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Effects of Phosphorus Deficiency on the Photosynthesis and Respiration of Leaves of Sugar Beet[1]

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ABSTRACT

Phosphorus deficiency was induced in sugar beet plants (*Beta vulgaris* L. var. F5855441), cultured hydroponically under standardized environmental conditions, by removal of phosphorus from the nutrient supply at the ten leaf stage 28 days after germination. CO_2 and water vapor exchange rates of individual attached leaves were determined at intervals after P cutoff. Leaves grown with an adequate nutrient supply attained net rates of photosynthetic CO_2 fixation of 125 ng CO_2 cm^{-2} sec^{-1} at saturating irradiance, 25 C, and an ambient CO_2 concentration of about 250 μl l^{-1}. After P cutoff, leaf phosphorus concentrations decreased as did net rates of photosynthetic CO_2 uptake, photorespiratory evolution of CO_2 into CO_2-free air, and dark respiration, so that 30 days after cutoff these rates were about one-third of the control rates. The decrease in photosynthetic rates during the first 15 days after cutoff was associated with increased mesophyll resistance (r_m) which increased from 2.4 to 4.9 sec cm^{-1}, while from 15 to 30 days there was an increase in leaf (mainly stomatal) diffusion resistance (r_l') from 0.3 to 0.9 sec cm^{-1}, as well as further increases in r_m to 8.5 sec cm^{-1}. Leaf diffusion resistance (r_l') was increased greatly by low P at low but not at high irradiance, r_l' for plants at low P reaching values as high as 9 sec cm^{-1}.

Much of the phosphorus found in the sugar beet plant is in the inorganic form and has many roles in cell metabolism (2). In particular, the coenzyme ATP, which acts as an intermediate energy transfer compound in such cell functions as photosynthesis, respiration, biosynthesis, stomatal opening, and the transfer of organic solutes across membranes, requires inorganic phosphate in its formation from ADP. It is to be expected, therefore, that deficiency of phosphorus would have wide ranging effects on cell function.

Phosphorus deficiency has been shown to diminish protein synthesis, increase carbohydrate content (7, 13, 21), and decrease moisture content (6, 17). Reports of the effects of phosphorus deficiency on photosynthesis (CO_2 uptake or O_2 evolution), however, are varied: moderate deficiency was found to diminish photosynthesis in spinach (4), tobacco (13), and subterranean clover (5), but had no effect initially on barley (10, 15), sunflower (22), or horsebean (1). Similarly effects of moderate phosphorus deficiency on respiration vary from little or none (3, 10, 16) to significant retardation (4, 22, 23).

The effects of phosphorus deficiency on photosynthesis and respiration have been determined mainly for plants other than sugar beets. Also, the data were obtained from plants grown in only partially controlled environments, so that other environmental variables may have interacted with phosphorus deficiency, thus complicating interpretation. In the present work sugar beet plants were grown in chambers under standardized cultural conditions so that the progressive effects of phosphorus deficiency on photosynthesis and respiration could be systematically studied independently of other environmental changes. CO_2 and water vapor exchange of leaves was determined using a leaf chamber in an open flow gas circuit of the type described by Terry *et al.* (24).

MATERIALS AND METHODS

Plant Culture. Sugar beet plants (*Beta vulgaris* L. var. F5855441) were cultured in growth chambers at a constant day/night temperature of 25 C. They were irradiated for 16 hr per day at 15.6 mw visible radiation per cm^2 by means of thirty 2.44 m long fluorescent 215-w lamps (General Electric No. F96T12/CW 1500), four 1.22 m long fluorescent lamps (same type), and 22 incandescent 60-w extended service lamps. The standard culture solution had the following composition: in millimoles per liter, 2.5 $Ca(NO_3)_2 \cdot 4\ H_2O$, 0.5 KH_2PO_4, 2.5 KNO_3, 1.0 $MgSO_4 \cdot 7\ H_2O$, and 0.5 NaCl, and, in milligrams per liter, 0.25 B, 0.25 Mn, 0.025 Zn, 0.01 Cu, and 0.005 Mo. Iron (2.5 mg per liter) was added as ferric-sodium ethylene diamine tetraacetate complex.

On day 0 seeds were planted 2 cm deep in vermiculite and supplied daily with culture solution. On day 14 the germinated seedlings with two true leaves were transplanted, three plants per pot, to culture solution in steel containers (57 cm diameter, 32 cm deep) lined with polyethylene. At day 17 the culture solution was aerated, and on day 21 the plants were selected for uniformity and thinned to one plant per pot.

Phosphorus deficiency was induced on day 28 (cutoff) by rinsing the roots of four plants with distilled water and transferring them individually to pots containing phosphorus-deficient culture solution which differed from the standard culture solution in that 0.5 mmole of K_2SO_4 per liter was added in place of KH_2PO_4. An equal number of plants was similarly transferred to standard culture solution. On day 35, in order to compensate for depletion due to plant uptake, concentrated stock solutions of nutrients were added in amounts to equal the initial nutrient contents. On day 42, the plants were transferred to containers with corresponding fresh complete or phosphorus-deficient culture solution, and on day 49 stock solutions were again added as on day 35. The experiments were terminated on day 56.

Determination of Leaf Gas Exchange. The apparatus which was employed for the determination of the parameters of leaf gas ex-

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change has been described elsewhere in detail (24); only a brief description of its salient features is presented here. The leaf was placed in a chamber mounted in an open flow gas circuit. Carbon dioxide and water vapor concentrations of the air entering and leaving the chamber were determined using an infrared gas analyzer (for CO_2) and dewpoint hygrometer and thermistor psychrometer (for water vapor). Leaf temperature was measured using two mobile thermistors which could be located at any point on the surface of the leaf.

Procedure. At about 4:00 PM on the day before leaf-gas exchange parameters were to be measured, the selected plant was brought from the growth chamber to a constant temperature laboratory (25 C), and an attached leaf was placed in the leaf chamber. The leaf chosen was generally the largest of the young expanding leaves and about 2 to 3 dm^2 in area. Air was supplied to the leaf which was placed in the dark over night. At 8:00 AM the following day, the CO_2 exchange rate (dark respiration, R_D) was determined over a 45-min interval. The leaf was then irradiated for 2 to 3 hr, during which time leaf temperature and CO_2 and water vapor exchange usually attained constant values which were recorded. Leaf temperature was maintained at 25 $\pm$ 0.5 C by means of a thermostated water jacket.

The ambient CO_2 concentration, C_a, at which gas exchange parameters were determined was approximately 500 ng CO_2 cm^{-3} air; subsequent gas exchange determinations were made over 60-min intervals at C_a values of 350, 200, and 100 ng CO_2 cm^{-3} air, and finally at a value of C_a attained when CO_2-free air was passed over the leaf. As the air inside the leaf chamber was well mixed, C_a was taken to be equal to the outlet air CO_2 concentration. At each value of C_a, and after determining the CO_2 exchange in normal air containing approximately 21% oxygen, CO_2 exchange was determined in oxygen-free air (nitrogen).

The experiment was terminated after measuring the evaporation rate and surface temperature of a piece of wet green blotting paper cut to the same size and shape as the leaf sample and placed in the leaf chamber under as nearly identical conditions as possible to those for the sample leaf.

Calculation of Gas Exchange Parameters. In the calculation of CO_2 and water vapor exchange parameters, we have followed the procedure and terminology (where possible) outlined by Slatyer (19). For convenience, the main concepts are restated here.

Transpiration rate, E, may be expressed in the following way:

$$E = \frac{C_w' - C_a'}{r_a' + r_l'} \quad (1)$$

where C_w' and C_a' are the concentrations of water vapor at the surfaces of the mesophyll cell walls and in the bulk air, respectively, r_a' the resistance to diffusion of water vapor from the leaf surface to the bulk air, and r_l' the resistance to diffusion from the surfaces of the mesophyll cell walls to the external leaf surface. C_w' was assumed to be the saturation vapor concentration at leaf temperature. Since the air within the chamber was thoroughly mixed, C_a' was obtained from the water vapor pressure of the air leaving the chamber. r_a' was determined from measurements of evaporation and surface temperature of the blotting paper leaf analog. Hence r_l' for the real leaf was obtained by substitution of the measured values in equation 1. Similarly, the net CO_2 exchange rate, F, may be expressed as

$$F = \frac{C_a - C_w}{r_a + r_l} = \frac{C_w - C_c}{r_m} \quad (2)$$

where r_m represents the resistance to CO_2 movement from the surfaces of the mesophyll cell walls to the intracellular site of photosynthesis and C_c, the CO_2 concentration at that site. The other terms of equation 2 correspond to those of equation 1, except that they refer to CO_2 not water vapor. In line with Slatyer's procedure, $r_a = 1.35\ r_a'$ and $r_l = 1.56\ r_l'$. The CO_2 compensation point, Γ, and the rate of photorespiratory evolution of CO_2 from the leaf into CO_2-free air, R_L, are defined as follows: $\Gamma = C_w$ when $F = O$, and $R_L = F$ when $C_w = O$. As the relation between F and C_w was found to be linear (Fig. 1), r_m was obtained as $\Delta C_w/\Delta F$. The values of F and r_m obtained in oxygen-free air are referred to as F^* and r_m^*, respectively.

Estimation of Leaf Phosphorus Concentrations. The phosphate phosphorus in leaf material which was soluble in 2% acetic acid is referred to as "soluble phosphorus." Its concentration was estimated by developing the blue phosphomolybdate color and reading its intensity by colorimeter. "Total phosphorus" concentrations were obtained by wet digestion of the leaf material, followed by the determination of phosphorus by the molybdenum blue method. For a more complete description of these two procedures, see Johnson and Ulrich (12), pages 49 to 53.

RESULTS

Changes in Gas Exchange Parameters with CO_2 Concentration. In order to determine what effects phosphorus deficiency has on the gas exchange of sugar beet leaves, it is necessary to establish the behavior of nondeficient control leaves. Parameters of leaf gas exchange are affected by such environmental variables as temperature, irradiation, and ambient CO_2 concentration. Figure 1 shows the relationships of three gas exchange parameters with CO_2 concentration: these parameters are (*a*) net CO_2 exchange rate in oxygen-free air, F^*; (*b*) net CO_2 exchange rate in normal air containing about 21% oxygen, F; and (*c*) the resistance of the leaf to the diffusion of water vapor, r_l'. These data show that F and F^* were linearly related with ambient CO_2 concentration (Fig. 1A), and with the internal leaf CO_2 concentration at the surfaces of the mesophyll cell walls (Fig. 1B), at least up to about 200 to 300 ng CO_2 cm^{-3} air; at higher concentrations there was a departure from linearity and the slopes of the curves decreased. The diffusion resistance, r_l', was unchanged by increase in CO_2 concentration up to 300 to 400 ng CO_2 cm^{-3} air, but increased at higher CO_2 concentrations.

Thus, because of the changing nature of the relationships of F, F^*, and r_l' with ambient CO_2 at concentrations above 300 to 400 ng CO_2 cm^{-3} air, it was decided that comparisons between control and phosphorus-deficient leaves should be made at an ambient CO_2 concentration of 300 ng CO_2 cm^{-3} air (at saturating irradiance and 25 C) (see Fig. 2).

Also, the data shown in Figure 1 are for two leaves from plants of greatly differing ages; despite this, the data for the two leaves are remarkably similar. This was achieved by routinely choosing leaves which were at a similar stage of development; this was toward the end of the phase of rapid leaf expansion when the maximum rates of photosynthesis were obtained. Thus differences in photosynthetic performance due to variation in leaf age were eliminated.

Effects of Phosphorus Deficiency with Time after Cutoff. When phosphorus was withheld from the nutrient solution (cutoff), soluble and total phosphorus concentrations in leaves decreased rapidly during the first week, then more slowly during the next 23 days (Fig. 2A). The rates of photosynthesis, F and F^* (Fig. 2B), and of light and dark respiratory CO_2 evolution, R_L and R_D, respectively (Fig. 2C), decreased steadily with time so that 30 days after cutoff the rates were of the order of one-third of the initial rates. The CO_2 compensation point, Γ, initially decreased, perhaps because the rate of respiratory CO_2 evolution dropped more sharply at first than did the rate of photosynthetic CO_2 uptake, but Γ eventually increased (Fig. 2D). The decrease in F and F^* up to 15 days after cutoff was associated mainly with increased

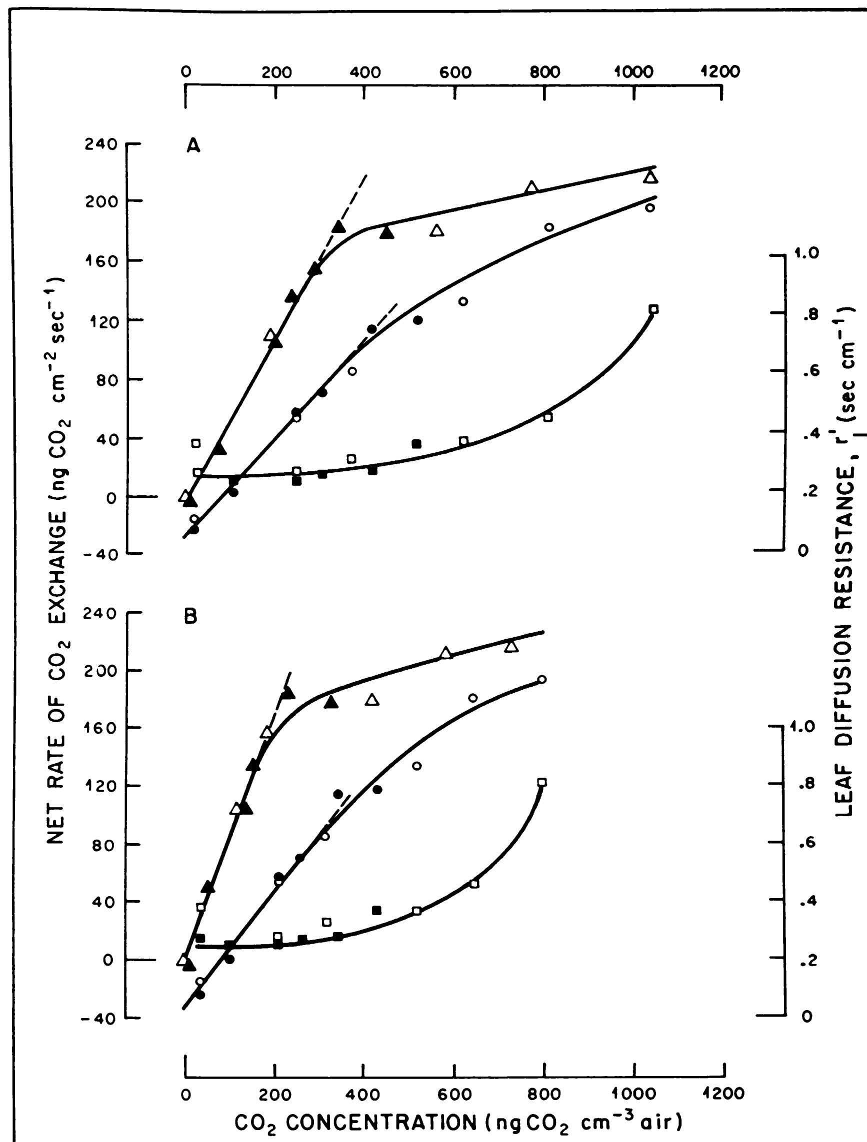

FIG. 1. Changes with CO_2 concentration for net CO_2 exchange rates of leaves at saturating irradiance and 25 C in oxygen-free air, F^* (△, ▲), and in normal air, F (○, ●), and for leaf diffusion resistance, r_l' (□, ■). A: With the ambient CO_2 concentration, C_a; B: with the internal leaf CO_2 concentration at the surfaces of the mesophyll cell walls, C_w. Data shown are for the sixth true leaf of a standard plant at 17 days after transplanting (▲, ●, ■) and for the 14th leaf of a different standard plant at 28 days after transplanting (△, ○, □). For the linear portions of the curves of net CO_2 exchange with CO_2 concentration shown in A: $F^* = 0.55\ C_a - 1.6$; $F = 0.33\ C_a - 26$; and in B: $F^* = 0.84\ C_w - 3.2$; and $F = 0.41\ C_w - 33$.

mesophyll resistance, r_m and r_m^*, respectively (Fig. 2F); decreases subsequent to this time were associated with increases in leaf diffusion resistance, r_l (Fig. 2E), as well as with increases in r_m. These data suggest, therefore, that photosynthetic and respiratory metabolic activities were the first processes to be affected by phosphorus deficiency and that this was followed by increases in leaf diffusion resistance due presumably to partial closure of stomata.

Changes with Irradiance. Changes in F^*, F, and r_l' for a leaf from a 26-day phosphorus-depleted plant with increase in irradiance are shown in Figure 3 (the data were obtained at an ambient CO_2 concentration of 250 μl l^{-1} air at 25 C). F and F^* were apparently saturated at about 30 to 40 mw visible radiation cm^{-2} for the control leaf. At low levels of irradiation, the diffusion resistance, r_l', of the phosphorus-depleted leaf reached the high values of 9 sec cm^{-1}, but decreased rapidly with increase in irradiation until at about 35 to 40 mw cm^{-2} it equalled the diffusion resistance of a standard-grown leaf (Fig. 3C). Thus at low levels of irradiation the diminished values of F and F^* of the phosphorus-depleted leaf were attributable in part to the very high leaf (mainly stomatal) diffusion resistance; at high levels of irradiance, however, phosphorus deficiency apparently had little effect on stomatal opening.

DISCUSSION

The data we obtained for the net CO_2 exchange of "normal" leaves, those leaves grown under standardized conditions with full nutrient supply, were comparable with recent data obtained by other workers for sugar beet. Under conditions of light saturation, 25 C, and an ambient CO_2 level of about 250 μl l^{-1}, we obtained net rates of CO_2 uptake of about 125 ng CO_2 cm^{-2} leaf surface sec^{-1} (45 mg CO_2 dm^{-2} hr^{-1}) in normal air, and 190 ng

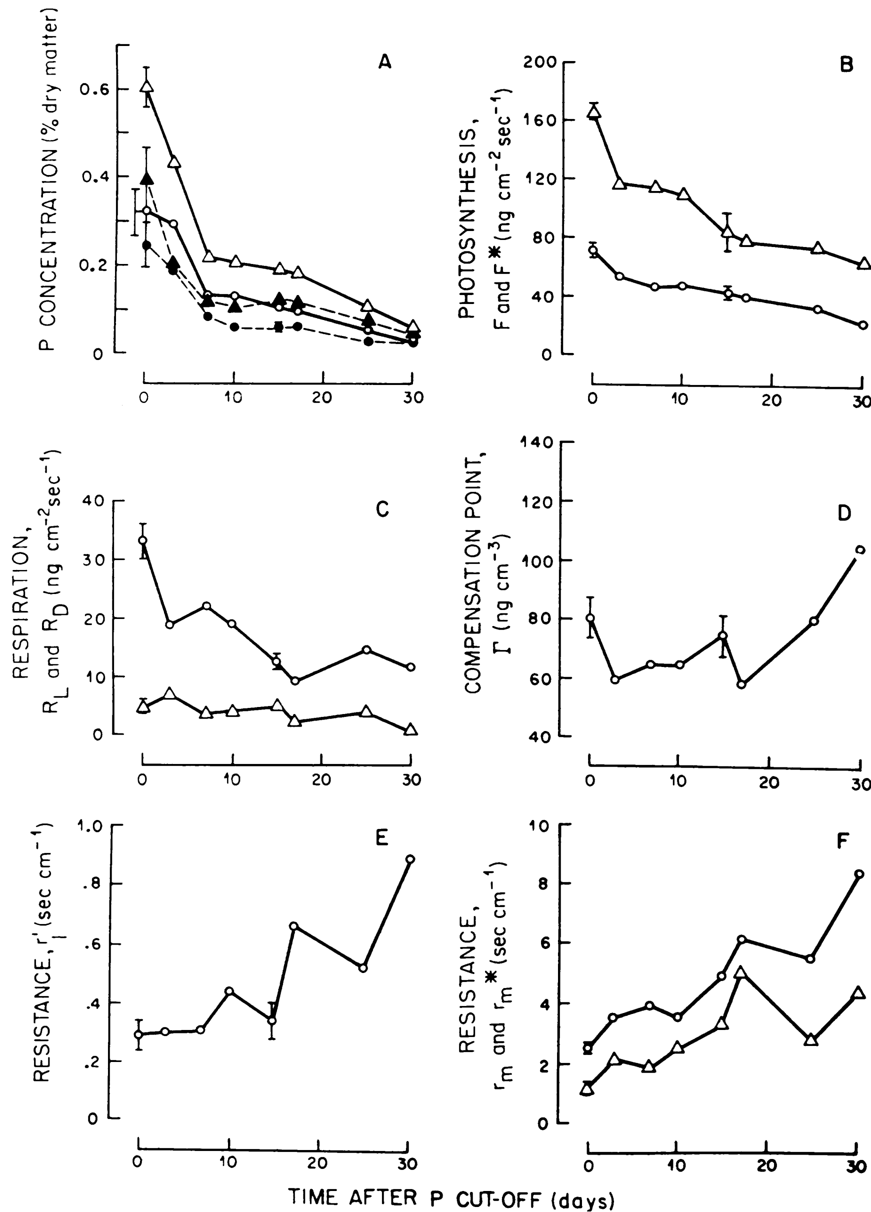

FIG. 2. Effects of phosphorus deficiency on leaf phosphorus and various leaf gas exchange parameters with time after cut-off. A: Changes in total (△, ○) and soluble (▲, ●) phosphorus concentrations of leaf blades (△, ▲) and petioles (○, ●); B: changes in rates of photosynthetic CO_2 uptake, F(○) and F^* (△); C: changes in rates of respiratory evolution of CO_2 in the light, R_L (○), and dark, R_D (△); D: changes in CO_2 compensation point, Γ; E: changes in leaf diffusion resistance, r_l'; F: changes in CO_2 mesophyll resistance in normal air (21% O_2), r_m (○), and in oxygen-free air, r_m^* (△). Vertical lines through points represent twice the standard error.

CO_2 cm^{-2} sec^{-1} (68 mg CO_2 dm^{-2} hr^{-1}) in oxygen-free air. Similar high rates for sugar beet were obtained by Hall (11) and Elmore (8). Respiration rates in the dark, R_D, (measured 15 hr after the dark period commenced) in our experiments were about 5 ng CO_2 cm^{-2} sec^{-1}, and agreed closely with those of Hall (11). Respiration rates in the light determined as the rate of CO_2 evolution in CO_2-free air, R_L, were about six times greater than respiration in the dark.

Rates of photosynthetic CO_2 fixation were diminished as soon as phosphorus concentrations in the leaf began to decrease, within 3 days after P cutoff. These diminished rates were associated with increased mesophyll resistance, r_m. Part of r_m is attributable to the "resistance" of the carboxylation process itself, the rate of which is partially determined by the rate of production of ATP and NADPH by the light reactions of photosynthesis. Evidence that P deficiency affects ATP and NADPH was obtained by Tombesi *et al.* (25), who showed that low P decreased the photosynthetic activity and the rate of ATP and NADPH formation of *Beta vulgaris* chloroplasts. Reduction in Hill activity with low P has been found in tomato chloroplasts (20) also indicating that production of ATP and NADPH would be slowed by low P. Phosphorus deficiency apparently does not affect photosynthesis through chlorophyll content since low P either increases or has no effect on the concentration of chlorophyll in leaf tissue (3, 4, 14, 16, 25). Eventually, with more severe phosphorus deficiency, stomatal resistance, r_l, increased, further reducing photosynthetic rates, but the initial effect of low P was on r_m.

Phosphorus deficiency diminished rates of respiratory CO_2 evolution in the dark, R_D, as well as in the light, R_L, to about one-third after 30 days. Rates of oxidative phosphorylation and of glycolysis, which together represent a large part of CO_2 evolution in the dark, have been shown to be diminished by low P in

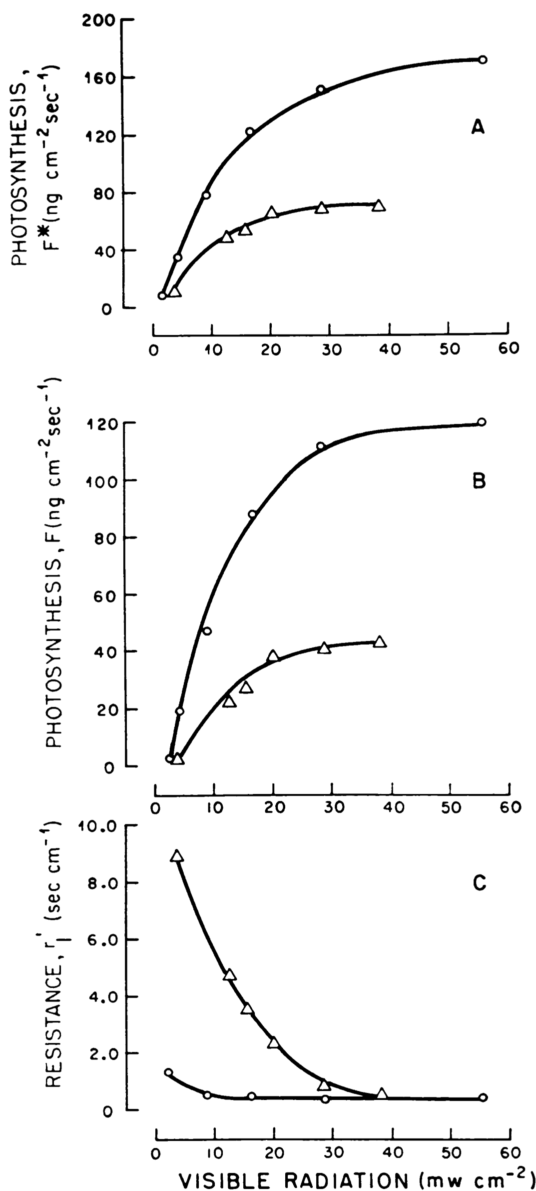

FIG. 3. Effects of irradiance on three gas exchange parameters of a leaf from a plant cultured under standardized environmental conditions (○), and for a leaf from a plant cultured for 26 days in a nutrient solution without phosphorus (△). A: Changes in photosynthetic CO_2 uptake in O_2-free air, F^*; B: changes in photosynthetic CO_2 uptake in normal air (21% O_2), F; C: changes in leaf diffusion resistance, r_l'.

corn (23). The effect of low P on light respiration could occur in several ways: for example, low P slowed down photosynthesis so there may have been less substrate for photorespiration. Alternatively since ATP and NADPH (produced via photophosphorylation) would be required to convert the by-products of photorespiration, such as serine, back into an intermediate of the Calvin cycle (9), then the reduced levels of ATP and NADPH at low P may have slowed photorespiratory CO_2 evolution due to product inhibition.

Ieteraction of irradiation intensity with leaf phosphorus content on the rates of photosynthesis, F and F^*, a phenomenon observed elsewhere (1, 14), was not apparent here, but there was an interaction effect on leaf diffusion resistance, r_l'. At low light r_l' increased from 1 sec cm^{-1} to 9 sec cm^{-1} with decrease in P supply, whereas at saturating irradiances there was no effect of P supply on r_l'. As the change in r_l' with light intensity was almost certainly attributable to changes in stomatal diffusion resistance, it appeared that the effect of low P on stomatal aperture in low light could be overcome in strong light. The mode of action whereby stomatal aperture was affected by low P was probably related in some way to ATP formation since ATP has been implicated in stomatal opening (26).

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LITERATURE CITED

1. ANDREEVA, T. F. AND V. M. PERSANOV. 1970. Effect of duration of phosphorus deficiency on photosynthesis intensity and leaf growth in connection with the productivity of horse beans. Sov. Plant Physiol. 17: 390–395.
2. ARNON, D. I. 1953. The physiology and biochemistry of phosphates in green plants. *In:* W. H. Pierre and A. G. Norman, eds., Soil and Fertilizer Phosphorus in Crop Nutrition, Chap. 4. Academic Press, Inc., New York. pp. 1–42.
3. BASLAVSKAYA, S. S. AND E. N. MARKAROVA. 1959. The effect of phosphorus on light and dark reactions of photosynthesis in *Scenedesmus quadricauda*. Sov. Plant Physiol. 6: 160–166.
4. BOTTRILL, D. E., J. V. POSSINGHAM, AND P. E. KRIEDEMANN. 1970. The effect of nutrient deficiencies on photosynthesis and respiration in spinach. Plant Soil 32: 424–438.
5. BOUMA, D. 1967. Growth changes of subterranean clover during recovery from phosphorus and sulphur stresses. Aust. J. Biol. Sci. 20: 51–66.
6. EATON, S. V. 1949. Effects of phosphorus deficiency on growth and metabolism of sunflower. Bot. Gaz. 110: 449–464.
7. EATON, S. V. 1950. Effects of phosphorus deficiency on growth and metabolism of soybean. Bot. Gaz. 111: 426–436.
8. ELMORE, C. D. 1966. Relationships of photosynthetic rates, leaf area development, leaf aging and net assimilation rate on plant growth. Master's thesis. University of Arizona, Tucson.
9. GOLDSWORTHY, A. 1970. Photorespiration. Bot. Rev. 36: 321–340.
10. GREGORY, F. G. AND F. J. RICHARDS. 1929. Physiological studies in plant nutrition. I. The effect of manurial deficiency on the respiration and assimilation rate in barley. Ann. Bot. 43: 119–161.
11. HALL, A. E. 1970. Photosynthetic capabilities of healthy and Beet Yellows Virus infected sugar beets (*Beta vulgaris* L.) Ph.D. thesis. University of California, Davis.
12. JOHNSON, C. M. AND A. ULRICH. 1959. Analytical methods for use in plant analysis. Calif. Agr. Exp. Sta. Bull. 766: 26–78.
13. KAKIE, T. 1970. Effect of phosphorus deficiency on the photosynthetic carbon dioxide fixation-products in tobacco plants. Soil Sci. Plant Nutr. 15: 245–251.
14. LINDEMAN, W. 1951. The influence of phosphate on the photosynthesis of *Lemna minor* L. Nd Akad. Wetensch. Proc. C. 54: 287–295.
15. NÁTR, L. 1970. Effect of mineral nutrient deficiencies on dry matter production, photosynthetic intensity and N, P and K quantities in plants. Physiol. Veg. 8: 573–583.
16. PIRSON, A. 1955. Functional aspects in mineral nutrition of green plants. Annu. Rev. Plant Physiol. 6: 71–114.
17. SCHMEHL, W. R. AND D. W. JAMES. 1971. Phosphorus and potassium nutrition. *In:* R. T. Johnson, J. T. Alexander, G. E. Rush, G. R. Hawkes, eds., Advances in Sugarbeet Production: Principles and Practices. The Iowa State University Press, Ames, Iowa. pp. 137–169.
18. SIPITANOS, K. M. AND A. ULRICH. 1969. Phosphorus nutrition of sugar beet seedlings. J. Amer. Soc. Sugar Beet Technol. 15: 332–346.
19. SLATYER, R. O. 1970. Comparative photosynthesis, growth and transpiration of two species of *Atriplex*. Planta 93: 175–189.
20. SPENCER, D. AND J. V. POSSINGHAM. 1960. The effect of nutrient deficiencies on the Hill reaction of isolated chloroplasts from tomato. Aust. J. Biol. Sci. 13: 441–455.
21. STEINBERG, R. A. 1951. Correlations between protein-carbohydrate metabolism and mineral deficiencies in plants. *In:* Truog, E., ed., Mineral Nutrition of Plants. University of Wisconsin Press, Madison. pp. 359–386.
22. STROGONOVA, L. E. 1968. Consumption of organic substances for respiration in plants cultivated under various conditions of mineral nutrition. Sov. Plant Physiol. 15: 229–236.
23. TAIRBEKOV, M. G., É. N. KAZANTSEV, AND S. V. TAGEEVA. 1965. Synthesis and decomposition of ATP as a function of the streaming of the cytoplasm in the plant cell. Biochemistry (USSR) 30: 1099–1105.
24. TERRY, N., L. J. WALDRON, AND A. ULRICH. 1971. An apparatus for the measurement of carbon dioxide and water vapor exchange of attached sugarbeet leaves. J. Amer. Soc. Sugar Beet Technol. 16: 471–478.
25. TOMBESI, L., M. T. CALÉ, AND B. TIBORNÉ. 1969. Effects of nitrogen, phosphorus and potassium fertilizer on the assimilation capacity of *Beta vulgaris* chloroplasts (I). Plant Soil 31: 65–76.
26. ZELITCH, I. 1969. Stomatal control. Annu. Rev. Plant Physiol. 20: 329–350.