Biol Fertil Soils (1993) 15:103–106

Biology and Fertility of Soils

Fitting plants to soil through mycorrhizal fungi: Mycorrhiza effects on plant growth and soil organic matter

M. Quintero-Ramos[1], D. Espinoza-Victoria[1], R. Ferrera-Cerrato[1], and G.J. Bethlenfalvay[2]*

[1]Centro de Edafología, Colegio de Postgraduados (CP), Montecillo, México 56230, Mexico
[2]Department of Agriculture, Agricultural Research Service, Western Regional Research Center (WRRC), Albany, CA 94710, USA

Received May 8, 1992

Summary. Vesicular-arbuscular mycorrhizal (VAM) fungi affect diverse aspects of plant form and function. Since mycorrhiza-mediated changes in host-plant responses to root colonization by different VAM fungi vary widely, it is important to assess each endophyte for each specific effect it can elicit from its host as part of the screening process for effectiveness. Three species of VAM fungi and a mixture of species were compared with non-VAM controls for their effects on soil organic matter contents and on nutrition and morphology in two varieties (native and hybrid) of corn (*Zea mays* L.) and one of sunflower (*Helianthus annuus* L.) in P-sufficient and N-deficient soil in pot cultures. Differences in soil organic matter due to the fungal applications were highly significant with all host plants. Native corn responded more to VAM colonization than the hybrid did; differences in treatments were significant in leaf area, plant biomass, and root:shoot ratio in the former, but not in the latter. Responses in the sunflower were similar to those in the native corn. Significant VAM treatment-related differences in shoot N and P contents were not reflected in shoot biomass, which was invariant. Correlations between plant or soil parameters and the intensity of VAM colonization were found only in soil organic matter with the native corn, in specific leaf area in the hybrid corn, and in plant biomass in the sunflower. The presence of the different endophytes and not the intensity of colonization apparently elicited different host responses.

Key words: *Helianthus annuus* – Mycorrhiza – Soil organic matter – VAM response – *Zea mays*

VAM fungi have been called biological fertilizers because of their effects on plant P nutrition (Azcón de Aguilar et al. 1979). Some are considered to be effective when they stimulate plant growth more than others (Abbott and Robson 1981). A definition of VAM effectiveness was introduced by Koide and Elliott (1989), and applies to P acquisition and utilization, in keeping with the important role of VAM fungi in plant P nutrition. However, the emphasis on P has masked other VAM effects, which are mentioned only in passing in VAM cost-benefit analyses (Raju et al. 1990). Another measure of VAM effects, mycorrhizal dependency, is based on plant dry weight (Bagyaraj 1992). Neither measure is helpful in evaluating VAM-fungal isolates for many other physiological and morphological effects on the host plant, or on the biotic and abiotic characteristics of the host soil. Root colonization is often used to relate the extent of the VAM condition to VAM effectiveness, because this parameter is easily measured, but the relationship between colonization and its contribution to VAM effectiveness (Vierheilig and Ocampo 1991) is often tenuous. A focus on plant growth, P uptake, and colonization is prevalent in the evaluation of VAM experiments. As a result, other VAM effects that may be present and important are often disregarded.

The purpose of the present experiment was to determine VAM effects in different host-endophyte combinations on plant and soil parameters in a soil sufficient in P, and to determine whether correlations occurred between plant and soil VAM responses and VAM root colonization in addition to those between plant P content and dry mass.

Materials and methods

The experiment was a 3×5 factorial with plants and VAM fungi as factors. The 15 treatments, each representing a different plant–fungus combination, were replicated five times. The results were evaluated by analysis of variance (actual probability values associated with the *F* statistic) and Duncan's multiple range test to show differences between individual treatments (at the arbitrary 5% level). In keeping with accepted practice (Nelson 1989), our interpretation of significance was not limited by the 5% level.

The growth medium was a mix of washed river sand and soil (1:1, v/v) with a final texture consisting of 75% sand, 10% silt, and 15% clay

* *Present address:* Horticultural Crops Research Laboratory, USDA-ARS, 3420 N.W. Orchard Avenue, Corvallis, OR 97330, USA

Correspondence to: G. J. Bethlenfalvay

and an organic matter content of 0.8% and pH 8.3. Nutrient concentrations were total N, 0.6 g kg^{-1}; $NaHCO_3$-extractable (Olson) P, 21 mg kg^{-1}; and Ca, K, and Mg (cmol kg^{-1}), 30.3, 0.7, and 5.8, respectively. The soil was low in organic matter, N, and Ca, high in K, Mg, and P, and was not fertilized during the experiment. A P-sufficient soil was used because the VAM P-response can mask other effects in P-deficient soils. The plants were grown in pot cultures (1.5 kg soil pot^{-1}) under greenhouse conditions as described previously (Espinoza-Victoria et al. 1992).

Infectivities of VAM-fungal inocula were determined in a preliminary experiment by the infection-unit method (Franson and Bethlenfalvay 1989), in order to apply inocula with equal numbers of propagules to all treatments, except to the non-VAM control plants. Two corn cultivars (*Zea mays* L., cv. Zac-58 SM18 Precoz, and cv. H-30, a hybrid) and one sunflower cultivar (*Helianthus annuus* L., cv. Scikus) were used. Zac-58 was of the natice race Cónico-Norteño (Wellhausen et al., 1951), and H-30 was a hybrid from the germplasm collection of the Centro de Genética, Colegio de Postgraduados, México. Mycorrhizal plants were inoculated with one of the three VAM fungi *Glomus etunicatum* Becker and Gerdeman, *G. mosseae* (Nicol. & Gerd.) Gerd. and Trappe, or *G. pallidum* Hall, or with all three of the fungi. Plants were harvested (corn 45 days and sunflower 40 days after planting) at growth stages when the soils in the pots were not yet root-bound, and following the lag phase of mycorrhizal development. Previous work had shown that the lag phase lasted 3–4 weeks under the conditions employed. Thus, VAM colonization and of plant responses were evaluated within the early stage of growth where VAM effects are particularly important for the development of annual plants.

Soil organic matter was by the wet combustion – volumetric method, plant N by Kjeldahl analysis, and plant P by the molybdenum blue method (Chapman and Pratt 1982). Plant biomass was determined after drying the material for 2 days at 70 °C. Leaf area was measured with a LI-COR, Inc., Lincoln, Nebraska, USA) leaf area meter. Specific leaf area was calculated as leaf area/leaf dry mass.

Table 1. Effects of vesicular-arbuscular mycorrhizal (VAM) fungi on plant and soil parameters

Treatment	Leaf area (cm^2)	Root dry mass (g)	Root:shoot ratio	Organic matter (%)
Native corn				
Ge	366^{ab}	0,92^{ab}	0.34^{ab}	1.49^{ab}
Gm	339^{b}	0.89^{b}	0.39^{a}	0.62^{c}
Gp	410^{a}	0.94^{ab}	0.35^{ab}	1.69^{a}
Mix	323^{b}	1.03^{a}	0.37^{ab}	1.21^{ab}
Control	358^{ab}	0,70^{c}	0.31^{b}	0.56^{c}
P value	0.046	<0.001	0.162	<0.001
Hybrid corn				
Ge	376^{a}	0.83^{a}	0.35^{a}	1.39^{a}
Gm	373^{a}	0.79^{a}	0.32^{a}	0.50^{b}
Gp	321^{a}	0.88^{a}	0.38^{a}	1.01^{a}
Mix	338^{a}	0.83^{a}	0.37^{a}	0.54^{b}
Control	350^{a}	0.66^{a}	0.35^{a}	0.58^{b}
P value	0.714	0.757	0.615	<0.001
Sunflower				
Ge	200^{b}	0.24^{ab}	0.16^{a}	1.54^{a}
Gm	312^{a}	0.25^{ab}	0.17^{a}	0.42^{c}
Gp	225^{b}	0.27^{a}	0.16^{a}	0.77^{b}
Mix	216^{b}	0.21^{b}	0.13^{b}	1.51^{ab}
Control	227^{ab}	0.12^{c}	0.09^{c}	0.58^{c}
P value	0.072	<0.001	<0.001	<0.001

Plants were colonized by the VAM fungi *Glomus etunicatum* (Ge); *G. mosseae* (Gm); *G. pallidum* (Gp); their mixture (Mix); or were non-VAM (Control). Numbers are the means of five replicates and are not significant ($P > 0.05$) by Duncan's multiple range test when followed (vertically) by the same letter. Probability values indicate levels of significance among VAM effects not restricted to the arbitrary 5% level

Results

The experiment provided inter- and intraspecific comparisons for plant responses to VAM fungi. Native corn was more responsive to colonization by VAM fungi than hybrid corn (Table 1). In hybrid corn, leaf area, plant biomass, and root:shoot ratios were invariant with VAM status, while significant differences were found between the treatments of native corn in the three parameters. The sunflower response was unlike that of corn, in that the treatments produced highly significant ($P < 0.001$) differences in root:shoot ratios. Like native corn, the sunflower varied in response to the fungi in leaf area and root dry mass, but the order of response to the fungi was different. There were significant differences in dry weight between the native and hybrid corn plants in all but the *G. mosseae* treatment (Fig. 1). The root zones of all three plants showed highly significant variations in soil organic matter content due to the presence of different fungi, or to their absence (Table 1). Significant correlations with VAM colonization for any of the parameters measured were found only for soil organic matter with native corn, specific leaf area in hybrid corn, and plant dry mass in sunflower (Fig. 2). The values were scattered in all other cases, indicating that colonization, and not its intensity, elicited variations in other plant responses.

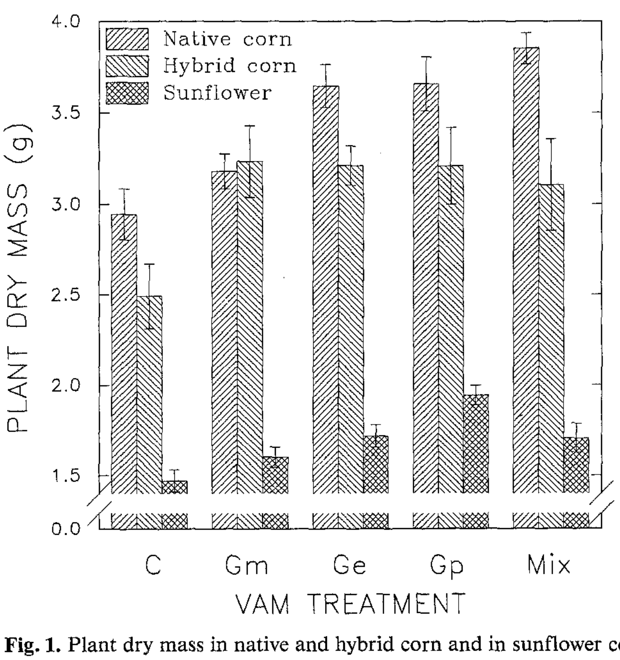

Fig. 1. Plant dry mass in native and hybrid corn and in sunflower colonized by different vesicular-arbuscular mycorrhizal (VAM) fungi in comparison with non-VAM control plants. Values are means and SE of five replicates. *C*, control; *Gm*, *Glomus mosseae*; *Ge*, *G. etunicatum*; *Gp*, *G. pallidum*; *Mix*, mixture of the three *Glomus* spp.

Highly significant ($P < 0.001$) differences in shoot N content were mainly due to the effect of *G. etunicatum* in both varieties of corn, and to that of the three fungi mixed in the sunflower (Table 2). Differences in P content were less pronounced, although significant ($0.1 > P > 0.07$). As with N, VAM-mediated differences in P content were found between some of the treatments in

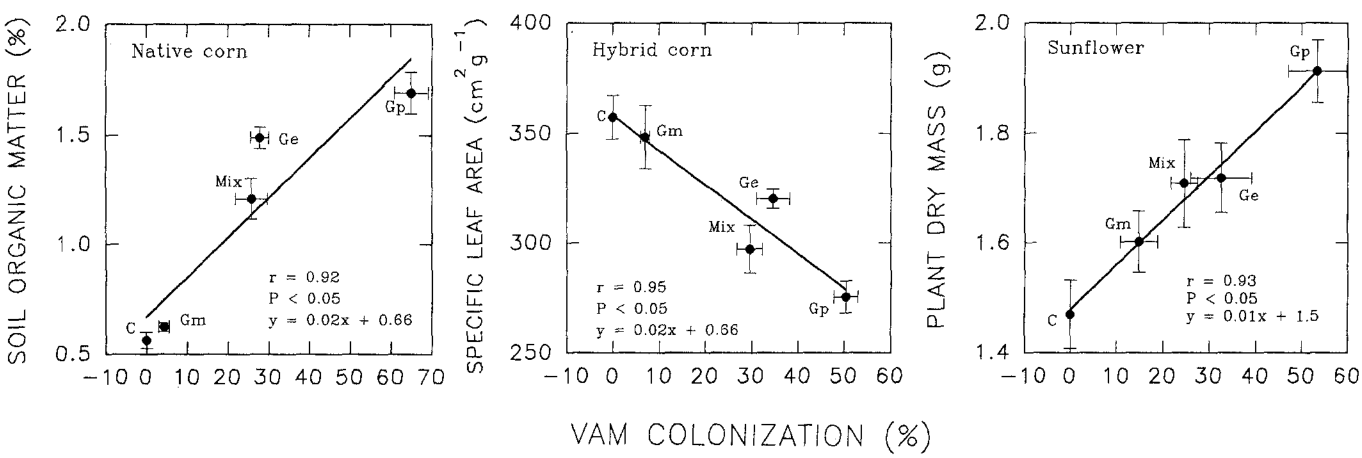

Fig. 2. Relationships between root colonization by vesicular-arbuscular mycorrhizal (VAM) fungi and plant and soil parameters

all three plants. Differences in shoot nutrient contents did not bring about significant differences (Duncan's multiple range test at 5%) between any of the treatments in shoot dry weight.

Analysis of variance (Table 3) showed significant plant effects except for VAM colonization, and significant fungal effects except for leaf area. For the root:shoot ratio, the effect was mainly due to the presence or absence of a VAM fungus, and not to differences among fungi. The significant interactions for shoot N content, root biomass, root:shoot ratio, and VAM colonization indicated that for these response variables incremental changes due to one factor were not accompanied by similar changes due to the other factor. In the case of N content, for instance, high levels of N associated with *G. etunicatum* in corn, but not in sunflower, followed a different pattern from that of N content associated with the three fungi mixed (high in sunflower, low in corn). Thus, the direction of change in N response was reversed, accounting for the high significance ($P = 0.006$) of the plant × fungus interaction for N. For P, however, the trend was similar in all three plants; levels of P tended to be high for *G. pallidum* and the mixture, low in the controls, and intermediate in *G. etunicatum* and *G. mosseae*, accounting for the low significance ($P = 0.368$) of the interaction for P.

Discussion

Mycorrhizal fungi are not a monolithic group, affecting plants only by their presence or absence, but highly variable organisms (Morton 1990) that can elicit a variety of host responses (Stahl et al. 1990). One effect of VAM fungi, of interest to both plant biology and agro-ecology, is a broadening of the ecological niche of plants (Allen et al. 1984) through changes in the availability of nutrients.

Our data show that the potential host–plant niche response mediated by VAM fungal is not limited to improved uptake of nutrients but may extend to alleviation of the effects of mineral excesses. Effects of individual VAM-fungal isolates on the uptake or exclusion of mineral elements other than P (Kothari et al. 1990) or on the

Table 2. Shoot biomass and nutrient contents of plants colonized by different vesicular-arbuscular mycorrhizal (VAM) fungi

Treatment	Shoot		
	Biomass (g)	N content (mg)	P content (mg)
Native corn			
Ge	2.72[a]	64.4[a]	3.98[ab]
Gm	2.27[a]	24.6[b]	3.98[ab]
Gp	2.71[a]	27.6[b]	4.26[ab]
Mix	2.82[a]	26.6[b]	4.96[a]
Control	2.24[a]	22.6[b]	3.44[b]
P value	0.067	<0.001	0.072
Hybrid corn			
Ge	2.36[a]	53.2[a]	3.52[b]
Gm	2.41[a]	21.6[b]	4.16[ab]
Gp	2.32[a]	23.8[b]	5.58[a]
Mix	2.28[a]	22.8[b]	6.00[a]
Control	1.83[a]	16.4[b]	4.82[ab]
P value	0.544	<0.001	0.074
Sunflower			
Ge	1.47[a]	30.2[ab]	4.50[b]
Gm	1.45[a]	23.4[b]	4.52[b]
Gp	1.67[a]	31.6[ab]	6.60[a]
Mix	1.60[a]	57.4[a]	5.08[ab]
Control	1.35[a]	22.8[b]	4.24[b]
P value	0.388	0.003	0.0964

See footnotes to Table 1

Table 3. Analysis of variance of host-plant and vesicular-arbuscular mycorrhizal (VAM) fungal effects on plant and soil parameters

Parameter	Effect		
	Plant	Fungus	Interaction
Leaf Area	<0.001	0.300	0.108
Shoot biomass	<0.001	0.043	0.748
Shoot N content	0.025	<0.001	0.006
Shoot P content	0.038	0.013	0.368
Root biomass	<0.001	0.021	0.010
Plant biomass	<0.001	0.024	0.779
Root : shoot ratio	<0.001	<0.001	<0.001
VAM colonization	0.113	<0.001	0.009
Organic matter	<0.001	<0.001	<0.001

Numbers denote probability values

alleviation of heavy metal toxicities (Heggo and Angle 1990) have been reported. A complete evaluation of VAM effects on plant nutrition by several isolates acting in concert is more elusive, because affinities of the isolates for each other and for the host plant must be considered, in addition to the nutritional effects each isolate may have on a given host individually (Allen 1991; Stahl and Christensen 1991). Superimposed on host–endophyte relationships, the tolerance of the fungi to soil conditions must be also be taken into account.

The importance of the soil factor is shown by the not yet fully appreciated VAM influence on plants that is mediated indirectly by improving soil structure (Tisdall 1991). Recent insights even suggest that there is specialization among VAM fungi in functions affecting plant vs. soil nutrition; VAM-fungal species of the genus *Gigaspora* appear to favor fluxes of C compounds from plants to the soil biota, resulting ultimately in enhanced soil aggregation, while *Glomus* spp. tend to favor plant growth through improved mineral nutrition (Miller and Jastrow 1992). Contributions by the fungi to soil organic matter play a key role in rhizosphere biology (Jakobsen and Rosendahl 1990), and therefore in nutrient availability (Marschner et al. 1987). Measurable changes by our VAM-fungal isolates on soil organic matter, even in this short-term experiment, suggest that they eventually affect plant nutrition not only through a direct regulation of mineral nutrient uptake or exclusion, but indirectly through a modification of soil structure.

Conclusions

Evaluations of VAM fungi should take into account the effects of individual isolates on specific plant and soil responses. These responses can vary even if the plants are grown in P-sufficient soil. Tests for VAM plant and soil responses under controlled conditions are necessary prior to field trials for specific VAM effects.

Acknowledgment. Work was funded by the Program in Science and Technology Cooperation, Office of the Science Advisor, Agency for International Development as Project No. 8.055, and was conducted in collaboration at CP and WRRC.

References

Abbott LK, Robson AD (1981) Infectivity and effectiveness of vesicular-arbuscular mycorrhizal fungi: Effect of inoculum type. Aust J Agric Res 32:631–639

Allen MF (1991) The ecology of mycorrhizae. Cambridge University Press, Cambridge

Allen MF, Allen EB, Stahl PD (1984) Differential niche response of *Bouteloua gracilis* and *Paspalum smithii* to VA mycorrhizae. Bull Torrey Bot Club 111:361–365

Azcón de Aguilar C, Azcón R, Barea JM (1979) Endomycorrhizal fungi and *Rhizobium* as biological fertilizers for *Medicago sativa* in normal cultivation. Nature (London) 279:325–327

Bagyaraj DJ (1992) Vesicular-arbuscular mycorrhiza: Application in agriculture. Methods Microbiol 24:359–373

Chapman HD, Pratt PF (1982) Methods of analysis for soils, plants and waters. Div Agric Sci Univ California Publ no. 4034, Berkeley, California

Espinoza-Victoria D, Quintero-Ramos M, Ferrera-Cerrato R, Bethlenfalvay GJ (1982) Fitting plants to soil through mycorrhizal fungi: Plant nutrition in host-endophyte combinations evaluated by the Diagnosis and Recommendation Integrated System. Biol Fertil Soils (in press)

Franson RL, Bethlenfalvay GJ (1989) Infection unit method of vesicular-arbuscular mycorrhizal propagule determination. Soil Sci Soc Am J 53:754–756

Heggo A, Angle JS (1990) Effects of vesicular arbuscular mycorrhizal fungi on heavy metal uptake by soybeans. Soil Biol Biochem 22:865–869

Jakobsen I, Rosendahl L (1990) Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. New Phytol 115:77–83

Koide R, Elliott G (1989) Cost, benefit and efficiency of the vesicular-arbuscular mycorrhizal symbiosis. Funct Ecol 3:252–255

Kothari SK, Marschner H, Römheld V (1990) Direct and indirect effects of VA mycorrhizal fungi and rhizosphere microorganisms on acquisition of mineral nutrients by maize (*Zea may* L.) in a calcareous soil. New Phytol 116:637–645

Marschner H, Römheld V, Cakmak I (1987) Root-induced changes of nutrient availability in the rhizosphere. J Plant Nutr 10:1175–1184

Miller RM, Jastrow JD (1992) The role of mycorrhizal fungi in soil conservation. In: Bethlenfalvay GJ, Linderman RG (eds) Mycorrhizae in sustainable agriculture. Am Soc Agron Spec Publ 54, Madison, Wisconsin, pp 45–70

Morton JB (1990) Species and clones of arbuscular mycorrhizal fungi (Glomales, Zygomycetes): Their role in macro- and microevolutionary processes. Mycotaxon 37:493–515

Nelson LA (1989) A statistical editor's viewpoint of statistical usage in horticultural science publications. HortScience 24:53–55

Raju PS, Clark RB, Ellis JR, Duncan RR, Maranville JW (1990) Benefit and cost analysis of phosphorus efficiency of VA mycorrhizal fungi colonizations with sorghum (*Sorghum bicolor*) genotypes grown at various phosphorus levels. Plant and Soil 124:199–204

Stahl P, Christensen M (1991) Population variation in the mycorrhizal fungus *Glomus mosseae*: Breadth of environmental tolerance. Mycol Res 95:300–307

Stahl P, Christensen M, Williams SE (1990) Population variation in the mycorrhizal fungus *Glomus mosseae*: Uniform garden experiments. Mycol Res 94:1070–1076

Tisdall JM (1991) Fungal hyphae and structural stability of soil. Aust J Soil Res 29:729–743

Vierheilig H, Ocampo JA (1991) Susceptibility and effectiveness of vesicular-arbuscular mycorrhizae in wheat cultivars under different growing conditions. Biol Fertil Soils 11:290–294

Wellhausen EJ, Roberts LM, Hernandez-Xolocotzi E, Mangelsdorf PC (1951) Razas de maíz en México. Folleto Técnico no. 5, Oficina de Estudios Especiales, Secretaría de Agricultura y Ganadería, Mexico City, Mexico