JOURNAL OF PLANT NUTRITION, 24(11), 1711–1729 (2001)

LEAF AREA DEVELOPMENT IN SOYBEAN AS AFFECTED BY PHOSPHORUS NUTRITION AND WATER DEFICIT

Flavio H. Gutiérrez-Boem[1,*] and Grant W. Thomas[2]

[1]Cátedra de Fertilidad y Fertilizantes, Facultad de Agronomía, Univ. de Buenos Aires, Av. San Martín 4453, 1417 Buenos Aires, Argentina
[2]Dept. of Agronomy, Univ. of Kentucky, N-122 Agric. Sci. Bldg. North, Lexington, KY 40546-0091, USA

ABSTRACT

Leaf area development is an important factor in crop production because it affects the amount of radiation intercepted and, therefore, plant growth. Even though phosphorus (P) deficit and water stress have been studied as isolated factors limiting leaf area development in soybean (*Glycine max* (L.) Merr.), little has been done on their possible interactions. A pot experiment was conducted to determine the effects of P supply and water deficit on leaf-area development in soybean, and whether P nutrition affects soybean response to water stress. Plants were grown in pots for 49 days. The soil used was a Sadler silt loam (fine-silty, mixed, mesic Glossic Fragiudalf), which contained $5.3\,mg\,P\,kg^{-1}$ (Mehlich III). Additional P was added at 0, 20,

*Corresponding author. E-mail: gutierre@mail.agro.uba.ar

and 80 mg P kg^{-1} soil. Water treatments consisted of keeping the soil-water content at 85% (well watered), 60% (mild water stress) and 35% (severe water stress) of the 10 kPa soil-water content. Phosphorus nutrition did not affect the response of soybean to water stress in most of the measured variables. Relative reductions due to water stress were similar at every P level for individual leaf area, whole-plant leaf area, aboveground biomass, stomatal conductance, and transpiration. Only plant development was slowed to a greater extent by water stress in P-deficient plants. Water stress had no effect on P nutrition, since it did not affect P concentration in plant tissue.

INTRODUCTION

Development of leaf area is a factor of primary importance in crop production because it affects the amount of radiation intercepted. The rate of leaf area development has a big influence on crop growth, especially prior to the closure of the canopy (1). Leaf area is a function of the number and size of leaves, and both, but especially size can be reduced by water stress (2) or nutrient deficiency (3). Reductions in crop production due to water and nutrient deficiency may be caused by decreased radiation interception, radiation use efficiency, or both, but photosynthetic metabolism is usually less affected than organ number and size (4). Reduced production of crops under these types of stress is frequently associated with inadequate development of leaf area, decreasing light interception (1).

Water stress effects on leaf area development in soybean (*Glycine max* (L.) Merr.) have been reported by several authors. Water stress may reduce leaf expansion rate, final leaf size, and rate of leaf appearance, all of these effects leading to a lower plant leaf area (5–7). Phosphorus deficiency in soybean has been shown to decrease whole plant leaf area, leaf area per unit leaf weight, individual leaf area, leaf appearance, and rate of leaf elongation (8–10).

Phosphorus nutrition may also affect plant response to water stress. In cotton (*Gossypium hirsutum* L.), P nutrition altered the relationship between leaf turgor and stomatal conductance, increasing the turgor threshold for stomata closure. Phosphorus-deficient plants closed their stomata at a leaf turgor of 0.31 MPa while high-P plants closed them when turgor was near 0 (0.06 MPa) (11). Stomata of low P plants also showed greater sensitivity to exogenous ABA and reduced their stomatal conductance by 59%, while high P plants only did so by 5% (11). When cotton plants were subjected to a drying cycle, leaf conductance of P-deficient plants was lower than it was in plants well supplied with P during the whole cycle until leaf turgor approached zero (12). Phosphorus

deficiency in cotton also significantly decreased root hydraulic conductance, an effect that was suggested as the cause for reduced leaf expansion (13). Clover (*Trifolium repens* L.) plants grown in high-P soil maintained a higher leaf water potential than low-P plants at a given soil matric potential when subjected to a soil drying cycle, and only low-P plants showed clear symptoms of water stress (14). Tiller and leaf appearance of wheat (*Triticum aestivum* L.) and stomatal conductance were reduced by a mild water stress only at the lowest P level, while plants well supplied with P were not affected (15).

Even though P deficit and water stress have been studied as isolated factors limiting leaf area development in soybean, little has been done on their possible interactions. The objective of this study was to determine the effects of P and water deficits on leaf-area development in soybean, and whether P nutrition affects soybean response to water stress. We hypothesized that soybean tolerance to water stress would be enhanced by adequate P nutrition.

MATERIALS AND METHODS

A greenhouse experiment was conducted using soybean (cv. CF492, maturity group IV, determinate growth). Germinated seeds inoculated with *Bradyrhizobium japonicum* were planted on February 1, 1997; they emerged 3 days later. One plant per pot was grown using cylindrical pots (15 cm diameter) with 2500 g of dry soil. Soil used was from the A horizon of Sadler silt loam (fine-silty, mixed, mesic Glossic Fragiudalf). Soil pH was 6.9. It contained 5.3 μg P, 106 μg potassium (K), 1563 μg calcium (Ca), 80 μg magnesium (Mg), and 1.4 μg zinc (Zn) per g of soil using the Mehlich III extraction (16). The soil water retention was determined at 10 and 1500 kPa, giving soil water contents of 36% and 8% by weight, respectively. Mean air temperature during the experiment was 23.7°C ($\pm$ 1.3). Daylength was kept constant at 16 h during the experiment by using supplementary artificial light.

Soil P treatments were established by the addition of 0, 20, and 80 μg P g^{-1} soil (as KH_2PO_4) and were denoted as P0, P20 and P80, respectively. Potassium was added as KCl to P0 and P20 to make the amount of K added equal among treatments (95 μg K g $soil^{-1}$). All nutrient additions were made as a solution that was thoroughly mixed with the soil 10 days before planting. Soil water treatments consisted of keeping the soil water content at 85% (well watered, WW), 60% (mild water stress, MS), and 35% (severe water stress, SS) of the soil water content at 10 kPa. Soil water content was kept at those levels by weighing and watering the pots daily. Pots were bottom sealed to avoid water loss by leaching. Water stress treatments were initiated 9 days after emergence (DAE). The experimental design was a completely randomized factorial with three levels of P

and three levels of water availability (i.e., nine treatments), and five replications per treatment.

Evapotranspiration was measured by recording daily pot weight before watering. Six extra pots without plants were used to measure evaporation from soil (two pots for each soil water level). Plant transpiration was calculated by subtracting evaporation from water consumed by each pot. Leaf expansion was monitored by measuring daily length and width of the terminal leaflet of all expanding leaves using a clear plastic ruler. Stomatal resistance of the latest fully expanded leaf was measured 16, 23, 30, and 44 days after emergence between 1:00 and 3:00 pm (eastern standard time) using a Li-Cor LI-1600 steady state porometer. Two harvests were done, one at the onset of the water stress (9 DAE) and the final one at 49 DAE. On both occasions the leaf area of every leaf was determined using a Li-Cor LI-3100 leaf area meter, and length and width of each terminal leaflet was measured. Leaf area during the experiment was calculated from the measured length and width using a regression relating the length × width product to the leaf area measured using the leaf area meter (Trifoliate leaf area = 1.931 length × width of terminal leaflet, $r^2 = 0.99$, $n = 663$, $p < 0.001$). As the leaf area of an individual leaf was formed during a period of mainly linear expansion, linear regressions of leaf expansion with time were fitted for each leaf using the following segmented function (Fig. 1):

$$A_{leaf} = a + bt\ (t < c) + bc\ (t \geq c)$$

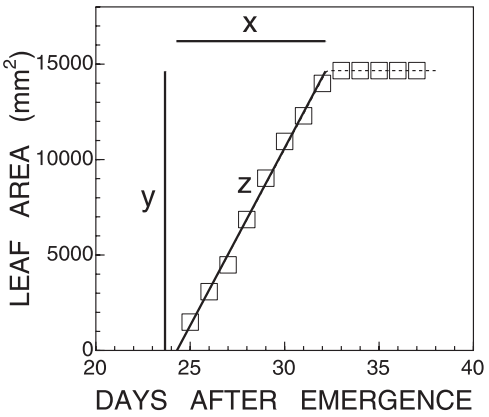

Figure 1. Change of leaf area of one leaf with time (fourth trifoliolate leaf of the P20-WW treatment, rep. 3). The length of line 'x' represents the period of leaf expansion (days), the length of line 'y' the final leaf area (mm^2), and the slope of 'z' the rate of leaf expansion ($mm^2\,day^{-1}$).

where A_{leaf} is the area of an individual leaf (mm^2), t the time (days), b the rate of leaf expansion ($mm^2\ d^{-1}$, slope of the line z in Fig. 1), and c is the day when the leaf reaches its final area. Terms ($t < c$) and ($t \geq c$) are equal to 1 or 0 when the condition is true or false, respectively. The period of expansion (length of line x in Fig. 1) is the number of days from leaf appearance to final leaf area (length of line y in Fig. 1) and is estimated by dividing the final leaf area by the expansion rate. For every leaf, the line fitted had a value of $r^2 > 0.94$.

At harvest, dry weight of leaf blades and stems (including petioles) was determined after oven drying at 65°C. Leaf weight ratio was calculated as the ratio between leaf blade and total aboveground dry matter. Phosphorus concentration of leaf blades and stems was measured in Kjeldahl digests by colorimetry using a Technicon II Autoanalyzer colorimeter (17).

Data collected were statistically analyzed by factorial ANOVA. When the interaction between main factors (P and water) was not significant ($p > 0.05$), contrasts (tested with the Student t test) were used to compare the means of the different treatments within each factor. When the interaction was significant, contrasts between water treatments at each P level were done. Individual leaf characteristics (rate, duration of expansion, and final area) were analyzed at each leaf position. Variances were stabilized when necessary using an empirical power transformation (18). In order to analyze relative effects of water stress at each level of P, some variables (e.g., biomass, leaf area) were expressed as relative values dividing the result of each plant by the mean of the WW treatment at the same P-level of the plant.

RESULTS AND DISCUSSION

Leaf Appearance and Expansion

To describe leaf expansion in a whole plant, we analyzed rates of appearance of new leaves, individual leaf expansion rates, and final leaf sizes. Plant development during vegetative growth is usually estimated measuring leaf appearance on the main stem (19). Figure 2a shows the effects of P and water availability on trifoliolate-leaf appearance on the main stem. The model that best fitted the data was one with 6 lines and 5 significantly different ($p < 0.05$) slopes (i.e., rates of leaf appearance). The water stress effect on rate of leaf appearance depended on the amount of P added. Severe water stress (SS) lowered the rate by 12, 23, and 26% at P80, P20, and P0 respectively. Mild water stress (MS) did not have any effect. Randall and Sinclair (6) observed a slight decrease (5%) in the appearance of leaves in soybean with water stress periods of 8 days. Larger decreases were observed when soybean was subjected to a 36-day drought (7). Temperature is considered the main environmental factor that determines plant development

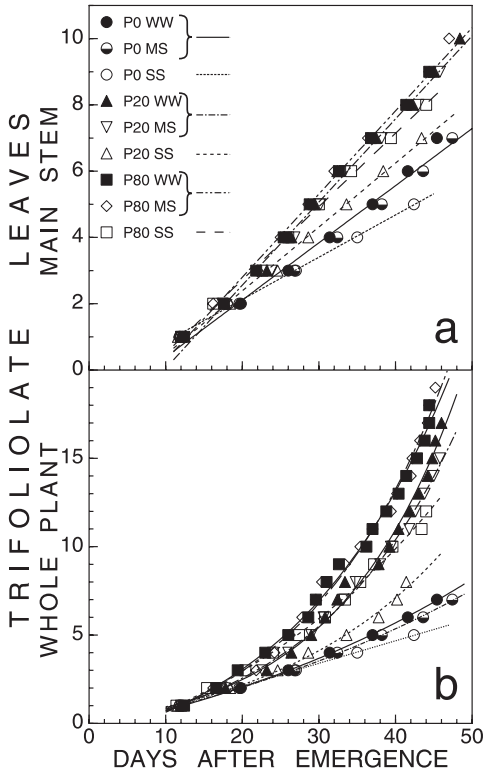

Figure 2. Trifoliolate-leaf appearance on (a) the main stem and (b) whole plants. Rates of leaf appearance on the main stem (slopes of the lines, leaf day^{-1}) were 0.17 for P0 WW and MS, 0.12 for P0 SS, 0.25 for P20 and P80 WW and MS, 0.19 for P20 SS, and 0.22 for P80 SS. Mean rates of leaf appearance on whole plants were 0.17, 0.17 and 0.12 for P0 WW, MS and SS; 0.44, 0.40 and 0.22 for P20 WW, MS and SS; 0.49, 0.51 and 0.33 for P80 WW, MS and SS, respectively.

during vegetative growth in soybean, and usually is the only one included in models for simulating that phase (20). Our results showed that both P deficiency and water stress may slow soybean development, and that these factors should be taken into account for plant development predictions during vegetative growth.

The final number of leaves on a plant depends not only on leaf appearance on the main stem, but also on leaf appearance on branches. Figure 2b shows the change in number of leaves with time for whole plants. There was no branching at P0, so all the leaves were on the main stem. Leaf appearance on lateral branches amplified the differences between SS and WW treatments at P20 and P80. The number of leaves grew exponentially due to lateral branching at P20 and P80

when plants were well watered or under a mild water stress. Phosphorus addition increased the number of lateral branches at every water level, while SS reduced it (Table 1). Branching was more sensitive to P-deficit and severe water stress than was leaf appearance on the main stem. Other authors have observed that branching and leaf appearance on axillary branches were more sensitive to other environmental factors than was leaf appearance on the main stem (21). Higher branching was observed in soybean when photosynthate supply was enhanced by growing plants at high irradiance or increased ambient CO_2 (21). Leaf number of wheat plants was reduced by a mild water stress only at the lowest P level, while plants well supplied with P were not affected (15). Contrary to what was reported for wheat, we did not observe any interaction between water stress and P nutrition on the final number of leaves in soybean (Table 1).

Individual leaf expansion was analyzed by dividing this process into rate of leaf expansion, period of leaf expansion, and final leaf area. These characteristics were evaluated for each leaf position on the main stem (Fig. 3, Table 2). The first trifoliolate leaf was not affected by water stress because it was already expanding when water stress treatments began. Starting from the second trifoliolate, individual-leaf area was reduced by both water and phosphorus deficits. Severe water stress caused a significant reduction in leaf area at every leaf position and P level, while MS had little or no effect on leaf area (Fig. 3a). Although the absolute reduction of leaf area due to water stress was different at each P level, these

Table 1. Effects of Phosphorus and Water Treatments on the Number of Branches, Trifoliolate Leaves, and Specific Leaf Area at Final Harvest (49 Days After Emergence)

	Branches				Leaves[†]				Specific leaf area			
	P0	P20	P80	Avg.	P0	P20	P80	Avg.	P0	P20	P80	Avg.
	[no.]				[no.]				[mm^2/mg^{-1}]			
WW	0	5	6.2	3.7	7	20.4	25.2	17.5	31.4	37.2	40.5	36.4
MS	0	4.6	5.8	3.4	7	18.0	23.6	16.2	34.1	35.8	38.9	36.3
SS	0	2.2	4.4	2.2	6	10.6	15.8	10.8	31.8	35.9	35.0	34.2
Avg.	0	3.9	5.4	3.1	6.6	16.3	21.5	14.8	32.4	36.3	38.1	35.6
ANOVA												
P:			**				**				**	
W:			**				**				ns	
P × W:			ns				ns				ns	
Contrasts	WW&MS vs. SS: **				WW&MS vs. SS: **				P0 vs. P20&P80: **			
Main effects	WW vs. MS: ns				WW vs. MS: ns				P20 vs. P80: ns			

[†]ANOVA performed only with P20 and P80 treatments.

*,**Significant at 0.05 and 0.01 probability level, respectively.

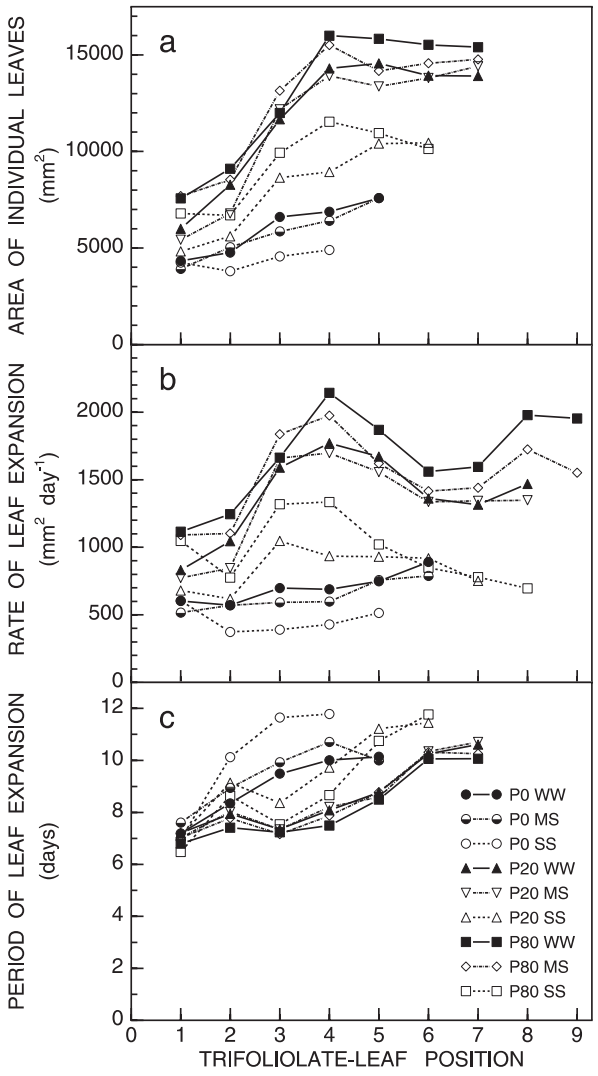

Figure 3. Effects of P and water treatments on (a) individual leaf area, (b) rate of leaf expansion and (c) period of expansion of leaves at different positions on the main stem.

differences were minimized when the reduction in leaf area was expressed relative to the well-watered treatment at each P level (Table 3). Therefore, the effect of water deficit could be calculated using the same coefficient for every P level. Reduced individual leaf areas due to water stress were also observed in soybean

Table 2. ANOVA for Individual Leaf Area, Rate of Leaf Expansion, and Period of Expansion of Trifoliolate Leaves at Different Main Stem Positions

	Leaf area						Rate of leaf expansion							Period of leaf expansion					
Leaf Position:	1	2	3	4†	5‡	6‡	1	2†	3†	4†	5	6‡	7‡	1	2†	3	4	5‡	6‡
Main factors and interaction																			
Phosphorus	**	**	**	**	ns	*	**	**	**	**	**	ns	**	*	**	**	**	ns	ns
Water	ns	**	**	**	**	**	ns	**	**	**	**	**	**	ns	**	**	**	**	**
P × W	ns	*	**	ns	ns	ns	ns	ns	*	ns	**	ns	ns	ns	ns	**	ns	ns	ns
Contrasts-Main effects																			
P0 vs. P20 & P80	**			**			**	**		**				**	**		**		
P20 vs. P80	**			**			**	**		**				ns	*		**		
WW & MS vs. SS				**	**	**		**		**		**	**		**		**	**	**
WW vs. MS				ns	*	ns		*		ns		ns	ns		ns		*	ns	ns
Contrasts-Simple effects																			
WW & MS vs. SS at P0		**	**						**		**					**			
at P20		**	**						**		**					**			
at P80		**	**						**		**					ns			
WW vs. MS at P0		ns	ns						*		ns					ns			
at P20		**	ns						ns		ns					ns			
at P80		ns	*						ns		**					ns			

† ANOVA performed with transformed data in order to stabilize variances.

‡Factorial ANOVA performed omitting PO treatments.

*,**Significance at the 0.05 and 0.01 probability levels, respectively.

Table 3. Effects of Severe Water Stress on Individual Leaf Area and Rate of Leaf Expansion at Each Phosphorus Level, Expressed as Relative Value to the Well Watered Treatment

	Individual leaf area				Rate of leaf expansion			
Leaf position	P0	P20	P80	Avg.	P0	P20	P80	Avg.
2	0.80	0.68	0.73	0.73	0.65	0.59	0.62	0.62
3	0.69	0.74	0.82	0.75	0.56	0.66	0.79	0.67
4	0.71	0.63	0.72	0.69	0.62	0.53	0.62	0.59
5		0.71	0.69	0.70	0.69	0.56	0.55	0.60
6		0.75	0.65	0.70		0.68	0.54	0.61
Average	0.73	0.70	0.72	0.72	0.64	0.60	0.63	0.62

in field experiments (5). Phosphorus deficit (P0) significantly decreased individual leaf area, and this effect was consistent across all leaf positions. Differences between P20 and P80 were also significant at several positions. Several other workers have observed reductions of leaf area in soybean growing in culture solutions with low-P concentrations (8–10).

The rate of leaf expansion was more sensitive to water and P stress than was the period of expansion, indicating that differences observed in final leaf area were due mostly to effects of P and water deficit on rate of leaf expansion (Fig. 3b). The rate of leaf expansion presented a similar pattern of variation as final leaf area: SS reduced the rate of leaf expansion at every P level and leaf position (from trifoliolate 2 to 6) while P addition increased the rate of leaf expansion. On the other hand, the period of expansion was prolonged by both water and P deficits (Fig. 3c). Both SS and P0 significantly increased the period of expansion at every leaf position. Effects of P and water were additive (no interaction) for most of the leaf positions. Lengthening of the period of expansion caused only a partial compensation for the reduction in the rate of leaf expansion because the period varied much less than did the rate. The rate of individual leaf expansion and individual leaf area were linearly related, and the rate of expansion explained 87% of the variation in the leaf area of trifoliolate leaves at positions 2 to 6 on the main stem ($n = 199$, $r^2 = 0.87$, $p < 0.001$).

Expansion of trifoliolate leaves on the main stem was more sensitive to water stress and P deficit than was the appearance of these leaves. Severe water stress reduced the rate of leaf expansion by 38% and the rate of leaf appearance by 12–26%. The higher sensitivity to water stress of leaf expansion over leaf appearance was also observed in soybean in field experiments. Under a mild water stress, leaf appearance was reduced by 5% and leaf expansion by 24% (6). Phosphorus deficit also reduced the rate of leaf expansion to a greater extent than

it did the rate of leaf appearance on the main stem. P0-WW treatment had 57% and 31% reductions in rates of expansion and appearance, respectively, when compared to P80-WW. Larger decreases in leaf expansion than in leaf appearance were also observed in soybean growing in low-P nutrient solution (8,10). This different sensitivity might be explained in terms of the sensitivity of cell division and expansion to both factors, water and phosphorus. Cell enlargement is more sensitive to water deficits than is cell division. Although cell division is not restricted at the beginning of leaf growth and cell division also implies cell enlargement, leaf differentiation and appearance is due mostly to cell division, while leaf expansion is mostly due to cell enlargement (2).

Whole-plant leaf area was reduced by both water and P deficits due to their effects on leaf appearance and expansion (Fig. 4a, Table 4). There was no significant interaction between P and water, although the reduction due to SS was relatively smaller at P0 (Fig. 4b). Reduced leaf area was due to both decreased number and size of leaves. The slightly smaller effect of SS at P0 was because of the complete inhibition of branching and lateral leaf appearance at P0, even when plants were well watered. Specific leaf area (i.e., leaf area per unit of dry matter of leaf blade, $cm^2\ g^{-1}$) (SLA) was only affected by P level (Table 1). When no P was

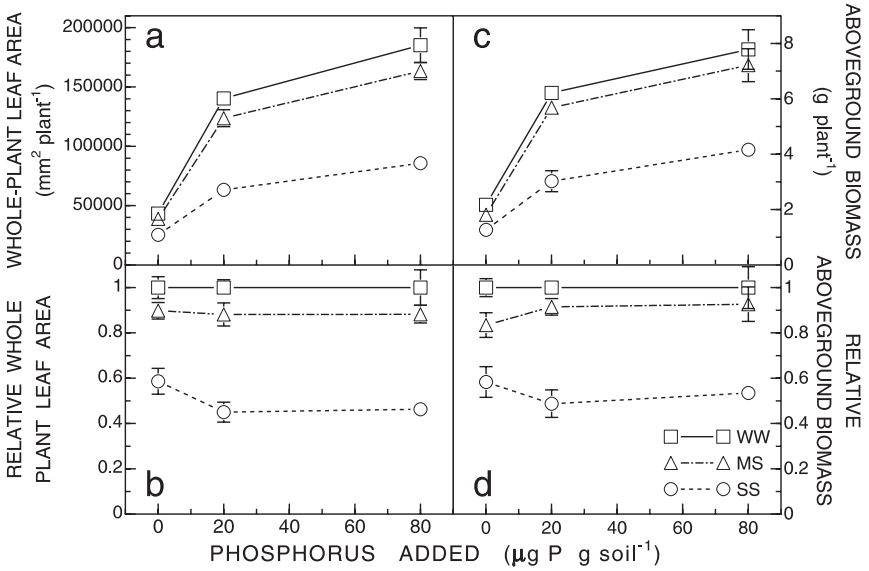

Figure 4. Effect of P and water deficits on (a) whole-plant leaf area, (b) leaf area relative to well watered treatments, (c) aboveground biomass and (d) aboveground biomass relative to WW treatments at final harvest. Vertical bars represent means ± 1 SE.

Table 4. ANOVA for Whole-Plant Leaf Area and Aboveground Biomass at Final Harvest (49 DAE), Stomatal Conductance (Average of 4 Measurements at 16, 23, 30, and 44 DAE), and Transpiration During the Period of Stress (9 to 49 DAE)

	Leaf area†	Biomass†	Stomatal conductance	Transpiration†
Main factors and interaction				
Phosphorus	**	**	**	**
Water	**	**	**	**
P × W	ns	ns	ns	ns
Contrasts-Main effects				
P0 vs. P20 & P80	**	**	**	**
P20 vs. P80	**	**	**	**
WW & MS vs. SS	**	**	**	**
WW vs. MS	*	ns	*	**

†ANOVA performed with transformed data to stabilize variances.
*,**Significant at 0.05 and 0.01 probability level, respectively.

added (P0) specific leaf area was reduced by 15%. This reduction of SLA is expected because P deficiency decreases leaf expansion more than it assimilates production. Soybean plants grown in a low-P nutrient solution had 85% less leaf area than control plants, while photosynthesis per unit of leaf area decreased by 55% (8). Phosphorus deficiency decreased photosynthesis per unit of leaf area by 30% and plant leaf area by 90% in soybean (10).

Aboveground Biomass and P Accumulation and Partition

Aboveground biomass was reduced by deficits of P and water (Fig. 4c). There was no interaction between P and water (Table 4) and the relative effect of water stress was similar at each P level (Fig. 4d). Mild water stress reduced biomass by 9% (not significant), while SS reduced biomass by 48%. P20 and P0 decreased biomass by 22 and 73%, respectively, compared to P80.

Partition of aboveground biomass towards leaf blades was slightly increased by P deficit. Percentages of aboveground biomass in leaf blades at final harvest were 59, 60, and 63 in P80, P20, and P0, respectively. There was no effect of water stress on biomass partition (Table 5). It is known that bigger and taller plants partition a greater proportion of aboveground dry matter to structural material in stems in order to provide mechanical support (22). Therefore, different partition to leaf blades could be an indirect effect of P treatments since P80 plants were more than 3.5 times as large as P0 plants.

Table 5. Effects of Phosphorus and Water Treatments on P Concentration in Leaves and Stems, Total P Accumulation, and Aboveground Biomass Partition to Leaves at Final Harvest (49 DAE)

	Leaf P				Stem P				P uptake†				Biomass partition to leaves			
	P0	P20	P80	Avg.	P0	P20	P80	Avg.	P0	P20	P80	Avg.	P0	P20	P80	Avg.
	[g kg^{-1}]								[mg pl^{-1}]				[g g^{-1}]			
WW	1.27	1.85	3.14	2.09	1.08	1.31	2.13	1.51	2.61	10.1	21.1	12.3	0.63	0.61	0.59	0.61
MS	1.38	2.01	3.05	2.14	1.05	1.39	2.15	1.53	2.27	10.0	19.1	10.5	0.63	0.61	0.59	0.61
SS	1.24	2.15	3.08	2.16	0.98	1.50	2.05	1.51	1.42	5.8	11.0	6.1	0.64	0.59	0.59	0.61
Average	1.29	1.99	3.09	2.13	1.04	1.40	2.11	1.52	2.10	8.6	17.1	9.3	0.63	0.60	0.59	0.61
ANOVA																
P:			**				**				**				**	
Water:			ns				ns				**				ns	
P × W:			ns				ns				ns				ns	
Contrasts	P0 vs. P20 & P80: **				P0 & P20 vs. P80: **				P0 vs. P20 & P80: **				P0 vs. P20 & P80: **			
Main effects	P20 vs. P80: **				P0 vs. P20: **				P20 vs. P80: **				P20 vs. P80: **			
									WW & MS vs. SS: **							
									WW vs. MS: ns							

†ANOVA performed with transformed data to stabilize variances.

*,**Significant at 0.05 and 0.01 probability level, respectively.

During vegetative growth, expanding leaves are the main sinks for new assimilates, and the number of growing points is expected to depend on assimilate supply (23). Plant growth rate (rate of aboveground dry matter accumulation) and the number of simultaneously expanding leaves during the period of water stress (DAE 9 to 49) were linearly related (Fig. 5). The reciprocal of the slope of the line (i.e., $50\,mg\,day^{-1}\,leaf^{-1}$) could be interpreted as the amount of assimilates needed for one leaf to grow (23). It seems that P deficiency and water stress affected the number of expanding leaves by reducing the assimilates available for growth, and not by modifying the allocation of new dry matter or the minimum amount of assimilate required for one leaf.

Phosphorus concentrations in leaves and stems were affected only by P level, increasing with P addition (Table 5). Phosphorus concentrations in leaves were 35 and 58% lower at P20 and P0, respectively, than at P80. Water stress, on the other hand, had no effect on P nutrition. Phosphorus concentrations in the P80 treatments were within the range of sufficiency reported by other authors, while concentrations at P20 and P0 may be considered 'low' and 'deficient', respectively (24,25). It is known that P diffusion in the soil is affected by soil water content and is a limiting factor in P absorption (26). Water stress reduced P tissue concentrations in field grown soybeans due to lowered P diffusion (27). The limited volume of soil explored by the roots in a pot could explain why soil-water content did not affect P nutrition in our study, since at high root densities

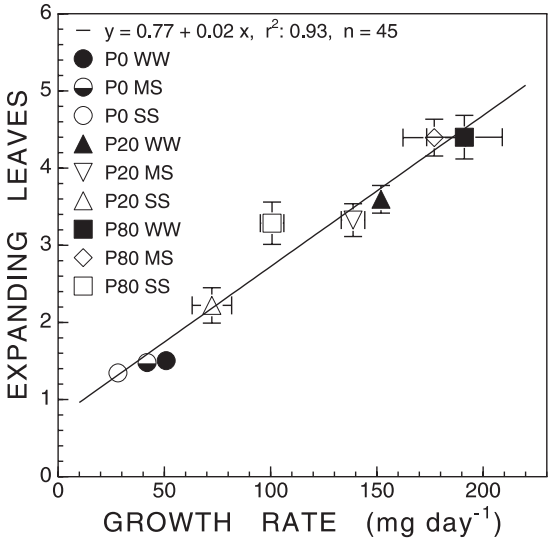

Figure 5. Number of simultaneously expanding leaves as a function of the rate of accumulation of aboveground dry matter. Bars represent means ± 1 SE.

the importance of P movement in plant uptake decreases. Total P uptake had a similar pattern of variation as dry matter, with a larger effect of P addition due to increased P concentration in dry matter along with P availability (Table 5). There was no interaction between P and water levels. Severe water stress had the same relative effect at each P level, reducing total P per plant by 45%. Phosphorus partition to leaf blades was not affected either by P-level or by water stress (data not shown), as the proportion of P in leaves was the same for all treatments (68%).

Stomatal Conductance and Transpiration

Stomatal conductance was reduced by both water and P deficits (Fig. 6a). Water stress reduced stomatal conductance by 8 and 40% for MS and SS, respectively, while phosphorus deficiency decreased it by 13 and 42% for P20 and P0, respectively. There was no interaction between water and P levels (Table 4). Contrary to what has been reported for wheat (15) and cotton (11), P nutrition did not affect stomatal response to water stress in soybean in our experiment. This invariable stomatal sensitivity to water stress under different P levels could be related to the similitude in the response to water stress at every P level that we observed in soybean.

Transpiration during the water stress period was affected by P nutrition and water stress (Fig. 6b, Table 4). Phosphorus deficiency decreased transpiration by 24 and 76% at P20 and P0, respectively, when compared to P80. Water stress caused a similar relative reduction at every P level, approximately 16 and 54% for MS and SS, respectively (Fig. 6c). The daily leaf area increase of whole plants was linearly related to transpiration rate. Figure 7 shows leaf area increase as a function of transpiration, with both variables expressed relative to the well-watered treatment at each P level. The phosphorus nutrition of the plants did not affect the relationship between relative transpiration and relative leaf area increase, as all plants lay on the same line, regardless of the P level. Other authors have used similar relationships between relative transpiration and relative leaf area expansion in order to predict the effect of water stress on leaf expansion in barley, calculating the relative transpiration from a soil moisture balance (28). Our results show that the same relationship may be used to predict leaf area expansion under water stress in plants with different levels of P nutrition.

CONCLUSIONS

Phosphorus nutrition did not affect the response of soybean to water stress in most of the measured variables. Relative reductions due to water stress were

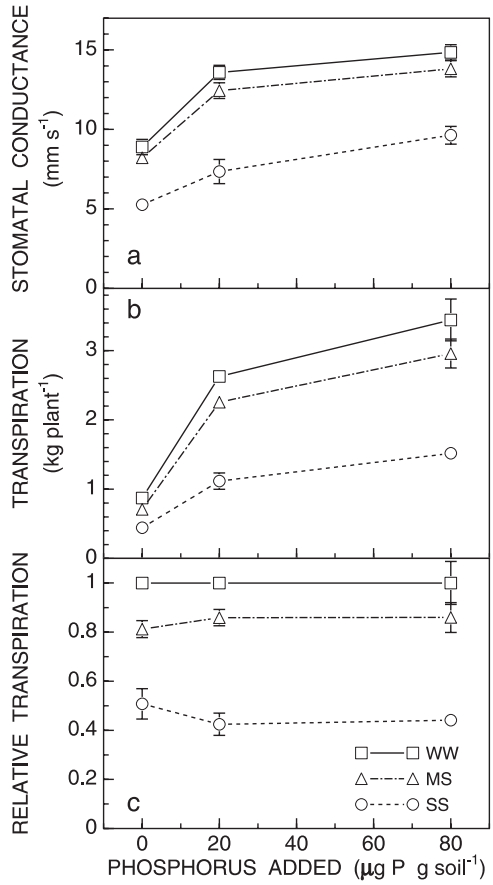

Figure 6. Effects of P and water deficits on (a) stomatal conductance (average of 4 measurements), (b) transpiration and (c) transpiration relative to well watered treatments during the period of water stress. Vertical bars represent means ± 1 SE.

similar at every P level for individual leaf area, whole-plant leaf area, aboveground biomass, stomatal conductance, and transpiration. Therefore, we rejected the hypothesis that soybean tolerance to water stress would be enhanced by adequate P nutrition. The effect of water stress on leaf expansion could be described in P-deficient plants using the same response function developed for plants well supplied with P. Only plant development was slowed to a greater extent by water stress in P-deficient plants. Water stress had no effect on P nutrition, since it did not affect P concentrations in plant tissue. Phosphorus deficiency decreased leaf appearance, expansion, and final area, biomass and P

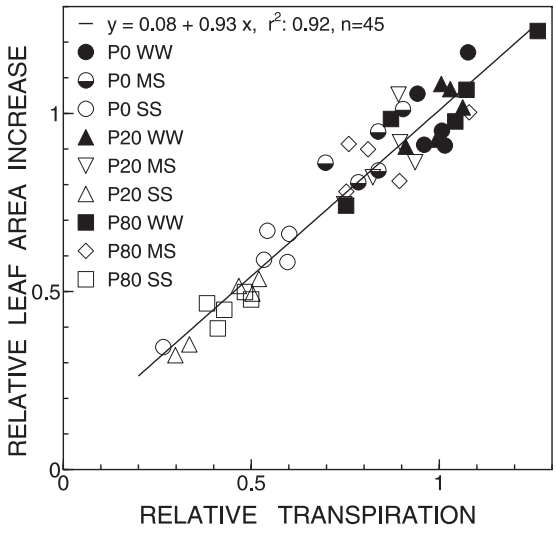

Figure 7. Relationship between daily whole-plant leaf area expansion and transpiration. Values are relative to the mean of the well watered treatment at each P level and averaged over the stress period (9 to 49 days after emergence). Each symbol represents one plant.

accumulation, P concentration in leaves and stems, and transpiration. Of the two components of individual leaf expansion (rate and period of expansion), the rate was more sensitive to water and P deficits, thus different leaf sizes were mainly a product of different leaf expansion rates. Individual leaf area was more sensitive to both stresses than was leaf appearance.

ACKNOWLEDGMENTS

We wish to acknowledge the support received from the CONICET, Argentina, and the University of Kentucky.

REFERENCES

1. Lawlor, D.W. Photosynthesis, Productivity and Environment. J. Exp. Bot. **1995**, *46*, 1449–1461.
2. Dale, J.E. The Control of Leaf Expansion. Annu. Rev. Plant Physiol. Plant Mol. Biol. **1988**, *39*, 267–295.

3. Longnecker, N. Nutrient Deficiencies and Vegetative Growth. In *Mechanisms of Plant Growth and Improved Productivity*; Basra, A.S., Ed.; Marcel Dekker: New York, 1994; 137–172.
4. Cornic, G. Drought Stress and High Light Effects on Leaf Photosynthesis. In *Water Deficits: Plant Responses from Cell to Community*; Smith, J.A.C., Griffiths, H., Eds.; Bios Scientific Publ.: Oxford, UK, 1994; 297–313.
5. Muchow, R.C.; Sinclair, T.R.; Bennett, J.M.; Hammond, L.C. Response of Leaf Growth, Leaf Nitrogen, and Stomatal Conductance to Water Deficits During Vegetative Growth of Field-Grown Soybean. Crop Sci. **1986**, *26*, 1190–1195.
6. Randall, H.C.; Sinclair, T.R. Sensitivity of Soybean Leaf Development to Water Deficits. Plant Cell Env. **1988**, *11*, 835–839.
7. Vendeland, J.S.; Sinclair, T.R.; Spaeth, V.; Cortes, P.M. Assumptions of Plastochron Index: Evaluation with Soya Bean under Field Drought Conditions. Ann. Bot. **1982**, *50*, 673–680.
8. Fredeen, A.L.; Rao, I.M.; Terry, N. Influence of Phosphorus Nutrition on Growth and Carbon Partitioning in *Glycine max*. Plant Physiol. **1989**, *89*, 225–230.
9. Israel, D.W.; Rufty, T.W., Jr. Influence of Phosphorus Nutrition on Phosphorus and Nitrogen Utilization Efficiencies and Associated Physiological Responses in Soybean. Crop Sci. **1988**, *28*, 954–960.
10. Qiu, J.; Israel, D.W. Carbohydrate Accumulation and Utilization in Soybean Plants in Response to Altered Phosphorus Nutrition. Physiol. Plant. **1994**, *90*, 722–728.
11. Radin, J.W. Stomatal Responses to Water Stress and to Abscisic Acid in Phosphorus-Deficient Cotton Plants. Plant Physiol. **1984**, *76*, 392–394.
12. Ackerson, R.C. Osmoregulation in Cotton in Response to Water Stress. Plant Physiol. **1985**, *77*, 309–312.
13. Radin, J.W.; Eidenbock, M.P. Hydraulic Conductance as a Factor Limiting Leaf Expansion of Phosphorus-Deficient Cotton Plants. Plant Physiol. **1984**, *75*, 372–377.
14. Singh, D.K.; Sale, P.W.G.; McKenzie, B.M. Water Relations of White Clover (*Trifolium repens* L.) in a Drying Soil, as a Function of Phosphorus Supply and Defoliation Frequency. Aust. J. Agric. Res. **1997**, *48*, 675–681.
15. Gutiérrez-Boem, F.H.; Thomas, G.W. Phosphorus Nutrition Affects Wheat Response to Water Deficit. Agron. J. **1998**, *90*, 166–171.
16. Mehlich, A. Mehlich 3 Soil Test Extractant: A Modification of Mehlich 2 Extractant. Commun. Soil Sci. Plant Anal. **1984**, *15*, 1409–1416.
17. Technicon Corp. *Technicon Auto Analyzer I*, Industrial Method 348R-6-31-5; Technicon: Tarrytown, NY, 1965.
18. Kuehl, R.O. *Statistical Principles of Research Design and Analysis*; Duxbury Press: Pacific Grove, CA, 1994; 686 pp.

19. Fehr, W.R.; Caviness, C.E. *Stages of Soybean Development*, Special Report No. 80; Cooperative Extension Service, Agriculture and Home Economics Experiment Station, Iowa State University of Science and Technology: Ames, Iowa, 1977.
20. Jones, J.W.; Boote, K.J.; Jagtap, S.S.; Mishoe, J.W. Soybean Development. In *Modeling Plant and Soil Systems*; Hanks, J., Ritchie, J.T., Eds.; ASA-CSSA-SSSA: Madison, WI, 1991; 71–90.
21. Hofstra, G.; Hesketh, J.D.; Myhre, D.L. A Plastochron Model for Soybean Leaf and Stem Growth. Can. J. Plant Sci. **1977**, *57*, 167–175.
22. Stutzel, H.; Charles-Edwards, D.A.; Beech, D.F. A Model of the Partitioning of New Above-Ground Dry Matter. Ann. Bot. **1988**, *61*, 481–487.
23. Charles-Edwards, D.A. On the Ordered Development of Plants. 1. An Hypothesis. Ann. Bot. **1984**, *53*, 699–707.
24. Jones, J.B., Jr. Interpretation of Plant Analysis for Several Agronomic Crops. In *Soil Testing and Plant Analysis, Part II, Plant Analysis*; Hardy, G.W., Halvorson, A.R., Jones, J.B., Munson, R.D., Rouse, R.D., Scott, T.W., Wolf, B., Eds.; Soil Science Society of America: Madison, WI, 1967; 49–58.
25. Small, H.G., Jr.; Ohlrogge, A.J. Plant Analysis as an Aid in Fertilizing Soybeans and Peanuts. In *Soil Testing and Plant Analysis*; Walsh, L.M., Beaton, J.D., Eds.; SSSA: Madison, WI, 1973; 315–327.
26. Nye, P.H.; Tinker, P.B. *Solute Movement in the Soil-Root System*; Blackwell Sci. Publ.: Oxford, UK, 1977; 323 pp.
27. Gutiérrez-Boem, F.H.; Thomas, G.W. Phosphorus Nutrition and Water Deficits in Field-Grown Soybeans. Plant Soil **1999**, *207*, 87–96.
28. Milroy, S.P.; Goyne, P.J. Leaf Area Development in Barley. Model Construction and Response to Soil Moisture Status. Aust. J. Agric. Res. **1995**, *46*, 845–860.