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Hydraulic Conductance as a Factor Limiting Leaf Expansion of Phosphorus-Deficient Cotton Plants

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JOHN W. RADIN* AND MARIE P. EIDENBOCK
United States Department of Agriculture, Agricultural Research Service, Western Cotton Research Laboratory, Phoenix, Arizona 85040

ABSTRACT

Suboptimal levels of phosphorus (P) strongly inhibited leaf expansion in young cotton (*Gossypium hirsutum* L.) plants during the daytime, but had little effect at night. The effect of P was primarily on cell expansion. Compared to plants grown on high P, plants grown on low P had lower leaf water potentials and transpiration rates, and greater diurnal fluctuations in leaf water potential. Hydraulic conductances of excised root systems and of intact transpiring plants were determined from curves relating water flow rate per unit root length to the pressure differential across the roots. Both techniques showed that low P significantly decreased root hydraulic conductance. The effects of P nutrition on hydraulic conductance preceded effects on leaf area. Differences in total root length, shoot dry weight, and root dry weight all occurred well after the onset of differences in leaf expansion. The data strongly indicate that low P limits leaf expansion by decreasing the hydraulic conductance of the root system.

In dicotyledonous plants, growth on suboptimal levels of N is characterized by a specific inhibition of leaf expansion (18-20). Low N decreases plant hydraulic conductance, thereby lowering the leaf ψ_w[1] during the daytime when transpiration generates large fluxes of water (18, 19). This increased water deficit, in turn, inhibits leaf expansion (18, 19). One consequence of this alteration is an increased root: shoot ratio (21). Low P also decreases hydraulic conductance (17, 23) and increases the root:shoot ratio (4, 13). These parallels between effects of N and of P suggest that hydraulic conductance may limit growth in P-deficient plants as it does in N-deficient plants. Here we present evidence that effects of P nutrition on hydraulic conductance determine its effects on growth of cotton plants.

MATERIALS AND METHODS

Plant Growth Conditions. Cotton (*Gossypium hirsutum* L. cv Deltapine 70) plants were grown in a growth chamber. In early experiments, plants were grown in 14-L pots containing sand. The pots were watered three times weekly with a modified half-strength Hoagland solution containing either 0.5 mM Pi as KH_2PO_4 (normal P) or 0.5, 0.25, 0.125, 0.0625, or 0 × normal P levels. In later experiments, plants were grown in liquid nutrient solution. Seeds were germinated in darkness in moist vermiculite for 4 d at 30° C, then the seedlings were suspended above 16-L pots containing vigorously aerated nutrient solution, with their root systems immersed. The chamber was set to maintain daily maximum and minimum temperatures of 31 and 20°C, respectively, with gradual transitions between the extremes. Daylength was 14 h with PAR at plant height of about 550 μE s^{-1} m^{-2}, determined with a LiCor LI-190SB quantum sensor attached to a LiCor LI-1600 steady state porometer (LiCor Instruments, Lincoln, NE).[2] Humidity was uncontrolled, but the RH rarely exceeded 45% during the afternoon.

Plants in liquid nutrient solution were grown only at 0.5 mM Pi (high P) or at 0 mM Pi (low P). Low P nutrient solution normally contained 0.5 mM KCl to maintain osmolarity, but tests did not reveal any effects of its omission. These low P seedlings had only the P from the seed, and P deficiency became progressively more severe with age.

Growth Measurements. Leaf expansion rates were followed in plants in both sand and liquid solution. Blade lengths of the youngest expanding leaves were measured with a ruler at both the start and the end of the light period to separate daytime and nighttime mean growth rates. Measurements were begun when the blade length was about 30 mm. Results are expressed as per cent increase in length per hour. When expressed in these units, expansion was uniform with leaf size until leaf length reached approximately 50 mm.

Cell size in the upper epidermis of a leaf was determined by spraying an acrylic resin on the surface, peeling it off after drying, and using its negative image of the surface for cell counts under a microscope (20). Cell size was calculated from the number of cells in a field of view and the known area of the field. Each datum is the mean of counts at four separate sites.

Root Lengths. Root systems were excised from plants grown in liquid nutrient solution and stained with methylene blue. The stained roots were spread over a grid of lines drawn on a glass plate and copied on a Xerox copier. Root lengths were calculated from counts of intersections of roots and grid lines on the Xerox copies (16). Each root was copied and counted in two positions on the grid, but differences between counts in the two positions were less than 5%. After copying, roots were dried for 24 h at 70°C in a forced draft oven, and weighed individually. For studies of the degree of root branching, primary laterals (with associated higher-order branches) were removed from the root systems and copied individually. Numbers and lengths of roots of each branching order were tabulated from the copies.

Transpiration and Water Potentials. Transpiration of individual leaves was measured with the LiCor LI-1600 steady state porometer set in the transpiration mode. Abaxial and adaxial rates of water loss were added for total transpiration rate. This method slightly overestimated transpiration into open air be-

[1] Abbreviations: ψ_w, water potential; π, osmotic pressure.

[2] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

cause the boundary layer resistance within the cuvette is estimated at 0.15 s/cm (3). In the growth chamber, wind speeds at the leaf surface were 20 to 40 cm/s, and crude calculations (6) indicate a boundary layer resistance of 0.3 to 0.4 s/cm.

Water potentials in the expanding leaves were determined as the xylem pressure potential in a pressure chamber. A standard pressure chamber (Soil moisture Equipment, Santa Barbara, CA) was fitted with a silicone rubber sealing ring especially designed for very short petioles.

In plants grown in sand without any added P, growth rates were highly variable due to residual P in the sand. For this reason, measurements of ψ_w and transpiration were carried out exclusively on plants in liquid nutrient solution.

Hydraulic Conductances. The hydraulic properties of roots were determined by several different methods. In the first method, root systems were excised from plants grown in liquid nutrient solution, and the roots were placed in a Soilmoisture pressure chamber filled with the same nutrient solution from which they had been removed. The chamber was connected to a cylinder of compressed air which was bubbled through the solution in the chamber to maintain aeration and stirring. The air flow rate was regulated by the tightness of the seal around the root. After several minutes of equilibration, exuded xylem sap was removed from the cut surface with a syringe, and a stopwatch was started. Subsequent exudate was collected and transferred to stoppered glass tubes. The amount of collected sap was determined by weighing the tubes. After a minimum of 50 μL of exudate had been collected at any constant pressure, the elapsed time was recorded, and the pressure was increased to a new value and the process restarted. With no external pressure, collection of 50 μL of exudate often required up to 60 min, especially from low P roots. When collection was ended at a pressure of 5 bars or more, then pressure was decreased stepwise and rates of exudation determined as before. The entire process was carried out at room temperature (23°C). After completion of the cycle, the root systems were removed from the chamber, dried, and weighed. Root dry weights were converted to estimates of length by the relationships shown in Figure 3. From these data, volume flow through roots was calculated (units of nL s^{-1} m^{-1}) and curves relating volume flow to pressure were constructed. The hydraulic conductance was taken to be the slope of the curve at high flow rates (ascending pressure).

The second method for determining hydraulic conductance was based upon volume flow through plants with intact root systems. In the late morning (about 5 h after the beginning of the light period), plants in liquid nutrient solution were pruned to remove all but a single leaf blade, and that leaf was further trimmed to different areas on different plants. In young plants (8 d after transfer to solution) the procedure was modified to use the expanded cotyledons, as not enough leaf area was otherwise available. In this case, both cotyledons were left in place and trimmed to a similar degree. The plants were then incubated *in situ* for about 3 h to reach a steady state. During this interval air temperature was held at 30°C. At the conclusion of this period, leaf transpiration rates, water potentials, and areas were measured. The root systems were excised, dried, and weighed, and the weights converted to lengths as before. Volume flow per unit length was calculated and plotted against ψ_w. Linear regressions were fitted only to those data points for which $\psi_w \leq -3$ bars. This restriction increased the likelihood of isolating the linear portion of the curve. Each regression included a minimum of eight plants with a suitable ψ_w. Hydraulic conductances of these 'intact' plants were determined from the slopes of the linear regressions.

The third method for determining hydraulic conductance was a simplification of the volume flow curves using excised roots. Procedures were similar to those described above, except that the roots were incubated at only two pressures, 3 bars and 5 bars. The roots were immersed in the solution and the pressure was increased to 3 bars and held there for 10 min to minimize transients. Exudation rates were then determined as before, and the pressure was increased to 5 bars and the process repeated. Again, root lengths were estimated from dry weights. Hydraulic conductances were calculated assuming linearity between volume flow and pressure over the two-bar range. Each reported value of hydraulic conductance is a mean of four replicate plants. Although measurements were made at various times of day, there was no identifiable effect of time of day on the results.

Osmotic Pressures of Exudates. The xylem exudate collected from pressurized excised roots was diluted 100-fold, and its electrical conductivity measured with a Wheatstone bridge (Yellow Springs Instruments) equipped with a standard conductivity cell. The readings were converted to osmolarities by comparison to a standard curve constructed using KNO_3+$MgSO_4$ (9:1 osmolar ratio). The mixture was chosen as an approximation of the mix of monovalent and divalent ions in xylem exudate (2). Osmotic pressures were then calculated assuming a π of 25 bars for a 1 osmolar solution.

Tissue Analyses. Levels of P in the leaves were determined by a modified Fiske-SubbaRow method (5) on H_2SO_4 digests of dried and ground plant material.

RESULTS AND DISCUSSION

Root and Shoot Growth. In plants grown in sand culture, low levels of P caused some specific changes from the normal growth pattern. Most notably, leaf area development was inhibited more than was dry matter accumulation. In one trial, for example, young plants grown without added P had 18% less shoot dry weight but 31% less leaf area. Subsequent experiments with solution-grown plants revealed that low P greatly increased the root:shoot ratio because of its effect on shoot growth rate. Root dry weight was unaffected by low P as late as 13 d after transfer to nutrient solution, when shoot dry weight was 20% less than that of high P plants (Table I). These effects are similar to earlier demonstrations that low P increases the root:shoot ratio (4, 13).

The growth changes seen here correspond closely to those associated with low N (18, 19). In N-deficient plants, leaf expansion is limited by hydraulic conductance (18, 19). If growth on low P is similarly restricted, then several consequences must be demonstrable. These include similarities in growth patterns under low P and during mild water stress; decreased leaf water potential or transpirational flux, or both, in intact low P plants; and effects of low P on hydraulic conductance preceding or coinciding with changes in leaf expansion. Each of these points will be examined.

Leaf Expansion. Leaves of high P plants grew most rapidly during the daytime, presumably because the temperature was higher than at night (1, 12). Low P markedly altered this diurnal growth cycle, specifically inhibiting daytime expansion (Fig. 1). Nighttime expansion rate was much less affected. This conversion to growth at night occurred when tissue P levels declined to about 25% of normal (Fig. 1).

Table I. *Root and Shoot Weights of Hydroponically Grown Cotton Plants*

Seedlings were transferred to nutrient solutions containing 0.5 mM (high) or 0 (low) Pi. Weights are means ± SE of our plants.

Time After Transfer	Root Dry Wt		Shoot Dry Wt	
	Low P	High P	Low P	High P
d	*mg/plant*			
8	45 ± 6	48 ± 2	183 ± 23	196 ± 8
13	112 ± 9	111 ± 21	374 ± 60	499 ± 88

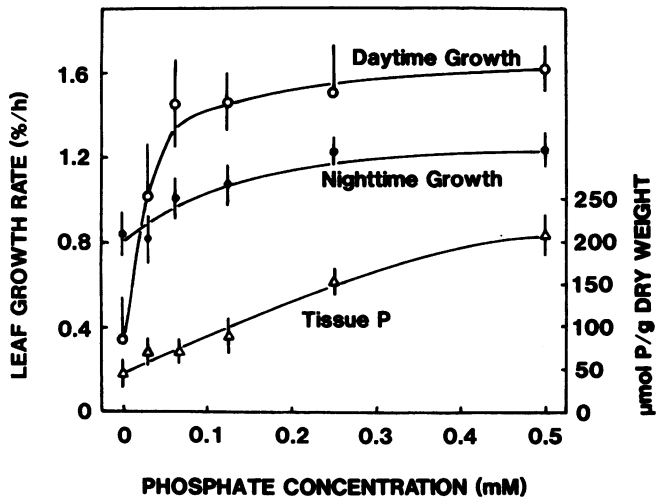

FIG. 1. Effects of nutrient phosphate level on elongation rates of expanding leaves and P concentrations of shoots. Plants were grown in sand culture.

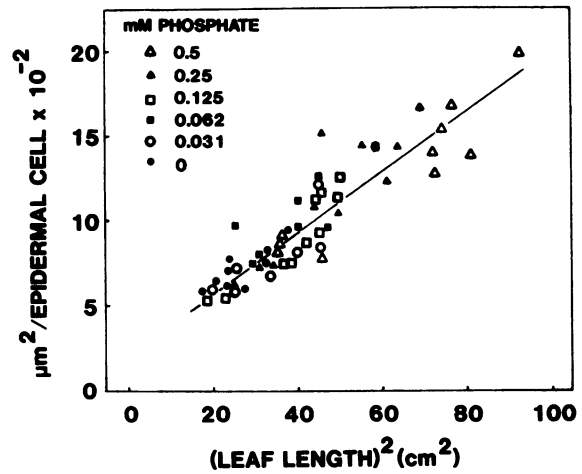

FIG. 2. Relationship between epidermal cell area and leaf size in expanding cotton leaves at the first node. The regression equation is $Y = 230.6 + 18.0\,X$ ($r = 0.914$).

During its later stages, leaf expansion results mostly from cell expansion, with little continuing cell division (14). In plots of epidermal cell area against the square of leaf length (proportional to leaf area), expanding leaves of all P treatments fell along a single regression line (Fig. 2). Thus, P nutrition did not alter the relationship between leaf size and cell size. Rather, it altered primarily the degree to which the cells could expand. Although this regression does not pass through the origin (indicating that some cell division continued during expansion), the deviation was small. In fact, for a 3-fold increase in leaf area (from 30 to 90 cm^2 on the abscissa) cell number increased by only 22% but cell area increased 2.45-fold. We conclude that P nutrition acted primarily on cell expansion.

Transpiration and Leaf Water Potentials. In plants grown in nutrient solution, low P decreased ψ_w and transpiration rates of expanding leaf blades both at night and during the day. In the afternoon, ψ_w was −6.1 and −7.5 bars for high and low P plants, respectively, and the transpiration rate was decreased 26% by low P (Table II). Daytime leaf elongation rate was decreased 53% by low P. Transpiration rates were greatly reduced at night, but the relative effects of P nutrition on transpiration and ψ_w were

Table II. *Rates of Elongation, Water Potentials, and Transpiration Rates of Young Expanding Leaves*

Seedlings were transferred to and grown for 13 d in nutrient solutions containing 0.5 mM (high) or 0 (low) Pi. Growth rates are means ± SE of six replicate plants. Other data are means ± SE of three to five replicates. Day and night values of ψ_w and transpiration were recorded about 8 h after and 1 h before the beginning of the 14-h photoperiod, respectively.

Treatment	Elongation	Water Potential	Transpiration
	% h^{-1}	*bars*	µg cm^{-2} s^{-1}
Low P			
Day	0.9 ± 0.1	−7.5 ± 0.1	11.4 ± 1.2
Night	0.8 ± 0.1	−4.9 ± 0.3	0.3 ± 0.1
High P			
Day	1.7 ± 0.2	−6.1 ± 0.2	15.4 ± 1.6
Night	0.8 ± 0.3	−4.4 ± 0.2	0.5 ± 0.2

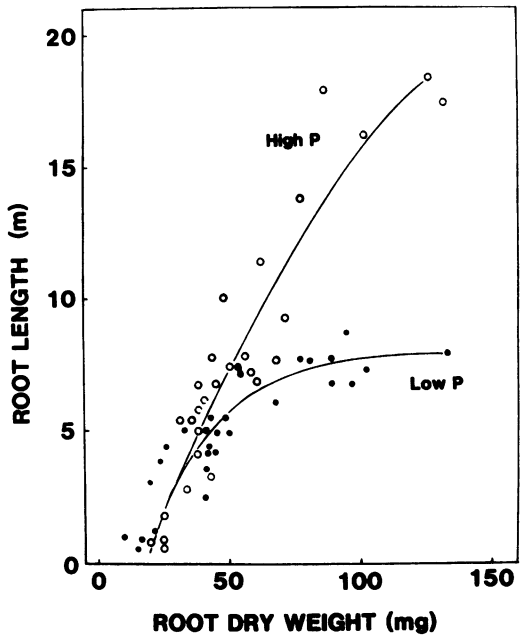

FIG. 3. Relationship between total length and dry weight of root systems. Plants were grown up to 15 d in nutrient solutions containing either 0.5 mM Pi (high P) or zero Pi (low P). Each point represents a single root system. At no age was there a significant effect of P nutrition on root dry weight.

unchanged from the daytime (Table II). At night, though, P nutrition did not affect leaf growth rate, indicating that at high ψ_w, the effect of P nutrition was minimized. The data show that the water status of the expanding low P leaves limited their growth during the daytime. Leaf expansion during the daytime ceased when the expanding leaf blade reached about −8 bars, indicating that the small differences in daytime ψ_w between high P and low P plants could have profoundly affected growth rates.

Hydraulic Properties of Roots. Two properties of root systems are of interest in the context of this work, length (or surface area) and hydraulic conductance. P nutrition did not initially affect total root length, but after several days of growth on low P nutrient solution total root length was noticeably less than on high P medium (Fig. 3). The length:weight ratio remained almost constant on high P but rapidly began to decrease in low P roots when their dry weights reached about 40 mg (7 d after transfer). This decrease corresponded to a strong effect of low P on growth of tertiary roots (second-order laterals), with no effect on length of secondary roots (first-order laterals) (Table III). Diameters of secondary and tertiary roots were, respectively, 0.47 ± 0.01 and

Table III. *Lengths of Secondary and Tertiary Roots of Hydroponically Grown Cotton Plants*

Seedlings were transferred to nutrient solutions containing 0.5 mM (high) or 0 (low) Pi. Lengths are means ± SE of two plants.

Time After Transfer	Secondary Roots		Tertiary Roots	
	Low P	High P	Low P	High P
d	*m/plant*			
4	0.4 ± 0.2	0.7 ± 0.3	0	0
7	3.0 ± 0.2	3.2 ± 0.9	0.1 ± 0.02	0.4 ± 0.2
9	4.0 ± 1.2	4.2 ± 0.3	0.6 ± 0.5	5.4 ± 1.1

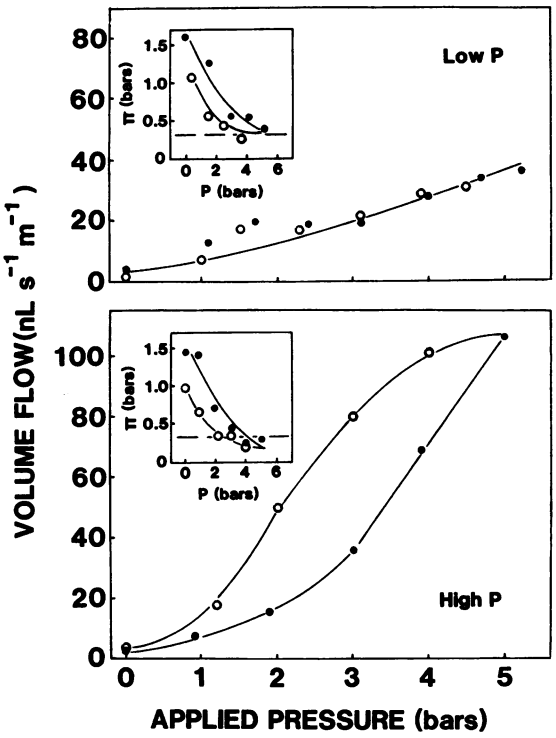

FIG. 4. Effect of applied pressure on volume flow through excised root systems. The cycle was initiated at zero applied pressure. (●), increasing pressure; (○), decreasing pressure. Insets: osmotic pressure of exudate collected from the cut surface. (---), the nominal external osmotic pressure.

0.34 ± 0.01 mm for low P plants and 0.42 ± 0.01 and 0.31 ± 0.01 mm for high P plants. From these data, low P did not significantly change root length or morphology until about 7 days after transfer to nutrient solution. After 7 d, however, it strongly inhibited growth and increased the average surface area:length ratio compared to high P roots. All these changes occurred without any differences in root dry weight (Table I).

Hydraulic conductance can be determined from the relationship between pressure applied to excised root systems and the volume flow through those roots (7, 8, 15). Two important effects of P nutrition were apparent from such curves. With high P, the curves were distinctly nonlinear over the range of 0 to 5 bars applied pressure, with a slope much greater at high flow than at low flow (Fig. 4). In addition, under the conditions of our tests, hysteresis with high P plants was pronounced when going from low to high flow and back to low (Fig. 4). With low P, the relationship in the same pressure range was less curvilinear, and there was little hysteresis (Fig. 4). As flow increased, in both cases the π of the exudate decreased to approximately the π of the external solution (Fig. 4, insets). Hydraulic conductance can be estimated from the slopes of the curves at high flow, because in this range there is little osmotic contribution to flow (7, 8, 15). By this criterion, low P decreased hydraulic conductance by about half (Fig. 4).

Volume flow curves were also developed for intact transpiring plants. Although the roots were not excised, plants were pruned to a single leaf (for the purposes of this experiment the cotyledons, if left attached, were treated as a single leaf) which was further trimmed to various areas to provide a wide range of flow through the roots. Although there was considerable variability, the relationships between flow and ψ_w were basically similar to the volume flow curves with excised roots (Fig. 5). Even with more than half of the original leaf area removed, the roots seemed to be operating mostly in the high-flow region of the curves. As a result, the relationships could be approximated by linear regressions. The regression coefficients (slopes), as measures of hydraulic conductance, again indicated that low P decreased hydraulic conductance significantly (Fig. 5).

The volume flow curves developed by either method were extremely time-consuming. Therefore, we simplified the procedure for excised roots to include only two points, at 3 bars and 5 bars applied pressure. With this technique, enough plants could be processed to obtain statistically valid estimates of hydraulic conductance. In both high P and low P plants, estimated hydraulic conductance decreased with plant age (Fig. 6). Differences between the treatments were already apparent only 2 d after transfer to nutrient solution (coincident with the emergence and growth of lateral roots), but the variability remained fairly high until several days later (Fig. 6). For comparison, hydraulic conductances of intact plants were also determined at 8, 10, and 13 d after transfer. Each of these linear regressions had a correlation coefficient significantly different from zero (95% confidence limits). At each of these three ages the values from intact plants fell very close to the curve established by the simplified procedure using excised roots (Fig. 6). Both methods revealed that, by 13 d after transfer, low P had decreased hydraulic conductance to less than 30% of the high P value (Fig. 6). It should be noted that low P increases the surface area:length ratio of root systems beginning about 7 d after transfer (Table III, and earlier discussion). Therefore, expressing conductances on the basis of length rather than surface area, as in Figure 6, tends to minimize the difference between treatments. We conclude that the simplified method provides data largely reflecting the behavior of transpiring plants, and that functional, rather than morphological, differences in root systems underlie the effects of P. We do not know the basis for the pronounced effect of age on hydraulic

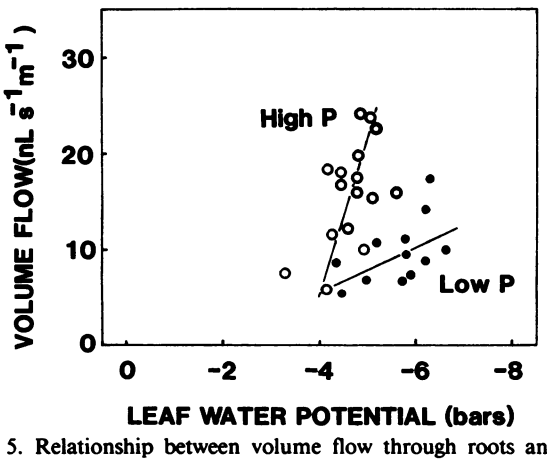

FIG. 5. Relationship between volume flow through roots and leaf water potential of transpiring plants. Leaf areas were altered to provide varying flow rates, as described in the text. The regressions are as follows: for low P, $Y = -4.3 + 2.4\,X$ ($r = 0.50$); for high P, $Y = -62.8 + 16.8\,X$ ($r = 0.59$).

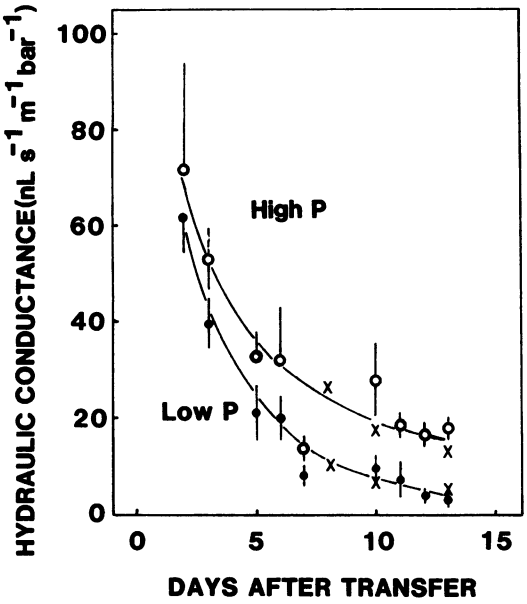

FIG. 6. Hydraulic conductances of excised root systems. Seedling plants were transferred to nutrient solutions containing 0.5 mM (high) or zero (low) Pi. Values shown are means ± SE of four replicates. The X symbols indicate estimates of hydraulic conductance from intact transpiring plants.

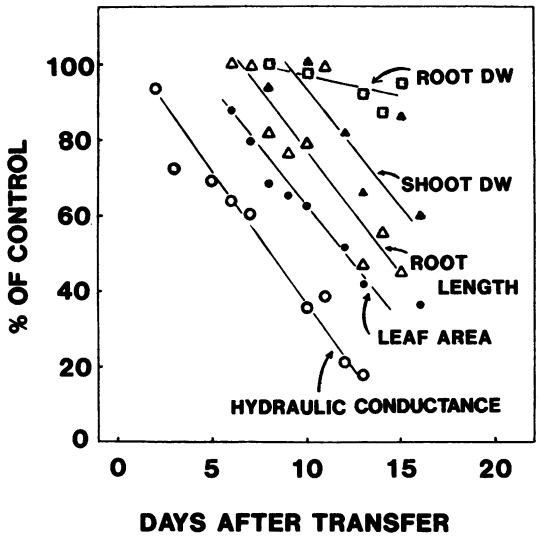

FIG. 7. Time courses for effects of low P on hydraulic conductance, leaf area, and other aspects of seedling growth. Hydraulic conductances are taken from Figure 6. All data are low P values as a percentage of the corresponding high P (control) values.

conductance (Fig. 6).

Sequence of Effects of Low P. The development of differences in hydraulic conductance was compared to the development of other effects of low P as the seedlings grew. For this purpose, hydraulic conductance of low P plants (Fig. 6) was replotted as a percentage of that of the high P treatment. Differences in hydraulic conductance clearly preceded any effects on leaf area development, which became apparent only 4 to 5 d after transfer (Fig. 7). Effects of low P which appeared even later included differences in root length (7 d), shoot dry weight (9 d), and root dry weight (12 d) (Fig. 7). The order of appearance of these effects implies some cause-and effect relationships, *i.e.* that hydraulic conductance limited leaf expansion by restricting water transport, and that leaf area differences then slowed the accumulation of total dry weight per plant. The cessation of root elongation earlier than root dry matter accumulation (Fig. 7) suggests that the two processes were controlled by different factors.

DISCUSSION AND CONCLUSIONS

This paper establishes that P deficiency decreases hydraulic conductance of cotton roots, and that this lowered hydraulic conductance then limits growth. The first conclusion rests upon the decreased leaf ψ_w and transpiration rates, and increased diurnal fluctuation in leaf ψ_w, of low P plants (Table II), as well as the decreased pressure-driven water flow through low P roots (Figs. 4–7). The second conclusion is supported by the specificity of the P effect for growth during the day and for leaf and cell expansion (Figs. 1, 2; Table I), and by the sequence of events during development of P deficiency (Fig. 7). Hydraulic conductance was altered whether measured in excised roots or intact transpiring plants. The altered diurnal growth curve, and the preferential effect on leaf expansion (and cell expansion) are consistent with a role of water stress in leaf development on low P (11).

The effect of P nutrition on hydraulic conductance was recognized earlier (17, 23). However, its proposed role in growth effects of P deficiency is new. With both low N (18, 19) and low P (Fig. 7), leaf area development is specifically inhibited by nutrient deficiency. This effect on transpiring surface area provides feedback to minimize differences in ψ_w. Such feedback, along with the restriction of growth effects to broadleaf plants (18), may have obscured the role of hydraulic conductance in growth regulation until now.

Despite the arguments above, we have not rigorously excluded some possible causes of growth inhibition other than hydraulic conductance. Direct effects of P on either cell wall extensibility (ability to undergo irreversible deformation) or the threshold turgor for growth could also account for the decreased leaf expansion on low P. The failure of P nutrition to affect leaf expansion significantly at night implies that these factors are unchanged, but the question remains open. This possibility deserves attention because low P decreases cytokinin content of plants (10, 24), and cytokinins have been implicated in the control of wall extensibility (25). However, low N, which parallels low P in its effects on leaf expansion (18–20), hydraulic conductance (18, 19), and cytokinin content (10, 24) did not measurably affect extensibility or the threshold turgor for growth (19).

Another possible effect of P nutrition is a change in wall elasticity which alters the relationship between ψ_w and turgor. In N-deficient sunflower, decreased elasticity of expanding leaf cells leads to a lower turgor at low ψ_w than occurs in high N plants, but the effect is minor (19). In fully expanded cotton leaves, P deficiency does not alter elasticity (unpublished data), but we do not know whether this finding extends to young expanding leaves as well.

The differing hysteretic behavior of low P and high P roots (Fig. 4) is a crucial point which allows some speculations about the effects of P nutrition. As one possible explanation of the divergent behavior, P nutrition may affect the rate of solute release (leakage?) from cortical cells and subsequent transport into the xylem. This idea is consistent with reports that P deficiency increases solute leakage into the external medium (9, 22). However, P nutrition did not greatly alter the electrical conductivity of collected exudate, which itself displayed hysteresis even when volume flow did not (Fig. 4). Furthermore, nominal π of the medium and the exudate were almost equivalent at high flow rates (Fig. 4). The simplest interpretation of these results is that gross hydraulic effects of P nutrition are unrelated to solute transport.

The existence of hysteresis shows, also, that the system was not at a steady state. Errors of interpretation introduced by this experimental deficiency would seem to be minor because the results with excised roots and with intact transpiring plants are similar (Figs. 4–6). Nonetheless, we note that the measurements provide only a crude approximation to the true (steady state) hydraulic conductance, and that precise quantitative inferences are not possible. Further studies of mechanisms by which P nutrition controls water transport may require more sophisticated techniques than those used here.

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