

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

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ABSTRACT

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The development of *Glomus fasciculatus*, a vesicular-arbuscular mycorrhizal (VAM) fungus, in soybean (*Glycine max*) roots was determined at 2- to 3-wk intervals for 18 wk. The symbiotic association was grown in 1.5-L pots containing an inert medium supplemented with 200 mg of hydroxyapatite ($\text{Ca}_{10}[\text{PO}_4]_6[\text{OH}]_2$) as the source of P. Fungal infection, estimated by visual observation of stained mycorrhizal segments was maximal 9 wk after planting. Endophyte biomass, measured by the determination of fungal chitin, increased linearly until week 15, and declined during the final 3 wk of the experiment. Fungal biomass (measured as percentage of mycorrhizal dry weight) peaked between 6 and 9 wk (20%)

and declined thereafter. Maximization of percent fungal biomass coincided in time with a reversal in increasing growth inhibition of mycorrhizae relative to uninfected control plant roots. Concentration of P in the mycorrhizae increased rapidly during the first 9 wk and slowly thereafter. The percent difference in P concentration of plant matter increased linearly with decreasing availability of P in the rooting medium. It was concluded that parasitic development of *G. fasciculatus* under the growth conditions used was due to the diversion of significant amounts of assimilate from the host to the endophyte and to competition for P by the symbionts during the first ontogenic stages of the association.

Stimulatory effects of vesicular-arbuscular mycorrhizal (VAM) fungi on plants growing under soil-nutrient conditions favoring mycotrophy (14) are of increasing interest in applied research (15,24). However, yield reduction following infection by VAM fungi was also occasionally observed, but never fully explained (25,26). Mutual advantages in the mycorrhizal association are improved P nutrition of the host (23) and a supply of photosynthate to the endophyte (18). Host-plant growth is enhanced when the benefits of increased P nutrition to the host outweigh the disadvantage of carbohydrate loss to the endophyte (13). Growth of that kind has been defined as mycotrophic (7) and is most likely to occur when P availability in the soil is low. In mycotrophic plants, a high concentration of P (%P) in VAM-plant dry matter relative to non-VAM plants of the same age was observed (12,17). This indicates that increased uptake of P is not the only effect on the host; the elimination of P stress would result in increased plant biomass with a concomitant internal dilution of P (25). The high %P was construed to be due to a loss of carbohydrates to the mycorrhiza, but whether the proportion of the additional energy requirement is due to growth- or maintenance respiration (21) of the endophyte alone (10) or to increased rates of infection-induced respiration in the mycorrhizal roots was not established (16,25).

The purpose of this investigation was to measure extraradical and intraradical (3) VAM fungal biomass in a simplified (sand/perlite) rooting medium, to assess the level of infection by VAM fungi in soybean roots, and to evaluate the effect of the endophyte biomass on the P and carbohydrate economy of the infected host-plant root (mycorrhiza).

MATERIALS AND METHODS

Growth conditions. Symbiotic associations between soybean (*Glycine max* (L.) Merr. 'Kent') plants and the VAM fungus *Glomus fasciculatus* (Thaxt. sensu Gerd.) Gerd. and Trappe were

grown in a greenhouse at Albany, CA, September 1980 to January 1981 under conditions described elsewhere (2).

Measurement of chitin in VAM fungi. Measurement of VAM fungal biomass was based on the determination of chitin (poly- β -[1,4]-*N*-acetylglucosamine) contained in fungal cell walls (1). Mycelium of *G. fasciculatus* external to the mycorrhiza was separated from the association after 9 and 12 wk of growth, dried at 80 C for 1 day, and weighed. The amount of chitin per unit dry weight of extraradical VAM fungal mycelium was used to calculate VAM fungal biomass inside and outside the mycorrhiza (3). The possibility of variation of chitin content in VAM fungi was discussed elsewhere (3,16). Fungal materials were suspended in 4 ml of a KOH solution (120 g KOH/100 ml H₂O) and autoclaved at 120 C for 1 hr. The resulting hydrolysis products were centrifuged in successive ethanol and water washes (4,22). The final pellet was resuspended in water to a volume of 1.5 ml, followed by deamination, complete hydrolysis to 2,5-anhydromannose, and chromophore development (27). Absorbance at 650 nm was measured on a Bausch & Lomb Spectronic 20 spectrophotometer.

Purified chitin derived from crabshells (Sigma Chemical Co., St. Louis, MO 63178) was used as the standard for the evaluation of fungal chitin. Standards were subjected to the same procedure described for fungal biomass. Different times of autoclaving were tested to establish the optimum time length (1 hr) to achieve depolymerization and deacetylation of chitin without destroying the resulting glucosamine (4). A standard curve based on glucosamine-HCl (GlcN-HCl) was prepared by using the same procedure as for chitin, but starting with the deamination step. Since colorimetric treatment of GlcN-HCl results in complete chromophore development, the GlcN-HCl standard was used to calculate the degree of chitin hydrolysis by comparing absorbances. To account for the difference in the molecular weights of the chitin monomer and GlcN-HCl, the chitin/GlcN-HCl conversion ratio of 0.94 was applied to express GlcN-HCl in terms of chitin. All values obtained from the chitin standard curve were recalculated to account for the incomplete (37.2%) hydrolysis of chitin after 1 hr of autoclaving (4).

Determination of intraradical VAM fungal biomass. Mycorrhizae washed free of extraradical mycelium were dried at 80 C for 1 day and ground in a Wiley mill fitted with a 420- μ m (40 mesh) screen. Ten milligrams of ground mycorrhiza was suspended

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in 4 ml of a KOH solution (120 g KOH/100 ml H₂O), autoclaved, and subjected to the same colorimetric procedure described for the VAM fungus alone. Standards were prepared as described above, except that 10 mg of dried and ground control plant roots was added to the purified chitin to account for color reactions due to host-plant materials (4) or chitin-containing organisms other than *G. fasciculatus*. Chitin contained in the mycorrhizae was calculated from standard curves containing purified chitin and 10 mg of uninfected host-plant roots. Intraradical VAM fungal biomass per plant was calculated from the chitin content of measured amounts of VAM fungus and from the chitin content and weight of the mycorrhizae.

Determination of extraradical VAM fungal biomass in the rooting medium. Mycorrhizae and roots of control plants were thoroughly washed over a 43- μ m mesh sieve. Washings were returned to the rooting medium, which was collected and dried for 2 days at 90 C. The dry rooting medium was thoroughly stirred to achieve a uniform distribution of hyphal fragments and spores. Duplicate, 40 cm³ samples taken from the stirred media were mixed with 30 ml of concentrated KOH and processed for chitin analysis as described above. To account for any chitin-positive contamination due to sources other than *G. fasciculatus*, the average absorbance from five replications of control-plant rooting medium was subtracted from the absorbance value obtained from each replicate of VAM-plant rooting medium. The standard curve used in calculating chitin in the rooting medium was based on different amounts of purified chitin mixed with 40 cm³ of nutrient solution-washed rooting medium (3). Extraradical fungal biomass was calculated from the chitin content of measured amounts of VAM fungus and the chitin content of the total rooting medium (3).

Other assays. Percent infection of the host-plant root system was estimated histologically by staining the mycorrhiza with trypan blue (5). Phosphorus content of plant matter and rooting medium was determined as described previously (2). Data were based on five replications.

RESULTS

Endophyte development. Total VAM fungal biomass increased linearly for 15 wk after planting and declined during the final 3 wk of the experiment (Fig. 1). The host-plant root system reached a level of VAM fungal infection between 60 and 80% 6 wk after planting and remained at this level (as measured by the histological method) during the rest of the experiment (Fig. 1). Fungal structures external and internal to the host root had similar rates of development for 6 wk (Fig. 2). The intraradical component continued to grow rapidly for 15 wk, while the extraradical component peaked at 9 wk and declined thereafter.

Endophyte-host interactions. The percentage of total fungal biomass in the mycorrhiza increased from zero to 20% between 2 and 6 wk after planting (Fig. 3). The percent difference in %P between mycorrhizae and control plant roots was calculated as $[(\%P \text{ mycorrhiza} - \%P \text{ control root}) / \%P \text{ control root}] \times 100$. It increased rapidly for the first 9 wk, and slowly thereafter. Multiple regression analysis of the %P difference data showed a significant difference ($P < 0.05$) between the initial 9-wk ($y = 5.4x - 33.7$) and the final 9-wk ($y = 1.2x + 3.2$) periods (Fig. 4). Differences in %P were directly proportional to P availability in the rooting medium, and increased with decreasing concentrations of available (NaHCO₃-extractable) P in the medium (Fig. 5). Percent differences in total P and in dry weight were calculated as for %P (Fig. 4). These differences showed a reversal at 9 wk from the initial trend towards growth inhibition in the mycorrhiza toward growth enhancement relative to control roots (Fig. 4).

DISCUSSION

Fungal biomass as a percentage of the mycorrhiza (Fig. 3) was higher than the previously estimated values (26). The endophyte, which comprised 20% of the total dry weight of the mycorrhiza 6 wk after planting, may act as a sink for carbohydrates or as a competitor for mineral nutrients. Respiration in plant tissues

infected by fungal biotrophs usually increases and may be two to four times higher than in uninfected tissues (6,28). In mycorrhizae, a 74% increase in respiration due to VAM fungal infection has been recorded (20). However, there is no agreement, whether increased

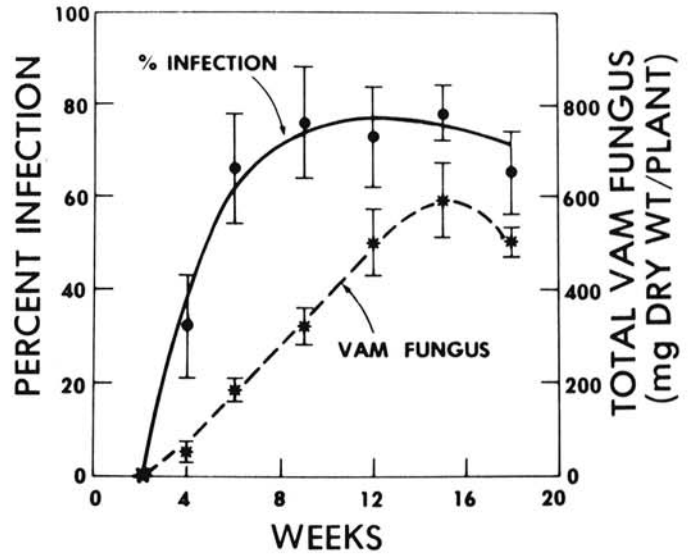


Fig. 1. Development of *Glomus fasciculatus*, a vesicular-arbuscular mycorrhizal (VAM) fungus, in soybeans. Percent infection was estimated by visual observation of stained mycorrhizal segments. Total (extraradical and intraradical) fungal biomass was determined by measurement of fungal chitin content in the mycorrhiza and in the rooting medium and by calculation of the proportion of chitin in dried isolates of extraradical fungal mycelium.

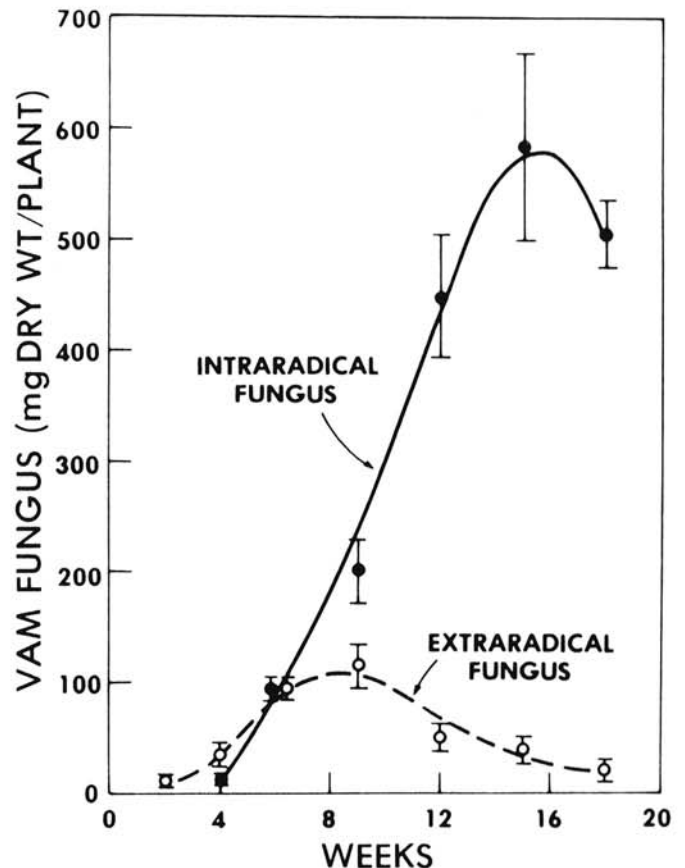


Fig. 2. Development of the extraradical and intraradical components of the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatus* in soybeans. Fungal biomass was determined as in Fig. 1. Data point of week 2 represents chitin contained in the inoculum.

respiration is due to the endophyte alone or to the effect of the biotroph on host tissue (10,19,28). The present data support the former view. If increased respiration is due to the effect of infection on host tissue, respiratory sink demand should increase with the product of percent infection (Fig. 1) and of the growing host root mass. The effect of this increasing sink demand should have been depression of mycorrhizal development relative to control roots past the recovery point between 6 and 9 wk after planting (Fig. 4). This was not the case. The reversal in the percent differences between mycorrhiza and control root at this time (Fig. 4) was preceded by a similar reversal in the percent dry weight of the endophyte (Fig. 3). This indicates that in spite of the continued rise in total VAM fungal biomass (Fig. 1) the proportionate decline of VAM fungus relative to host plant dry weight represented a decrease in sink demand that permitted a gradual recovery of the

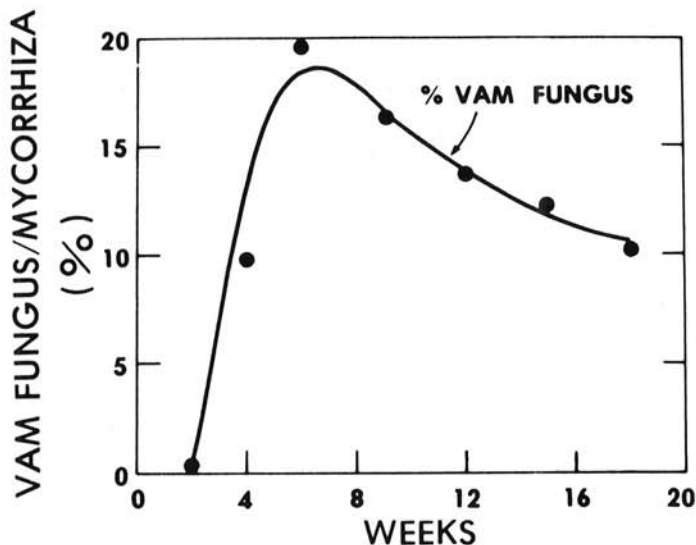


Fig. 3. Total biomass of the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatus* as percent of mycorrhizal dry weight in soybeans.

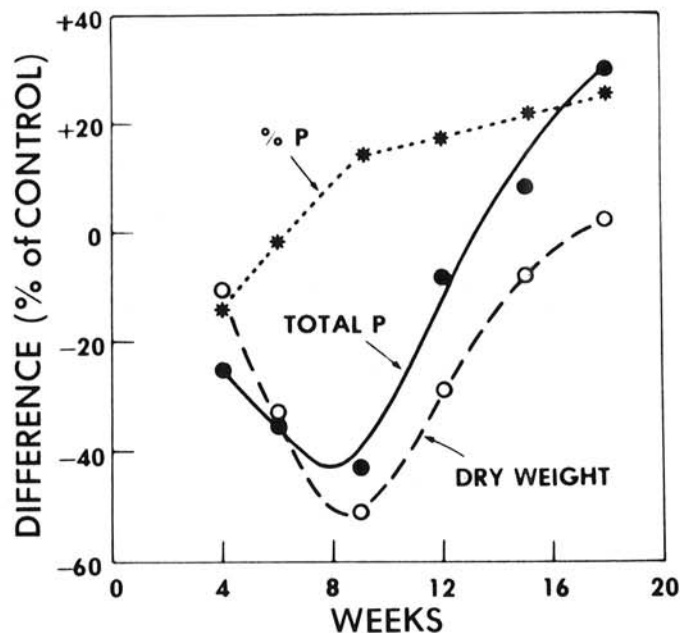


Fig. 4. Percent difference in total P, P concentration (%P), and dry weight between mycorrhiza and uninfected control plant root. Percent differences were computed as [(mycorrhiza - control root)/control root] × 100. The mycorrhiza was formed by infection of soybean roots with *Glomus fasciculatus*, a vesicular-arbuscular mycorrhizal fungus.

mycorrhiza from its most depressed level between the assays at 6 and 9 wk (Fig. 4). Respiration rates of fungi have been shown to be many times higher than those of higher plants on a unit weight basis (11). Thus, the rapid increase in VAM fungal biomass relative to that of the host root (Fig. 3) up to approximately the 7th week after planting may have accounted for a significant loss in host plant energy reserves as a result of both growth- and maintenance respiration (21) of the endophyte. After this critical period, recovery of the mycorrhiza (Fig. 4) coincided with the decline in the percentage of VAM fungus in the mycorrhiza (Fig. 3).

Changes in the percent difference in %P with time and the phase change at 9 wk in the two-phase linear regression line of %P (Fig. 4) indicated an involvement of P availability to the host in growth inhibition. The rapid increase in %P in the mycorrhizae relative to control roots up to 9 wk (Fig. 4) is interpreted as an accumulation of P in the endophyte either as a result of storage (8) or of retention in the rapidly expanding mycelium. During this time, competition for P between host and endophyte (9) may have resulted in increasing P stress in the host. Coincidence of the recovery phase of the mycorrhizae following the assay at 9 wk with the slower rate of %P accumulation (Fig. 4) indicated accelerated release of P to the host from the endophyte. The linear increase of the percent difference in %P with the decline in available (NaHCO₃-extractable) P in the growth medium (Fig. 5) showed a shift to an increasing advantage in P uptake by the mycorrhiza over non-VAM roots. The decline of available P to a value between 11 (week 9) and 16 μg P/g rooting medium (week 6) appeared to be critical for the reversal of growth inhibition. This level of available P may therefore represent the limit of the plant's capacity to utilize P effectively in the absence of the endophyte under the conditions of this experiment.

It is concluded that reduced dry weight of the mycorrhiza compared to control plant root during the early part of the association's ontogeny was due to the utilization of significant amounts of assimilate and to competition for P by the VAM fungal endophyte. The reversal in growth depression of the host root was related to the level of P availability to the association.

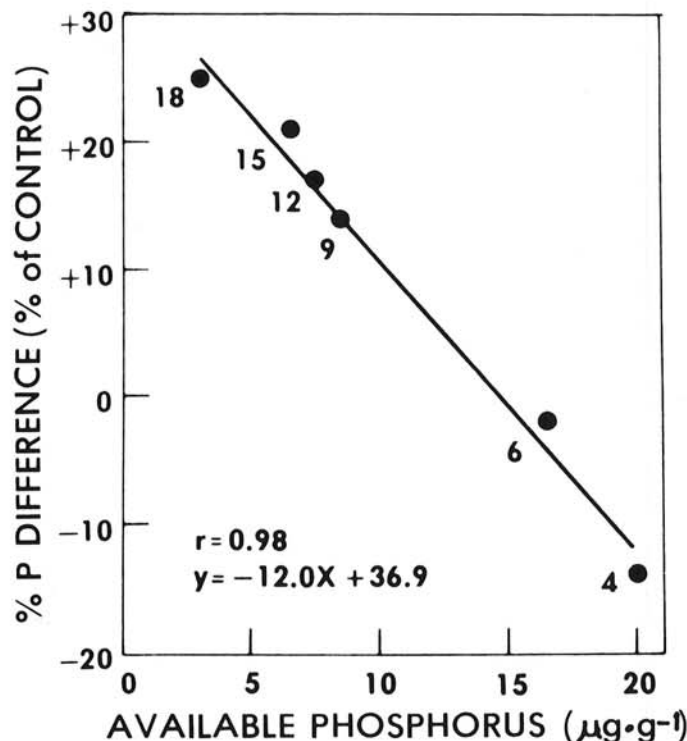


Fig. 5. Correlation of available (NaHCO₃-extractable) P in the rooting medium with percent difference in P concentration. Numbers next to data points indicate times of assay (weeks after planting). The mycorrhiza was an association between soybean roots and *Glomus fasciculatus*, a vesicular-arbuscular mycorrhizal fungus.

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