

# Light Effects in Mycorrhizal Soybeans

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## ABSTRACT

Soybean (*Glycine max.* L. Merr.) plants were grown in an experiment with a  $3 \times 3$  factorial design using different levels of light (170, 350, and  $700 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) and P as factors. Plants were grown in a greenhouse in pot cultures using a soil low in plant-available P under three P regimes: no additional P, P added as  $\text{KH}_2\text{PO}_4$ , or P uptake enhanced by colonization of the host plant with the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum* (Thaxt. *sensu* Gerd.) Gerd. and Trappe. Development of the VAM fungal endophyte and of plants under all three P regimes was depressed by limiting light. However, the growth response of VAM plants to increasing light relative to non-VAM plants in the absence of additional P increased while the response relative to non-VAM plants with additional P decreased slightly. The highly significant interaction between the factors ( $P < 0.001$ ) of the experiment was due to differences in the magnitude and direction of simple effects of the factors. The implications of these differences in terms of source-sink relationships of the symbionts and the value of different non-VAM controls in interpreting VAM effects are discussed.

The cost-benefit relationship in the symbiotic association formed by plants and VAM<sup>1</sup> fungi appears to center on the dependence of the endophyte on C compounds derived from the host plants (5, 10, 18) and on modified P uptake by the root in the presence of VAM fungi (17). The controlling factors of this relationship are not well known (17, 19). Conditions inhibiting photosynthesis were shown to be detrimental to the development of the VAM-fungal endophyte (6, 8), but recently a more complex relationship had been observed, which was affected by host plant and fungal species as well as by light levels and P availability (7).

One problem in evaluating host reactions to colonization by VAM fungi is the difficulty in growing non-VAM controls which are physiologically and morphologically comparable to VAM plants. Most of the literature up to this time reports comparisons of VAM plants with non-VAM plants grown in the absence of additional P. Such non-VAM control plants are useful in accentuating differences in plant nutrition and development, but are inadequate as a physiological reference due to their stunted growth. Non-VAM plants provided with additional P to achieve a nutritional status equivalent to VAM plants are therefore preferable (18). However, such plants may not be true controls as they may lack plant responses to VAM infection (4).

The mechanisms by which the plant responds to sink demands for carbohydrates by the mycosymbiont are controversial (2, 20). Compensatory  $\text{CO}_2$  fixation to offset the additional C require-

ment of root symbionts has been observed under certain conditions (10, 15), but was ascribed recently to a lowering of dry matter in leaves, rather than to higher rates of photosynthesis in VAM plants (5, 18). Such a differential export of leaf C by VAM plants, however, is in turn controversial (13).

The present work was undertaken to determine the responses of host plants and their VAM fungal endophyte to conditions limiting photosynthesis and to relate these responses to non-VAM plants either in the absence of additional P, or treated with sufficient P to approximate VAM-plant dry weight.

## MATERIALS AND METHODS

**Biological Materials.** Soybean (*Glycine max.* L. Merr. cv Amsoy) seeds were selected for uniformity by weight and germinated for 3 d at  $28^\circ\text{C}$ . Two seedlings of equal radicle length were planted in plastic growth tubes (2.5-cm diameter and 12-cm long) in soil mixed with VAM-fungal inoculum or were left uninoculated as controls. Upon observing VAM-fungal infection in the roots of additional plants at 2 weeks, the seedlings and soil were transplanted from the growth tubes to pots.

The VAM fungus used was the Gerdemann isolate of *Glomus fasciculatum* (Thaxt. *sensu* Gerd.) Gerd. and Trappe (obtained from Abbott Laboratories). The amount used per pot consisted of  $20 \text{ cm}^3$  of soil containing approximately 60 spores and 140 heavily infected (80%) root fragments approximately 1-cm long. All controls were initially watered with washings ( $43 \mu\text{m}$  sieve) of the inoculum free of *G. fasciculatum*.

**Growth Conditions.** Plants were grown in 1.5 L white plastic pots in a greenhouse at Albany, California, July to September 1982. Temperature and RH varied within the day/night ranges of  $32^\circ/16^\circ\text{C}$  and 50/95%, respectively. Daylength was extended to 16 h by Sylvania<sup>2</sup> 1000-w metal halide lamps mounted vertically in parabolic reflectors. Plants were screened from the combined incidence of sunlight and supplementary light by different thicknesses of white cloth, to achieve maximum diurnal levels of PPFD of 700, 350, or  $170 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ . The growth medium was a medium textured, red brown, 'Josephine series' soil of low ( $4 \mu\text{g}$  P/g soil) available ( $\text{NaHCO}_3$ -extractable) P content. It was sterilized with ethylene oxide to inactivate native VAM-fungal propagules and was mixed with  $10 \text{ g CaCO}_3$  per pot to eliminate Mn toxicity to the plants. Soil pH after  $\text{CaCO}_3$  (1% w/w) addition was 6.9 (water extract). After transplant, some uninoculated plants (+P controls) received a nutrient solution initially  $0.2 \text{ mM}$  in  $\text{KH}_2\text{PO}_4$ . Other uninoculated plants (-P controls) and VAM plants were watered with the same nutrient solution less P. Phosphorus applied to +P controls was periodically increased to achieve similar growth in VAM and +P control plants at the highest level of PPFD used. Growth comparisons were made by counting leaves and measurement of leaf areas. All +P controls

<sup>1</sup> Abbreviations: PPFD, photosynthetic photon flux density; VAM, vesicular-arbuscular mycorrhizal.

<sup>2</sup> Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the United States Department of Agriculture.

(at all light levels) received nutrient solution of the same P concentration. The final P concentration was 1.0 mM. The nutrient solution consisted of 0.5 mM  $K_2SO_4$ , 0.25 mM  $MgSO_4$ , 2.0 mM  $NH_4NO_3$ , 1.5 mM  $CaCl_2$ , and 0.02 mM Fe supplied as FeEDDHA- (ferric ethylenediamine di-[*o*-hydroxyphenyl] acetic acid). Micronutrients were according to Johnson *et al.* (9) at one-quarter strength, less Mn. Excess nutrient solution was applied initially at 3-d intervals. The frequency of watering was increased as determined by demand of the growing plants.

**Evaluation and Assays.** Plants were harvested during the 12th week of growth over a 3-d period. Dry weights of plant parts were measured after drying at 70°C for 2 d. Plant P content was determined according to Allen (1). Individual P determinations were made for each replication at the 700  $\mu E \cdot m^{-2} \cdot s^{-1}$  PPFD level for leaves of the VAM plants and the +P and -P controls, and for the roots of VAM plants at all levels of PPFD. Confidence intervals were determined from these measurements. For the other treatments, replications were pooled. Available ( $NaHCO_3$ -extractable) P in the soil was determined according to Murphy and Riley (12) as modified by Watanabe and Olsen (22). Intraradical VAM-fungal biomass was determined by the chitin assay (2, 3) and per cent colonization of the host's root system was estimated from a large number of stained root segments as described previously (3). Determination of the extraradical fungal biomass (16) was not feasible in the soil used. Vesicle formation was estimated by counting the number of infected root segments and calculating the percentage of such segments which contained at least one vesicle.

The experimental design was 3 × 3 factorial, with three P regimes (VAM, +P control, -P control) and three PPFD exposures (170, 350, 700  $\mu E \cdot m^{-2} \cdot s^{-1}$ ). There were six replications per treatment for a total of 54 plants. The significance of differences between levels was calculated by Duncan's Multiple Range Test. The significance of main effects and interaction between factors was determined by analysis of variance on total plant dry weight. Six replications were used in all calculations, except for some of the P determinations which were pooled.

## RESULTS

**Effect of P Treatment and PPFD on the Plant.** Total plant, root, and leaf dry weights were not significantly different for +P controls and VAM plants at the highest light level ( $P > 0.05$ ). They were much lower for -P controls than for VAM plants at all light levels (Table I). At intermediate and low PPFD, roots of +P controls had significantly greater dry weights than those of VAM plants, while root dry weights of VAM plants and -P controls were not significantly different. The changes with PPFD in VAM-plant dry weights relative to +P or -P controls were different (Fig. 1). Total plant and leaf dry weights of VAM plants relative to +P controls decreased slightly ( $P > 0.05$ ) with increasing PPFD, while they increased relative to -P controls. Roots were larger in VAM plants relative to both controls with increasing PPFD. Root-to-leaf dry weight ratios increased with increasing light for all three P treatments (Table I). The ratios were highest for -P controls and lowest for VAM plants at all levels of PPFD. Changes in root-to-leaf dry weight ratios with increasing light were greatest in -P controls and similar in +P controls and VAM plants.

Leaf P concentrations tended to decrease with increasing PPFD and were significantly lower at the highest than at lower light levels for all three P treatments (Table II). A similar trend held for roots, with a significant decrease in P concentrations from the lowest to the highest level of PPFD in VAM and +P control plants. The -P controls had significantly lower concentrations in both root and leaf than VAM and +P controls. Differences in P content of VAM- and +P-control-plant leaves were not significant, while in the roots +P controls had signifi-

Table I. Dry Weights and Root/Leaf Ratios of Soybean Plants under Different P and Light Regimes

Plants were inoculated with the VAM fungus *Glomus fasciculatum* or treated with P in the nutrient solution (+P control) or left untreated (-P control). Three levels of PPFD were achieved by screening. Numbers are means of 6 replications. Numbers in a column followed by the same letter are not significantly different ( $P > 0.05$ ).

Phosphorus Treatment	PPFD		
	170	350	700
	$\mu E \cdot m^{-2} \cdot s^{-1}$		
	Leaf dry wt (g)		
VAM	2.07a	3.79a	5.49a
+P control	1.45b	3.27b	4.91a
-P control	0.81c	1.08c	0.96b
	Root dry wt (g)		
VAM	0.32a	0.92a	2.43a
+P control	0.50b	1.22b	2.68a
-P control	0.39ab	0.87a	1.12b
	Total plant dry wt (g)		
VAM	4.36a	9.16a	13.52a
+P control	3.58b	8.17b	12.45a
-P control	2.22c	3.35c	3.29b
	Root/leaf ratios		
VAM	0.15	0.24	0.44
+P control	0.34	0.37	0.55
-P control	0.48	0.80	1.17

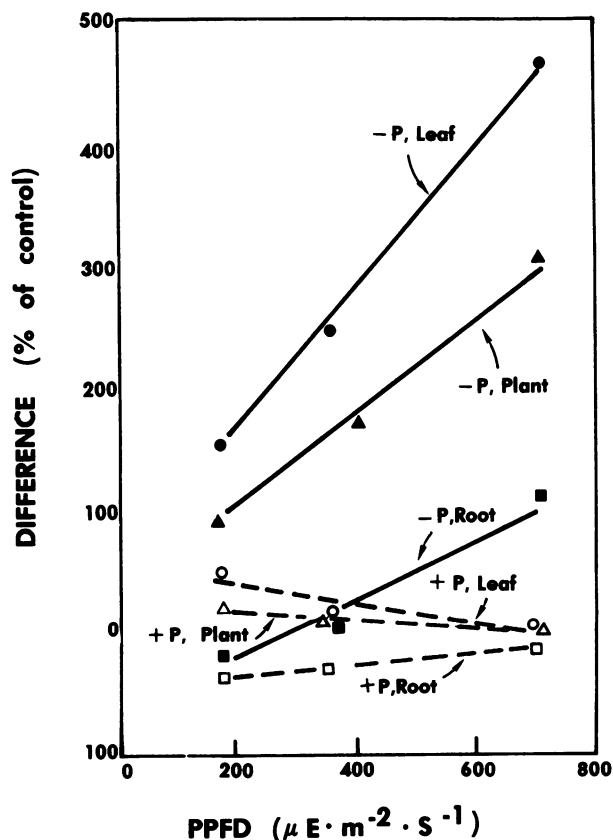


FIG. 1. Per cent change with PPFD in VAM plant parameters relative to non-VAM plants treated with additional P (+P control) or not treated (-P control). Per cent difference was calculated as  $([VAM \text{ plant} - \text{control}] / \text{control}) \times 100$ . The VAM plants were colonized by the fungus *Glomus fasciculatum*.

Table II. Phosphorus Content of Soybean Leaves and Roots under Different P and Light Regimes

Plants were inoculated with the VAM fungus *Glomus fasciculatum* or treated with P in the nutrient solution (+P controls) or left untreated (-P controls). Numbers followed by the same letter in a line (a,b) or column (x,y,z) are not significantly different ( $P > 0.05$ ).

Phosphorus Treatment	PPFD		
	170	350	700
	$\mu E \cdot m^{-2} \cdot s^{-1}$		
	Leaf P concentration (%)		
VAM	0.32ax	0.27ax	0.18bx
+P control	0.35ax	0.30ax	0.19bx
-P control	0.13ay	0.13ay	0.07by
	Root P concentration (%)		
VAM	0.20ax	0.18ax	0.11bx
+P control	0.17ay	0.14by	0.13bx
-P control	0.08az	0.07az	0.06ay

Table III. Analysis of Variance in Total Plant Dry Weights due to Light and Phosphorus Effects

Source	Degrees of Freedom	Sums of Squares	Mean Square	F	Significance Level
					%
Total	53	897.3			
Light (A)	2	359.9	179.9	291.9	0.1
Phosphorus (B)	2	385.3	192.6	312.5	0.1
A x B	4	124.4	31.3	50.5	0.1
Error	45	27.7	0.6		

Table IV. VAM Fungal Parameters of Soybean Plants Grown under Different Light Regimes

Per cent colonization of roots by the VAM fungus *Glomus fasciculatum* was determined by staining in large numbers of root segments. Relative degree of vesicle formation was estimated as the percentage of colonized root segments containing at least one vesicle. Chitin content of the VAM roots was measured colorimetrically. Total intraradical VAM-fungal biomass was calculated from the chitin content of isolated VAM fungal mycelium and of the VAM roots. Numbers are means of 6 replications. Numbers followed by different letters are significantly different ( $P < 0.05$ ).

VAM-Fungal Parameters	PPFD		
	170	350	700
	$\mu E \cdot m^{-2} \cdot s^{-1}$		
Colonization (%)	63.7a	84.5b	91.4c
Vesicle formation (%)	27.3a	56.1b	86.0c
Chitin/root (mg/g)	4.8a	6.5b	8.4c
Fungal biomass (mg/plant)	16.5a	67.3b	233.5c
Fungus/root ratio	5.1a	7.3b	9.6c

cantly less P than VAM plants at the two lower PPFD levels.

The analysis of variance of total plant dry weight showed highly significant main effects due to both factors and highly significant ( $P < 0.001$ ) interactions between the factors (Table III).

**Effect of PPFD on the VAM Fungus.** All VAM fungal parameters increased significantly with increasing light at all levels of PPFD (Table IV). Vesicle formation at high PPFD was 3-fold that at low light. While the chitin content of VAM roots and fungal biomass as per cent of VAM-root dry weight at high light were less than double that at low light, fungal biomass per host plant was four times greater at intermediate and 14 times greater at high light than at low light (Table IV). The relationships

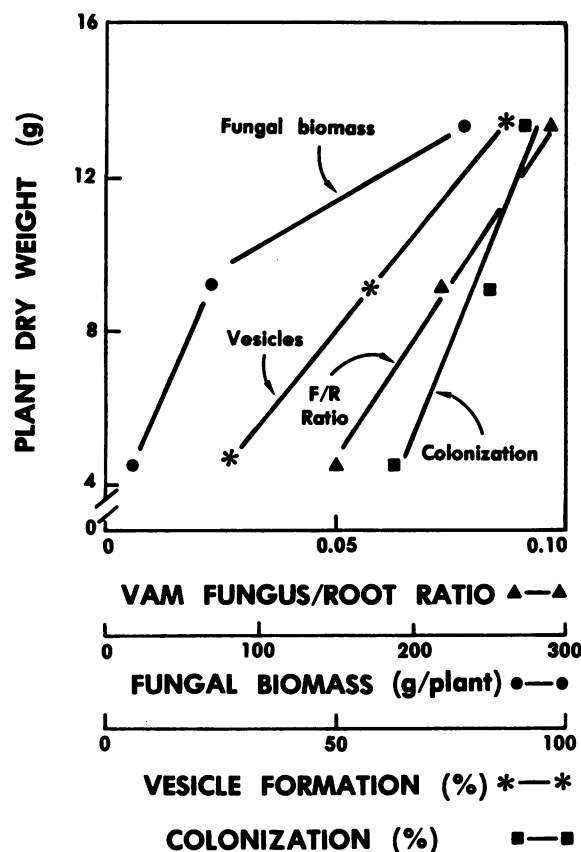


Fig. 2. Soybean plant dry weight as a function of VAM fungal development. Fungal parameters were calculated as in Table IV.

between plant dry weight and VAM fungal colonization, vesicle development, and the VAM fungus/root ratio were linear (Fig. 2). The rate of change in fungal biomass was greater between low and medium than between medium and high light (Fig. 2).

DISCUSSION

Light saturation of photosynthesis in soybeans varies widely with growth conditions and cultivars (14). In a preliminary experiment in our greenhouse using full sunlight plus supplementary light (PPFD  $1500 \mu E \cdot m^{-2} \cdot s^{-1}$ ) we found no significant differences in VAM plant development as compared to plants grown at one-half of that PPFD. To determine the effect of light stress on the VAM association, light levels of PPFD of  $700 \mu E \cdot m^{-2} \cdot s^{-1}$  or less were used in this experiment.

The decrease in growth enhancement in VAM plants relative to -P controls as light (photosynthesis) became more limiting (Fig. 1) supports the view that the endophyte acts on its host as a sink for carbohydrates (2). The contrary trend in VAM-plant values relative to +P controls (Fig. 1) suggests that VAM plants under low light may have an advantage over +P controls, which comparable plants under high light do not possess. The significant increase in VAM-plant dry weights at low and medium light over the +P controls (Table I) at comparable tissue (shoot + root) P concentrations (Table II) indicates a relationship that cannot be explained by viewing the VAM fungus as a simple P source and C sink. The changes in the root/leaf ratios with P treatment offer an explanation. Lowest ratios in VAM plants at all light levels signify a smaller investment by the VAM plant in root mass than that which must be expended by non-VAM plants to achieve the same uptake capability (Table I). The ability of a plant to apportion a greater share of its resources to growing leaves is expected to be increasingly advantageous as light be-

comes more limiting. This requirement is best satisfied in this experiment by VAM plants (lowest root/leaf ratios) and least by the -P controls (largest root/leaf ratios).

The difference in the P concentrations of VAM plants and +P controls at high and low light (Table II) may mean that under light (carbohydrate) stress P is more readily available to the plant in the absence of VAM fungi where the additional energy requirement of active transport of P (11) from endophyte to host (VAM plants) does not exist. The effect of the large increase in fungal biomass with increasing light on source-sink relationships within the symbiosis is difficult to evaluate, as measurement of the extraradical hyphae (16), the mineral-uptake organs of the endophyte, was not feasible in the soil used. Also, a portion of the intraradical mycelium consisted of vesicles (Table IV), which do not appear to be directly involved in P transfer to the host (17). The rapid development of vesicles with increasing light indicated a greater availability of carbohydrates for the formation of these storage organs (17) by the endophyte. Linear relationships between light and fungal or plant parameters (Tables I and IV) show that light has a direct effect on both symbionts by affecting the availability of photosynthate. It is likely that there is also an indirect, growth-stimulating effect of light on the host plant mediated by increased P uptake due to the enhanced development of the VAM-fungal endophyte (Fig. 2). The apparent difference in the rates of increase in fungal biomass between low and medium light and medium and high light (Fig. 2) may be the result of correspondingly greater increase in root mass at the lower than at the higher light levels (Table I). Thus, large increases in colonized root length are reflected importantly in VAM-fungal biomass (and effectiveness), even if per cent colonization over different periods of growth or different growth conditions remains small (21).

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