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Phosphorus Nutrition Influence on Leaf Senescence in Soybean[1]

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ABSTRACT

Remobilization of mineral nutrients from leaves to reproductive structures is a possible regulatory factor in leaf senescence. The relationship between P remobilization from leaves of soybean (*Glycine max* [L.] Merr. cv McCall) during reproductive development and leaf senescence was determined by utilizing soil P treatments that supplied deficient, optimum, and supraoptimum soil P levels. The soil P treatments simulated field conditions, being initiated at the time of planting with no subsequent addition or removal of P. It was hypothesized that P deficiency would accelerate leaf senescence and that supraoptimum P nutrition would delay the timing or rate of leaf senescence relative to plants grown with optimum P. Supraoptimum soil P led to a two- to fourfold increase in leaf P concentration compared with optimum P, and during senescence there was no net P remobilization from leaves for this treatment. Leaf P concentration was similar for plants grown at optimum or deficient soil P, and there was significant net P remobilization from leaves of both treatments in one of the two experiments. As indicated by changes in leaf N, carbon dioxide exchange rate, ribulose 1,5-bisphosphate carboxylase/oxygenase activity, and chlorophyll concentration, leaf senescence patterns were similar for all soil P treatments. Thus, it can be concluded that leaf senescence was not affected by either P deficiency or enhanced leaf P concentration resulting from supraoptimum soil P. The results suggest that P nutrition in general, and specifically P remobilization from leaves, does not exert any regulatory control on the process of leaf senescence.

Mineral nutrient remobilization from leaves to reproductive structures has long been considered a factor in the process of leaf senescence (20). In particular, leaf N remobilization has been widely studied due to the large amount of N in the leaves and because a large portion of the leaf N comprises the enzymes of the photosynthetic apparatus (7). In addition to N, other mineral nutrients, including P, are also remobilized from the leaves during reproductive growth (13, 14).

Growing plants at deficient soil P levels leads to decreased biomass production and seed yield (15, 17, 18, 22, 26). Studies have indicated that leaf P is extensively remobilized during reproductive growth and that this remobilization may be an important factor in leaf senescence and yield production (12, 21, 26).

In a previous study of vegetative growth of tobacco (*Nicotiana tabacum* L.), we demonstrated that soil P levels could be manipulated such that leaf P concentration remained relatively constant until maximum biomass was produced, after which further increases in soil P levels resulted in progressive increases in leaf P concentration (6). These treatments simulated the conditions in which plants would experience P deficiency or supraoptimum P levels under field conditions and appeared to be an ideal way to determine the role of P nutrition in reproductive development and leaf senescence in soybean. We utilized these soil P treatments to produce soybean plants that were (a) P limited and (b) produced similar biomass and seed yield but differed two- to threefold in total plant and leaf P content (5). It was demonstrated that normal seed growth and development could occur independently of net leaf P remobilization. Visually, canopy senescence was not markedly altered by any of the soil P levels. However, because of differences in leaf age, it is difficult to evaluate leaf senescence visually or from a sample consisting of the total plant leaf mass. In this study, we report the effect of these same soil P treatments on senescence of a leaf near the top of the plant. Two hypotheses were tested: (a) deficient soil P would lead to more rapid leaf senescence, and (b) supraoptimum soil P that markedly increases leaf and total plant P concentration would delay leaf senescence.

MATERIALS AND METHODS

Plant Culture

A detailed description of the plant culture methods used for these experiments was previously reported (5). Briefly, two greenhouse experiments, planted on January 16, 1990 (experiment 1) and March 7, 1990 (experiment 2), were conducted using the soybean (*Glycine max*) cultivar McCall. Plants were grown in 20 by 20 cm pots containing soil that was very low in available P; there were two plants per pot. Plants were not nodulated due to the high level of N fertility.

Three soil P treatments were established by blending $Ca(H_2PO_4)_2$ with the soil prior to planting. The P treatments were equivalent to 134, 538, and 2150 kg P ha^{-1}, denoted as P1, P2, and P3, respectively, for experiment 1. For experiment 2, P1 and P2 treatments were the same as for exp. 1, whereas the P3 treatment was decreased to 1612 kg P ha^{-1}. Soil test results for extractable P at the beginning and end of the experiments were as reported previously (5). As a reference,

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the P2 treatment resulted in soil P tests that are considered to be very high.

Sampling

Trifoliolate leaves from the third or fourth node from the apex of the plant were sampled at growth stages R5 (beginning seed), R6 (full seed), R7 (physiological maturity), and just prior to R8 (full maturity) (8) so that attached leaves could be sampled. Leaf area was determined and leaf discs were taken and immediately frozen at −80°C for enzyme assay. Leaf discs were also taken for Chl determination. The remaining leaf tissue was frozen and lyophilized prior to dry weight determination. The tissue was then frozen with liquid N and ground to a powder for N and P analysis. CER[2] was determined at several times during the course of the experiment using leaves at the same node position as was used for tissue analysis. At each sampling time, eight leaves, two from each of four pots (replications), were sampled for each P treatment. The experimental design was a randomized complete block design with four replications. The data were statistically analyzed by analysis of variance and, when a significant F test was obtained, the LSD ($\alpha = 0.05$) test was used to compare P treatments within and across sampling times.

Total N and P

Subsamples (50 mg) of dry, ground tissue were acid-digested by the method of Nelson and Sommers (23) and aliquots of the digests were used for N and P analysis. Total N was determined colorimetrically by analyzing for NH_4^+ as previously described (1). Total P was determined by the method of Fiske and Subbarow (9).

CER

CER was determined with a Li-CoR LI 6000 portable photosynthesis system. A 1073 mL leaf chamber was used and CER was determined on 1250 mm^2 of leaf area. Measurements were made on sunny days between 10:30 AM and 12:30 PM Eastern daylight saving time.

Rubisco Activity

Rubisco activity was determined by homogenizing 113 mm^2 of leaf area in 1.0 mL of medium containing 100 mM Tricine-NaOH (pH 8.0), 5 mM $MgCl_2$, 0.1 mM EDTA, 5 mM DTT, 5 mM isoascorbate, 1 mM PMSF, 20 μM leupeptin, and 1% (w/v) casein. Aliquots (50 μL) of the resuspended crude extracts were assayed for total (fully activated) Rubisco activity at 30°C as previously described (25) except that Triton X-100 and casein were not included in the assay medium. Assays were terminated after 30 s and incorporation of $NaH^{14}CO_3$ into acid-stable products was determined as previously described (25).

[2] Abbreviation: CER, carbon dioxide exchange rate.

Chl

Chl was extracted overnight from 226 mm^2 of leaf tissue with 5 mL of methanol. Chl concentration was determined as previously described (4).

RESULTS AND DISCUSSION

We previously reported that the soil P levels used in this study did not alter reproductive plant ontogeny (5). P deficiency (P1 treatment) reduced biomass and seed yield to approximately 30% of that produced by the P2 and P3 treatments in both experiments even though, due to the more favorable light environment, plant biomass and seed yield were threefold greater for experiment 2 (5).

Leaf P concentrations were similar for the P1 and P2 treatments (Fig. 1) even though plant growth was much greater for P2 than P1 (5). The P3 treatment resulted in leaf P concentrations that were two- to fourfold greater than the other treatments, depending on the experiment. These effects for the single leaf that was sampled for this study were similar to those observed for the total plant leaf mass (5). The P3 treatment essentially eliminated net leaf P remobilization during seed development (Fig. 1). Net leaf P remobilization during seed development was observed for the P1 and P2 treatments in experiment 2. Comparison of the P2 and P3 soil P treatments provided a straightforward means to test the hypothesis that increased leaf P concentration would delay leaf senescence. However, the hypothesis that P deficiency would cause accelerated leaf senescence appeared to be untestable. As with a previous experiment with tobacco (6), soil P levels that caused reductions in biomass did not reduce leaf P concentrations relative to plants grown with just enough P to produce maximum biomass. Plant growth was apparently regulated according to the P supply, possibly mediated by changes in root hydraulic conductivity (24), such that the leaf P concentration remained relatively constant until biomass

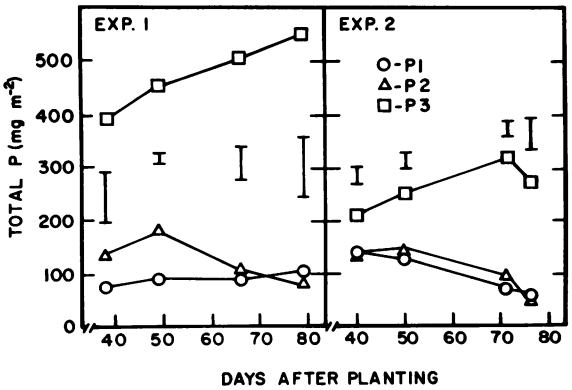

Figure 1. Total phosphorus concentration of leaves sampled from soybean plants that were grown at three different levels of soil P. The results of two separate experiments are reported. Soil P levels were 134, 538, and 2150 kg P ha^{-1} for P1, P2, and P3, respectively, for experiment 1. For experiment 2, the P3 treatment was 1612 kg P ha^{-1}. F LSD ($\alpha = 0.05$) values for individual sampling times were indicated on the figure only when a significant F value was obtained in the analysis of variance.

production reached a maximum. Leaf P concentration did not increase until the soil P level exceeded that required for maximum growth.

Leaf senescence is often characterized by a large decrease in leaf N during the seed filling period (2, 3, 7, 19, 28, 29). For all soil P treatments used in this study, there was a large decline in N concentration during the seed filling period (Fig. 2). The time course and extent of leaf N loss was similar for all soil P treatments even though leaf N concentration was higher, particularly for experiment 2, for the P-deficient treatment (P1). These data were in agreement with data for the whole plant leaf mass (5) and indicate that N and P remobilization from leaves during the seed filling period are independent processes. Furthermore, the data suggest that N remobilization may not be subject to manipulation by varying soil N levels as we have demonstrated for P.

Relative to the effect on growth (5), P deficiency had only a minor effect on CER (Fig. 3). CER was depressed 25 to 30% by low P in experiment 1, but for experiment 2 there were no treatment differences. Other studies have also demonstrated variability in the effect of P stress on CER in soybean (10, 11). Because leaf P concentration was similar for the P1 and P2 treatments, it is perhaps not surprising that CER was not influenced to a great extent by soil P levels. Our results for tobacco also indicated that CO_2 fixation at saturating levels of light and CO_2 was not markedly affected by deficient soil P until the level of soil P was such that growth was almost completely inhibited (6). The CER results indicate that biomass differences between the P1 and P2 treatments (5) were due to leaf area development and not photosynthesis per unit leaf area. In general, CER declined during the seed filling period in a similar manner for all P levels (Fig. 3). Thus, based on N remobilization (Fig. 2) and CER, leaf senescence appeared to proceed in a typical manner for all soil P treatments.

The temporal changes in Rubisco activity (Fig. 4) during the seed filling period were similar to changes in leaf N and CER (Figs. 2 and 3) and were not affected by soil P level. Temporal changes in Chl were also not affected by soil P level even though low soil P resulted in higher Chl concentrations at any given sampling time (Fig. 5).

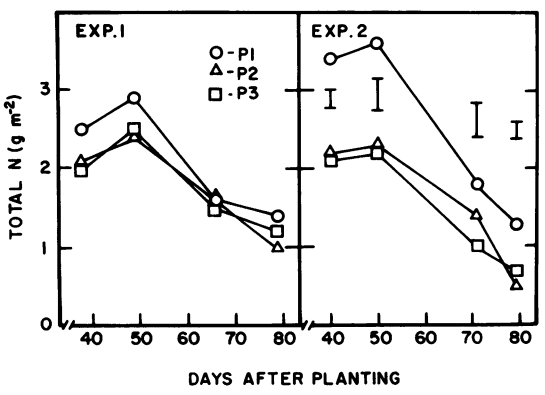

Figure 2. Total nitrogen concentration of leaves sampled from soybean plants that were grown at three different levels of soil P. Soil P levels and statistics are as described in Figure 1.

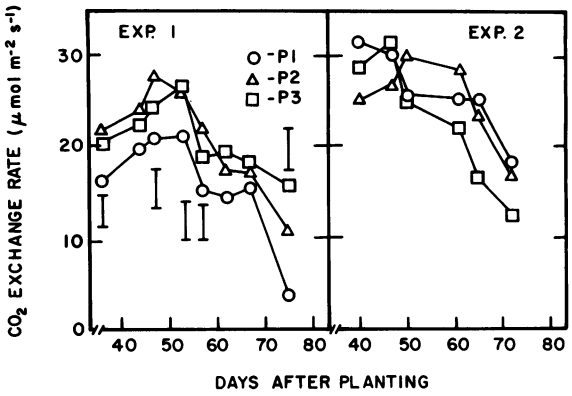

Figure 3. CER of leaves sampled from soybean plants that were grown at three different levels of soil P. Soil P levels and statistics are as described in Figure 1.

The depressed CER for the P1 treatment in experiment 1 (Fig. 3) was not related to depressed Rubisco activity (Fig. 4). This could possibly be explained by the results of Fredeen *et al.* (11), who reported that decreased photosynthesis at low P nutrition levels was more closely associated with changes in ribulose-5-phosphate kinase activity than with Rubisco activity. Also, the increased leaf N concentration for the P1 treatment, especially for experiment 2, did not result in enhanced Rubisco activity. This suggested that the increased leaf N for the P1 treatment was apparently not utilized for synthesis of Rubisco. The increased leaf N may have been accounted for by increased levels of vegetative storage proteins (27). P stress has also been shown to result in higher concentrations of soluble reduced N in leaves of soybean plants grown on inorganic N (16).

The cumulative results of these experiments provided evidence that soil P levels that either markedly depressed plant growth or that led to greatly enhanced leaf P concentration did not affect the senescence process as determined by several classical indicators of leaf senescence. Because low soil P (P1 treatment) did not alter leaf P concentration compared with

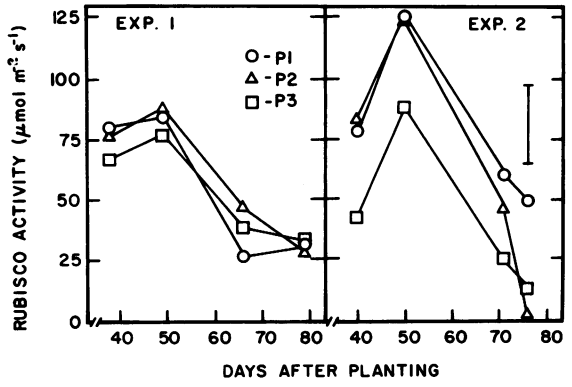

Figure 4. Rubisco activity of leaves from soybean plants that were grown at three different levels of soil P. Enzyme assays were conducted using fully activating conditions. Soil P levels and statistics are as described in Figure 1.

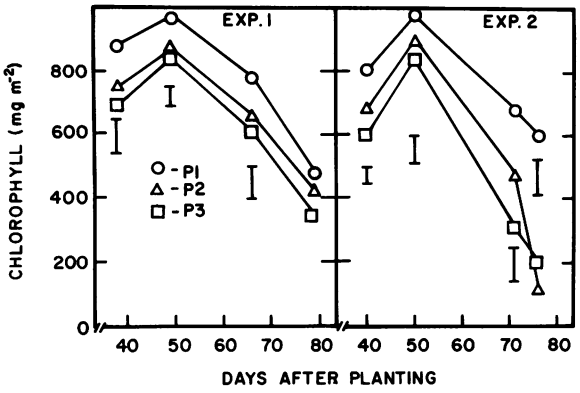

Figure 5. Chl concentration of leaves sampled from soybean plants that were grown at different levels of soil P. Soil P levels and statistics are as described in Figure 1.

plants supplied with optimum soil P (P2 treatment), it was not surprising that leaf senescence was not altered by deficient P. Studies that have demonstrated an effect of P nutrition on leaf senescence have used P removal treatments imposed after plants had been grown on adequate P for much of the life cycle (12, 26). Thus, leaf senescence is influenced when P stress treatments are imposed after the plants have attained P uptake rates, leaf area development, leaf P concentration, seed numbers, etc., in proportion to an optimum level of P nutrition. However, treatments in which P nutrition is instantaneously altered do not reflect the way in which plants would experience deficient or supraoptimum P levels under field conditions. Similar to the soil-based plant culture system used in the present study, field-grown plants would be subjected to P deficiency or excess at the time of planting without further addition or removal of P.

If P remobilization is a factor in the senescence process, then enhancing the leaf P concentration would be expected to delay senescence. Data for the P3 treatment clearly demonstrated that enhanced leaf P concentration did prevent net P remobilization (Fig. 1 and ref. 5), but leaf senescence was not altered. Thus, even though leaf P does decline in some cases during the seed filling period in soybean (13, 14, 18), this decline is not a factor in the regulation of the senescence process.

It has been consistently demonstrated that monocarpic leaf senescence is associated with declines in leaf N as manifested by degradation of the photosynthetic apparatus (2, 3, 7, 19, 28, 29). The results of this study were consistent with this observation. Even when leaf N concentration was significantly increased by the P1 treatment in experiment 2 (Fig. 2), there was no effect on N remobilization or leaf senescence. Efforts to understand the mechanism of monocarpic leaf senescence should probably continue to be directed to delineating the factors that control the degradation of the photosynthetic apparatus.

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