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Parasitic and mutualistic associations between a mycorrhizal fungus and soybean: The effect of phosphorus on host plant-endophyte interactions

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Soybean [*Glycine max* (L.) Merr. cv. Kent] plants were colonized by the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum* (Thaxt. *sensu* Gerd.) Gerd. and Trappe in pot cultures using an inert medium and a nutrient solution. Phosphorus was provided initially as 0, 25, 50, 100 or 200 mg hydroxyapatite [HAP, $Ca_{10}(PO_4)_6(OH)_2$] per pot. Under the low (0 mg HAP) and high (100 and 200 mg HAP) P regimes, VAM plants showed 20, 25 and 38% growth retardation, respectively, relative to non-colonized controls. At 50 mg HAP, VAM plant growth was significantly enhanced (14%). Dry weight and P content of both VAM and control plants increased with increased P availability throughout the HAP gradient. Intraradical VAM fungal biomass increased linearly with increasing P availability. Extraradical VAM fungal biomass was smaller than the intraradical component of the fungus at the lowest and highest levels of P addition in the growth medium. The ratio of extra- to intraradical mycelium, a suggested index of VAM fungal effectiveness, was greatest for the 50 mg HAP treatment, coinciding with growth enhancement of the host plant. This enhanced growth of the host at an intermediate P level was apparently a result of increased P uptake by the endophyte.

Additional key words – *Glomus fasciculatum*, *Glycine max*, growth enhancement, mycorrhiza, symbiosis.

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Introduction

A symbiosis is mutualistic when both symbionts benefit from the association. Such a state of co-existence depends on a balance between the respective requirements of each symbiotic partner (Gerdemann 1974). In the vesicular-arbuscular mycorrhizal (VAM) association the fungal endophyte always benefits, for it is an obligate biotroph (Warner and Mosse 1980). Its effect as a carbohydrate sink on its host plant may be negligible when its biomass relative to the host is small (Tinker 1975a) or when excess photosynthetic capacity exists to supply the additional demand for reduced carbon by the VAM fungus (Paul and Kucey 1981). However, when VAM fungal biomass is large (Bethlenfalvay et al. 1982c) or when conditions for photosynthesis are less than optimal (Daft and El Giahmi 1978), VAM fungi may become parasites significantly inhibiting host plant growth (Bethlenfalvay et al. 1982b, d). Benefits accrue to the host when its fungal endophyte increases the uptake of relatively immobile mineral nutrients (especially P) from a limiting supply. The resulting enhanced host-plant growth has been redefined by Cooper (1975) as mycotrophic, at variance with a previous, less restrictive usage of the term (Stahl 1900). When available P concentration is either above or below the level sup-

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porting mycotrophy, the host is either independent of its VAM fungal endophyte for P nutrition (Bowen 1978) or may have to compete with the microflora for P (Barber and Loughman 1967). In either case it suffers a loss of carbohydrate to the endophyte without deriving the benefits of increased P uptake.

It is proposed here that within a span of available P concentrations from severely limiting up to but not including such high levels of available P as to inhibit VAM fungal colonization, host plant growth may be inhibited when P is either less or more available than needed for mycotrophy. The purpose of this investigation was to test this hypothesis.

Abbreviations – HAP, hydroxyapatite; PPFD, photosynthetic photon flux density; VAM, vesicular-arbuscular mycorrhizal.

Materials and methods

Growth conditions. Soybean [*Glycine max* (L.) Merr. cv. Kent] plants were grown in 1.5 l white plastic pots in a greenhouse at Albany, California, May to June 1981. Temperature and relative humidity varied from day to day, but did not exceed or fall below the day/night ranges of 30°/15°C and 45/95%, respectively. On sunny and overcast days photosynthetic photon flux density (PPFD) averaged 500 and 300 μmol m^{-2} s^{-1}, respectively. The majority of the days during the experimental period were overcast or partly overcast due to coastal fog. Daylength was extended to 16 h by Sylvania 1 000 W metal halide lamps mounted vertically in parabolic relectors and arranged to provide uniform supplementary PPFD of 400 μmol m^{-2} s^{-1} at plant emergence level. The growth medium consisted of 1.25 l of sterile perlite: sand mixture (2:1, v/v) covered by a 2.5 cm layer of perlite. This was watered with a nutrient solution (pH 6.8) consisting of 1.5 m*M* $CaCl_2$, 0.5 m*M* K_2SO_4, 0.25 m*M* $MgSO_4$ and 2.0 m*M* NH_4NO_3. The concentration of Fe was 20 μ*M*, supplied as FeEDDA [ferric ethylenediamine di(*o*-hydroxyphenyl)acetic acid]. Micronutrients were according to Johnson et al. (1957) at one-quarter strength. Phosphorus was added as finely ground HAP (obtained from Sigma Chemical Company, Saint Louis, MO 63178, U.S.A., as Type VI calcium phosphate). Ninety-eight percent of the HAP particles were within a 0.9 to 0.06 mm size range, with the median particle diameter approximately 0.5 mm (standard error = ± 0.03). Five P regimes were used, consisting of 0, 25, 50, 100, or 200 mg of HAP per pot. The sand was not acid washed, and was the source of P (1.8 μg available P/g medium) to plants at the lowest P regime. Hydroxyapatite equilibrated with nutrient solution to give a concentration of 3 μ*M* P. Plants were watered with nutrient solution five times a week, and once with deionized water.

Biological materials. Seeds were germinated for 2 days at 28°C. Seedlings were selected for uniformity and were inoculated with the Gerdemann isolate of *Glomus faciculatum* (Thaxt. *sensu* Gerd.) Gerd. and Trappe or left uninoculated as controls. The inoculum (obtained from S. Woodhead, Abbott Laboratories, Long Grove, IL 60047, U.S.A.) consisted of 10 g of soil containing approximately 30 spores and 80 root fragments partially infected by *G. fasciculatum*. Control plants were initially watered with washings (43 μm sieve) of the inoculum free of *G. fasciculatum*. Plants were harvested over a 4-day period during the eighth week after planting.

Assays. Intraradical and extraradical fungal biomass was determined by chitin analysis as described previously (Bethlenfalvay et al. 1981, 1982a). This method relies on the measurement of the amount of chitin per unit weight of extraradical VAM-fungal mycelium. This chitin concentration is used in calculating VAM-fungal biomass from the chitin found in the mycorrhizae and the soil containing extraradical VAM hyphae. Chitin due to organisms other than VAM fungi is accounted for by the use of internal standards and non-VAM controls. Percentage of host-root colonization was determined by microscopic inspection of a large sample of stained root segments (Bethlenfalvay et al. 1981). Available ($NaHCO_3$-extractable) P in the substrate was determined according to Murphy and Riley (1962) as modified by Watanabe and Olsen (1965), and total P according to Shelton and Harper (1941). Plant P content was determined according to Allen (1940). Replications of plant samples were pooled for the P determination with the exception of one P regime (50 mg HAP), on which confidence intervals were determined. Dry weights of plant parts were measured after drying at 80°C for 1 day. Percent differences in dry weight between VAM and control plants were calculated as [(VAM plant – control)/control] × 100. Four replications were used, and positional differences in the greenhouses were minimized by daily rotation of plants. Numbers reported in the tables are means and standard deviations of 4 replications.

Results

Development of the host plant

Plant dry weight increased with increasing concentrations of available P for both VAM and control plants throughout the range of P addition (Tabs 1, 3). The rates of increase, however, were different; VAM plants responded more to availability at the low range of P amendment (0 to 50 mg HAP/pot) and controls at the high range (50 to 200 mg HAP/pot). Controls had significantly higher dry weights than VAM plants at the 0, 100 and 200 mg HAP treatments, while at 50 mg HAP VAM plants had significantly more biomass than con-

Tab. 1. Dry weight of soybean plants. Plants were inoculated with the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum* or left uninoculated as controls. Growth medium was amended with various amounts of P as hydroxyapatite (HAP). *, ** and *** denote significant differences between VAM- and control plants at the $0.01 < P < 0.05$, $0.001 < P < 0.01$ and $P < 0.001$ levels, respectively.

Phosphorus (mg HAP/pot)	Dry weight (g)			
	Shoot		Root	
	VAM plant	Control	VAM plant	Control
0	1.41±0.12	1.81±0.14**	0.66±0.11	0.81±0.08
25	1.90±0.13	1.90±0.24	0.85±0.09	0.82±0.08
50	2.30±0.04	2.02±0.14**	0.99±0.03	0.84±0.10*
100	2.83±0.23	3.75±0.27**	1.18±0.05	1.51±0.15**
200	4.64±0.45	7.42±0.29***	1.51±0.17	2.63±0.09***

Tab. 2. Phosphorus concentration of soybean plants. Plants were inoculated with the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum* or left uninoculated as controls. Growth medium was amended with various amounts of P as hydroxyapatite (HAP). Standard deviations were ± 0.005 for VAM and control plants at 50 mg P addition. * denotes significant difference at $0.01 < P < 0.05$ between VAM- and control plants.

Phosphorus (mg HAP/pot)	P Concentration					
	%				mg/plant	
	Shoot		Root			
	VAM plant	Control	VAM Plant	Control	VAM Plant	Control
0	0.06	0.07	0.06	0.06	1.25	1.76*
25	0.07	0.08	0.06	0.07	1.85	2.09
50	0.08	0.09	0.07	0.08	2.53	2.49
100	0.08	0.11*	0.07	0.09*	2.09	5.49*
200	0.10	0.14*	0.09	0.11*	6.00	12.93*

trols (Tab. 1). Thus, in terms of percentage difference, VAM plant growth was enhanced at 50 mg HAP, and inhibited above (100 mg HAP) or below (0 mg HAP) the mycotrophic-growth level (Fig. 1).

The percentage of P for plant tissues increased with increasing P amendment, and was significantly lower in VAM plants than in controls at 100 and 200 mg HAP (Tab. 2). Differences between VAM- and control plants in total P per plant were not significant ($P > 0.05$) for the 25 and 50 mg HAP treatments, but controls had significantly more total P than VAM plants at the lowest and highest levels of P availability. Total P contained initially in the sand and perlite alone was 17.8 mg P per pot. Available ($NaHCO_3$-extractable) P was significantly lower at harvest in control-plant growth media amended with 100 and 200 mg HAP than in VAM-plant pots (Tab. 3), reflecting the higher levels of P uptake by the controls. At lesser amounts of P amendment, differences in available P were not significant between control- and VAM-plant growth media.

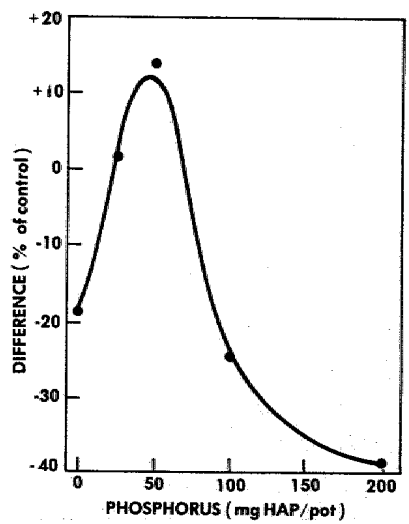

Fig. 1. Relative host-plant response to colonization by the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum*. Percentage differences between VAM plants and uncolonized controls were calculated as [(VAM plant − control)/control] × 100. Plants were grown under different P regimes determined by addition of hydroxyapatite (HAP) to the growth medium.

Development of the VAM-fungal endophyte

The intraradical VAM fungal mycelium increased linearly with increasing P amendment (Fig. 2). Extraradical mycelium was significantly ($0.01 < P < 0.05$) lower than intraradical mycelium at the highest and

Tab. 3. Available ($NaHCO_3$-extractable) P in the growth medium of soybean plants. Plants were colonized by the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum* or left uncolonized as controls. Treatments consisted of P addition to the medium by various amounts of hydroxyapatite (HAP). Determinations were made prior to planting (0 wk) and at harvest (8 wk). *denotes significant difference at $0.01 < P < 0.05$ between VAM- and control plants.

	P concentration (µg P/g medium)		
	0 wk	8 wk	
Phosphorus (mg HAP/pot)		VAM plant	Control plant
0	1.8±0.6	0.9±0.5	1.0±0.4
25	4.7±0.6	1.8±0.7	1.7±0.7
50	8.0±0.5	3.1±0.3	2.9±0.3
100	16.7±0.3	5.7±0.5	4.2±0.4*
200	30.4±2.2	9.5±0.9	8.0±0.4*

lowest levels of P amendment, and significantly higher ($0.01 < P < 0.05$) at 50 and 100 mg HAP. Total VAM-fungal biomass doubled from the mycotrophic P level of 50 mg HAP to the level of highest inhibition of 200 mg HAP. The ratio of extraradical to intraradical mycelia was maximal around the 50 mg HAP treatment (Fig. 3). The ratio of VAM fungal biomass to host-plant root dry weight increased sharply between the 0 and 50 mg HAP additions, and slowly thereafter (Fig. 4). This development was closely paralleled by VAM fungal colonization of the host root system.

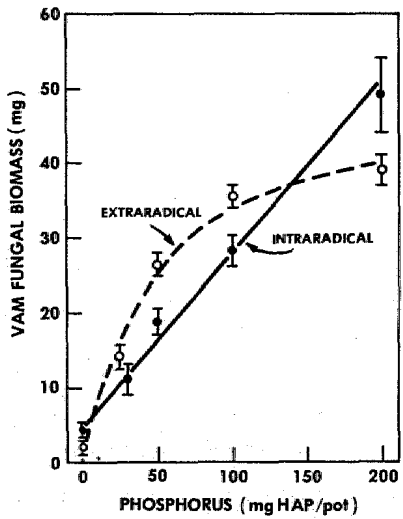

Fig. 2. Development of fungal biomass. Extraradical and intraradical mycelia of the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum* were measured by chitin assay of the growth medium and of the host-plant root, respectively. Vertical bars denote ± SE.

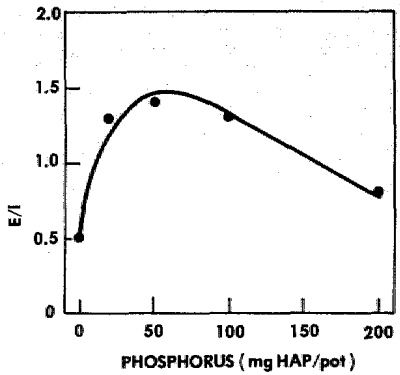

Fig. 3. Ratio of extraradical to intraradical mycelia of the vesicular-arbuscular mycorrhizal fungus *Glomus fasciculatum*.

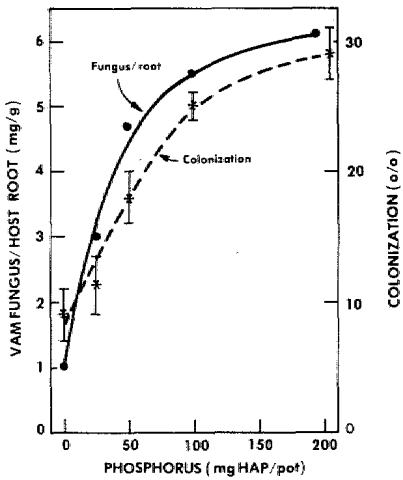

Fig. 4. Colonization and fungal biomass as percentage of host root. The extent of colonization of the host-plant root system by the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum* was determined histologically on a large sample of stained root segments. The ratio of fungal biomass to host-plant dry matter was calculated from the values of (extra-and intraradical) VAM fungal mycelium and host-root dry weights.

These two expressions for the assessment of VAM fungal development were significantly correlated (r = 0.93).

Discussion

The positive response of VAM- and control plant dry weight (Tab. 1) and P content (Tab. 2) to HAP amendment of the medium showed that P was limiting

throughout the P-treatment gradient. Plant P concentrations were within the range observed elsewhere (Lambert et al. 1979), but were lower in VAM plants than in controls (Tab. 2), contrary to the observations of other workers (Stribley et al. 1980). We are not sure why this was so and can only ascribe this deviation from the norm to the wide range of variation found in host response to different symbiotic partners and growth conditions (Allen et al. 1981, Carling and Brown 1980). Phosphorus stress decreased with increasing P availability, but plant (Menge et al. 1978) and soil (Nelsen et al. 1981) P-concentration levels inhibitory to VAM fungal colonization were not attained (Fig. 4). Within this range of internal (Tab. 2) and external (Tab. 3) P concentrations, VAM and control plants exhibited different growth patterns. Dry matter of VAM plants was significantly lower than that of controls at low and high levels of P availability, and was significantly higher at an intermediate level (Fig. 1, Tab. 1), verifying a suggestion by Crush (1975) that endophyte-host relationships vary between parsitism and mutualism depending on soil available-P levels.

The depression of VAM-plant growth at 0 mg HAP (Fig. 1) may be attributed to competition for P by host and microsymbiont. Phosphorus contained in the thin mineral coatings of sand grains is generally regarded as having limited availability for most plants alone (Jehne and Thompson 1981). As the addition of slightly soluble P was increased in subsequent treatments (25 and 50 mg HAP per pot), VAM plants were more effective in exploiting available P than controls (Fig. 1) due to the greater P-uptake efficiency of the extraradical VAM fungal mycelium (Sanders et al. 1977). Thus, at the 50 mg HAP treatment, mycotrophic growth of the host occurred. The growth advantage so imparted to the host apparently occurs only under conditions of growth limitation due to P stress (Ross 1971) but is restricted to a certain range of suboptimal P concentrations. This advantage conferred on the host by the endophyte is a result of the thorough permeation of the medium by the extraradical mycelium. In our essentially non-sorbing medium, where P concentrations depend on the distance between HAP grains, VAM-fungal hyphae are able to tap pockets of high P concentration by decreasing the distance between P source and the nearest absorbing surface (Tinker 1975b). The level of P initially available at 50 mg HAP (8.0 µg P/g medium, Tab. 3) was within the range previously observed to trigger mycotrophic growth in soybean plants grown in a medium in which P availability decreased with time (Bethlenfalvay et al. 1982b).

With P additions in excess of 50 mg HAP, the spacing of HAP grains was apparently close enough to be effectively exploited by the host-plant root alone. The rapid increase in control-plant dry weight (Tab. 1) and P content (Tab. 2) may, therefore, be the result of the more extensive root system of the controls in this range of P availability (100 and 200 mg HAP) than at lower levels of P in the soil.

The cause for retardation of host plant growth at a level of P availability above that favoring mycotrophic growth, but below that causing inhibition of VAM fungal colonization, is controversial (Tinker 1975a). It has been attributed to a diversion of carbohydrate from host to endophyte (Buwalda and Goh 1982). Growth responses to the presence of VAM fungi similar to the ones reported here were obtained in a separate experiment conducted under the same conditions of P availability, but with the plants relying on symbiotic N_2 fixation as the main source of N (Bethlenfalvay et al. 1982d). However, inhibition of the host plants was more pronounced at all levels of HAP addition than in the present work, apparently as a result of increased competition for carbohydrates by *Rhizobium*, which has a high energy requirement (Phillips 1980).

Growth patterns of extra- and intraradical VAM fungal mycelia (Fig. 2) appeared to be related to the response of host-plant to endophyte. Proliferation of the extraradical mycelium, the mineral uptake organ and major plant-to-soil interface, was most pronounced relative to the intraradical mycelium around the 50 mg HAP treatment (Fig. 3). The ratio of extraradical to intraradical mycelium, previously suggested as a measure of endophyte effectiveness (Bethlenfalvay et al. 1982a), thus paralleled the development of VAM plants relative to controls (Figs 1, 3).

It is concluded that for a given host-endophyte combination, under a given set of growth conditions, a level of available-P concentration exists which promotes mycotrophic growth of the host. It appears that such growth is related to a high ratio of uptake (extraradical) to storage-exchange (intraradical) organs of the endophyte. It is further suggested that growth depression of the host is determined by the amount of endophyte biomass acting as a carbohydrate sink and by competition by the symbionts for limiting factors such as P or carbohydrates.

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