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Response of Mycorrhizal and P-Fertilized Soybeans to Nodulation by *Bradyrhizobium* or Ammonium Nitrate[1]

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ABSTRACT

Management of N_2-fixing bacteria or P-scavenging endomycorrhizae may lead to decreased fertilizer use on extensively-cropped lands. To measure the effectiveness of these microsymbionts, soybean [*Glycine max* (L.) Merr. cv. Amsoy 71] plants were grown in a growth chamber in a soil [Josephine silty clay loam (mesic Typic Haploxerult)] low in plant-available N and P. Plants were inoculated with different *Bradyrhizobium* strains or received nutrient solutions of different N concentrations (0.0, 1.0, 2.0, 4.0 m*M* N) and P adequate for maximum plant growth under these conditions. Other plants were infected with a vesicular-arbuscular mycorrhizal (VAM) fungus and a *Bradyrhizobium* strain and received no N or P in the nutrient solution. The purpose of this study was to determine the growth response of soybean to N fertilization or nodulation by *B. japonicum* under conditions of high P availability or VAM-assisted P uptake. Nodulated non-VAM soybean plants had dry weights and development similar to that of the 4.0 m*M* N fertilizer treatment. Total N and Mn, leaf area, and leaf P of nodulated plants were higher than in the comparable N-fertilized plants in the absence of P stress. Soybeans infected with both the VAM fungus and *Bradyrhizobium* were similar in total dry weight, leaf area, and development to plants that received 1.0 or 2.0 m*M* N. They, however, contained more leaf N, more root Cu and Zn, and less Mn and P than the 2.0 m*M* N treatment. It is concluded that a number of host characteristics of nodulated plants are due to the altered functional aspects of the symbiosis and not N input alone. The presence of the VAM fungus can decrease nutrient stress in environments limited in P, Zn and Cu, elements essential in N_2 fixation.

Additional index words: *Glomus*, N nutrition, Nodule activity, Root nodule.

LEGUMINOUS crop plants form mutualistic associations with the bacterium *Bradyrhizobium* and with vesicular-arbuscular mycorrhizal (VAM) fungi (9). In soils marginal in N and P, these microsymbionts play a crucial role in legume development and yield (29) since enhanced nodulation and N_2 fixation result from improved P nutrition (8). Under conditions where P is plentiful but N is not, improved growth and nutrition of nodulated legumes depends on the effectiveness (19) of symbiotic N_2 fixation. Nodulated hosts are usually compared to N-deficient, non-inoculated controls to assess N input over the lifetime of the symbiotic association (22) or to nonnodulating, near-isogenic lines (6). For studies involving metabolic processes in N_2-fixing associations, non-inoculated plants must be given fertilizer N to provide controls that are morphologically and nutritionally similar (15). Plants grown with NH_4NO_3 under a saturating P regime (23) permitted observation of developmental characteristics as a function of N nutrition alone. These observations formed the baseline for comparisons of individual levels of N application with *Bradyrhizobium* ("slow-growing" *Rhi-*

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zobium) effects in the presence or absence of a VAM fungus. While such controls grown on NH_4NO_3 may be equivalent to *Bradyrhizobium*-inoculated plants in some morphological or nutritional characteristics, they will not be physiologically equivalent (28).

When P and N are limiting, normal host-plant growth depends on P uptake assisted by a VAM fungus (30), efficient N_2 fixation even under P stress (7), and microsymbiont compatibility (20). Synergistic effects between a VAM fungus, such as *Glomus fasciculatum*, and *Bradyrhizobium* with respect to N_2 fixation and mycorrhizal colonization (27) have led to improved host productivity, N and P nutrition, and nodule formation (30). These latter effects increase photosynthesis (32) which, in turn, stimulates nodule and VAM activity (3, 16) in an auto-catalytic cycle. Micronutrients essential in nodulation and N_2 fixation (31) are often increased by mycorrhizal colonization. Hormonal effects, triggered by the presence of either symbiont (1, 26) may also contribute to the effectiveness of the tripartite symbiosis.

The purpose of this study was to determine the growth response of soybean to N fertilization or nodulation by different strains of *B. japonicum* under conditions of high P availability or P uptake mediated by a VAM fungus at low levels of available P.

MATERIALS AND METHODS

Experimental Design

Plants were subjected to two P regimes by providing P through either phosphate fertilizer or a VAM fungus to facilitate P uptake. Nitrogen was provided for non-VAM plants by nutrient solutions containing one of four different NH_4NO_3 concentrations or the plants were inoculated with one of three strains of *Bradyrhizobium*. Plants colonized by VAM received no NH_4NO_3 and were either inoculated with the same *B. japonicum* strains as the non-VAM plants or were not inoculated. Thus, there were seven N treatments under the P fertilizer regime and four N treatments among VAM plants. There were six replications of each treatment in a randomized-block design for a total of 66 plants.

Biological Materials

Soybean [*Glycine max* (L) Merr. cv. Amsoy 71] seeds (0.5 to 0.6 g) were surface sterilized and planted as described elsewhere (23). Plants were inoculated with one of three *B. japonicum* strains (110, 136, or 61A118, originally obtained from H.H. Keyser, USDA-ARS, Beltsville, MD 20705), or with the Gerdemann isolate of *G. fasciculatum* (Thaxt. *sensu* Gerd.) Gerd. and Trappe, or with both *Glomus* and *Bradyrhizobium*. Plants that received the VAM fungus were inoculated with 60 $\times 10^3$ mm^3 of Josephine soil containing between 300 and 350 spores of a *G. fasciculatum* isolate originally obtained from Abbott Laboratories (Long Grove, IL 60047).[3]

Nutrient Solutions

Plants colonized with the VAM fungus were furnished with a basal (N- and P-free) solution which consisted of 1.5 m*M* $CaCl_2$, 0.5 m*M* K_2SO_4, 0.25 m*M* $MgSO_4$, 25 μM H_3BO_3, 20 μM FeEDDHA, 2.0 μM $ZnSO_4$, 0.5 μM $CuSO_4$, 0.4 μM H_2MoO_4, and 0.6 μM $CoCl_2$. Plants not inoculated with the VAM fungus or *Bradyrhizobium* received solutions containing N as either 0.0, 0.5, 1.0, or 2.0 m*M* NH_4NO_3 and P as 1.0 m*M* KH_2PO_4. Plants inoculated with *B. japonicum* only received the N-free solution. The pH of all fertilizer solutions was adjusted to 6.9 with 0.01 *N* KOH. Pots were watered to field capacity three to five times a week with the nutrient solutions.

Soil

The soil used was a Josephine silty clay loam (mesic Typic Haploxerult). It contained, per g of soil, 12.8 μg NH_4^+-N, 4.5 μg NO_3^--N, 1.6 mg Kjedahl N, 4 μg Olsen-available P, 0.33 mg total P, and it retained 0.95 g P g^{-1} P added. Soil was sterilized with ethylene oxide and limed (10 g $CaCO_3$ kg^{-1} soil) as described previously (23). Soil pH after liming was 7.0 (1:5 $CaCl_2$ extract). Each pot received 1.25 kg of soil which was initially watered with a leachate of the VAM inoculum filtered free of VAM propagules so as to establish the same microflora in VAM and control treatments.

Growth Conditions

Plants were grown in a walk-in style growth chamber (Sherer Model 511-38). The light/dark period was 16/8 h and temperature and relative humidity were 28/22°C and 70/80%, respectively. Photosynthetic photon flux density varied from 650 to 550 $\mu mol\ m^{-2}\ s^{-1}$ from the center to the edge of the growth platform. Plants were rotated within a block three times a week to minimize positional effects. The platform grating and growth chamber floor were disinfected once a week with 0.70 L L^{-1} ethanol to reduce cross-contamination by *Bradyrhizobium* strains.

Evaluations and Assays

All plants were harvested 9 weeks after planting. In all cases this time corresponded to the full bloom stage for soybeans. Nodule activity (24) of excised roots (ATP-dependent H_2 production and C_2H_2 reduction) was determined by gas chromatography following two 30-min incubations (H_2 evolution and C_2H_2 reduction) of excised roots as described previously (22). Leaf areas were determined using a Li-Cor L-1500 leaf area meter (Lincoln, NE 68504). Leaf number and leaf area were measured separately for primary (main stem) and secondary (lateral branch) leaves. Leaf, stem, root, and nodule weights were measured after drying for 2 days at 70°C. Plant analysis for N (Kjeldahl), P (perchloric acid digest) and micronutrients (dry ashing and atomic adsorption spectrophotometry) were determined by standard methods (23). Percent colonization of the host-plant root system was established histologically by staining the mycorrhiza with trypan blue and scoring the roots (4). The chitin content of 20 mg of VAM roots was determined spectrophotometrically, and the fungal biomass was estimated as described in a companion article (23). An analysis of variance (ANOVA) was performed on the plant and soil data for the P-fertilized, non-VAM soybeans. Regression analyses were carried out on data from the NH_4NO_3-supplemented treatments to determine plant response as a function of N input. Differences to nodulation by *Bradyrhizobium* strains were evaluated using Duncan's Multiple Range Test (DMRT). The procedures of the Statistical Analysis Systems were used in all evaluations (2). Data for plant growth as a function of soluble N concentration were used to generate response curves. Means and variances for data of nodulated plants were projected onto

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[3] Reference to a company and/or product by USDA is only for the purposes of information and does not imply approval or recommendation of the products to the exclusion of others which are also suitable.

these curves to determine the equivalent N input and to establish confidence intervals for comparison of nodulated hosts with N-fertilized plants. A separate ANOVA was performed on data from VAM-colonized soybeans, and a comparison between different treatments was carried out using DMRT. A third ANOVA was run on data from all nodulated treatments along with DMRT to assess the impact P source had on N_2 fixation.

RESULTS

Dry Weight

In the case of N fertilized soybeans, dry weight increased linearly with increasing NH_4NO_3 concentrations (Table 1) for leaf ($y = 2.0x + 6.1$, $r^2 = 0.99$; $p < 0.01$) stem ($y = 2.3x + 5.8$, $r^2 = 0.98$, $p < 0.01$) and root ($y = 0.8x + 5.6$, $r^2 = 0.96$, $p < 0.01$). Non-VAM plants inoculated with *B. japonicum* strains were 60% to 100% larger than plants receiving no NH_4NO_3 (-N control). Soybeans infected with *Bradyrhizobium* only had stem and leaf dry weights equivalent to plants that received between 3.0 and 4.0 m*M* N, while root growth was equivalent to plants given from 2.0 to 3.0 m*M* N. Strains 136 and 61A118 were more efficient than strain 110 at promoting dry weight increases (Table 1). Plants inoculated with strain 136 had significantly ($p < 0.05$) larger shoot to root ratios than the 4.0 mM N treatment regardless of the source of P. Dry weights of nodulated VAM plants were 50 to 60% greater than those of the non-nodulated VAM control (Table 1). However, nodulated VAM plants were generally only two-thirds as large as plants from the P-fertilized set inoculated with the same *Bradyrhizobium* strain.

Developmental Stage and Leaf Area

Soybeans inoculated with *Bradyrhizobium* had as many nodes as the 4.0 m*M* N-fertilized plants (Table 1). Nodulated VAM-plants had fewer nodes than nodulated plants given soluble P. Total leaf area increased linearly with N fertilization ($y = 575x + 692$, $r^2 = 0.93$, $p < 0.01$) as did primary leaf area ($y = 240x + 964$, $r^2 = 0.94$, $p < 0.01$) and secondary leaf area ($y = 334x - 2.8$, $r^2 = 0.92$, $p < 0.01$). The total leaf area of nodulated plants was equivalent to plants receiving between 3.8 and 4.5 m*M* N. Nodulated VAM soybeans had 50% the total leaf area of P-fertilized, nodulated plants and were similar in leaf area to those of the 2.0 m*M* N treatment. The average area per leaflet increased linearly with N input ($y = 0.82x + 18.9$, $r^2 = 0.89$, $p < 0.01$), and it was significantly greater in *Bradyrhizobium*-inoculated than in N-fertilized plants. The increase in the average area per leaflet was enhanced in the presence of VAM. The proportion of secondary to primary leaves increased linearly with solution N concentration ($y = 0.16x + 0.09$, $r^2 = 0.96$, $p < 0.01$) and with nodulation, but was low in the presence of the VAM fungus. Specific leaf area was significantly greater in nodulated plants compared to the N-fertilized plants. Nodulated VAM soybeans had as much leaf area as the 2.0 m*M* N treatment, although the N-fertilized plants had more leaves and relatively more secondary leaves.

Elemental Composition

The N contents of nodulated plants were equivalent to plants that received between 4.0 and 5.0 m*M* N (Table 2). The N concentration of the nodules was more than twice that of the roots and was 30% greater than the leaf N levels in nodulated plants. Nodulated VAM soybeans had leaf and nodule N contents similar to the corresponding tissue N levels for the P-fertilized nodulated plants. There was a differential redistribution of N in the nodulated plants. Total N increased linearly with solution N ($y = 104x + 97$, $r^2 = 0.98$, $p < 0.01$). Total N in the leaves of these plants was equivalent to plants that were given between 4.3 and 5.2 m*M* N while the total N in roots was equivalent to that of plants that received between 2.8 and 3.8 m*M* N. The seed contributed 25 ± 5 mg N in the -N control (0.0 m*M* N treatment); the remaining 100 mg N was assimilated from soil N. Phosphorus-fertilized soybeans inoculated with *Bradyrhizobium* assimilated an average of 468 mg N over the

Table 1. Yields and leaf area parameters for soybean plants grown under various N and P regimes.

Treatment	Dry weight	Shoot/Root ratio	Nodes	Total leaf area	Average area/leaflet	Specific leaf area	Primary to secondary leaf area ratio
	g plant^{-1}		Nodes plant^{-1}	m^2	mm^2 leaf^{-1}	m^2 kg^{-1}	
P-fertilized plants (non-VAM)							
N concentration (m*M*)							
0.0	18.1	2.43	15.5	0.122	1980	20.1	0.15
1.0	22.6	2.46	16.6	0.138	1960	18.1	0.20
2.0	27.7	2.73	17.6	0.183	1970	18.8	0.36
4.0	37.8	3.40	19.2	0.344	2270	26.0	0.76
Bradyrhizobium strains							
110	30.7 c*	3.69 ab	19.0 a	0.312 c	2970 a	29.8 a	0.57 ab
136	36.5 ab	3.90 a	19.1 a	0.393 a	3020 a	31.1 a	0.71 a
61A118	33.9 b	3.32 c	18.8 a	0.361 ab	2710 ab	27.7 ab	0.81 a
VAM plants (no added P)							
Control (no *Bradyrhizobium*)	12.6 z	2.42 z	14.8 z	0.096 z	2080 z	21.5 z	0.01 z
110	18.5 y	3.54 y	17.9 y	0.169 y	3990 y	29.4 xy	0.10 yz
136	19.1 xy	3.60 y	17.7 y	0.188 y	4010 y	32.4 y	0.20 y
61A118	20.6 x	3.33 y	17.6 y	0.186 y	3470 y	28.1 x	0.11 yz

* Mean values (6 replications) for each parameter having common letters within a column are not significantly different at the 0.05 level by Duncan's Multiple Range Test (DMRT).

seed and the soil contribution (Table 2). The total N input of 189 mg N by *Bradyrhizobium* in VAM associations represented a 40% decrease relative to the amount of N taken up by P-fertilized, nodulated soybeans.

Leaf P concentrations for nodulated soybeans were significantly greater than for any N-fertilized plants (Table 2). Nodule and root P contents were similar for non-VAM plants, but in VAM plants nodule P content was three times higher than root P contents. Nodulated VAM soybeans had markedly lower leaf and root P contents than the P-fertilized plants. Total P did not show a good linear increase with N fertilization ($y = 3.4x + 51$, $r^2 = 0.51$, $p > 0.05$). Total P uptake in plants inoculated with *Bradyrhizobium* was equivalent to plants that were given between 4.2 and 5.4 m*M* N (Table 2). The N-limited VAM control took up 10 mg P in excess of the 2 mg P in the seed, while nodulated VAM plants assimilated approximately 13 mg P.

The uptake of micronutrients was modified by the presence of both microsymbionts (Table 3). Root Fe content was higher in nodulated VAM plants than in non-VAM plants, while the Fe content of the leaves was lower. Nodule Fe content was higher than in either roots or leaves. Nodulated roots had higher Mn contents than non-nodulated roots, but shoot Mn in nodulated VAM plants was lower than non-VAM plants. Nodulation did not affect Zn concentration in roots but lowered the concentration of Zn in the leaves. Mycorrhizal plants had higher Zn and Cu concentrations than non-VAM plants. Copper concentrations were generally lower in the leaves of nodulated plants compared to leaves from non-nodulated plants.

Nodulation and Nitrogenase Activity

Inoculation of non-VAM plants with strains 136 and 61A118 resulted in greater nodule dry weights than inoculation with strain 110 (Table 4). Acetylene-

Table 2. Elemental composition for soybean plants grown under various N and P regimes.

	N content				P content			
Treatment	Leaf	Root	Nodule	Total N	Leaf	Root	Nodule	Total P
	mg g^{-1}			mg plant^{-1}	mg g^{-1}			mg P plant^{-1}
P fertilized plants (non-VAM)								
N concentration (m*M*)								
0.0	8 d	14	ND†	126	3.3	7.0	ND†	55.6
1.0	10 d	15	ND	179	2.9	4.8	ND	53.0
2.0	14 c	17	ND	278	2.5	3.6	ND	58.5
4.0	26 b	20	ND	530	2.8	4.1	ND	71.3
Bradyrhizobium strains								
110	35 a*	20 a	48 a	543 b y	5.2 a	3.5 cd	3.4 b	81.8 ab wx
136	36 a	21 a	47 a	647 a z	3.9 b	4.2 bc	4.0 a	82.3 ab wx
61A118	32 a	21 a	44 b	593 ab yz	4.7 a	3.3 d	3.5 b	86.2 a w
VAM plants (no added P)								
Control	9 z	14 z	ND†	93 w	1.2 z	1.7 y	ND†	12.4 z
110	30 x	20 y	44 y	276 x	1.6 y	1.2 z	2.9 y	14.8 y
136	31 x	18 y	47 x	279 x	1.8 y	1.1 z	3.4 x	16.4 y
61A118	27 y	19 y	42 y	290 x	1.3 z	1.0 z	2.8 y	15.0 y

* Mean values for each parameter having common letters within a column are not significantly different at the 0.05 level by DMRT.

† Plant provided with NH_4NO_3 were not inoculated and formed no nodules.

Table 3. Micronutrient content for plant parts from soybean plants grown under various N and P regimes.

	non-VAM (P-fertilized)							VAM-plants			
	N concentration (m*M*)				*Bradyrhizobium* strains†				*Bradyrhizobium* strains		
Micronutrient	0.0	1.0	2.0	4.0	110	136	61A118	Control	110	136	61A118
	μg g^{-1}										
Fe											
Leaf	157	172	168	191	156 b y*	173 ab y	175 ab y	211 x	139 z	124 z	116 z
Root	1570	2150	2170	1800	1950 ab y	2010 ab y	1860 ab y	1810 y	2320 y	2730 z	2650 z
Nodule	ND†	ND	ND	ND	4610 a x	3390 ab y	3010 b y	ND	4410 x	3550 y	4130 x
Mn											
Leaf	178	190	196	180	219 a y	202 ab y	226 a y	261 x	134 z	115 z	138 z
Root	193	184	179	152	258 a z	252 a z	247 a z	161 y	230 z	268 z	250 z
Nodule	ND†	ND	ND	ND	321 a z	210 ab y	179 b y	ND	225 y	183 y	212 y
Zn											
Leaf	44	53	41	43	28 b vw	25 b vw	21 b v	95 z	45 y	37 xy	30 wx
Root	21	19	17	18	19 a z	22 a yz	20 a z	32 x	33 x	31 x	27 xy
Nodule	ND†	ND	ND	ND	25 a xy	18 a y	20 a y	ND	30 x	21 xy	23 xy
Cu											
Leaf	25.4	28.6	20.4	18.9	14.7 ab yz	11.9 b z	10.8 b z	37.1 w	18.9 x	17.3 xy	13.6 yz
Root	10.2	8.5	9.2	8.3	16.4 a y	11.7 b z	11.3 bc z	23.0 x	16.1 yz	17.4 yz	15.2 yz
Nodule	ND†	ND	ND	ND	10.3 a y	9.8 a y	7.8 a y	ND	9.5 y	9.0 y	8.5 y

* Mean values for each parameter having common letters within a row are not significantly different at the 0.05 level by DMRT. The letters a, b, c are used to compare nodulated and non-nodulated non-VAM plants; v, w, x, y, z are used to compare nodulated VAM and non-VAM plants.

† Plants provided with NH_4NO_3 were not inoculated and formed no nodules.

Table 4. Symbiotic N_2 fixation parameters for soybean plants inoculated with *Bradyrhizobium* strains, or infected with both VAM fungi and inoculated with *Bradyrhizobium*.

Rhizobium strains	Nodule dry weight	C_2H_4 produced	H_2 evolved	Symbiotic N†
	g plant^{-1}	—μmol plant^{-1} h^{-1}—		mg plant^{-1}
P fertilized (non-VAM)				
110	0.63 b*	18.3 b	0.72 c	417 bc
136	0.85 a	42.6 a	0.47 c	520 a
61A118	0.82 a	22.5 b	5.33 a	467 ab
VAM plants (no added P)				
Control				
110	0.39 c	8.1 d	0.16 d	175 d
136	0.41 c	16.6 c	0.03 d	182 d
61A118	0.42 c	7.9 d	1.65 b	197 d

* Mean values for each parameter having common letters within a column are not significantly different at the 0.05 level by DMRT.

† Symbiotic N = Total N − (seed N + assimilated soil N).

dependent C_2H_4 production was significantly higher in roots colonized with strain 136, whereas H_2 evolution was significantly greater for nodules colonized by strain 61A118 regardless of the P source (Table 4). Acetylene reduction and symbiotic N (total N less seed N and assimilated soil N) were correlated in non-VAM plants ($r = 0.83$, $p < 0.05$) and in VAM plants ($r = 0.73$, $p < 0.05$).

Fungal Colonization

Inoculation of soybean plants with *G. fasciculatum* resulted in over 40% colonization of all VAM-plant roots (Table 5). Soybeans nodulated with strains 136 and 61A118 had significantly higher fungal colonization that the VAM controls or plants inoculated with strain 110. Inoculation with strain 61A118 resulted in the largest amount of fungal proliferation in terms of VAM-fungal biomass. *Bradyrhizobium* strain 61A118 thus had the greatest effect in stimulating VAM-fungal growth, and hosts infected with this strain had the highest VAM biomass to nodule weight ratio (Table 5).

DISCUSSION

The effects of beneficial microsymbionts on their host plants have been traditionally studied in terms of biomass or yield enhancement (20, 22) or by determining the activity of an individual process such as C_2H_2 reduction (30), with nutrient-deficient controls serving as the non-symbiotic plants. While nutritional equivalency may be achieved by supplementing controls with N and P, host responses to nodulation (26) or VAM fungal colonization (5) will be lacking. Such host-responses were noted in the divergence in N utilization and storage (see also 25), leaf development, shoot to root ratio, and the allocation of nutrients to the leaves. This finding indicates that the supply-demand relationships in these symbiotic plants are necessarily different from the N and P fertilized plants. Dry weight N concentration can vary according to the efficiency of the N_2-fixing symbiosis (12) or the additional respiratory burden associated with N_2 fixation in nodulated plants (28). These differences may affect such essential physiological processes as photosynthetic activity (10), the utilization of carbohydrates by the endophytes (3, 11) and compensatory CO_2 fixation in response to the additional demand by the endophytes (15).

Table 5. Fungal characteristics for soybean roots colonized with VAM fungi and inoculated with *Rhizobium*.

Treatment	VAM-fungal colonization	Intradical VAM-fungal biomass	% Fungal biomass	VAM/Nodule ratio†
	%	mg plant^{-1}	%	
Control	42.0 c*	195 c	5.2 c	0.0 d
110	43.6 c	207 c	5.6 c	0.54 c
136	56.8 b	267 b	7.0 b	0.68 b
61A118	62.9 a	350 a	7.7 a	0.84 a

* Means having common letters within a column are not significantly different at the 0.05 level by DMRT.

† VAM-fungal biomass (mg)/nodule weight (mg).

Colonization by one or both of the two endophytes altered the uptake and distribution of several micronutrients in the host plant. The decreased Mn uptake by VAM plants is of interest since acid soils often contain phytotoxic levels of Mn and Al that can be detrimental to N_2 fixation (13) or the growth of most *Rhizobium* strains (14). Plants inoculated with *B. japonicum* increased Mn uptake to toxic levels (21), but dual colonization by *Bradyrhizobium* and the VAM fungus decreased Mn to less than critical concentrations. Low concentrations of available Mn could be expected in VAM hyphae or vesicles since Fe or Mn is inactivated through the precipitation with phosphates (18). It was uncertain whether the increases in Cu and Zn concentrations were the result of higher requirements for these elements by the VAM symbiosis, or if accumulation was incidental to the greater uptake capability of the fungus. The enhanced Cu and Zn uptake did not appear related to N_2 fixation since the levels of these micronutrients were lower in nodule than in root.

Nodule dry weights were a function of P availability and to some extent *B. japonicum* strain differences (17). As P fertilization increased, nodule weights doubled, but nodule activity increased only 20%. It was concluded that nodule growth, rather than nitrogenase activity responded to the increase in P availability. The dependence of nodulation on the VAM fungus in tripartite associations was shown by the approximately 3-fold increase in nodule P over root P concentrations as compared to no difference in the P-fertilized non-VAM plants. The nodule appears to be a powerful sink for P (and Fe) whose requirements tend to be satisfied even under limiting conditions (31). Hydrogen evolution relative to C_2H_2 reduction was not affected by VAM-colonization, so there was no change in net N_2 fixation as has been noted in *Phaseolus-Rhizobium-Glomus* symbiosis (4).

The improved growth and N nutrition in these tripartite associations may have been due to VAM-mediated host effects, rather than improved nodulation or N_2 fixation. Strain 61A118 was least efficient at N_2 fixation (greatest H_2 evolution), but this strain stimulated P uptake in non-VAM plants and also the greatest proliferation of the VAM fungus. Differences between strains of *B. japonicum* in phosphate storage and utilization (7) probably plays a role in the host response. Rapid colonization by a VAM fungus would result in an enhanced P status, but would lower the level of carbohydrate in the roots. *Brady-*

rhizobium strains unable to store P but capable of storing C as poly-β-hydroxybutyric acid could have a competitive advantage under these conditions.

These data demonstrate that several morphological and nutritional differences occur in response to the minerals and microsymbionts to which a plant is exposed. The measurement of these attributes in the field should allow further identification of factors influencing these symbioses and would allow correlation of the data with more detailed laboratory results.

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