Investigation of the Role of Phosphorus in Symbiotic Dinitrogen Fixation[1]

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ABSTRACT

The interactive effects of phosphorus supply and combined nitrogen (nitrate) on dry matter and nitrogen accumulation by nodulated soybean (*Glycine max* L. Merr.) plants, and the relative effects of phosphorus supply on nodule number, mass, and function in comparison to host plant growth were used to investigate the role of phosphorus in symbiotic dinitrogen fixation. Mixed positive and negative phosphorus by nitrogen source interactions indicated that severe phosphorus deficiency markedly impaired both host plant growth and symbiotic dinitrogen fixation and that symbiotic dinitrogen fixation has a higher phosphorus requirement for optimal functioning than either host plant growth or nitrate assimilation. In the whole plant phosphorus concentration range of 0.8 to 1.5 grams per kilogram dry weight, plants supplied with 20 millimolar nitrate accumulated significantly more dry matter and nitrogen than symbiotic plants without nitrate. This suggested that the higher phosphorus requirement for symbiotic dinitrogen fixation is internal rather than being associated with differences in the ability of roots in the two nitrogen regimes to absorb phosphorus from the external solution. Increasing the phosphorus concentration in plants solely dependent on dinitrogen fixation resulted in highly significant (P = 0.0001) increases in whole plant nitrogen concentration as well as highly significant increases (P = 0.0001) in whole plant dry matter and nitrogen accumulation. This indicated a greater responsiveness of symbiotic dinitrogen fixation than of host plant growth to improvement in phosphorus nutrition. The large increases in whole plant nitrogen concentration were associated with about 3.5-fold increases in the ratio of nodule mass to whole plant mass and about 2-fold increases in specific acetylene reduction (nitrogenase) activity of the nodules. The large increase in nodule mass (>30-fold) between the 0 and 2.0 millimolar phosphorus levels resulted from 11- and 3-fold increases in nodule number per plant and average mass of individual nodules, respectively. Root mass per plant over the same concentration range increased 3.5-fold. These results indicate that phosphorus has specific roles in nodule initiation, growth, and functioning in addition to its involvement in host plant growth processes.

Mineral nutrients may influence symbiotic dinitrogen fixation of leguminous plants at any of four phases of the overall process: (a) host plant growth, (b) growth and survival of rhizobia, (c) infection and nodule development, and (d) nodule function (19). The relative requirements of host plant growth and of the sym-

biotic dinitrogen fixation process for a mineral nutrient have been assessed by determining (a) the nature of the interaction between the supply of a mineral nutrient and supply of combined nitrogen on growth of symbiotic plants, and (b) the effect of supply of a mineral nutrient on concentrations of nitrogen in tissues of symbiotic plants; the size, mass, distribution, and number of nodules; and nitrogenase activity of nodules (19).

The influence of phosphorus on symbiotic dinitrogen fixation in leguminous plants has received considerable study but its role in the process remains unclear. Robson *et al.* (20) observed that combined nitrogen increased the growth response of nodulated subterranean clover (*Trifolium subterraneum* L.) plants to phosphorus. Thus, they concluded that phosphorus increased symbiotic dinitrogen fixation in subterranean clover by stimulating host plant growth rather than by exerting specific effects on rhizobial growth and survival or on nodule formation and function. Studies with stylosanthes (*Stylosanthes humilis* H.B.K.), soybean (*Glycine max* L. Merr.), and pea (*Pisum sativum* L.) plants have suggested more specific effects of phosphorus on nodule initiation, growth, development and function (5–7, 9, 13). In most of these latter studies, the interactive effect of combined nitrogen and phosphorus supply on growth of symbiotic plants was not examined. Conclusions were based on the observations that increases in phosphorus supply to plants dependent on symbiotic dinitrogen fixation as the only source of usable nitrogen increased nodule number, mass, and relative growth rate more than host plant growth and increased the specific nitrogenase (acetylene reduction) activity of nodules.

The objective of this study was to reconcile conflicting reports on the role of phosphorus in the symbiotic dinitrogen fixation process by examining (a) the interactive effects of phosphorus supply and combined nitrogen on growth and nitrogen accumulation by nodulated plants and (b) the relative effects of phosphorus supply on nodulation parameters in comparison to host plant growth.

MATERIALS AND METHODS

Plant Culture and Inoculation. Soybean (*Glycine max* L. Merr.) plants were grown in outdoor pot culture from late May until November 1985. Plants were exposed to natural light intensity, temperature, and daylength conditions throughout the entire course of the experiment.

Nutrient solutions were prepared in tap water available at the site. The composition of the nutrient solution was as described by McClure and Israel (16), except that potassium dihydrogen phosphate was used as the sole source of phosphorus and was varied to produce solutions containing 0, 0.1, 0.25, 0.5, 1.0, and 2.0 mM phosphorus. A 0 and a 20 mM potassium nitrate series, each containing the six phosphorus concentrations resulted in 12 nutritional treatments. The pH of all nutrient solutions was adjusted to 6.0, and the potassium concentrations in the minus

nitrogen solutions were made equal to those in the 20 mM potassium nitrate solutions by addition of 10 mM potassium sulfate. Corrections were not made for differences in the amount of potassium added with the potassium dihydrogen phosphate because all solutions contained a large excess of potassium (>22 mM). The phosphorus concentration in the tap water, which was determined each time a new group of solutions was prepared, did not exceed 4.2 μM. The phosphorus concentrations cited as treatment levels refer to the amount added as potassium dihydrogen phosphate.

Seeds of Ransom soybeans (maturity group VII) were germinated for 72 h at 30°C and 95% RH. Roots of seedlings of both nitrate-supplied and nonnitrate-supplied plants were dipped in inoculum of *Bradyrhizobium japonicum* strain USDA 110 just before transplanting. Just after transplanting, 1.0 ml of inoculum was applied to the Perlite[2] at the base of each seedling. Inoculum was grown to stationary phase (about 10^9 CFU/ml)[3] in yeast extract-mannitol medium (23). Two seedlings were transplanted into each 6 L pot of Perlite, containing 300 g of calcium carbonate in the form of crushed oyster shells to control rhizosphere acidification (12). Two weeks after transplanting the seedlings were thinned to 1/pot.

From 1 to 9 DAT, each pot was supplied 0.25 L of appropriate nutrient solution at 0800 h and 0.5 L of water at 1400 h daily. From 10 to 60 DAT, pots were flushed each day with 1.5 to 2.0 L of water at 0800 h and at 1400 h and 0.5 L of appropriate nutrient solution was applied to each pot after the 1400 h flushing. From 60 to 97 DAT, each pot received 0.5 L of appropriate nutrient solution after both the 0800 and 1400 h flushings. Pots were supported in racks about 1 m above the ground, and excess water or nutrient solution was allowed to drain through the pots to the ground.

To make valid comparisons of dry matter and nitrogen accumulated by plants solely dependent on symbiotic dinitrogen fixation and those supplied with nitrate, all plants were supplied one of six minus-nitrogen solutions containing different phosphorus levels until nodules began to fix N_2, as indicated by regreening of leaves after a transient yellowing. At this time, 20 DAT, one-half of the plants supplied each phosphorus concentration were changed to a 20 mM potassium nitrate nutrient solution containing the same phosphorus concentration for the remainder of the experiment.

Sufficient plant material was cultivated to permit four replications of 12 nutritional treatments (six phosphorus levels with each nitrogen source, strain USDA 110 and strain USDA 110 plus 20 mM nitrate) and two sampling dates (40 and 97 DAT). These sampling dates correspond to vegetative and beginning seed fill stages of the developmental cycle. The experiment was arranged in a randomized complete block design with the 24 combinations of nutritional treatments and sampling dates randomized within each block.

Sampling and Analytical Procedures. Acetylene reduction assays were performed on the excised root systems of 40-d-old plants using the methods of Sloger (22). Nodulated root systems were incubated for 30 min at 25°C in 1 L jars containing 0.1 atm of acetylene. Duplicate samples were removed from the jars after the 30-min incubation and stored in 5 ml vacutainers until gas chromatographic analysis could be performed. Ethylene and acetylene in 0.5 ml samples were separated and quantified with a Carle model 311 H gas chromotgraph equipped with a flame

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[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.

[3] Abbreviations: CFU, colony-forming unit; DAT, days after transplanting.

ionization detector and a column of Porapak N (182 × 0.32 cm). After incubation, nodules were counted, weighed, and dried. Nitrogenase activities are presented as micromoles of ethylene formed per h per plant and per gram fresh weight of nodule tissue.

At the 40 DAT sampling, N_2-fixing plants were separated into leaflet, stem plus petiole, root and nodule fractions. The small amount of nodule tissue on nitrate-supplied plants was not separated from the roots. At the 97 DAT sampling, plants from all treatments were separated into leaflet, stem plus petiole, pod and root plus nodule fractions. All plant material was dried at 65°C for 72 h, weighed and ground to pass a 1 mm screen.

The total nitrogen concentration of tissue samples was determined by a Kjeldahl procedure that included a salicylic acid predigestion step to convert nitrate to ammonium (18) and that employed a zirconium-copper catalyst (10). After alkalinization of digests, ammonia was steam distilled into 0.32 N boric acid and quantified by titration with 0.01 N potassium bi-iodate.

For determination of total phosphorus concentration, tissue samples (100 mg) were ashed overnight at 500°C, and the ash was dissolved in 3 ml of 4 N HCl and diluted to 25 ml with redistilled water. Appropriate aliquots were analyzed for phosphorus by the ammonium molybdate method of Murphy and Riley (17).

Statistical Methods. Data were subjected to analysis of variance using the General Linear Models procedure of the Statistical Analysis System (11). Phosphorus concentrations and nitrogen sources were designated as fixed effects and separate analyses of variance were conducted for data collected at each sampling date. When appropriate, $LSD_{0.05}$ values are presented in tables and figures for comparison of treatment means.

RESULTS

Phosphorus Uptake and Tissue Concentrations. With both sources of nitrogen nutrition, phosphorus uptake increased across the entire range of nutrient solution phosphorus concentrations (Fig. 1, A and B). At 40 DAT, USDA 110 inoculated plants with or without 20 mM nitrate had absorbed similar amounts of phosphorus at the three lowest treatment levels (0, 0.1 and 0.25 mM), while at the three highest treatment levels plants supplied nitrate had significantly more phosphorus than those solely dependent on dinitrogen fixation (Fig. 1A). At 97 DAT, inoculated plants with or without 20 mM nitrate had absorbed similar amounts of phosphorus at all except the highest external phosphorus concentration (Fig. 1B).

The increase in total phosphorus uptake in response to increasing phosphorus supply was associated with significant increases in the phosphorus concentration in all plant organs at both sampling dates (Tables I and II). At the 0.1 and 0.25 mM phosphorus concentrations the nodules contained a higher phosphorus concentration than any other part of symbiotic plants at 40 DAT (Table I). As the nutrient solution phosphorus concentration was increased from 0.1 to 2.0 mM, the phosphorus concentration in the nodules increased 39% while that of leaflets, stem plus petioles and roots of symbiotic plants increased 138, 206, and 449%, respectively. The percentage increases in phosphorus concentration for the respective parts of nitrate-supplied plants were 217, 227, and 361 (Table I).

Dry Matter and Nitrogen Accumulation. Highy significant (P = 0.0001) increases in whole plant dry weight and nitrogen accumulation occurred for plants with or without 20 mM nitrate in response to increasing phosphorus concentration in the nutrient solution (Fig. 1, C and D; Fig. 2, A and B). After 40 d of growth, plants with and without 20 mM nitrate had accumulated similar amounts of dry matter and nitrogen at the 0 and 0.1 mM phosphorus concentrations (Figs. 1C and 2A). At the three highest phosphorus levels, plants supplied 20 mM nitrate accu-
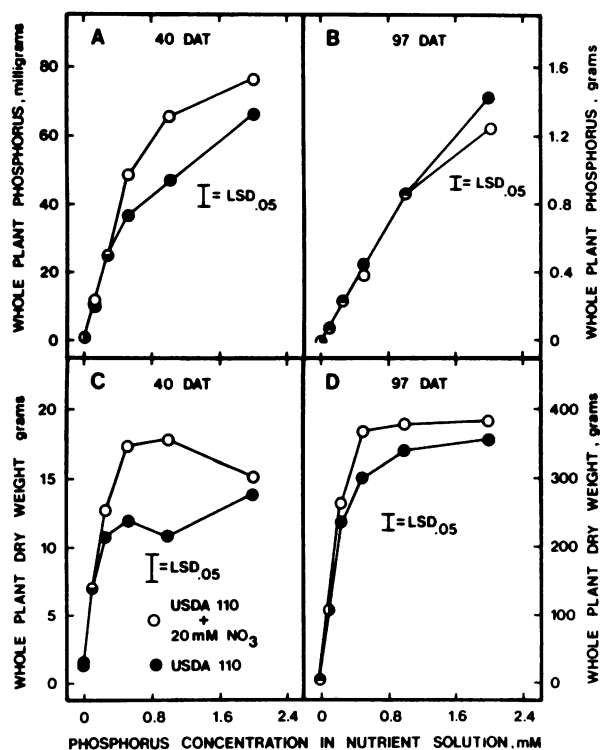
FIG. 1. Effect of phosphorus supply and source of nitrogen nutrition on whole plant phosphorus content and whole plant dry matter accumulation at two sampling dates. Since phosphorus concentration by nitrogen source treatment interactions were significant (P = 0.0001), the LSD$_{0.05}$ values can be used to compare any two treatment means.

mulated significantly more dry matter and nitrogen than plants solely dependent on symbiotic N$_2$ fixation (Figs. 1C and 2A). At 97 DAT (Figs. 1D and 2B) plants with and without 20 mM nitrate had accumulated similar amounts of dry matter and nitrogen at the two lowest phosphorus concentrations (0 and 0.1 mM). At

the intermediate phosphorus concentrations (0.25 and 0.5 mM) nitrate-supplied plants accumulated significantly greater amounts of dry matter and nitrogen than plants solely dependent on symbiotic N$_2$ fixation. At the highest phosphorus levels, the amounts of dry matter and nitrogen accumulated by plants solely dependent on symbiotic N$_2$ fixation approached those of plants supplied 20 mM nitrate (Figs. 1D and 2B).

Phosphorus supply had highly significant effects (P = 0.0001) on the whole plant nitrogen concentration of plants with or without 20 mM nitrate. At 40 DAT, whole plant nitrogen concentration of plants solely dependent on symbiotic N$_2$ fixation was increased more by improvement in phosphorus nutrition (117%) than that of plants supplied 20 mM nitrate (18%) (Fig. 2C). At 97 DAT, whole plant nitrogen concentration of plants solely dependent on symbiotic N$_2$ fixation increased 56%, whereas that of plants supplied 20 mM nitrate decreased 28% with improvement in phosphorus nutrition (Fig. 2D). The increases in whole plant nitrogen concentration for plants solely dependent on N$_2$ fixation in response to increased phosphorus supply were a reflection of highly significant (P ⩽ 0.01) increases in the concentration of nitrogen in all plant parts at both 40 DAT and at 97 DAT (data not shown).

Nodulation and Nitrogenase Activity. Alleviation of phosphorus deficiency caused highly significant increases (P = 0.0001) in nodule mass per plant, nodule number per plant, and average mass per nodule at d 40 (Table III). Maximum levels for all of these parameters, except nodule number, were approached at a nutrient solution phosphorus concentration of 0.5 mM. A 32.8-fold increase in nodule mass per plant at the 0.5 mM concentration compared to the 0 phosphorus concentration resulted from a 8.5-fold increase in nodule number per plant and a 3.9-fold increase in average mass per nodule (Table III).

After 40 d of vegetative growth, highly significant effects (P = 0.0001) of phosphorus concentration in the nutrient solution on whole plant and specific nitrogenase activities were observed (Table III). Near maximum values of both parameters were apparent for plants supplied the 0.5 mM phosphorus concentration. Whole plant and specific nitrogenase activities were 58.9-

Table I. Effect of Phosphorus Supply and Source of Nitrogen Nutrition on the Phosphorus Concentration in Ransom Soybean Plants at 40 DAT

Nitrogen Source	Phosphorus Concentration					
	Nutrient solution	Leaflets	Stems + petioles	Roots	Nodules	Whole plant
	mM	g P/Kg dry wt				
USDA 110	0	0.83	0.72	0.66	ND[a]	0.74
	0.10	1.76	1.58	1.38	4.45	1.74
	0.25	2.37	1.95	1.70	4.79	2.28
	0.50	3.23	3.03	2.45	5.46	3.18
	1.00	3.79	4.50	4.77	5.95	4.36
	2.00	4.19	4.84	7.58	6.18	5.30
USDA 110 + 20 mM NO$_3^-$	0	1.04	0.87	0.87	ND[b]	0.92
	0.10	1.55	1.45	1.26	ND[b]	1.43
	0.25	2.38	1.75	1.64	ND[b]	2.00
	0.50	3.30	2.40	2.41	ND[b]	2.82
	1.00	4.06	3.36	3.42	ND[b]	3.70
	2.00	4.91	4.75	5.81	ND[b]	5.09
	LSD$_{0.05}$[c]	0.24	0.30	0.30	0.31	0.24

[a] Not determined because of insufficient material for phosphorus analysis. [b] Not determined because nodules were not separated from roots of plants that received 20 mM nitrate. [c] Since phosphorus concentration by nitrogen source treatment interactions were significant (P ⩽ 0.002), the LSD$_{0.05}$ values given for leaflet, stem plus petiole, root, and whole plant phosphorus concentrations can be used to compare any two treatment means.

Table II. *Effect of Phosphorus Supply and Source of Nitrogen Nutrition on Phosphorus Concentration in Ransom Soybean Plants at 97 DAT*

Nitrogen Source	Phosphorus Concentration				
	Nutrient solution	Leaflets	Stems + petioles	Pods	Roots + nodules	Whole plant
	mM			*g P/Kg dry wt*		
USDA 110	0	0.88	0.41	1.60	0.61	0.71
	0.10	0.94	0.48	1.50	0.65	0.83
	0.25	1.21	0.53	2.05	0.72	1.01
	0.50	1.96	0.95	2.42	1.16	1.49
	1.00	2.76	2.22	3.44	2.09	2.56
	2.00	3.38	4.27	4.19	4.39	3.99
USDA 110 + 20 mM NO_3^-	0	0.98	0.44	1.88	0.55	0.77
	0.10	0.83	0.39	1.50	0.51	0.78
	0.25	1.02	0.44	1.65	0.59	0.88
	0.50	1.28	0.64	2.19	0.74	1.08
	1.00	2.26	1.75	3.38	3.19	2.31
	2.00	2.73	3.33	4.00	3.59	3.27
	$LSD_{0.05}$[a]	0.18	0.23	0.26	0.34	0.16

[a] Since phosphorus concentration by nitrogen source treatment interactions were significant (P ≤ 0.01), the $LSD_{0.05}$ values for all parameters can be used to compare any two treatment means.
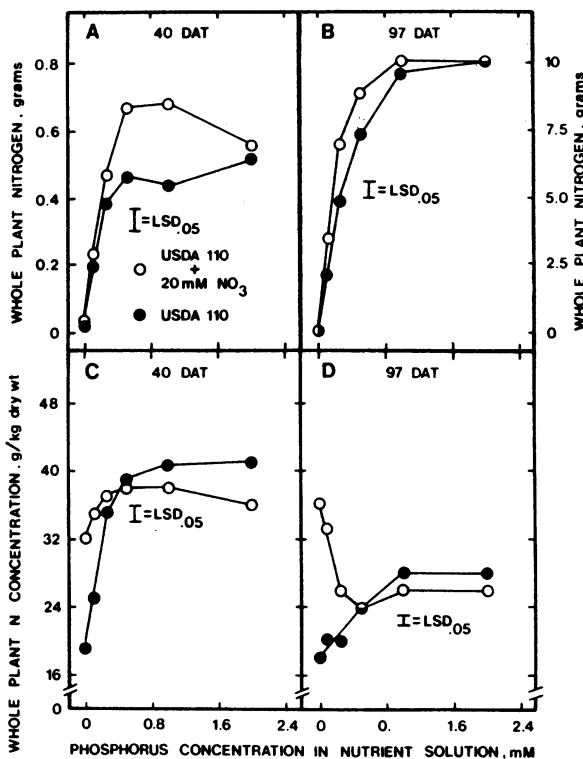

FIG. 2. Effect of phosphorus supply and source of nitrogen nutrition on whole plant nitrogen accumulation and whole plant nitrogen concentration at two sampling dates. Since phosphorus concentration by nitrogen source treatment interactions were significant (P = 0.0001), the $LSD_{0.05}$ values can be used to compare any two treatment means.

and 1.8-fold higher, respectively, for plants supplied 0.5 mM phosphorus than for those supplied no phosphorus (Table III). The total acetylene reduction activities of plants supplied 20 mM nitrate were 20 to 25% of that for plants solely dependent on symbiotic N_2 fixation at the three highest phosphorus levels (data not shown).

DISCUSSION

From the work of Robson (19) and others (1–3, 14), a conceptual basis can be established for evaluating the interactions between combined nitrogen and supply of a mineral nutrient and the related impact on growth. Several possible interactions with nodulated leguminous plants are illustrated in Figure 3. A completely negative interaction occurs when a mineral element is not required for growth of plants supplied combined nitrogen but is required for growth of nodulated plants without combined nitrogen (Fig. 3A). An incompletely negative interaction occurs when a low mineral nutrient supply limits growth of nodulated plants with and without combined nitrogen (Fig. 3B). However, in this case, growth of nodulated plants without combined nitrogen is limited to a greater extent at low nutrient supply than growth of nodulated plants with combined nitrogen. In addition, growth of symbiotic plants without combined nitrogen is more responsive to an increase in nutrient supply than growth of plants with combined nitrogen (Fig. 3B). A positive interaction (Fig. 3C) occurs when growth of nodulated plants with and without combined nitrogen is restricted to a similar extent at low nutrient supply and when growth of nodulated plants with combined nitrogen is more responsive to increased supply of nutrient than growth of nodulated plants without combined nitrogen. A mixed interaction may result from an occurrence of a positive interaction in the low to intermediate range of nutrient supply and of a negative interaction in the intermediate to adequate range of nutrient supply (Fig. 3D).

Robson (19) has emphasized that the timing of combined nitrogen application in interaction experiments is critical, because there is a lag between the time of inoculation and initial infection and the time that nodules become functional in N_2 fixation. Although not emphasized previously, it is also important to use a rhizobial strain that is highly efficient in fixing N_2 in symbiosis with the host plant at adequate mineral nutrient levels. Lack of attention to either of these matters could lead to a false positive interaction between combined nitrogen and the supply of nutrient.

In most studies only interpretations of interactive effects of combined nitrogen and a mineral nutrient on dry matter accumulation have been made (1–3, 14, 19). In the present study, interactive effects of combined nitrogen and phosphorus supply

Table III. *Effect of Phosphorus Supply on Nodulation and Dry Weight of Ranson Soybean Plants Solely Dependent on N₂ Fixation at 40 DAT*

| P Concentration in Nutrient Solution	Nodule Fresh Wt	Nodule Number	Average Fresh Wt per Nodule	Nodule Dry Wt Whole Plant Dry Wt	Root Dry Wt	Nitrogenase Total Activity	Nitrogenase Specific Activity
mM	$g/plant$	$per\ plant$	mg	$ratio$	$g/plant$	$\mu mol\ C_2H_4/h/plant$	$\mu mol\ C_2H_4/g\ fresh\ wt/h$
0	0.18	58	3.1	0.023	0.79	1.2	6.7
0.10	2.00	268	7.5	0.054	2.10	21.9	11.0
0.25	3.58	382	9.4	0.064	2.90	39.0	10.9
0.50	5.91	492	12.0	0.078	2.60	70.7	12.0
1.00	5.23	444	11.8	0.083	2.30	83.2	15.9
2.00	6.09	629	9.7	0.087	2.80	89.9	14.8
LSD₀.₀₅	0.60	114	2.3	0.0077	0.46	18.0	3.1
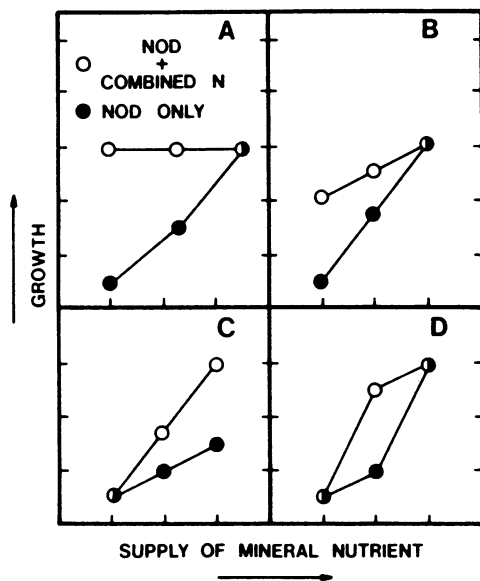
FIG. 3. Illustration of several possible interactions between combined nitrogen and supply of mineral nutrient on growth of nodulated leguminous plants.

on both dry matter and nitrogen accumulation were examined, since phosphorus supply had such profound effects on the concentration of nitrogen in symbiotic plants without nitrate (Fig. 2, C and D). Interactions between combined nitrogen (20 mM nitrate) and phosphorus supply for dry matter and nitrogen accumulation were positive and highly significant (P = 0.0001) after 40 d of growth and mixed (*i.e.* positive and negative) and highly significant (P = 0.0001) after 97 d of growth (Figs. 1 and 2). The positive interactions after 40 d of growth probably resulted from imposing the 20 mM nitrate treatments before nodules became fully functional in N₂ fixation. Thus, it is appropriate that more attention be given to the mixed interactions observed at 97 DAT (Figs. 1D and 2B).

The mixed interactions at 97 DAT (Figs. 1D and 2B) were positive in the severely deficient to moderately deficient phosphorus concentration range (between 0 and 0.5 mM) and negative in the moderately deficient to adequate phosphorus concentration range (between 0.5 and 1.0 mM). A similar mixed interactive effect of combined nitrogen and calcium on dry matter accumulation by nodulated subterranean clover plants has been observed by Loneragan (14). The equivalent accumulation of dry matter and nitrogen by plants with both nitrogen regimes at the severely deficient phosphorus concentration (0 mM) indicated that both host plant growth processes and the symbiotic N₂ fixation process were markedly impaired (Figs. 1D and 2B). Significantly greater accumulation of dry matter and nitrogen by nitrate-supplied plants than by plants solely dependent on N₂

fixation occurred at the moderately deficient phosphorus concentration (0.5 mM). But, similar accumulation of dry matter and nitrogen occurred in plants in the two nitrogen regimes at high phosphorus concentrations (1 and 2.0 mM) (Figs. 1D and 2B). Thus, the data indicate that the symbiotic N₂ fixation process has a higher requirement for phosphorus than host plant growth. Since nitrate was used as the combined nitrogen source, these interactions (Figs. 1D and 2B) also suggest that symbiotic N₂ fixation has a higher phosphorus requirement than the nitrate-assimilation process.

Phosphorus uptake by and the whole plant phosphorus concentration of N₂-fixing plants were equal to or greater than those of nitrate-supplied plants across the entire range of external phosphorus concentration after 97 d of growth (Fig. 1B; Table II). These observations indicate that the higher phosphorus requirement for symbiotic N₂ fixation than for nitrate assimilation did not result from differences in the ability of plant root systems in the two nitrogen regimes to absorb phosphorus at low external phosphorus concentrations. Examination of the relationship between whole plant phosphorus concentration and whole plant nitrogen accumulation (Fig. 4A) reveals that, in the 0.8 to 1.5 g/Kg dry weight whole plant phosphorus concentration range, plants supplied 20 mM nitrate accumulated significantly more nitrogen than symbiotic plants without nitrate. Collectively, these results (Figs. 1B and 4A) are consistent with the interpretation that plants solely dependent on symbiotic dinitrogen fixation
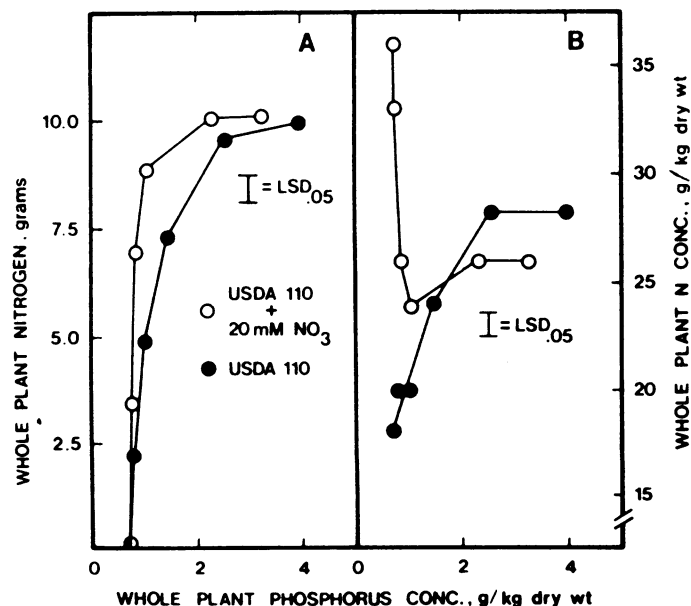

FIG. 4. Relationships between whole plant phosphorus concentration and whole plant nitrogen accumulation, and whole plant nitrogen concentration at 97 DAT.

have a higher internal phosphorus requirement for optimal growth than plants supplied 20 mM nitrate.

At 97 DAT, increases in phosphorus concentration in symbiotic plants without nitrate resulted in concomitant increases in their nitrogen concentration, and whole plant nitrogen concentration attained a maximum level at a whole plant phosphorus concentration of 2.6 g/Kg dry weight (Fig. 4B). In contrast, the nitrogen concentration in plants supplied 20 mM nitrate decreased with increases in whole plant phosphorus concentration and approached a constant value at about 1.0 g/Kg dry weight (Fig. 4B). Increases in nitrogen concentration in symbiotic plants without combined nitrogen with improvement in phosphorus nutrition is a widely reported phenomenon, having been observed in experiments with Phaseolus lathyroides, Phaseolus atropurpureus, Lotononis bainesii, Desmodium intortum, Vigna luteola (4); Stylosanthes humilis (4, 8); Trifolium subterraneum (20); Pisum sativum (13) and Glycine max (21 and this report). The increases in nitrogen concentrations in symbiotic plants without combined nitrogen with improvement in phosphorus nutrition indicate a greater responsiveness of the symbiotic N_2 fixation system to the increased phosphorus supply than of overall host plant growth.

While the interactive effects of combined nitrogen and phosphorus supply on dry matter and nitrogen accumulation after 40 d of growth are difficult to interpret, it is informative to relate the effects of phosphorus supply on nodule number, mass, and functioning at 40 DAT (Table III) to the nitrogen nutrition of symbiotic plants without nitrate (Figs. 2 and 4). The dramatic increases in whole plant nitrogen concentration with improvement in phosphorus nutrition (Fig. 2, C and D; Fig. 4B) were associated with 3.4- to 3.8-fold increases in the ratio of nodule mass to whole plant mass (Table III) and 1.8- to 2.4-fold increases in specific nitrogenase activity of the nodules (Table III). Whole plant nitrogen concentration was maximized when nodule mass approached 8% of whole plant mass (Fig. 2, C and D; Fig. 4B; Table III). The increase in ratio of nodule mass to whole plant mass indicated greater stimulation of nodule growth by improvement in phosphorus nutrition than of host plant growth (Fig. 1, C and D) or of root growth (Table III). The increase in nodule mass per plant in response to increase in phosphorus supply was a consequence of an approximate 10-fold increase in nodule number per plant and an approximate 3-fold increase in the average mass of individual nodules (Table III). The greater responsiveness of nodule number per plant (10.8-fold) than of root mass per plant (3.5-fold) between 0 and 2.0 mM phosphorus concentrations (Table III) suggests that phosphorus has a specific effect on early stage(s) of nodule development.

Collectively, results of this study with soybean are consistent with the conclusion that phosphorus has specific roles in nodule initiation, growth, and functioning in addition to its role in host plant growth. Other studies with soybean and with other leguminous plants are also consistent with this interpretation (5–7, 9, 13). On the other hand, Robson et al. (20) interpreted the positive interactive effects of phosphorus supply and combined nitrogen on growth of nodulated subterranean clover plants to indicate that increasing the phosphorus supply increased N_2 fixation by stimulating host plant growth rather than by exerting specific effects on rhizobial growth and survival or on nodule growth and functioning. However, an alternative explanation is also consistent with their data. The positive interaction between phosphorus supply and combined nitrogen could have resulted from the Rhizobium strain used as symbiont having low N_2 fixation capability even at adequate phosphorus supply. The lower nitrogen concentration in plants solely dependent on symbiotic N_2 fixation than in plants supplied combined nitrogen at high phosphorus supply (20) is consistent with this interpretation. In the present study the whole plant nitrogen concentration was

significantly greater for plants solely dependent on N_2 fixation than for nitrate-supplied plants when high phosphorus concentrations were present in the nutrient solution (Fig. 2, C and D). This indicates that the growth response of symbiotic plants to increasing phosphorus supply was not limited by the dinitrogen fixation capacity of the nodule symbiont (B. japonicum USDA 110). Indeed, this particular isolate of strain USDA 110 has been shown to have superior N_2 fixation capability in symbiosis with soybean (15).

Experiments to identify the specific steps in the nodulation and fixation processes which are enhanced by improvement in phosphorus nutrition are in progress.

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