The *Glycine-Glomus-Bradyrhizobium* symbiosis. IX. Nutritional, morphological and physiological responses of nodulated soybean to geographic isolates of the mycorrhizal fungus *Glomus mosseae*.

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The objective of the work was to determine differences in plant response to geographic isolates of a vesicular-arbuscular mycorrhizal (VAM) fungus, and to demonstrate the need for such determinations in the selection of desirable host-endophyte combinations for practical applications. Soybean (*Glycine max* (L.) Merr.) plants were inoculated with *Bradyrhizobium japonicum* and isolates of the VAM-fungal morphospecies *Glomus mosseae* (Nicol. & Gerd.) Gerd. and Trappe, collected from an arid (AR), semiarid (SA) or mesic (ME) area. Inoculum potentials of the VAM-fungal isolates were determined and the inocula equalized, achieving the same level of root colonization (41%, $P>0.05$) at harvest (50 days). Plants of the three VAM treatments (AR, SA and ME) were evaluated against nonVAM controls. Significant differences in plant response to colonization were found in dry mass, leaf K, N and P concentrations, and in root/shoot, nodule/root, root length/leaf area and root length/root mass ratios. The differences were most pronounced and consistent between the AR and all other treatments. Photosynthesis and nodule activity were higher ($P<0.05$) in all VAM treatments, but only the AR plants had higher ($P<0.05$) photosynthetic water-use efficiency than the controls. Nodule activity, evaluated by H_2 evolution and C_2H_2 reduction, differed significantly between treatments. The results are discussed in terms of nutritional and non-nutritional effects of VAM colonization on the development and physiology of the tripartite soybean association in the light of intraspecific variability within the fungal endophyte.

Key words – *Bradyrhizobium japonicum*, *Glycine max*, *Glomus mosseae*, nitrogen fixation, partitioning, phosphorus nutrition, photosynthesis, potassium nutrition, soybean, vesicular-arbuscular mycorrhiza, water-use efficiency.

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Introduction

Vesicular-arbuscular mycorrhizal (VAM) fungi are usually classified on the basis of morphology (Hepper et al. 1988). Some species are known to have world-wide distribution (Schenck and Pérez 1988). Geographical isolates, having evolved in association with different soils, climates and host plants (Pirozynski and Malloch 1975), probably form environmentally adapted populations (Lambert et al. 1980), although they may be indistinguishable under the microscope. Such VAM-fungal isolates have been found to elicit different host-plant responses in the field and have been referred to as 'ecotypes' (Morton 1988).

The promise of VAM fungi as biological fertilizers (Azcón G. de Aguilar et al. 1979) has focussed interest on their role in agrobiology (Jeffries 1987). Such a role requires a goal-oriented selection of organisms best suited for the requirements of specific agricultural situations (Abbott and Robson 1982, Bethlenfalvay et al. 1988). If host response to VAM fungi is influenced by intraspecific variability within the endophyte, collection

for specific agricultural applications will have to be based on an assessment of the edaphic and floristic characteristics of the native site and on the criteria of the prospective site of utilization. Selection of suitable isolates for agricultural use, in turn, should be based on an experimental determination of host response under laboratory and field conditions (Abbott and Robson 1985).

The purpose of this work was to make such an experimental determination under controlled conditions, demonstrating physiological differences in host-plant response to colonization by different geographic isolates of the same VAM-fungal morphospecies.

Abbreviations – AR, arid; CER, CO_2 exchange rate; ME, mesic; PUE, phosphorus-use efficiency; RE, relative efficiency of N_2 fixation; SA, semiarid; VAM, vesicular-arbuscular mycorrhizal.

Materials and methods

Experimental design

The experiment had a completely random design with 4 treatments and 6 replications. Plants were either inoculated with one of 3 isolates (designated AR, SA and ME, based on the arid, semiarid and mesic climates of the geographic origins of the fungi) of a VAM fungus or left to grow non-symbiotically (nonVAM).

Biological materials

Soybean (*Glycine max* (L.) Merr. cv. Hobbit) seeds were surface sterilized, germinated for 2 days at 30°C, selected for uniformity and planted in 1.5 l pots with or without an inoculum of the VAM fungus *Glomus mosseae* (Nicol. & Gerd.) Gerd. and Trappe. The AR and SE isolates of *G. mosseae* were collected from *Juniperus californica* Carr. in a sandy wash of the Anza-Borrego desert of Southern California (Bethlenfalvay et al. 1983) and from *Oryzopsis hymenoides* (R. & S.) Ricker of the Wyoming big sagebrush community of northwestern Nevada (Young and Evans 1974). The ME isolate was obtained from N. C. Schenck (isolate # 156, International Culture Collection of VAM fungi, Univ. of Florida, Gainesville, FL, from Arredondo fine sand of pH 7.6 at the Univ. of Florida Agronomy Farm). The isolates were cultured on *Sorghum bicolor* L. Inoculum potentials of the soil cultures (inocula) were determined by the infection unit method (Franson and Bethlenfalvay, 1989) based on a microscopic count of successful encounters (infection units) between fungus and root. Inocula of each isolate, in amounts calculated to produce the same number of infection units [1000 units(g root FW)$^{-1}$] after 14 days of growth, were mixed into the soil prior to planting. The plants were also inoculated at planting with the diazotrophic bacterium *Bradyrhizobium japonicum*, Nitragin strain 61A118. The inoculum consisted of 10 ml of the bacterial suspension [10^9 cells (ml)$^{-1}$] in yeast-mannitol broth. All plants also received a wash of the combined VAM inocula free of VAM-fungal propagules (43 μm screen) to equalize the microbiota of treatments.

Growth conditions

Plants were grown in a walk-in type growth chamber under a day/night regime of 16/8 h, 27/21°C and 60/90% relative humidity. Photosynthetic photon flux density was 800 μmol m^{-2}s^{-1} at shoot tip, provided by General Electric 1500 mA cool white fluorescent tubes and 90 W incandescent bulbs. Utilization of only a central part of the platform minimized light differences across the growth area. To allow for any other positional effects, the plants were re-randomized weekly.

The growth medium was a Balcom series (Yolo County, CA) heavy silt loam (Typic Xerorthent) with a pH of 8.0, organic matter content of 17 g kg^{-1} and a sand:silt:clay content of 20:56:24%. Plant-available nutrient concentrations (μg g^{-1} soil) were: K, 114; P, 3.3; S, 6.0; B, 0.39; Cu, 1.6; Fe, 4.0; Mn, 3.5; Mo, 0.01; and Zn, 3.8. Total nutrients were (mg g^{-1} soil): Ca, 22.2; K, 13.0; N, 0.7; P, 0.8; S, 0.1. The soil was mixed with fine sand (2:1, v:v, soil:sand), sterilized by autoclaving, mixed with the VAM inoculum, potted, wetted from below and allowed to stand moist for 10 days prior to planting. Plants were watered twice a week with deionized water.

Measurements and analyses

Plants were harvested after 50 days of growth. Root, nodule and leaf dry weights were determined after drying 70°C for 2 days. Nutrient analyses of leaf (K, N and P) and root and nodule (P) tissues were performed by standard methods (Chapman and Pratt 1982). The third through sixth trifoliate leaves were used for all comparative leaf date, except for net CO_2 exchange rates (CER). At the time of harvest the sixth leaf was fully and the seventh leaf approximately half expanded and the lower leaves had dropped off from the plants of some of the treatments. The fifth trifoliate leaf was used to determine CER, transpiration and leaf conductance rates with a LI-COR LI-6000 Portable Photosynthesis System (Brown and Bethlenfalvay 1987). Nodule activity (H_2 evolution and C_2H_2 reduction) and VAM-fungal colonization (grid-line intersect method) were measured as described previously (Bethlenfalvay et al. 1985). Leaf area and root length were determined with a LI-3000 area meter (LI-COR, Inc., Lincoln, NE, USA) and a Comair Root Length Scanner (Hawker de Haviland, Ltd., Victoria, Australia), respectively.

The relative efficiency of N_2 fixation (RE), an expression for the allocation of electrons to the reduction of H^+ to H_2 and of C_2H_2 to C_2H_4 by nitrogenase (Schubert and Evans 1976) was calculated as 1 – (mol H_2 evolved/ mol C_2H_2 reduced). A high RE value denotes little loss

Tab. 1. Symbiotic plant responses. Soybean plants were colonized by one of 3 isolates of the VAM fungus *Glomus mosseae,* or left uncolonized as controls. Leaf data reflect the 3rd through 6th trifoliate leaves only. Numbers are the means of 6 replications and are significantly different (*P*<0.05) when followed by different letters, by Duncan's multiple range test.

Growth parameter	VAM isolate			nonVAM control
	AR	ME	SA	
Plant dry mass (g)	5.14a	3.50b	3.31bc	2.85c
Leaf				
area (cm^2)	144 a	99 b	102 b	100 b
dry mass (g)	1.10a	0.72b	0.79b	0.73b
Shoot dry mass (g)	3.38a	2.09b	1.97b	1.56b
Root				
length (m)	88.8 a	93.2 a	98.2 a	98.8 a
dry mass (g)	1.55a	1.31a	1.25a	1.24a
colonization (%)	43.5 a	38.2 a	41.2 a	
col. length (m)	37.8 a	35.5 a	35.6 a	
Nodule				
number/root	129 a	92 ab	78 bc	59 c
dry mass (g)	0.21a	0.11b	0.10b	0.05c

of reducing power to H$_2$ evolution. Nodule P-use efficiency (PUE) was calculated as the ratio of N$_2$ reduced/total nodule P. The reduction of N$_2$ to ammonia was estimated as (mol C$_2$H$_2$ reduced/3) (RE) (Bethlenfalvay and Phillips 1977).

Nutritional and morphological parameters of interest were evaluated for statistically significant differences among the 4 treatments by Duncan's multiple range test (*P*<0.05). Percent growth enhancement by the VAM treatments vs the nonVAM control was evaluated by Student's t-test as non-ranked comparisons. Leaf gas exchange values of the VAM plants were tested individually against those of the controls by Student's t-test. The relationships between leaf conductance, photosynthesis, transpiration and water-use efficiency were evaluated by regression analysis.

Results

Root colonization by the AR isolate was associated with increases in all plant parameters measured, except for root length and root mass, relative to the control (Tab. 1). The ME isolate affected only total plant and nodule mass and nodule number, and the SA isolate affected nodule mass only. Both the percentage of root colonization and the length of colonized roots were invariant with treatment, indicating that the number of viable propagules applied as well as host-endophyte compatibilities were very similar for the three isolates.

Leaf P concentrations among VAM plants were not different (*P*>0.05) but were all significantly higher than in the nonVAM plants (Tab. 2). In the roots, P concentration was highest in AR and lowest in the controls (*P*<0.05). Root P levels were not reflected in those of the nodules, which were similar throughout. Leaf N concentrations were higher (AR, ME, *P*<0.05; SA, *P*<0.1) in the VAM plants than in the control. Leaf K

concentrations were significantly higher in AR, and significantly lower in ME and SA than in the controls.

Growth response (Fig. 1) to the three isolates varied widely in dry matter (AR, 80%, *P*<0.001; ME, 23%, *P*<0.05; SA, 16%, 0.05<*P*<0.1). Morphogenic relationships between plant parts (ratios of root mass/shoot mass, root length/leaf area, root length/mass, nodule mass/number, nodule mass/root mass), which are indicators of resource allocation among plant parts or the symbiotic organs, were significantly different for the AR vs the control (Tab. 3). The ME and SA treatments were different from the control only in the root/shoot and the nodule/root dry mass ratios. The root/shoot ratios were inversely related to the growth response. While such a relationship is frequently observed in VAM plants (Smith 1980), and is, in general, recognized as a stress-related phenomenon (Warenbourg and Morrall 1978), the relationship between root length and mass has received less attention (Fitter 1985a). The significant (*P*<0.05) difference in the root length/mass ratio between AR and the other treatments (Tab. 3) does not become apparent from the root length and mass data (Tab. 1) alone. The low root length/mass ratios of the AR plants suggest a morphological readjustment by the host of the most efficient isolate to produce a coarser root system, perhaps in response to

Tab. 2. Nutrient concentration (mg g^{-1}) in VAM and nonVAM soybeans. Explanation of symbols as in Tab. 1.

Nutrient concentration		VAM isolate			nonVAM control
		AR	ME	SA	
Leaf	N	22.3 a	22.6 a	21.3 ab	19.6 b
	K	6.8 a	3.2 c	2.8 c	4.2 b
	P	0.80a	0.80a	0.8 a	0.67b
Root	P	1.15a	0.98b	0.98b	0.82c
Nodule	P	2.80a	3.03ab	3.25b	2.93ab

Fig. 1. Soybean plants were inoculated with geographic isolates of the vesicular-arbuscular mycorrhizal (VAM) fungu *Glomus mosseae*. Isolates were collected from an AR site in California, an ME site in Florida and an SA site in Nevada. All VAM plants and the nonVAM controls were nodulated by *Bradyrhizobium japonicum*. Significant ($P<0.05$) responses in plant dry mass were mediated by the AR and ME isolates.

the increased uptake capability (Smith 1980) of its fungal component. The lack of such a pronounced readjustment in the other two VAM treatments appears to be related to impaired K nutrition (Tab. 2) resulting from VAM colonization.

The large nodule mass/root mass ratio of the AR plants is associated with their higher root P content (Tab. 2), but the reason for the small specific nodule mass (nodule weight/number) of the AR vs the other treatments is obscure and cannot be explained on nutritional grounds by the present data. The low root length/leaf area ratio of the AR treatment is also problematic, because of its potential impact on plant water balance. It should favor rapid desiccation, an effect which was not observed. Increased water transport by the VAM-fungal hyphae is a possible explanation, but its occur-

rence has been disputed by others (see Fitter 1985b) on theoretical grounds.

Transpiration and CER were significantly ($P<0.05$) higher in the ME and SA plants than in the controls (Fig. 2). Transpiration, photosynthetic water-use efficiency and CER increased linearly with leaf conductance for the control, SA and ME treatments. Plants of the AR treatment diverged from this linear relationship. They had the same leaf conductance and transpiration rates ($P>0.2$) as the controls, but their photosynthetic water-use efficiency was significantly higher than that of plants of the other 3 treatments. The higher CER values of VAM plants were reflected in their higher N and P concentrations, but not in the K concentrations of the SA and ME plants (Tab. 2).

Two measures of nodule activity, C_2H_2 reduction and

Tab. 3. Relative morphological development of plant organs in symbiotic soybean plants. Leaf area reflects 3rd through 6th trifoliate leaves only. Explanation of symbols is as in Tab. 1.

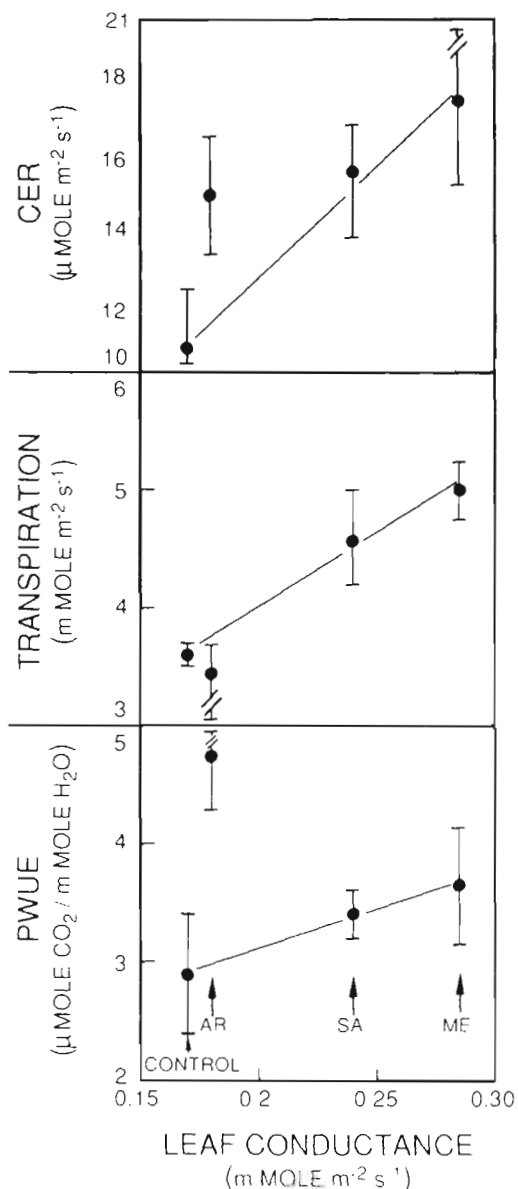| Growth Parameter	VAM isolate			nonVAM
	AR	ME	SA	
Root/shoot (g/g)	0.44a	0.63b	0.66b	0.86c
Root length/leaf area (m/cm²)	0.62a	0.94b	0.96b	0.99b
Root length/root mass (m/g)	57.3 a	71.1 b	78.6 b	79.7 b
Nodule mass/number (g)	0.77a	0.89b	0.89b	1.07b
Nodule mass/root mass (g/g)	0.17a	0.08b	0.08b	0.04c

Fig. 2. Leaf gas exchange in soybeans colonized by geographic isolates of the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus mosseae*. Transpiration and CER and photosynthetic water-use efficiency (PWUE) of the VAM treatments were evaluated against nonVAM controls by Student's t-test. Linear regression analysis of the controls and the SA and MA treatments showed significant relationships (CER, r=0.997, P<0.05; Transpiration, r=0.995, P<0.07; PWUE, r=0.999, P<0.05). The AR value was off the line in each case. Symbols represent means ± SE. Treatment designations are as in Fig. 1.

H_2 evolution, varied significantly among treatments and were highest in AR and lowest in the control (Tab. 4). Two measures of nodule efficiency indicated that the nodules of nonVAM plants were more effective than those of VAM plants in utilizing P, and that electron allocation by nitrogenase to C_2H_2 reduction was also highest in the nonVAM plants. These observations were in conflict with the higher levels of leaf N of VAM plants (Tab. 2). Given the sufficient and essentially treatment-invariant nodule P concentrations, higher nodule efficiency should have translated into higher leaf N concentrations. While this conflict underscores the well-known unreliability of nodule gas-exchange measurements as indicators of nitrogen fixation and nitrogen nutrition per se, they serve adequately and conveniently to document responses of nodule activity to the fungal co-endophyte.

Discussion

Suggestions of intraspecific variation in VAM fungi are few in the literature, but differences in host responses in the field have been reported in terms of biomass enhancement (Carling and Brown, 1980, Haas and Krikun, 1985), water relations (Stahl and Smith 1984), heavy metal tolerance (Gildon and Tinker 1983), and the development of the extraradical fungal mycelium (Graham et al. 1982). As shown by our results, differences in host responses to isolates of a VAM-fungal morphospecies are not restricted to single parameters but are pervasive in both plant form and function. This strongly suggests that geographically isolated populations of a VAM-fungal species develop distinct characteristics capable of eliciting different symbiotic responses. While such populations may still be the source of considerable variability, perhaps down to the level of the individual, multinucleate spore, it is important nevertheless that isolates be given unique identification (Sylvia and Burks. 1988) to avoid confusion about isolate characteristics.

Use of the term 'ecotype' (Stahl and Smith 1984) to describe such populations further adds to the confusion. The ecotype concept, as introduced (Turesson 1922), redefined (Clausen 1939), misused (see Gregor 1942)

Tab. 4. Nodule activity and efficiency in VAM and nonVAM soybeans. For definitions of the relative efficiency of nitrogen fixation (RE) and P-use efficiency see Materials and methods. Explanation of symbols as in Tab. 1.

Parameter	VAM isolate			nonVAM control
	AR	ME	SA	
H₂ evolution (µmol h⁻¹ plant⁻¹)	1.8 a	0.7 c	1.0 b	0.3 d
C₂H₂ reduction (µmol h⁻¹ plant⁻¹)	1.8 a	1.3 b	1.4 b	0.9 c
RE (1-H₂ evolved/C₂H₂ reduced)	0.00d	0.46b	0.29c	0.66a
P-use efficiency [µmol N₂ (g nodule P)⁻¹]	0.02c	0.60b	0.42b	1.35a

and put into classical taxonomic context (Heslop-Harrison 1964), establishes a relationship between environment and heredity and is not applicable to VAM fungi, which do not reproduce sexually and cannot be crossed. We suggest 'edaphotype' to designate such intraspecific variants of these soil fungi that are of different edaphic origin and elicit distinct physiological responses from plants under uniform (same new host, same new soil) conditions. We derive the term from the definition of edaphology ("the study of soil from the point of view of higher plants", sensu Brady, 1974) and apply it with the expectation that further study will address the relative levels of variation within and between such geographically separated populations.

Plant response to VAM fungi has often been thought of exclusively as a P response. Currently, a case is being made for regarding mycorrhizae as agents broadening the ecological range of plants, especially relating to stress conditions (Schönbeck 1987). Of these, P stress is often the most obvious and its alleviation by VAM fungi may therefore mask other effects. The present data also showed a significant and essentially edaphotype-invariant increase in plant P nutrition. However, given the uniformity of leaf P and N concentrations, differences in the growth enhancement of the AR vs the ME and SA plants must be ascribed to another factor. Of those measured, only the low K levels of the ME and SA plants are related to the differences. These results confirm previous observations (Menge et al. 1982, Powell 1975) documenting both depression and stimulation of VAM-mediated K accumulation.

Differences in the relative development of plant organs are influenced by source-sink relationships within the symbiotic association (Bayne et al. 1984) and by nutrient stress, especially as it relates to root vs. shoot development (Marschner 1986). Alleviation of nutrient (P and K) stress was most pronounced in the AR plants (Tab. 2), a condition which was reflected in their low root length/mass (coarse root system) and root/shoot ratios (Tab. 3). While P stress was also relieved in the ME and SA plants, the differences between the AR and the ME and SA plants reflect the imposition of K stress in the latter.

When measured, CER and transpiration have always been enhanced in VAM plants (Smith and Gianinazzi-Pearson 1988) and are accompanied by increased leaf conductance (Allen et al. 1984), but are not necessarily related to improved P nutrition (Brown and Bethlenfalvay 1988). Leaf K concentrations apparently did not affect the CER/transpiration relationship, since both processes were highest (among VAM plants) in the ME plants of low leaf K content and lowest in the AR plants of high leaf K content. It is noteworthy that the edaphotype originating from the most arid of the 3 sites was the only one to have a significant effect on photosynthetic water-use efficiency.

As with photosynthesis, the edaphotype-dependent change in RE and nodule PUE was apparently not a nutritional effect, in view of similar P concentrations in the nodules and in the leaves of the VAM treatments. While differences in plant responses attributable to the ME and SA edaphotypes were few, the differences in nodule response to these fungi were pronounced. This observation indicates a wide and varied symbiotic response range to VAM-fungal edaphotypes. As to the mechanism of these responses, analogy with responses to plant pathogens offers at least 2 models. One is a direct release of stimulatory or inhibitory substances by the endophyte to its symbiotic partners (Knight and Langston-Unkefer 1988), another the elicitation of reactions (Anderson 1988) from an otherwise dormant part of the host's genome, resulting in the production of secondary metabolites.

The relationships in form and function of the treatments may shift during the ontogeny of the associations and may be altered under different external conditions. Nevertheless, the results can best be interpreted in terms of real differences in host and co-endophyte reaction to VAM colonization and demonstrate two salient points: 1. Morphologically indistinguishable edaphotypes of VAM-fungal morphospecies can elicit different host responses under identical conditions (same new host, same new soil, same inoculum potential, same new soil microbiota); 2. Differences in host response are pervasive and not necessarily related to P nutrition.

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