Effect of Phosphate Fertilization on Yield of Mycorrhizal and Nonmycorrhizal Soybeans

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Cooperative investigations of Plant Science Research Division, ARS, USDA, and the North Carolina Agricultural Experiment Station, Raleigh; published with approval of the Director of Research as Journal Series Paper No. 3481. U.S. Regional Soybean Laboratory Journal Series No. 697.

The author is indebted to J. A. Harper for technical assistance.

Accepted for publication 6 July 1971.

ABSTRACT

Yields from soybeans grown in fumigated soil were not related to phosphate fertilization levels when plants were infected by the mycorrhizal fungus, *Endogone*; yields from nonmycorrhizal soybeans similarly fertilized were directly related to phosphate applications. At low, medium, and high phosphate, yield increases due to *Endogone* mycorrhizae were 122, 67, and 12%, respectively. Concentrations of N, P, Ca, and Cu in foliage of mycorrhizal plants were greater than those from nonmycorrhizal plants at the various phosphate levels. Phosphorus concentration in foliage of mycorrhizal plants at the lowest phosphate level was greater than that in nonmycorrhizal plants at the highest phosphate level. Nonmycorrhizal soybean roots appear to be inefficient phosphate-absorbing organs, and mycorrhiza may aid plants in functions other than phosphate uptake.

Additional key words: Glycine max.

Phytopathology 61: 1400-1403.

Vesicular-arbuscular (VA) mycorrhizae formed by *Endogone* spp. enhance mineral uptake, especially that of phosphorus, in many species of plants (2); recently, *E. mossae* was shown to absorb and translocate locate ^{32}P to mycorrhizal onion roots (3).

Soybean yields and concentrations of P, Ca, N, Cu, and Mn in foliage were recently reported greater from *Endogone*-infected soybean plants (*Glycine max* [L.] Merr.) than from nonmycorrhizal plants (10). Foliar P content and yields were increased 100 and 40%, respectively, by the *Endogone* species. Phosphate fertilization on soils of medium P levels (22-44 kg/hectare) caused only small (10%) soybean yield increases (6, 9), and this " . . . failure to increase grain yields consistently through phosphorus fertilization has probably discouraged detailed studies" (8). This study attempts to relate previous soil fertility studies with soybeans to studies of mycorrhizal responses of other crops by examining the growth and yield of mycorrhizal and nonmycorrhizal soybeans provided with various soil P levels.

MATERIALS AND METHODS.—The experiment was conducted in eighteen 1- × 1.5-m fiberglass bins filled with Norfolk sandy loam (10). Twenty days prior to planting, the top 20 cm of soil in each plot was turned, and six equally spaced holes (15 cm diam) were dug 0.9 m to the clay subsoil. After 8 ml of chloropicrin were injected with a fumigun into the bottom of each hole, the plots were covered with polyethylene plastic, and 90 ml of methyl bromide were dispensed under the covering. Plots remained covered for 3 days.

Mycorrhizal plots were infested with 800 g of soil containing about 44,000 chlamydospores of an *Endogone* species (10). Intercellular hyphae of this species form arbuscules in cortical roots cells (Fig. 1) and vesicles which develop into chlamydospores. Chlamydospores also form on hyphae on the root surface and in the rhizosphere.

Original soil P levels (weak acid-extractable) in half the plots was 74 kg P/hectare; in the other half, 246 kg P/hectare. Super-triple phosphate (0-46-0) was applied at 44 kg P/hectare and 176 kg P/hectare, and some plots received no P. Treatments were divided as equally as possible among plots of the two original P levels. Three plots of each P fertilizer level were infested with *Endogone*; three were not infested. Soil pH ranged from 5.6 to 5.9, and 16.4 kg of K/hectare were applied as KCl to all plots.

Fertilizer and *Endogone* inoculum were mixed to a depth of 30 cm 2 days prior to planting. Cultivar Lee soybean seed were surface-disinfested in 1.5% NaOCl, dried, and treated with commercial *Rhizobium japonicum*. Each plot was planted on 28 May with two 1.5-m rows 51 cm apart. One month later, plants were thinned to 30/row.

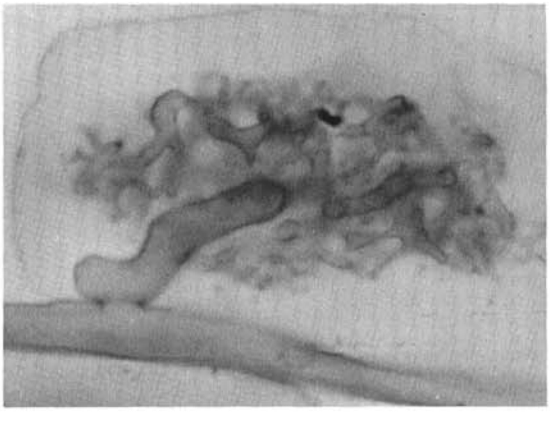

Fig. 1. Arbuscule of *Endogone* in a cortical cell of a soybean root. Note the intercellular hypha.

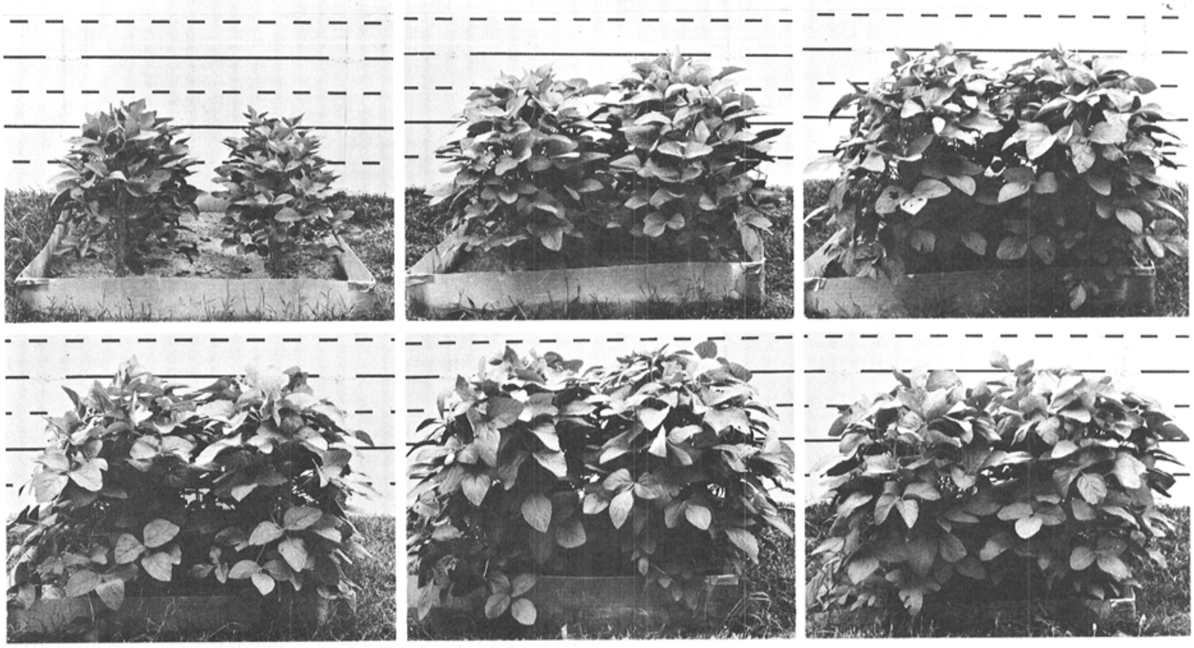

Fig. 2. Effect of *Endogone* on the response of soybeans to phosphate fertilization (7/14/70). Bottom row: *Endogone*-infested soil was added to plot soil; top row: noninfested plots. (Left to right) Plots were fertilized with no phosphate, 44, and 176 kg P/hectare.

Soil samples containing root fragments were taken to a 20-cm depth twice from each plot during the season. Root fragments extracted 10 weeks after planting were floated free, stained with acid fuchsin, and microscopically examined for structures of *Endogone*. After harvest, root fragments from 1,000-g soil samples were extracted by flotation and sieving. The roots were weighed and blended in water to free the chlamydospores which were recovered by a process of sieving and decanting. Spores in aliquots of the resulting suspension were counted, and the number of spores calculated per unit weight of root.

Data on elemental foliage composition, plant growth, and seed yield were obtained. Leaf samples were taken at random from the exposed foliage of plants in each plot 98 days after planting, and elemental analyses were performed by the Department of Soil Science, North Carolina State University, Raleigh. After maturity (27 October), plants were excised at ground level, air-dried, and weighed. Seed from each plot were threshed, cleaned, and weighed. Two hundred seed from each plot were weighed to obtain a measure of seed size. Analyses for the percentage of seed protein and oil were conducted by the U.S. Regional Soybean Laboratory, Urbana, Ill.

RESULTS.–Seven weeks after planting, nonmycorrhizal plants with no supplemental phosphate were noticeably smaller than those in other treatments (Fig. 2). Growth response to phosphate fertilization of nonmycorrhizal plants was greater than that of mycorrhizal plants, and the beneficial response to mycorrhizae was greatest in plants at low phosphate.

As the season progressed, differences in plant growth among treatments became more apparent. Leaves of nonmycorrhizal plants in low phosphate plots were abnormally yellow, whereas foliage of mycorrhizal plants was dark green.

Phosphate fertilization had little or no effect on the elemental composition of leaves from mycorrhizal plants (Table 1). In nonmycorrhizal plants, concentrations of N, P, and Ca in leaves increased with increasing phosphate fertilization. Concentrations of N, P, Ca, and Cu were greater in leaves of mycorrhizal plants at the lowest phosphate level than those in leaves from nonmycorrhizal plants at the highest phosphate level. Manganese concentrations tended to be greater in mycorrhizal than in nonmycorrhizal plants.

Differences in plant dry weight and seed yield between mycorrhizal and nonmycorrhizal plots decreased as phosphate levels increased (Table 2). At the lowest phosphate level, mycorrhizal plants outyielded nonmycorrhizal plants by 122%, whereas at the highest phosphate level, the 11.9% yield increase caused by the fungus was not statistically significant. Seed yield was correlated with dry weight of plants. Ratios of seed weight:total dry plant weight for mycorrhizal plants were larger than those for nonmycorrhizal plants (Table 2). This ratio for the mycorrhizal plants at the highest phosphate level was noticeably less than it was for the other mycorrhizal treatments. Seed from all mycorrhizal treatments were heavier than those from nonmycorrhizal plots; however, seed weight of the latter was not altered by the phosphate fertilization levels.

Except at the zero phosphate level, protein content of seed from nonmycorrhizal plants was less than

TABLE 1. Interaction of various phosphate fertilization levels and *Endogone* on the elemental composition of soybean leaves

Phosphate fertilization (kg P/hectare)	*Endogone*	Per cent[a]					μg/mg[a]		
		N	P	K	Ca	Mg	Fe	Cu	Mn
0	0[b]	3.23 a	0.10 a	1.30 a	0.49 a	0.15 a	231 a	6.0 a	306 a
0	+	4.37 c	0.21 c	1.50 a	0.71 c	0.18 a	258 a	10.8 c	379 a
44	0	3.50 a	0.14 b	1.50 a	0.55 b	0.13 a	225 a	5.9 a	305 a
44	+	4.23 bc	0.21 c	1.37 a	0.74 c	0.17 a	244 a	9.7 bc	389 a
176	0	4.06 b	0.16 b	1.53 a	0.61 bc	0.18 a	233 a	6.0 a	322 a
176	+	4.39 c	0.22 c	1.40 a	0.73 c	0.14 a	231 a	8.6 b	373 a

[a] Numbers with different letters within columns are statistically different (5% level).
[b] 0 = No *Endogone* sp. added. + = *Endogone* sp. added, 44,000 spores/plot.

that of seed from mycorrhizal plants (Table 2). Seed from mycorrhizal plants contained less oil than did seed from nonmycorrhizal plants (Table 2).

No *Endogone* structures were found in root samples of nonmycorrhizal plants taken on 4 August. In contrast, root fragments from *Endogone*-infested plots contained vesicles and arbuscules; on the outside, roots bore copious hyphae with attached vesicles and spores. Root samples collected on 17 November yielded 5,000, 4,950, and 4,000 chlamydospores/g of root tissue from mycorrhizal plots fertilized with 0, 44, and 176 kg P/hectare, respectively. These spore counts are not significantly different. Roots from six of nine plots not infested with *Endogone* averaged 450 chlamydospores/g.

DISCUSSION.–These results from field-simulated conditions confirm and extend the conclusions from earlier greenhouse experiments with a large variety of host species that VA mycorrhizae increase P uptake by plant roots (2, 3, 5). The 122% increase in seed yield from mycorrhizal plants at the lowest phosphate level verifies the earlier finding that *Endogone* plays an important role in promoting soybean growth and yield (10).

Initial levels of soil P in the present test were equal to, or far exceeded, those in experiments of previous workers who obtained only slight yield increases (less than 20%) from phosphate applications to soils with initial P levels of 40 to 90 kg/hectare (6, 9, 11). The failure to obtain larger soybean yield increases from phosphate applications in previous field tests has probably been due to the high P-absorbing efficiency of indigenous VA mycorrhizae on soybean roots in soil with low P levels.

The decrease in the seed weight:plant weight ratio from mycorrhizal plants at the highest rate of phosphate fertilization is suggestive of, and possibly related to, the report of Bureau et al. (1) which showed slight yield depressions with increasing phosphate fertilization. These results and the increasing yield responses from nonmycorrhizal plants associated with increasing P fertilization suggest that applications of phosphate to mycorrhizal soybeans may reduce soybean yields; whereas yields of slightly mycorrhizal or nonmycorrhizal soybeans would respond to phosphate fertilization if soil P levels were not excessive.

Since phosphate fertilization did not affect the size of seed from either mycorrhizal or nonmycorrhizal plants, this character may be insensitive to phosphate fertilization and to plant P levels. Since seed from mycorrhizal plants were larger and contained more protein than seed from nonmycorrhizal plants, the mycorrhizal relationship may benefit the host via avenues other than P nutrition alone. The very low yields from the low-phosphate non-

TABLE 2. Interaction of various phosphate fertilization levels and *Endogone* on growth and yield of soybean in fumigated plots (1 X 1.5 m)

Phosphate fertilization	*Endogone*	Seed[a]				Total plant wt[a]	Ratio seed yield: total plant wt
		Yield	Size	Protein	Oil		
kg P/hectare		*g*	*g/100 seed*	*%*	*%*	*g*	*%*
0	0[b]	540 a	14.1 a	41.8 a	22.5 b	1271 a	42.5
0	+	1202 d	15.1 b	42.4 a	21.6 a	2561 c	46.9
44	0	700 b	14.1 a	40.4 b	22.8 b	1752 b	39.9
44	+	1172 d	15.3 b	42.2 a	21.7 a	2492 c	47.0
176	0	940 c	14.2 a	40.2 b	22.7 b	2282 c	41.6
176	+	1052 cd	15.2 b	42.8 a	21.8 a	2406 c	43.7

[a] Numbers with different letters within columns are significantly different (5% level).
[b] 0 = No *Endogone* sp. added. + = *Endogone* sp. added, 44,000 spores/plot.

mycorrhizal plants probably induced seed with high protein content.

The positive correlation of foliage P with increasing applications of phosphate fertilizer in nonmycorrhizal plants, and the lack of such correlation in mycorrhizal plants, indicates that nonmycorrhizal soybean roots are relatively inefficient in absorbing phosphate. This theory is supported by the fact that P content in foliage of mycorrhizal plants at the lowest soil phosphate level was greater than in that of nonmycorrhizal plants at the high soil phosphate levels. Uptake of soil phosphate by mycorrhizal roots is attributed to extensive hyphae in the soil which absorb and translocate phosphate to roots. Within the plant, intercellular hyphae and arbuscules introduce phosphate into host processes (3).

The higher concentrations of Ca, N, and Cu in leaves of mycorrhizal plants may indicate that *Endogone* also promotes their uptake by soybean (10). A relationship between mycorrhizal development and processes related to N fixation has been suggested (10). However, since concentrations of Ca and N in nonmycorrhizal plants were directly related to phosphate fertilization, accumulation of these elements may be associated with increased phosphate absorption per se rather than with fungal activity.

Although the decrease in numbers of chlamydospores associated with increased phosphate fertilization was not statistically significant, the trend of less mycorrhizal development with increasing fertilization agrees with the results of previous workers (4, 7). Recovery of *Endogone* chlamydospores late in the season from some noninfested plots was probably due to the survival of some chlamydospores after soil fumigation. The level of infestation after fumigation, however, was too low to have any measurable effect on plant responses.

LITERATURE CITED

1. BUREAU, M. F., H. J. MEDERSKI, & C. E. EVANS. 1953. The effect of phosphate fertilizer material and soil phosphorus level on yield and phosphorus uptake of soybeans. Agron. J. 45:150-154.
2. GERDEMANN, J. W. 1968. Vesicular-arbuscular mycorrhiza and plant growth. Annu. Rev. Phytopathol. 6:397-418.
3. GRAY, L. E., & J. W. GERDEMANN. 1969. Uptake of phosphorus-32 by vesicular-arbuscular mycorrhizae. Plant Soil 30:415-422.
4. HAYMAN, D. S. 1970. Endogone spore numbers in soil and vesicular-arbuscular mycorrhiza in wheat as influenced by season and soil treatment. Brit. Mycol. Soc. Trans. 54:53-63.
5. HAYMAN, D. S., & B. MOSSE. 1971. Plant growth responses to vesicular-arbuscular mycorrhiza I. Growth of Endogone-inoculated plants in phosphate-deficient soils. New Phytol. 70:19-27.
6. KAMPRATH, E. J. 1964. Optimum soil fertility levels for soybeans and corn. Plant Food Rev. 10:4-6.
7. MOSSE, B., & G. W. JONES. 1968. Separation of Endogone spores from organic soil debris by differential sedimentation in gelatin columns. Brit. Mycol. Soc. Trans. 51:604-608.
8. OHLROGGE, A. J. 1960. Mineral nutrition of soybeans. Advances Agron. 12:229-263.
9. OHLROGGE, A. J., & E. J. KAMPRATH. 1968. Fertilizer use on soybeans, p. 273-295. *In* L. B. Nelson [ed.]. Changing patterns in fertilizer use. Soil Sci. Soc. Amer.
10. ROSS, J. P., & J. A. HARPER. 1970. Effect of Endogone mycorrhiza on soybean yield. Phytopathology 60:1552-1556.
11. WELCH, C. D., N. S. HALL, & W. L. NELSON. 1949. Utilization of fertilizers and soil phosphorus by soybeans. Soil Sci. Soc. Amer. Proc. 14:231-235.