

Mycotrophic growth and mutualistic development of host plant and fungal endophyte in an endomycorrhizal symbiosis

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Summary Soybean plants colonized by the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum* were grown in pot cultures utilizing a composite greenhouse rooting medium. Development of fungal mycelia inside and outside the host root and total fungal biomass were determined from assays of fungal chitin. Growth and phosphorus uptake by VAM plants and uncolonized controls were compared. Mycotrophic growth in VAM plants occurred during the final six weeks of the 19-week growth period, when the concentration of available soil P fell below 10 µg P/g soil. Growth enhancement was most pronounced in the reproductive organs. The data suggest a relationship between the initiation of the reproductive phase in the host and the cessation of growth in the endophyte. Source-sink relationships and P availability appear to be factors influencing interactions between the symbionts.

Introduction

Response of the host plant to colonization by vesicular-arbuscular mycorrhizal (VAM) fungi depends on many biotic and environmental factors^{18, 21, 24, 25, 30}. Perhaps the most important of these is the availability of P^{13, 26} and most work on the VAM symbiosis centers on increased uptake of P by VAM fungi and concomitant growth enhancement of the host (mycotrophy) because of the practical implications of this phenomenon in agriculture¹². Unfortunately, the complexity of interacting factors in the agricultural ecosystem makes it difficult to pinpoint simple cause-effect relationships between the host plant and its VAM fungal endophyte^{8, 25}. Results of work carried out using pot cultures, where conditions of interest are under the experimenter's control, are more suitable to establish such relationships³⁰. Although such work has been generally found to be of limited predictive value to field conditions²⁵, it is important for a basic understanding of the physiology and ecology of VAM systems. One factor complicating the evaluation of reports on host response to VAM fungi grown in diverse, locally available soils or artificial substrates is the timing of the response during the ontogeny of the symbiotic association. Host response may be lacking or range from severe growth depression to marked enhancement during the symbiotic associations lifespan^{3, 4, 26} and a one-time harvest and assay may lead to incorrect inferences about the overall behavior of the system²⁹.

It therefore seemed worthwhile to determine the developmental patterns of the symbionts of a mycorrhizal association grown in a widely used and recognized

greenhouse potting mix, and to correlate these patterns with each other and with the change in P availability in the mix.

Materials and methods

Growth conditions

Soybean [*Glycine max* (L.) Merr. cv. Kent] plants were grown in 1.5 l plastic pots in a greenhouse at Berkeley, California, U.S.A., December 1980 through March 1981. Temperature and relative humidity varied from day to day within the day/night ranges of 32/15°C and 55/95%, respectively. Photosynthetic photon flux density (PPFD) averaged 450 $\mu\text{mole}/(\text{m}^2 \text{ s})$ (μE) at noon on sunny days and 280 μE on overcast days. Daylength was extended to 18 h by Sylvania 1000 W metal halide lamps mounted vertically in parabolic reflectors and arranged to provide supplementary PPFD of 400 μE at plant emergence level. The rooting medium was a University of California Type C soil mix^{16,17} amended to consist of the following components: 33% clay loam, 17% Canadian Sphagnum peat moss, 17% Sacramento River Delta peat soil, 25% fine (0.1–0.25 mm) sand, and 8% coarse (0.5–1.0 mm) sand. The soil mix had a pH of 6.8, and was amended by 1.3 kg/m^3 oyster-shell lime (CaCO_3), 0.6 kg/m^3 dolomite lime [$\text{MgCa}(\text{CO}_3)_2$], 0.5 kg/m^3 single superphosphate (18–20% available P_2O_5) and 0.2 kg/m^3 KNO_3 . Soil was steam sterilized. Pots were flushed once a week with deionized water, and five times a week with a nutrient solution of pH 6.7. The solution consisted of 1 mM CaCl_2 , 0.75 mM K_2SO_4 , 0.25 mM MgSO_4 , 2 mM NH_4NO_3 , and micronutrients equivalent to one-quarter strength Johnson's solution¹⁴.

Seeds were germinated for 2 days at 28°C. Seedlings were selected for uniformity and either inoculated at planting with 10 g of soil containing approximately 60 spores of the Gerdemann isolate of *Glomus fasciculatum* (Thaxt. *sensu* Gerd.) Gerd. and Trappe, or left uninoculated as controls. The inoculum (obtained from S. Woodhead, Abbott Laboratories, Long Grove, Illinois, U.S.A.) also contained approximately 90 root segments 0.3 to 1.0 cm in length and 80% colonized by *G. fasciculatum*. Control plants were initially watered with root- and spore-free washings (43 μm sieve) of the inoculum. Pots were rotated frequently to minimize positional effects. Plants were harvested at 2- or 3-week intervals. Five replications were used.

Assays

Removal of the mycorrhiza from the soil separated much of the extraradical VAM fungal mycelium from the intact VAM structure. Mycorrhizal roots were washed thoroughly over a 43 μm sieve to remove remaining extraradical hyphae and spores from the root surface. The sievings were examined microscopically for spores and returned to the soil, which was mixed thoroughly and stored moist at 4°C until analysis. Host-plant roots which included the intraradical mycelium were used to estimate the percentage of VAM fungal colonization histologically and to determine the intraradical VAM fungal biomass by analyzing host roots for chitin. The histological assay consisted of staining 50 randomly selected, 1-cm root segments and determining the level of infection over the length of each segment microscopically⁶. The chitin assay was based on a quantitative determination of the degradation products of this fungal cell-wall constituent spectrophotometrically⁶. Control roots were subjected to the same analyses as VAM roots to check for colonization or to account for contamination by chitin-containing organisms other than *G. fasciculatum*. Average chitin values thus obtained for the five controls were subtracted from the chitin value of each of the five replicates of mycorrhizae. Extraradical VAM fungal biomass was determined by analyzing the soil for chitin according to Pacovsky and Bethlenfalvay²². The biomass of VAM fungal mycelium was expressed as dry mass⁶.

Plant parts were weighed after drying at 80°C for two days. Dry plant roots and shoots (leaf, stem and pods) were ground in a Wiley Mill (40-mesh screen). Percent P content was subsequently determined by the molybdenum blue method after digestion with perchloric acid¹. Replications of

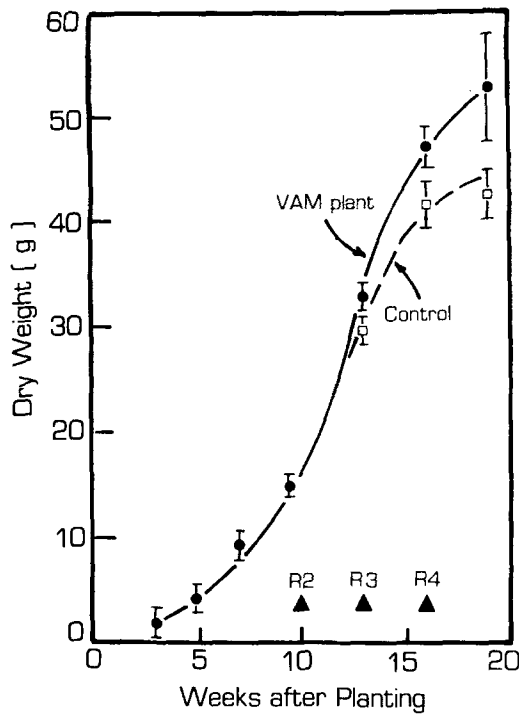


Fig. 1. Dry weights of soybean plants colonized by the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum* or left uninoculated as controls. Annotations along abscissa represent soybean plant reproductive stages as defined by Fehr¹¹ and denote the full bloom (R2), beginning pod (R3) and full pod (R4) stages. Data points for VAM and control plants through week 10 were not significantly different ($P > 0.05$).

plant materials of the same harvest were pooled for the P assay, after intra-replicate variation was determined to have standard deviations less than 5%. Percent-P values of the pooled replications were taken to represent the means. Statistical significance ($p < 0.05$) in percent P between VAM and control plants was computed from the difference of means and standard deviations. Available P in the soil was determined by the NaHCO_3 -extraction method^{20,32}, with replicates pooled as above. Mycorrhizal biomass (g) was defined as the host-plant root system including the extraradical and intraradical fungal mycelia. It was determined by adding the biomass of the extraradical mycelium calculated from the soil-chitin assay²² to the measured dry weight of host-plant root including the intraradical mycelium. Host plant root tissue alone was determined by subtracting the calculated dry mass of the intraradical mycelium⁶ from the dry weight of the host-plant root plus intraradical mycelium.

Results

Development of the host plant

Significant growth enhancement in terms of total dry matter was first observed 13 weeks after planting (Fig. 1). Individual plant parts showed different response patterns to VAM fungal colonization than the entire plant (Table 1). Host plant

Table 1. Dry weights of soybean plants colonized by the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum* or left uncolonized as controls

Growth stage ^{1,1}	Time planting week	Plant dry weight g							
		Leaf ^a		Pod		Shoot ^b		Root	
		VAM	Control	VAM	Control	VAM	Control	VAM ^c	Control
V3	3	1.06	1.05	-	-	1.53	1.55	0.53*	0.47
V6	5	1.79	1.75	-	-	2.96	2.86	1.03*	0.91
V8	7	3.29	3.15	-	-	6.49	5.93	2.21**	1.94
V12, R1	10	5.00	4.82	-	-	10.68	10.19	3.74**	3.32
R3	13	10.62	10.07	0.70	0.61	24.16*	22.39	8.07***	7.29
R4	16	13.37	12.42	7.51**	5.85	37.53**	32.79	9.50	9.03
R6	19	-	-	17.48**	12.52	44.38**	32.48	10.46	10.06

^a Plants at week 19 were senescent. Leaves not abscised at this harvest were included in shoot weights.

^b Shoots include leaf, pod and stem weights.

^c VAM root dry weights include the biomass of the intraradical mycelium.

*, ** and *** denote significant differences between VAM and control parts by one-tailed t-test at the $P < 0.05$, $P < 0.01$ and $P < 0.001$ levels, respectively.

Table 2. Leaf to root dry-weight ratios of soybean plants colonized by the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum* or left uncolonized as controls

Leaf/root ratio	Time after planting, week					
	3	5	7	10	13	16
VAM plant ^a	1.87***	1.60**	1.35**	1.23**	1.28*	1.26 NS
Control	2.24	1.89	1.62	1.46	1.38	1.29

^a Root dry weights of VAM plants include extra- and intraradical VAM fungal biomass.

***, **, * and NS denote statistical significance by one-tailed t-test at the $P < 0.001$, $P < 0.01$, $P < 0.05$ levels, respectively.

roots (including the intraradical mycelium) had significantly higher dry weights than control roots up to the full pod (R4) stage¹¹ of soybean development. Shoots of VAM plants, on the other hand, did not develop significantly more dry matter than controls until the beginning pod (R3) stage (week 13). Leaves of VAM plants were not significantly different from those of the controls throughout the time course, but differences between VAM- and control-plant pods past the beginning pod (R3) stage were highly significant.

Parts of VAM plants had greater dry weights than those of control plants during the entire experimental period, but enhancement of the pods (Fig. 2) and of the mycorrhizae (Fig. 3) was most marked. Enhancement of the mycorrhizae (host plant root plus extra- and intraradical mycelium) relative to control roots was maximal at the onset of flowering and declined thereafter (Fig. 3). Development of host-plant root tissue alone (extra- and intraradical VAM fungal

Table 3. Phosphorus content of soybean plants colonized by the vesicular-arbuscular mycorrhizal VAM fungus *Glomus fasciculatum* or left uncolonized as controls

Time after planting week	Phosphorus, %			
	Root		Shoot ^b	
	VAM ^a	Control	VAM	Control
3	0.23*	0.27	0.29	0.29
5	0.17*	0.21	0.22*	0.25
7	0.15*	0.17	0.18*	0.20
10	0.14	0.14	0.18*	0.20
13	0.11	0.11	0.12*	0.14
16	0.10	0.09	0.07	0.08
19	0.10*	0.07	0.05	0.06

^a Numbers represent % P of host-plants roots including the intraradical mycelium.

^b Shoots include leaves, stem and pods.

* Significant differences between VAM- and control-plant parameters at the $P < 0.05$ level.

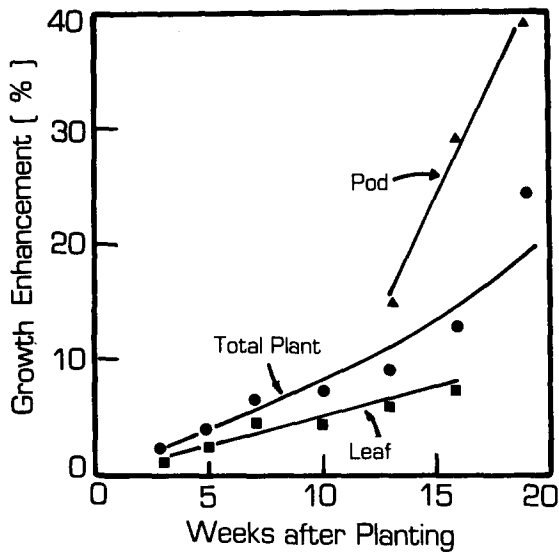


Fig. 2. Host-plant growth response to colonization by *Glomus fasciculatum*. Enhancement of host biomass production was calculated as $[(\text{host} - \text{control})/\text{control}] \times 100$.

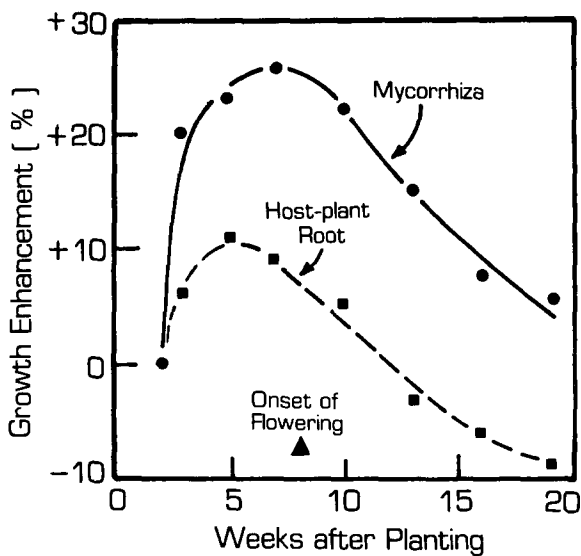


Fig. 3. Response of soybean plant root systems to colonization by *Glomus fasciculatum* relative to uninoculated controls. Mycorrhizal values included the extra- and intraradical VAM-fungal mycelia. Values for the host plant root system alone excluded all VAM-fungal mycelia. Fungal biomass was calculated from chitin analysis. Enhancement of the mycorrhizal root system or the host-root tissues alone was calculated as $[(\text{host} - \text{control})/\text{control}] \times 100$, or $[(\text{mycorrhiza} - \text{control})/\text{control}] \times 100$.

biomass subtracted) was enhanced initially, but became negative relative to control roots between full bloom and early pod development (weeks 10 and 13, Fig. 3). Leaf to root ratios were significantly lower in VAM than in control plants between weeks 3 and 13 (Table 2).

Percent P in VAM-plant roots was significantly lower than in controls during the first 7 weeks and in the shoots during weeks 5 to 13 (Table 3). At the last harvest VAM plant roots had significantly higher P concentration than controls. Extraradical VAM fungal mycelium had a P concentration of 0.24%. Total P per plant was not significantly different ($P > 0.05$) between VAM and control plants up to the harvest at week 13 (Fig. 4). Available soil P decreased over the time course from 34 to less than 4 $\mu\text{g P/g}$ soil. At week 19, the concentration of available P in control-plant soil was at approximately twice that in VAM-plant soil (Fig. 4).

Development of the endophyte

Colonization of soybean roots by *G. fasciculatum* started between weeks 2 and 3 after planting and reached a plateau of approximately 70% infection after week 13 (Fig. 5). Total (extra- and intraradical) fungal biomass (Fig. 6) had a developmental pattern similar to that of VAM fungal colonization. There was more extra- than intraradical mycelium initially, but this situation was reversed after week 10 (Table 4). This development coincided with flowering (R2) in the host plant (Fig. 1). At the final harvest, intraradical VAM fungal biomass was almost five times that of the extraradical component. Fungal biomass as a percentage of the mycorrhiza was maximal following full bloom (R2, week 10) and declined thereafter (Fig. 6). Flowering and the onset of sporulation coincided.

Table 4. Biomass of extra- and intraradical mycelia of the vesicular-arbuscular mycorrhizal fungus *Glomus fasciculatum* in soybeans

Time after Week	Fungal biomass ^a , g	
	Extraradical mycelium	Intraradical mycelium
3	0.041 ± 0.005	0.004 ± 0.001
5	0.089 ± 0.038	0.017 ± 0.003
7	0.229 ± 0.029	0.092 ± 0.018
10	0.314 ± 0.093	0.236 ± 0.048
13	0.257 ± 0.036	0.899 ± 0.068
16	0.249 ± 0.049	0.931 ± 0.105
19	0.204 ± 0.025	0.983 ± 0.209

^a Determined by the chitin assay and calculated from chitin/extraradical mycelium ratios. Numbers represent means and standard deviations of replications.

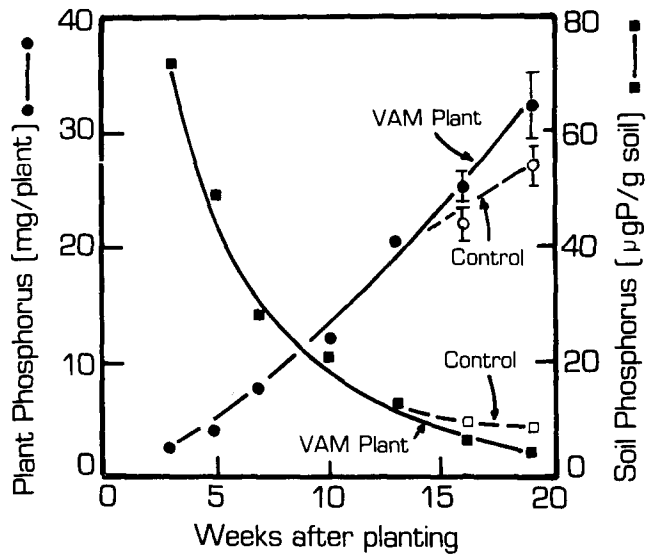


Fig. 4. Phosphorus content of soybean plants colonized by the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum* or of uncolonized controls, and of the soil in which the associations were grown. Plant P values included total P of the extraradical mycelium. Values for soil P represent available (NaHCO_3 -extractable) P concentrations. Values of plant and soil P for VAM and control plants were not significantly ($P > 0.05$) different up to 13 weeks.

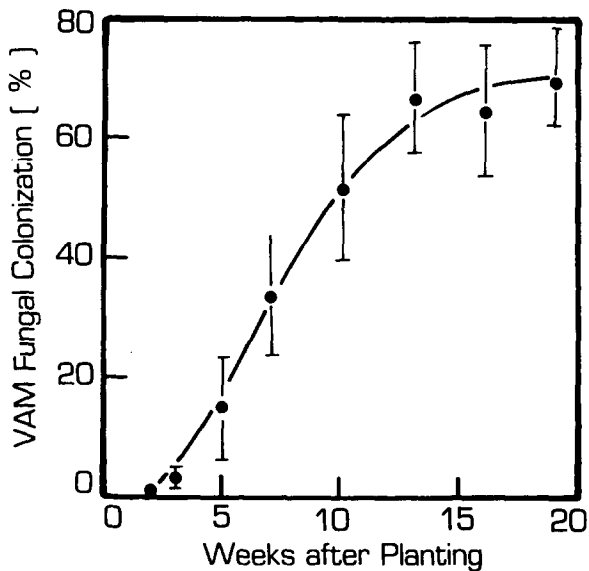


Fig. 5. Percent colonization of soybean-plant roots by the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum*. The extent of colonization was estimated from large samples of randomly selected, stained root segments.

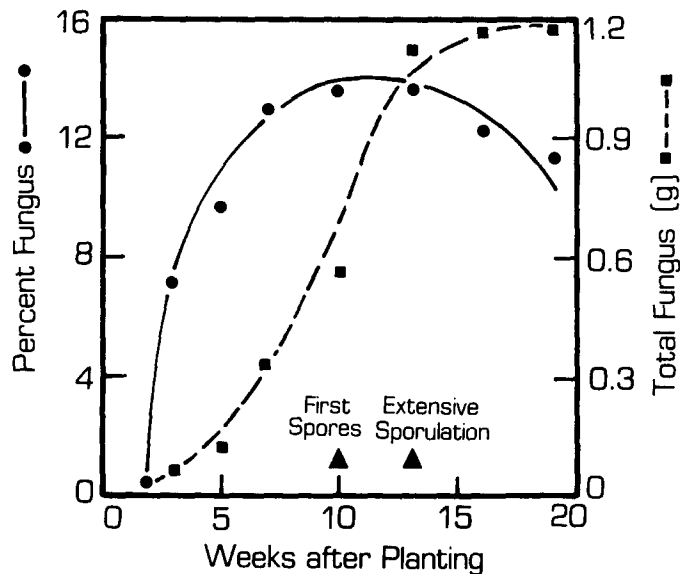


Fig. 6. Development of *Glomus fasciculatum* in soybean roots. Total (extra- and intraradical) vesicular-arbuscular mycorrhizal (VAM) fungal biomass was calculated from the chitin content of fungal structures. Percent fungus represents the ratio of total fungal biomass to mycorrhizal dry weight.

Discussion

Host plant growth response to VAM fungal colonization varies widely in its timing. In soybeans, growth enhancement was observed as early as six weeks after planting¹⁰ or delayed to 10 weeks following growth inhibition³. While growth response is generally related to soil nutrient levels¹⁹, relationships between growth stage and development of the symbiotic partners are rarely reported^{27,30}. The juxtaposition of host and endophyte developmental patterns presented here do not represent cause-effect relationships, yet are indicative of interactions between the symbionts.

Enhancement of total dry weight (Fig. 1) and P (Fig. 4) at later stages of plant development appeared to be related to the decline in available P in the soil³ (Fig. 4). Flowering, between weeks 8 and 10 marked the turning point in the initial enhancement of mycorrhizal roots relative to controls (Fig. 3) and also coincided with beginning spore formation (Fig. 6). Attempts have been made to connect physiological changes in the host, such as flowering, with developmental stages of the endophyte, such as the rapid-spread phase⁹, but without subsequent verification⁷. Such interactions, are likely to vary with different host-endophyte combinations, and to depend on the timing of changes in source-sink relationships. Pod development showed the greatest enhancement (Fig. 2) and thus may be regarded as a significant factor in determining intersymbiont relationships. The early pod stage (R3), during which a powerful new sink was

initiated, coincided with the cessation of VAM fungal growth (Fig. 6) and of colonization (Fig. 5). Thus initiation of the reproductive phase in the host plant, which in soybeans is related to a self-destructive mobilization of nutrients from the vegetative structures²⁸, appears to be a factor controlling growth of the VAM fungal endophyte. It is possible that this control is exerted through the apportioning of nutrients. The present data support similar observations made previously in a hydroponic system utilizing available P in the nutrient solution⁴. Decreased allocation of photosynthate to the endophyte is a likely cause for the decrease in the proportion of VAM fungus to total mycorrhizal biomass after week 10 (Fig. 6). The onset of sporulation, however, has not been correlated with carbohydrate stress in previous work¹⁵. The significant enhancement of VAM plants (Fig. 1) at a stage of development when both host-plant root tissue (Fig. 3) and the extraradical mycelium (Table 4) declined indicates that the extent of the uptake organs for nutrients is less crucial for mycotrophic growth than the availability of nutrients. In the soil used here, P availability below 10 µg P/g soil (week 10) was the threshold for mycotrophy (Fig. 4), as was previously observed for a sand-perlite medium³. Up to this time P concentration in control plants was significantly higher than in VAM plants (Table 3), even though total P was not significantly different (Fig. 4) due to the higher biomass of the latter (Table 1). With decreasing available-P concentrations in the soil, P concentrations in VAM control plants equalized and at week 19 VAM-plant roots had a higher concentration of P than controls. This shift in P concentrations in favor of the mycorrhizal system with decreasing P availability may be indicative not only of its higher efficiency in P uptake when P levels are low³¹, but also of the advantage the non-mycorrhizal system may have when P is readily available⁵. Leaf/root ratios in VAM plants were significantly lower than in controls up to week 13 (Table 2) as a result of greater mycorrhizal biomass, while leaf masses remained equal (Table 1). As the fungal endophyte represents an additional sink for photosynthates, VAM plants may have compensated for the increased carbohydrate demand²³ by higher rates of photosynthetic production².

It is concluded that the developmental patterns and interactions observed in soybean plants colonized by *G. fasciculatum* and grown under the conditions described can be explained by an interplay of P and photosynthate availability and allocation.

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