

Parasitic and Mutualistic Associations Between a Mycorrhizal Fungus and Soybean: Development of the Host Plant

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We thank L. A. Andres and A. W. Thayer of the Biological Control of Weeds Facility, USDA, Albany, CA 94796.

for providing plant-growing facilities and advice on pest control for this project. We are indebted to G. Secor for making plant phosphorus analyses.

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Accepted for publication 11 December 1981.

ABSTRACT

Bethlenfalvay, G. J., Brown, M. S. and Pacovsky, R. S. 1982. Parasitic and mutualistic associations between a mycorrhizal fungus and soybean: Development of the host plant. *Phytopathology* 72: 889-893.

Soybean plants (*Glycine max* 'Kent') inoculated with the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatus* were grown in 1.5-L pots in a perlite/sand rooting medium containing 200 mg of hydroxyapatite ($\text{Ca}_{10}[\text{PO}_4]_6[\text{OH}]_2$) as the source of P. Plants were harvested at 2- or 3-wk intervals for 18 wk. Fungal biomass as percent total dry weight (of the host and symbiont) was calculated from measurements of fungal chitin and had a maximum value of 7.3% at 6 wk after planting. Total and percent P content of VAM-infected and uninfected control plants were determined, and the decrease of available (NaHCO_3 -extractable) P in the rooting medium was monitored. As a result of development of the

endophyte, growth inhibition of VAM plants relative to the controls increased up to the ninth wk. It was reversed after 9 wk, and, after 15 wk, VAM plants had significantly greater dry weight and P contents than the controls. The data suggest that early growth inhibition was a result of carbohydrate demand on the host by the endophyte, at a time when the shoot-to-root ratio and photosynthetic source capacity of the host were low. Available P in the rooting medium of VAM plants decreased to 10 μg P/g rooting medium during the 3 wk preceding the reversal in growth inhibition, indicating that mycotrophic growth of the host induced by increased P uptake by the endophyte commenced at this P concentration.

"Pathogenesis can be viewed as a battle between a plant and a pathogen refereed by the environment," and may be broadly defined as a malfunction that results in unsatisfactory plant performance (26). In the mutualistic, vesicular-arbuscular mycorrhizal (VAM) association, where the host plant and its fungal endophyte live together in an intimate, balanced relationship, pathological symptoms occur only when the balance is disturbed (12). Most workers agree that the environmental factor primarily responsible for tipping the balance in endomycorrhizae towards growth inhibition or growth enhancement of the host is the availability of P (3,11,18). Growth enhancement occurs when P levels are low ("mycotrophy," see ref. 8), and VAM fungal colonization is inhibited when P levels are high (6). At intermediate levels of P availability, a shift was observed from growth reduction at first to enhancement (7). Growth reduction in the host plant was associated with soil fertility levels high enough to render the mycorrhizae superfluous, but not so high as to inhibit infection (5). Reduction of host plant biomass as a result of VAM fungal infection has been ascribed to P toxicity (17), temperature effects (10) or to demands made upon the host plant for photosynthate by the endophyte (9,11). However, the role of VAM fungi as a significant sink for photosynthesis is controversial (4,23).

The purpose of this investigation was to determine the reaction of soybean plants to infection by the VAM fungus *Glomus fasciculatus* (Thaxt. sensu Gerd.) Gerd. and Trappe (13) under conditions of P availability that favored fungal development in nonmycotrophic plants and to assess the effect of fungal biomass on host development and P nutrition. An inert (sand/perlite) rooting medium was chosen for this experiment to minimize soil-P interactions and to focus on host-endophyte growth relationships. This report discusses effects of the symbiosis on the entire host

plant. Effects on the endophyte and the mycorrhiza (infected host root) are treated in a companion article (2).

MATERIALS AND METHODS

Growth conditions. Soybean (*Glycine max* (L.) Merr. 'Kent') plants were grown in 1.5-L white plastic pots in a greenhouse at Albany, CA, September 1980 to January 1981. Temperature and relative humidity varied seasonally and from day to day within the day/night ranges of 28/15 C and 50/95%, respectively. Photosynthetic photon flux density (PPFD) averaged 500 $\mu\text{einsteins}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$ (μE) at noon on sunny days and 300 μE on overcast days. Daylength was extended to 18 hr by Sylvania 1,000-W metal halide lamps mounted vertically in parabolic reflectors and arranged to provide supplementary PPFD of 400 μE at plant emergence level. The growth medium consisted of 1.25 L of perlite/sand mixture (2:1, v/v) covered by a 2.5-cm layer of perlite. This was watered with a nutrient solution (pH 6.2), which was equivalent to 0.25-strength Johnson's solution (15) except in P. Macronutrients in the solution consisted of 1 mM CaCl_2 , 0.75 mM K_2SO_4 , 0.25 mM MgSO_4 , and 2 mM NH_4NO_3 . The concentration of Fe was 20 μM supplied as ferric ethylenediamine di-(*o*-hydroxyphenyl) acetic acid. Phosphorus was added as 200 mg of hydroxyapatite ($\text{Ca}_{10}[\text{PO}_4]_6[\text{OH}]_2$) mixed with the rooting medium at planting. 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 50 spores of *G. fasciculatus*, or left uninoculated as controls. The inoculum (obtained from S. Woodhead, Abbott Laboratories, Long Grove, IL 60047) also contained approximately 110 root segments less than 1 cm long and 60–80% infected with *G. fasciculatus*. Control plants were initially watered with root- and spore-free washings (43 μm sieve) of the inoculum. Washings were from an amount of soil equivalent in weight to the inoculum used. Pots were rotated regularly to minimize positional effects. Plants were harvested at 2 or 3 wk intervals. Plants were senescing towards the end of the experiment. Only live and attached leaves were weighed and analyzed at each harvest.

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Assays. Fungal biomass external and internal to the host-plant root was calculated according to Bethlenfalvay et al (2). Plant parts were dried for 1 day at 80 C, weighed and ground in a Wiley mill fitted with 0.50-mm (40-mesh) screen. Plant P content was determined colorimetrically by the molybdenum-blue method after digestion with perchloric acid (1). Total P in each component of the potting medium was determined at planting and in the mixed medium after each harvest according to Shelton and Harper (21). Available (NaHCO_3 -extractable) P was determined according to Murphy and Riley (19) as modified by Watanabe and Olsen (25). Differences in dry weight and total P content between VAM and control plants were calculated as percent of control $([\text{VAM plant} - \text{control}]/\text{control}) \times 100$.

RESULTS

Effect of VAM fungal infection on host plant biomass. No infection of soybean plants by *G. fasciculatus* was observed in stained (3) roots 2 wk after planting. Fungal biomass relative to total host plant dry weight was maximal (7.3%) at 6 wk and declined thereafter (Fig. 1). Dry weights of VAM plants were significantly lower than those of control plants between 4 and 5 wk of growth (Fig. 2). After 16 wk VAM plants significantly exceeded the controls in dry weight. Growth inhibition in VAM plants increased for 9 wk (Fig. 3) as compared with controls (Fig. 1). After 9 wk a reversal occurred; the percent difference between VAM and control plants decreased. After 16 wk, growth enhancement was observed as compared with controls (Figs. 1 and 2). Shoots and roots of VAM plants showed an identical pattern of growth inhibition between 4 and 15 wk (Table 1). At 18 wk, the shoots of VAM plants had significantly greater dry weight than those of the controls. The shoot plus pod to root dry weight ratio increased linearly more than twofold between 4 and 18 wk in VAM plants (Fig. 4) and was similar to that of the controls throughout the

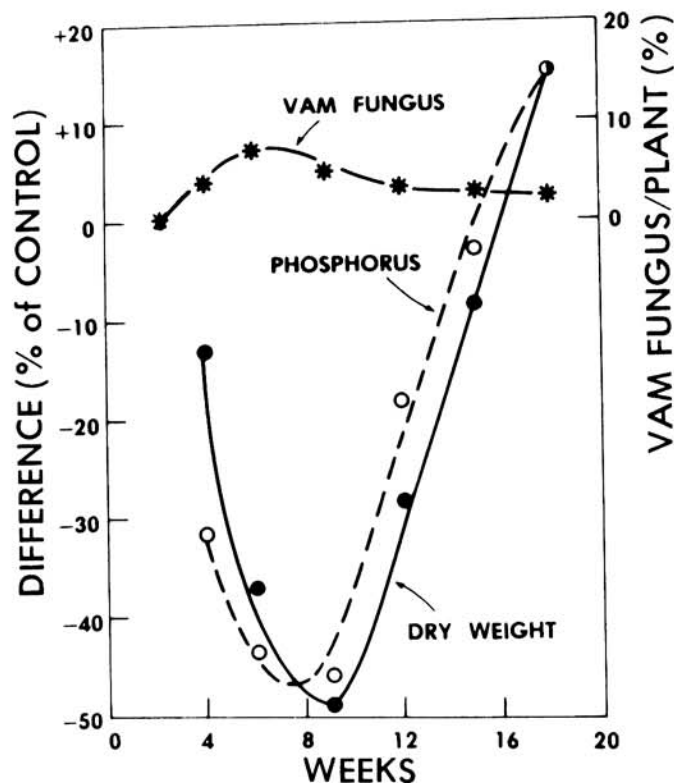


Fig. 1. Percent difference between Kent soybeans inoculated with the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatus* and uninoculated controls, and fungal biomass as percent of association dry weight. Difference for dry weight or total P content was computed as percent of control $([\text{VAM plant} - \text{control plant}]/\text{control plant}) \times 100$. VAM fungal biomass external and internal to host roots was calculated from chitin analysis.

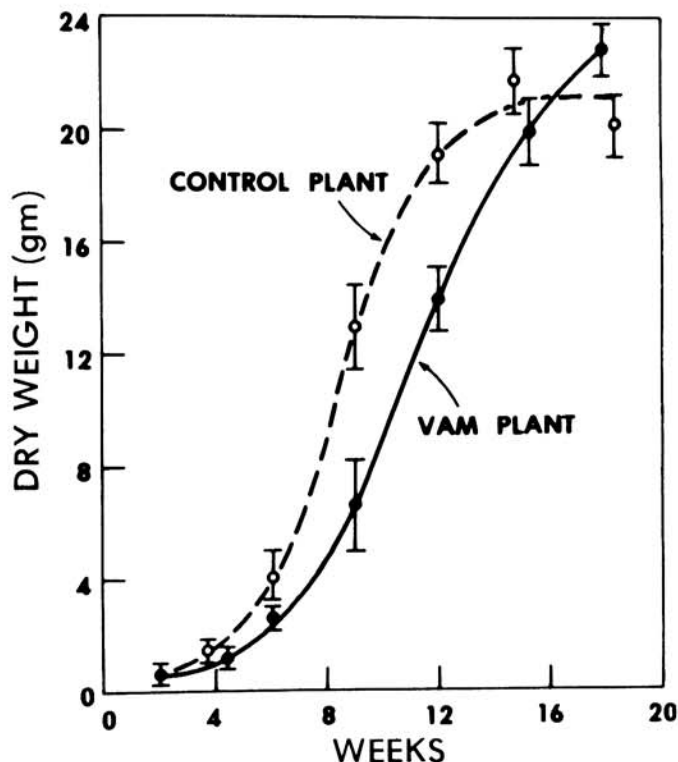


Fig. 2. Development of Kent soybeans inoculated with the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatus* and of uninoculated control plants.



Fig. 3. Comparison of a 9-wk-old Kent soybean plant inoculated with the vesicular-arbuscular mycorrhizal fungus *Glomus fasciculatus* with an uninoculated control plant. Plants were grown in 15-cm-diameter pots.

growth period (not shown).

Effect of VAM fungal infection on plant P content. Percent P in the shoots of the controls was significantly higher than in VAM plants during the first 6 wk of growth but was not different thereafter (Table 2). In the roots of VAM plants percent P was significantly lower after 4 wk, the same after 6 wk, and significantly higher after this period than in the controls. Total P in the entire plant followed the same pattern as dry weight, except that VAM plants were significantly lower in P than controls between 4 and 15 wk (Fig. 5). The shoot plus pod to root P ratios for controls were higher than for VAM plants and their increase was nonlinear over

TABLE 1. Plant part dry weights of Kent soybeans inoculated with the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatus* and of uninoculated controls during host-plant ontogeny

Week	Treatment	Dry weight (grams per plant part) ^a		
		Shoot	Root	Pod
4	VAM	0.8 ± 0.2 NS	0.5 ± 0.1 NS	...
	Control	0.9 ± 0.1	0.5 ± 0.1	...
6	VAM	1.7 ± 0.3***	0.9 ± 0.2*	...
	Control	2.7 ± 0.6	1.4 ± 0.5	...
9	VAM	4.6 ± 1.3***	1.9 ± 0.4***	0.03 ± 0.01
	Control	9.0 ± 1.2	4.0 ± 0.5	0.05 ± 0.02
12	VAM	10.1 ± 0.9***	3.7 ± 0.4***	0.11 ± 0.04*
	Control	13.5 ± 1.1	5.2 ± 0.7	0.18 ± 0.04
15	VAM	14.2 ± 1.0*	4.8 ± 0.7 NS	1.0 ± 0.3 NS
	Control	15.3 ± 0.8	5.2 ± 0.5	1.2 ± 0.3
18	VAM	13.3 ± 1.2*	5.0 ± 0.2 NS	4.9 ± 0.3 NS
	Control	10.9 ± 1.8	4.9 ± 0.5	4.4 ± 0.3

^a Values are means and standard deviations of five replications. Asterisks *** and * indicate statistically significant differences $0.001 < P < 0.01$ and $P < 0.05$, respectively, by Student's *t*-test. NS indicates no statistical significance ($P > 0.05$).

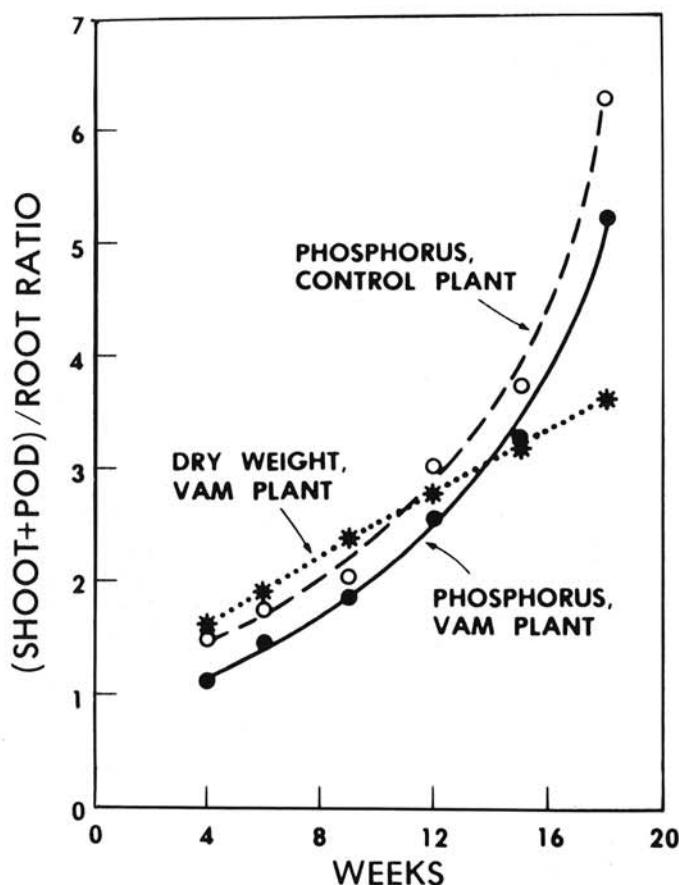


Fig. 4. Ratios of plant parts for dry weight and total P content in Kent soybeans inoculated with the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatus* or in uninoculated controls.

the experimental period (Fig. 4).

Phosphorus availability. Total P contained in the seed, hydroxyapatite, sand, and perlite was initially 1.0, 37.0, 16.5, and 0.3 mg per pot, respectively. Only a small fraction (1.5 mg) of the P contributed by the sand was available (NaHCO_3 -extractable) P. Hydroxyapatite equilibrated with nutrient solution to yield a P concentration of $3 \mu\text{g P/ml}$ solution within the pH range of 5 to 7. Available P ($\mu\text{g P/g}$ medium) decreased linearly with total P ($\mu\text{g P/g}$ medium) in the rooting medium from 4 to 18 wk during the experiment (Fig. 6). Measured amounts of P per pot decreased linearly with time during the experiment (Table 3) and were comparable to amounts calculated from total initial P (54.8 mg P/pot) and P removed by the plant up to each time of harvest (Fig.

TABLE 2. Phosphorus content of Kent soybeans inoculated with the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatus* and of uninoculated controls during host-plant ontogeny

Plant part	Week	P content ($\mu\text{g/g}$ of substrate) ^a	
		VAM plants	Control plants
Shoot	4	980 ± 84	1,380 ± 84***
	6	940 ± 55	1,140 ± 182*
	9	880 ± 84	880 ± 84 NS
	12	800 ± 71	780 ± 84 NS
	15	720 ± 84	680 ± 71 NS
	18	540 ± 55	580 ± 84 NS
Root	4	1,280 ± 130	1,480 ± 130*
	6	1,220 ± 84	1,200 ± 122 NS
	9	1,120 ± 130	980 ± 48*
	12	860 ± 152	700 ± 71*
	15	800 ± 141	660 ± 55*
	18	600 ± 100	480 ± 45*
Pod	15	1,980 ± 80	1,980 ± 160 NS
	18	1,820 ± 80	1,840 ± 60 NS

^a Values are means and standard deviation of five replications. Asterisks *** and * indicate statistically significant differences by Student's *t*-test $0.001 < P < 0.01$ and $P < 0.05$, respectively. NS designates no statistical significance ($P > 0.05$).

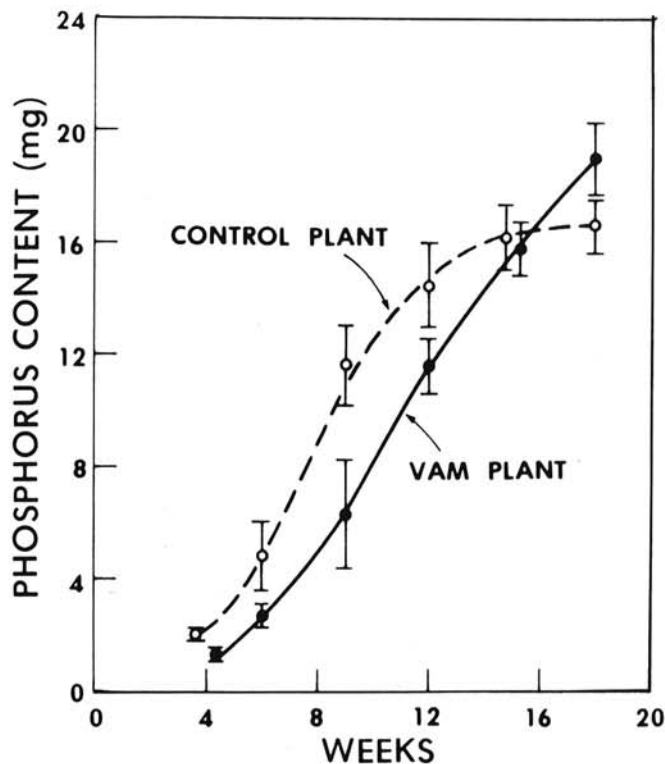


Fig. 5. Total phosphorus in Kent soybeans inoculated with the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatus* and in uninoculated controls.

5). The difference was attributed to leaching due to occasional excess watering.

DISCUSSION

The data describe the development of soybean plants infected with *G. fasciculatus* under a P regime that did not eliminate fungal infection yet was adequate for nonmycotrophic plant growth. Initial growth depression followed by growth enhancement under such conditions was reported earlier (6), but plant response data were not supported by measurements of total fungal biomass or changes in soil P levels. In the present experiment, a dramatic reversal in the trend from negative to positive percent differences between VAM and control plants occurred at the midpoint in the ontogeny of this symbiotic association (Fig. 1). This was preceded by changes in several variables. The reversal in the course of host plant development from growth depression to growth enhancement after 9 wk was ascribed to these changes.

The proportion of fungal endophyte as percent of total association (host plus fungus) biomass was maximal (7.3%) after 6 wk (Fig. 1). This proportion was greater than previous estimates for VAM plants (6,24). Such low estimates of fungal biomass led to the

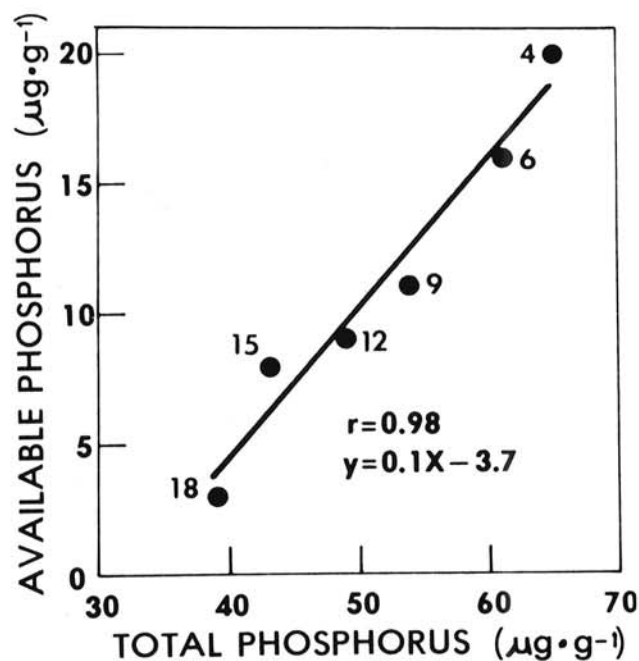


Fig. 6. Change in available (NaHCO_3 -extractable) phosphorus in the rooting medium as a result of P uptake by Kent soybean plants inoculated with the vesicular-arbuscular mycorrhizal fungus *Glomus fasciculatus*. The rooting medium ($1,250 \text{ cm}^3$) consisted of a mixture of perlite and sand (2:1, v/v) and 200 mg of hydroxyapatite ($\text{Ca}_{10}[\text{PO}_4]_6[\text{OH}]_2$). Total initial P due to all components was 54.8 mg per pot. Numbers represent micrograms of P per gram of medium by extraction procedures detailed in Materials and Methods.

TABLE 3. Phosphorus depletion in the rooting medium as a result of uptake by soybean plants

Time (wk)	Total P (mg/ pot)	
	Measured ^a	Computed ^b
4	54.8	58.5
6	51.4	52.1
9	45.4	48.5
12	40.7	42.9
15	35.9	39.0
18	32.7	33.8

^a Determined according to Shelton and Harper (21).

^b Computed as the difference of total P input (54.8 mg/ pot) minus plant P content (Fig. 5).

conclusion that the diversion of significant amounts of photosynthate to the endophyte was unlikely (23). This view was recently supported by findings that only 4% of the C fixed by the host was utilized by the fungal endophyte (20). In this case, however, the percentage of VAM fungus in the association was several times smaller (14) than the one reported here. Changes in the percentage of fungal biomass under our conditions appear to be related to the changes in growth inhibition of the host plant (Fig. 1). Competition for photosynthate between host and endophyte is one explanation for this relationship, and different symbionts and growth conditions may be factors influencing the extent of C allocation to the endophyte. Observations that support this view are the coincident increase in fungal biomass and decrease in VAM-plant dry weight relative to the controls (Fig. 1). Thus the greatest effect of sink demand by the VAM fungus on the association's energy balance was reflected as growth inhibition of the host before week 9. Since shoot to root ratios determine to a large extent the amount and proportion of carbohydrates available to different plant parts (16), the low shoot to root dry weight ratios during the early part of the association's ontogeny (Fig. 4) indicate lowered source capacity to supply the additional photosynthate required by the fungus and the infected host-root tissue. As the proportion of VAM fungus in total association biomass declined (Fig. 1), and as shoot (source) development relative to root growth increased (Fig. 4), the effect of the endophyte in inhibiting host growth apparently diminished, resulting in the observed reversal of growth inhibition in the VAM plants.

Growth inhibition in VAM plants and its reversal are intricately connected to the balance of P in the plant-soil fertilizer system (8). Shoots (up to week 9) and roots (up to week 6) of the controls had significantly higher percent P than VAM plants (Table 2), indicating an impairment of P uptake by infected host-plant roots during this initial period. Such an effect was ascribed to competition for P by the fungus and the host root. Since the fungus depletes the soil of available P and subsequently sequesters it, uptake of P by the host may be reduced and its growth inhibited in comparison with fungus-free plants (7). This view is supported by the low shoot to root P ratios of VAM plants compared to controls observed in the present experiment (Fig. 4), which indicate storage of P throughout the time course (24) and preferential use of P by the endophyte for its own development at least during the early stages of its establishment. Equalization of percent P in VAM- and control-plant shoots after 9 wk (Table 2) and the gradual gain of total P and dry weight in VAM-plant shoots (Tables 1 and 3) over the controls indicate the initiation or acceleration of P transfer from endophyte to host following an initial period of P stress.

The supply of total P appeared to be adequate throughout the time course, for at the final harvest total P remaining in the pots was still 58% higher than P assimilated by the plants during the entire experiment (Fig. 5, Table 3). Available P, however, decreased more rapidly than total P (Fig. 6) and was near $15 \mu\text{g P/g}$ substrate at 6 wk when percent P in the mycorrhiza became equivalent to the P concentration in the control roots (Table 1). Within the next 3 wk, the level of available P decreased to $10 \mu\text{g P/g}$ substrate, an event that coincided with the reversal in the trend to more negative percent differences (Fig. 1). This coincidence suggests that the effectiveness of the endophyte as a mutualistic, rather than parasitic, partner in the association depended on a sufficiently low concentration of available P, and that this concentration was at $10 \mu\text{g P/g}$ substrate under the conditions of this experiment.

The negative host-plant growth response to extensive endophyte (sink) development under conditions of relatively high P availability initially, and the reversal to a positive response after an apparent transition to mycotrophic growth observed in this experiment, supports the concept of opposing effects (22). Under this concept, enhanced P uptake is stimulatory and photosynthate demand by the endophyte is detrimental to the host whose growth response to the endophyte is determined by the balance between these effects. The data suggest a causal connection between P availability, the development of a significant sink for photosynthate as a result of VAM fungal proliferation, and growth

inhibition of the host plant. Following a decrease in P availability to a level favoring mycotrophic plant growth, P transfer from the endophyte to the host increased stimulated by increasing P deficiency in the host. Under these conditions the additional P uptake capability of the mycorrhiza may eventually lead to host plant growth enhancement.

The results show that a delicate balance exists between the level of P availability, endophyte development, and crop response. It may be inferred that under field conditions, this growth response in VAM plants should vary with the amount of P fertilizer applied. This response may range from mycotrophic growth enhancement at low levels of P, through growth inhibition induced by parasitic VAM activity at intermediate levels of P, to nonmycotrophic growth enhancement of the host at high levels of P.

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