3 and L4 were measured every 2 days, from leaf tip emergence until collar visible, in two tagged plants per experimental unit. The individual leaf area (m^2) was calculated as the product of leaf width, leaf length and a shape factor (0.7). The value of the shape factor was calculated at each harvest by measuring the area of individual leaves with a leaf area meter LI-3100 (LI-Cor, Lincoln, NE). Leaf expansion rates (LERs) during the *quasi*-linear phase, and the duration of the *quasi*-linear phase of leaf expansion were calculated using an optimisation model (Eqs. (2a) and (2b)) that fitted the experimental data iteratively by means of a curve-fitting software, Genstat 3.1 (Genstat 5 Committee, 1987),

$$\mathrm{ILA} = a + \mathrm{LER} \times x \quad \text{if} \quad x \leq c \tag{2a}$$

$$\mathrm{ILA} = a + \mathrm{LER} \times c \quad \text{if} \quad x > c \tag{2b}$$

where ILA is the individual leaf area (m^2), a the y-axis intercept, LER the leaf expansion rate ($m^2\ {}^{\circ}Cd^{-1}$), x the thermal time since leaf emergence (°Cd), and c is the cumulated thermal time when leaf expansion stopped (°Cd). The duration of the *quasi*-linear leaf expansion period (DUR, °Cd) was calculated as $c+a/\mathrm{LER}$. The emergence of main stem tillers was recorded in tagged plants following the methodology of Klepper et al. (1982).

Total above-ground plant dry weight, and its partitioning into stems+sheaths, and leaf blades was monitored by sampling the subplots four times. Sample 1 was taken at 27 DAE, sample 2 at 34 DAE, sample 3 at 48 DAE, and sample 4 at 61 DAE. These samples were taken in homogeneous areas of the subplots by cutting all the plants within a 0.17 m^2 frame. Leaf blade and stem areas were determined using an area meter (LI 3100, Li-Cor, NE). Plant parts were oven-dried at 65°C to a constant weight, weighed and ground for P determination. Phosphorus in plant material was determined after digestion with a nitric–perchloric mixture by the molybdovanado-phosphoric acid method. Percent water-soluble carbohydrates (%WSC) in leaves and stems were determined by the anthrone method after extraction with hot water (Yemm and Willis, 1954). Samples were collected between 08:00 and 09:00 h to minimise the effects of diurnal variation in carbohydrate concentrations. In studies with C_{14}, this procedure recovers more than 95% of sugars from tissue (Wardlaw and Willenbrink, 1994), and is particularly effective in temperate grasses and winter cereals which are mainly fructosan-accumulators (Smith, 1969).

During the experimental period, 3–5 plants per plot were harvested at 5–7 days intervals and their main stem dissected to determine the developmental stage of the apex (Gardner et al., 1985), the developmental stage of the crop was frequently recorded following the scale of Feekes (1941).

Radiation use efficiency (RUE) was calculated as the ratio between the increase in above ground biomass and cumulated intercepted radiation between samplings 1 and 2, 2 and 3, and 3 and 4.

Differences among treatments were tested by ANOVA (split-plot design), using Genstat 3.1 (Genstat 5 Committee, 1987), values of LSD (P=0.05) for the P+S comparison of means are also presented.

3. Results

3.1. Weather

Mean air temperature during the experimental period was 10.4°C with a maximum of 28.6°C and a minimum of −5.3°C. Soil temperature under the shade was on average 3°C lower than in non-shaded treatments; air temperatures were similar for shaded and non-shaded treatments. Mean daily total radiation during the experimental period was 9.9 MJ m^{-2} per day. During the experimental period, the accumulated amount of rainfall was 133 mm.

3.2. Crop growth and leaf area development

The crop grew exponentially during the experimental period irrespective of the treatments imposed (Fig. 1). Low P supply reduced leaf area index (LAI) and the production of above ground biomass at all stages (P<0.05). Shading reduced leaf area at samplings 1 and 3 (P<0.05), and above ground biomass at all sampling times (P<0.05). The interaction between shading and the level of P, on the size of the leaf area was not statistically significant. At samplings 2 and 4, the effect of shading on above ground biomass production was more important at higher (P3) than at lower (P1, P2) levels of P supply (P<0.05) (Fig. 1).

Shading did not alter the development of the crop. Irrespective of the shading treatment, at 61 DAE, P1 plants were at Feekes 6 (first node detectable) and P2 and P3 plants at Feekes 7 (second node detectable) (Table 1). One week later (68 DAE), the terminal spikelet was present in all the treatments. The number of main stem leaves per plant and the number of tillers per unit of area were significantly reduced by both P and shading (Table 1).

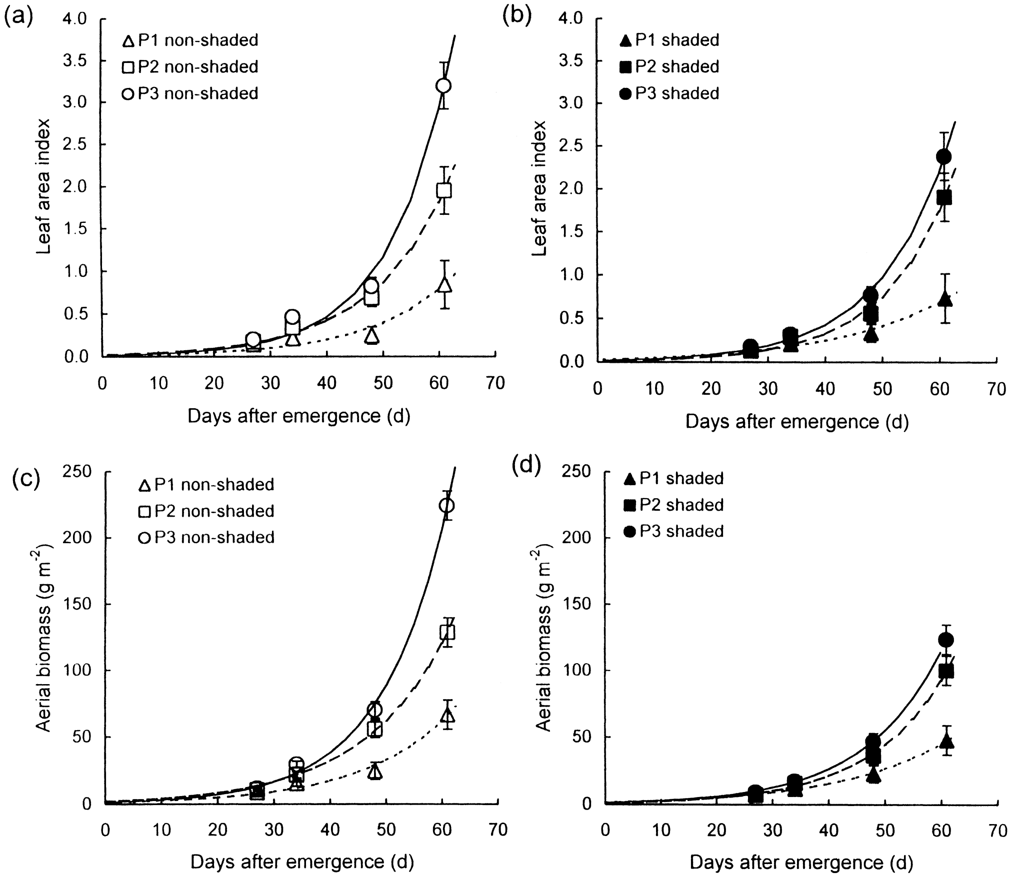

Fig. 1. LAI (a, b) and above ground biomass (c, d) of a wheat crop grown at different levels of phosphorus supply and two levels of incident radiation non-shaded (a, c) and shaded (b, d). Bars are standard errors for the difference of the means.

Table 1
Feekes stage, number of leaves on the main stem (MSLN) and number of tillers per plant in wheat plants grown under three levels of P supply and two levels of radiation intensity at 61 DAE

	Non-shaded			Shaded			LSD[a]	P[b]	S[c]	P×S
	P1	P2	P3	P1	P2	P3				
Feekes	6	7	7	6	7	7				
MSLN	6.2	7.5	7.5	6.0	6.5	7.0	0.7	*	**	NS[d]
Tillers	0.8	1.9	2.2	0.07	0.9	1.5	0.6	**	***	NS[d]

* Significant at 0.05 probability level.
** Significant at 0.01 probability level.
*** Significant at 0.001 probability level.
[a] Least significant differences for P+S comparisons.
[b] Effects of the phosphorus level.
[c] Effects of the shading level.
[d] Not significant.

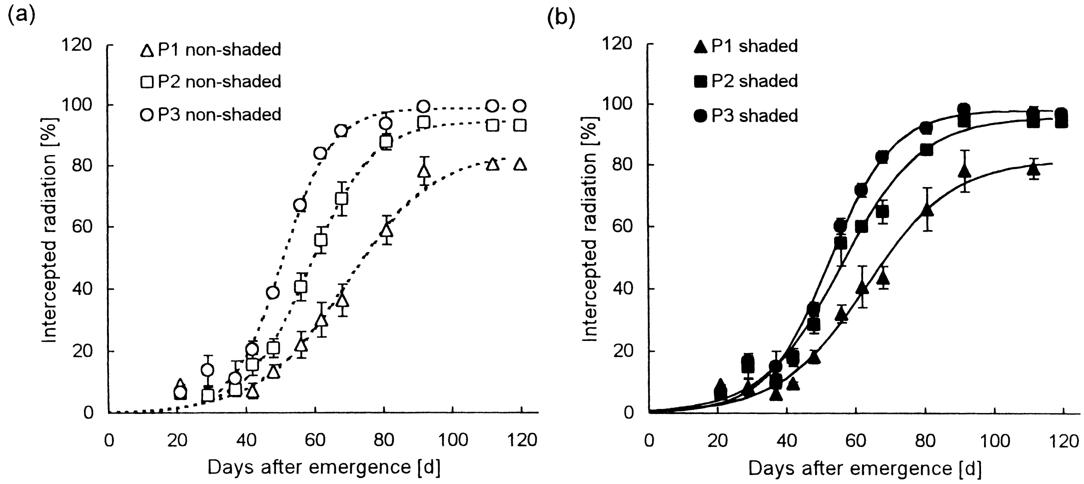

Fig. 2. Percent of intercepted radiation of a wheat crop grown at different levels of phosphorus supply and two levels of incident radiation non-shaded (a) and shaded (b). Bars are standard errors for the difference of the means.

Compared to the P3 treatments at 61 DAE, Q ($Q_{61\mathrm{DAE}}$) was reduced by 63 and 46% in P1 non-shaded and P1 shaded plots, respectively (Fig. 2). The maximum percent radiation interception ($Q_{\max}$, %) was significantly reduced by P deficiency (Fig. 2 and Table 2), shading did not affect the values of $Q_{\max}$ or $Q_{61\mathrm{DAE}}$. Phosphorus deficiency reduced $Q_{\max}$ by lowering Q_{rate} ($P<0.001$), though $Q_{\mathrm{DUR}50\%}$ was slightly increased ($P<0.001$).

The coefficients for the extinction of radiation within the canopy (K) were calculated from plots of $-\ln(1-Ir/100)$ versus LAI, the linear regressions always explained more than 94% of the variability. The level of fertilisation with P did not affect the value of K (Table 2). However, the effect of shading the crop on K was different at different levels of P supply. The value of K in P1-shaded plants was significantly higher than for the rest of the treatments ($P<0.05$).

Table 2
Coefficients after fitting Eq. (1) to the data on intercepted radiation, and extinction coefficient for PAR

	Non-shaded			Shaded			LSD[a]	P	S	P×S
	P1	P2	P3	P1	P2	P3				
$\max_{ir}$[b]	1.5	2.4	3.2	1.5	2.0	2.8	0.6	***	NS[c]	NS
rr_{ir}[d]	0.09	0.14	0.47	0.15	0.19	0.19	0.4	NS	NS	NS
l_t[e]	44	41	36	38	33	36	9	NS	NS	NS
Q[f]	80	92	97	79	93	95	6.2	***	NS	NS
$t_{\max}$[g]	85	81	70	82	80	75	10	**	NS	NS
K[h]	0.37	0.4	0.46	0.66	0.38	0.47	0.15	NS	*	*

** Significant at $P<0.01$.
*** Significant at $P<0.001$.
[a] Least significant differences for P×S.
[b] Maximum rate radiation interception.
[c] Not significant.
[d] Average relative growth rate during the exponential phase of light interception.
[e] Duration of the exponential phase.
[f] Maximum value of intercepted radiation.
[g] Total duration until Q is reached.
[h] Light extinction coefficient.

3.3. Area of individual leaves

Leaf area of individual leaves increased with leaf number (Fig. 3 and Table 3). Higher order leaves were bigger as the rate of leaf expansion increased with leaf number. The duration of leaf expansion changed little from leaf 1 to leaf 4. Significant differences in the size of individual leaves among treatments were observed as early as on L2. The leaves were larger in shaded plots, and smaller at lower levels of P (Table 3). Phosphorus deficiency tends to increase the value of DUR; however, due to the effects of P on LER, phosphorus deficiency reduced the final size of individual leaves (Table 3). The relative effect of P deficiency was less important in leaves from shaded plots. At P1, the area of individual leaves was reduced by about 21–47% in non-shaded plots, and by about 18–28% in shaded plots, when compared to P3 non-shaded and shaded plots, respectively (Table 3).

The relative values of LER ($\mathrm{LER_{rel}}$) for leaves L2, L3 and L4, with respect to the controls P3 in shaded and non-shaded plants, were asymptotically related to the concentration of P in the leaves. However, the response to leaf P% was different for shaded and non-shaded plants (Fig. 4, Eqs. (3a) and (3b)). These relationships presented a threshold value of leaf P%, defined as the value of leaf P% when $\mathrm{LER_{rel}}=0.9$, of 0.4% for non-shaded plots, and of 0.27% for shaded ones.

$$\begin{aligned}&\text{Non-shaded}:\\&\mathrm{LER_{rel}} = 1 - \exp\{-8.8(\text{Leaf P\%} - 0.13)\},\\&R^2 = 0.88,\quad P < 0.001,\quad n = 9\end{aligned}\tag{3a}$$

$$\begin{aligned}&\text{Shaded}:\\&\mathrm{LER_{rel}} = 1 - \exp\{-18(\text{Leaf P\%} - 0.15)\},\\&R^2 = 0.84,\quad P < 0.001,\quad n = 9\end{aligned}\tag{3b}$$

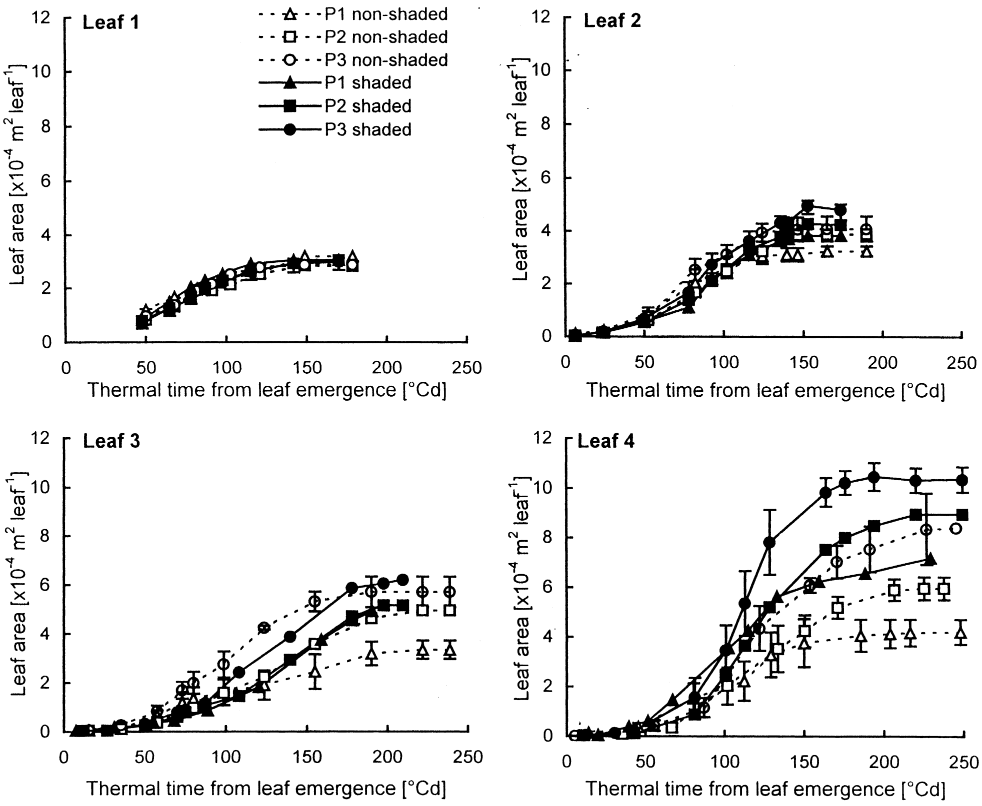

Fig. 3. Individual leaf area size of leaves 1–4 of wheat plants grown at different levels of phosphorus supply and two levels of incident radiation, as a function of cumulated thermal time. Bars are standard errors for the difference of the means.

Table 3
Final leaf area, LER, and duration of the leaf expansion of main stem leaves 1–4, of wheat grown at three levels of phosphorus supply (P1, P2, P3), and two levels of incident radiation

MSLN[a]	P1		P2		P3		LSD	P	S	P×S
	N-S[b]	S[c]	N-S[b]	S[c]	N-S[b]	S[c]				
Final leaf area ($\times10^{-4}$ m^2 per leaf)										
1	3.2	3.0	2.8	3.0	2.9	3.0	0.5	NS	NS	NS
2	3.2	3.8	3.9	4.2	4.1	4.8	1.0	*	NS	NS
3	3.3	4.9	4.9	5.1	5.7	6.2	1.8	*	NS	NS
4	4.4	7.2	6.0	9.0	8.4	10.3	2.3	**	***	NS
Leaf expansion rate ($\times10^{-4}$ m^2 °Cd^{-1})										
1	0.02	0.03	0.02	0.03	0.03	0.03	0.01	NS	NS	NS
2	0.04	0.05	0.04	0.05	0.06	0.05	0.02	NS	NS	NS
3	0.03	0.05	0.04	0.05	0.05	0.05	0.01	**	NS	NS
4	0.03	0.06	0.07	0.11	0.09	0.16	0.05	**	**	NS
Duration of leaf expansion (°Cd)										
1	158	92	120	118	98	118	53	NS	NS	NS
2	93	79	88	81	78	99	28	NS	NS	NS
3	128	107	114	102	102	115	15	NS	NS	NS
4	117	137	80	80	91	68	40	**	NS	NS

[*] Significance level $P<0.05$.
[**] Significance level $P<0.01$.
[***] Significance level $P<0.001$.
[a] Main stem leaf number.
[b] Non-shaded.
[c] Incident radiation (45%).

Shaded leaves tended to be larger but lighter than non-shaded leaves (Fig. 5). The specific leaf area (SLA, m^2 kg^{-1}) of the shaded L4 was on average 18% higher than that of non-shaded L4 (Table 4). The concentration of water-soluble carbohydrates in leaves (%WSC), at the time leaf 4 reached its maximum size was lower in shaded and in high-P plots (Table 4). As observed with total dry weight, effects of shading on the dry matter used in the synthesis of structural components of leaves (dry matter less water-soluble carbohydrates) was different for the different levels of P ($P<0.05$) (Fig. 6). At low or intermediate levels of *P*, shading reduced the structural dry matter content in leaves by 16 and 10%, while at P3, the reduction was of 35%. In leaves, the accumulation of WSC (g m^{-2}), at the time leaf 4 reached its maximum size, was not affected by P treatments, and was reduced by 65% on average, by shading. The values of LER of leaf 4 were inversely related to %WSC (Fig. 7), and the relationship between SLA and %WSC was different in shaded and non-shaded plants (Fig. 8). Irrespective of the %WSC and for most P treatments, shaded plants had higher values of SLA than non-shaded ones. The SLA at 0%WSC, calculated from extrapolation of the regression lines in Fig. 8 was 18% higher in shaded than in non-shaded treatments.

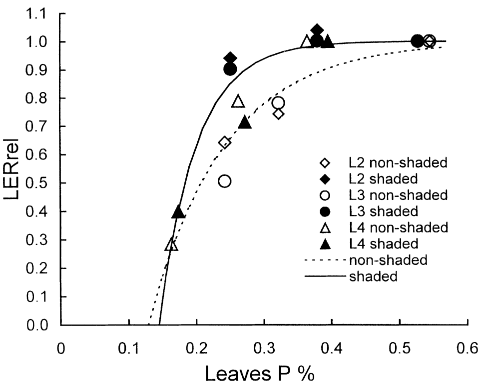

Fig. 4. Relative values of LER with respect to the control non-shaded and shaded treatments, as a function of the concentration of P in the leaves. Lines are fitted by Eqs. (3a) and (3b).

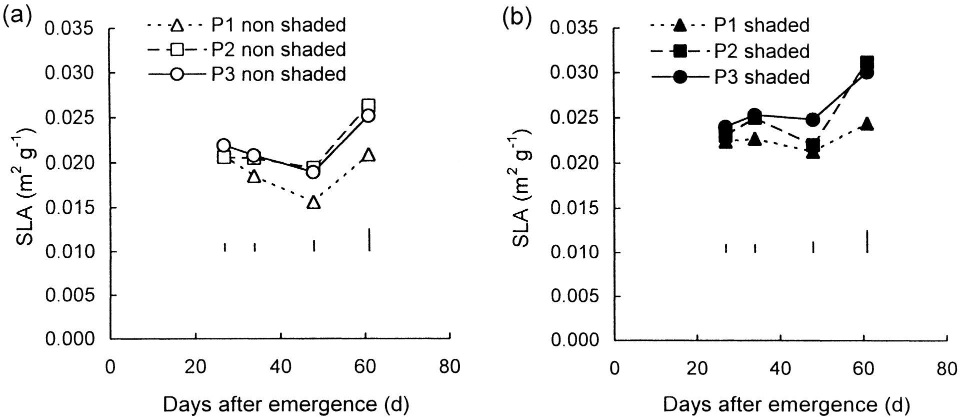

Fig. 5. Specific leaf area of leaf 4 as a function of DAE for non-shaded (a) and shaded (b) wheat crops growing at different levels of P supply. Bars are standard errors for the difference of the means.

3.4. Radiation use efficiency

RUE tended to decrease with time and was significantly affected by both P and shading treatments (Table 4). Phosphorus deficiency reduced RUE by 40% in P1 non-shaded plants. Reductions of 65% on the incident radiation increased RUE on average by 33%. Irrespective of the shading treatment, the relative values of RUE ($\mathrm{RUE_{rel}}$) for the different harvest intervals were asymptotically related to the concentration of P in the leaves (Eq. (4) and Fig. 9). This relationship presented a threshold value of leaf P%, defined as the value of leaf P% when $\mathrm{RUE_{rel}}=0.9$, at a leaf P% equal to 0.34:

$$\mathrm{RUE_{rel}} = 1 - \exp\{-8.4(\text{Leaf P\%} - 0.06)\} \qquad (4)$$

From Eqs. (3a), (3b) and (4), and at a concentration of P in leaves of about 0.15% (Fig. 10), canopy assimilation, i.e. RUE, was still 50% of the maximum observed at high P, while leaf expansion, i.e. LER, was close to zero.

4. Discussion

Varying the supply of P and the level of incident radiation generated substantial changes in leaf expansion, and above ground biomass accumulation, and small effects on the timing of the phenostages of the

Table 4

Specific leaf area ($\mathrm{m^2\,g^{-1}}$), concentration of water-soluble carbohydrates (%), radiation use efficiency (RUE, $\mathrm{g\,MJ^{-1}}$) for the periods between harvest 1 and 2, 2 and 3, and 3 and 4, in wheat plants grown under three levels of P supply and two levels of radiation intensity at 61 DAE

	Non-shaded			Shaded			LSD[a]	P[b]	S[c]	P×S
	P1	P2	P3	P1	P2	P3				
SLA-L4	0.022	0.024	0.024	0.027	0.028	0.028	0.004	NS	**	NS[d]
%WSC	26.2	12.9	9.7	16.0	3.9	5.9	7.2	**	**	NS
$\mathrm{RUE_{2-1}}$	2.7	4.4	4.6	2.1	3.1	3.6	2.9	NS	NS	NS
$\mathrm{RUE_{3-2}}$	1.2	2.3	2.0	1.8	2.1	2.8	0.9	*	NS	NS
$\mathrm{RUE_{4-3}}$	1.0	1.2	1.3	1.2	1.7	1.8	0.3	*	**	NS

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

[a] Least significant differences for P+S comparisons.

[b] Effects of the phosphorus level.

[c] Effects of the shading level.

[d] Not significant.

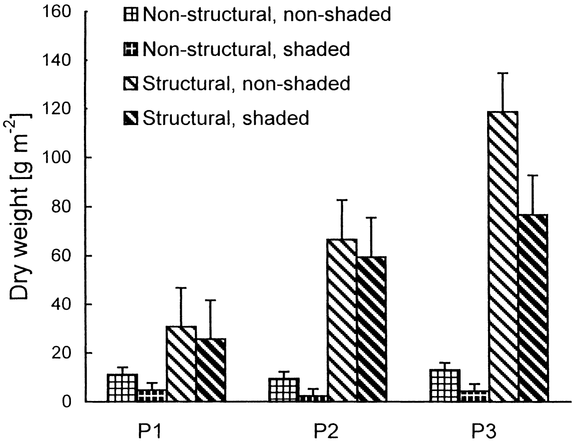

Fig. 6. Non-structural and structural dry weight in leaves of wheat plants grown at different levels of phosphorus supply and two levels of incident radiation. Bars are standard errors for the difference of the means (n=4).

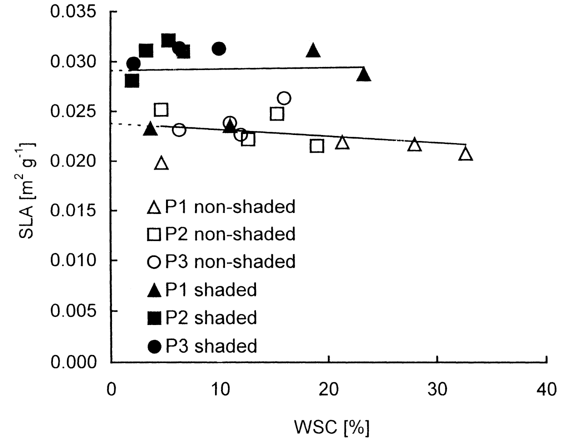

Fig. 8. Specific leaf area of leaf 4 as a function of the concentration of water-soluble carbohydrates in leaves for non-shaded and shaded wheat crops growing at different levels of P supply.

crop. The increase in the duration of the leaf area expansion period at low P was probably due to a larger phyllochron and to a delayed and limited tiller emergence (Rodríguez et al., 1998a,b).

P limitation reduced the fraction of intercepted radiation as a consequence of a reduced leaf area expansion, and not through effects on the light extinction coefficient. However, as mentioned by Elliot et al. (1997), leaves of non-shaded low-P plants tended to be more erect, and the canopy to present a lower value of K, which is probably related to the effects of P on the individual size of the leaves. In shaded plots, K did not decrease but increased in P1 plants. A higher value of K indicates a canopy having a higher efficiency for intercepting radiation per unit of LAI ($m^2\,m^{-2}$). Values of K at low LAI are usually higher than those for more developed canopies (Green, 1987; Goudriaan, 1988; Meinke, 1996). In this work, the values of LAI in P1 plants were low and similar irrespective of the shade treatment, yet their K presented a differ-

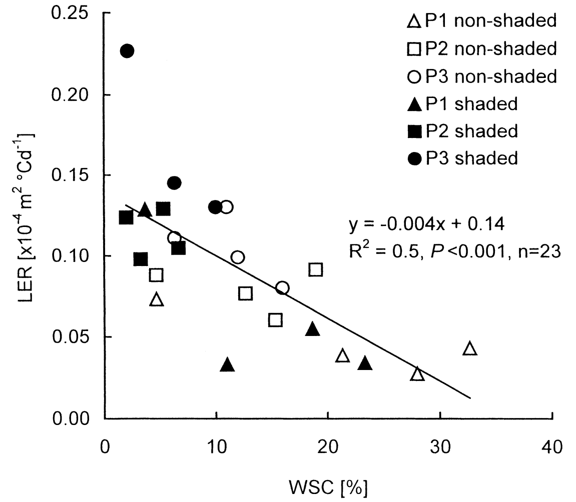

Fig. 7. LER of leaf 4 as a function of the concentration of water-soluble carbohydrates in leaves of non-shaded and shaded wheat crops growing at different levels of P supply.

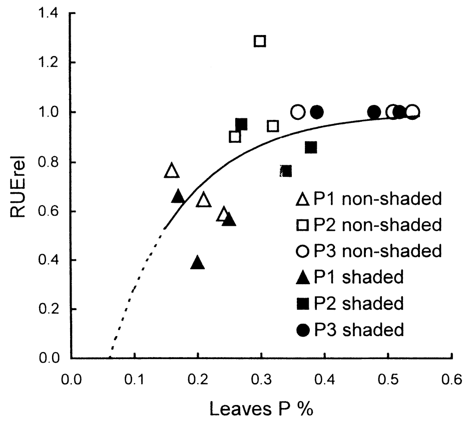

Fig. 9. Relative values of RUE with respect to the control non-shaded and shaded treatments, as a function of the concentration of P in the leaves. Lines are fitted by Eq. (4).

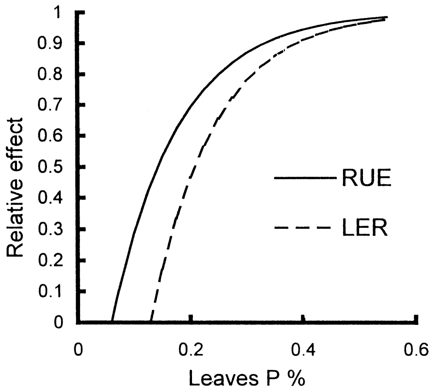

Fig. 10. Relative effect of the concentration of phosphorus in leaves on the relative values of RUE and LERs.

ence of 1.8-fold indicating that differences in *K* were not related to the size of the LAI.

Phosphorus and shade treatments reduced light interception by reducing the emergence of leaves, and tillers, and in the case of P deficiency also by reducing the size of the individual leaves. The effects of P deficiency on the emergence of leaves and tillers were consistent with those in previous works (Sato et al., 1996; Elliot et al., 1997; Rodríguez et al., 1998a,b). The P limitation reduced the individual leaf size through a reduced LER, while the duration of leaf expansion remained relatively unaffected agrees with most existing data of leaf area expansion under stress conditions (Porter, 1984; Ong and Baker, 1985; Rodríguez et al., 1998a,c). As observed before (Fischer, 1975; Schnyder and Nelson, 1989), leaves expanding under low radiation environments were larger but thinner than those expanding at full radiation. Greater areas of shaded leaves have been associated with an increased length and a lower dry matter import of the elongation zone (Schnyder and Nelson, 1989). Since the extension zone in wheat is not exposed to direct light, the effects of shading on its size must be indirect. Shaded plants had lower %WSC and lower structural dry weight, while the accumulation of structural material in high-P shaded leaves was less than in low-P treatments. Since values of LER were also negatively related to %WSC in leaves, it is not likely that leaf expansion in low-P plants is limited by the availability of assimilates. The negative relationship between LER and %WSC is far from indicating any cause–effect relationship, however, it clearly demonstrates that at a low P supply, the availability of carbon for growth was not limiting the values of LER. The range of values of %WSC observed in this work is large and in agreement with values obtained by other authors, for wheat and other species (Housley and Pollock, 1985; Kuhbauch and Thome, 1989).

Phosphorus deficiency did not affect the content of WSC (g m^{-2}) in non-shaded or shaded leaves (Fig. 6). Consequently, the higher %WSC observed in low P leaves and stems was probably due to a direct limitation of P on the activity and number of sinks for assimilates (Kuhbauch and Thome, 1989). Phosphorus deficiency can directly limit the size of individual leaves by producing fewer cells per leaf primordia, and/or by limiting cell elongation. Cell division can be limited by phosphorus deficiency. Jacob and Lawlor (1991) observed that in low P wheat plants, the number of cells per leaf was reduced by 10-fold. Alternatively, LER could have been driven by some unknown leaf epidermal property, e.g. cell wall elasticity, which may change in response to a low plant P status (Fredeen et al., 1989). Cell wall properties can limit expansion under nutrient deficiency (Pritchard et al., 1990; Palmer et al., 1996).

The values of LER relative to the high P plants ($\mathrm{LER_{rel}}$) were asymptotically related to the concentration of P in the leaves, yet the relationship was different for non-shaded and shaded plots. We believe that differences in the response of $\mathrm{LER_{rel}}$ to leaf P, between non-shaded and shaded plants, could arise if shading altered the anatomical structure of the leaves. "Structural SLA", i.e. the value of SLA when %WSC approaches zero, was higher in shaded than in non-shaded leaves, with less resources per unit of leaf area needed to form these leaves. Nevertheless, under most field conditions, levels of radiation, as low as those we had, in our shaded plot are unlikely, and consequently we believe that Eq. (3a) can be used for predictive purposes.

The values of RUE of P3 non-shaded plants are within the range of those reported by Kiniry et al. (1989) for the preanthesis period of wheat. P deficiency can reduce RUE, as calculated here, if partitioning towards roots increases at low P, and/or if the overall efficiency of the canopy for CO_2 fixation decreases. Phosphorus deficiency consistently decreased the value of photosynthesis at high irradiance, of recently expanded leaves of P-deficient wheat

(Rodríguez et al., 1998a), and in sunflower plants (Rodríguez et al., 1998c). Similar results were observed in this work for leaf 4 by Rodríguez et al. (1999), who reported that the value of photosynthesis at high irradiance was reduced by 30 and 16% for P1 and P2 plants, respectively.

At 61 DAE light interception, above ground biomass and LAI were reduced more strongly by P deficiency in non-shaded than in shaded plants. Since P deficiency limits photosynthesis through a reduced regeneration of ribulose 1,5-biphosphate (RuBP) (Brooks et al., 1988; Rao and Terry, 1995), assimilation and growth in low P plants will be particularly affected at high levels of radiation, when photosynthesis depends most on RuBP regeneration. At low levels of radiation, photosynthesis depends on the levels of available energy rather than on RuBP regeneration. In low P leaves, the capacity for RuBP regeneration can be reduced if the availability of fixed carbon, the initial activity of the Calvin cycle enzymes, and/or the supply of ATP and NADPH are limited. Under moderate P-deficient conditions, Rao and Terry (1995) proposed that RuBP regeneration is most likely to be limited by the supply of Ru5P, and/or the initial activity of the Ru5P kinase. Root–shoot partitioning can be modified by P deficiency if the strength of aerial sinks for assimilates is relatively more inhibited than roots. Using simulation techniques, Rodríguez et al. (1998a,c) showed that a direct limitation to leaf expansion by low P increased the allocation of assimilates to the roots modifying the root–shoot ratio.

In this work, irrespective of the shading treatments, the relative values of RUE were asymptotically related to the concentration of P in the leaves. This relationship presented a threshold value of leaf P% of 0.34, that was similar to the critical threshold for photosynthesis reported by us in wheat (Rodríguez et al., 1998a), and sunflower (Rodríguez et al., 1998c), and by others in gamelia (Cromer et al., 1993), and eucalyptus (Kirschbaum and Tompkins, 1990). The response of $\mathrm{RUE}_{\mathrm{rel}}$ and $\mathrm{LER}_{\mathrm{rel}}$ to leaf P% in Eqs. (3a) and (4), clearly indicates a higher sensitivity of leaf expansion over assimilate production in wheat grown under P-deficient conditions. We believe that Eqs. (3a) and (4) could be used in the construction of simulation models for the prediction of the growth of wheat when grown at sub-optimal levels of P supply.

5. Conclusions

In this work, we have shown that leaf expansion in wheat grown at low levels of P supply is not likely to be limited by the availability of carbon for growth (trophic limitation). Whether leaf expansion is reduced by some leaf epidermal property, that may change in response to a low plant P status, remains to be tested.

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