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# Response of "criollo" maize to single and mixed species inocula of arbuscular mycorrhizal fungi

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## Abstract

We tested the effect of two single species inocula and a mixed inoculum of the native population of arbuscular mycorrhizal (AM) fungi on the growth response of "criollo" maize (*Zea mays* L.). To determine the inocula that produced the highest response on maize growth, we conducted a greenhouse experiment at 3 levels of P fertilization (0, 40 and 80 kg $ha^{-1}$). Inoculation with *Glomus mosseae* (Nicolson and Gerdemann) Gerd. and Trappe (LMSS) produced the greatest shoot growth rates at the two lowest P fertilization levels. Inoculation with *Acaulospora bireticulata* Rothwell and Trappe (ABRT) and the native population (NP) resulted in similar shoot growth rates at all P levels. These rates were higher than the non-mycorrhizal control rate at the lowest P level but lower than the control at the highest P level. Also, ABRT and NP had significantly lower shoot growth rates than the inoculation treatment with *G. mosseae* at all P levels. The non-mycorrhizal control had the lowest growth rate at the lowest P level but its growth rate increased linearly with increased P fertilization. Inoculation with *G. mosseae* and *A. bireticulata* produced similar colonization rates which were lower than the native population colonization rate. There was no correlation between colonization and shoot growth rates.

## Introduction

Arbuscular mycorrhizal fungi play an important role in the phosphorus nutrition of plants by increasing the absorption zone for P uptake through external hyphae that extend into the soil (Harley and Smith, 1983). The effectiveness of mycorrhizal fungi has been defined as their relative ability to stimulate plant growth (Abbott and Robson, 1981). Different species and isolates of the same species of mycorrhizal fungi can vary considerably in the benefits they confer to host plants (Bethlenfalvay et al., 1989). Native fungi may adapt to the prevailing local conditions at the sites (Lambert et al., 1980). Native populations can be effective for crops in some sites but not in others (Dodd et al., 1983), or only some of the constituent species may be effective.

In the study of the effects of mycorrhizal fungi on plant growth, it is essential to compare the response curves of mycorrhizal and non-mycorrhizal plants to phosphate additions (Abbott and Robson, 1982). This allows the selection of those fertilization levels that optimize the response to inoculation. Comparison of mycorrhizal colonization curves with sequential harvests also permits the development of colonization and its relation to growth responses to be monitored during the experiment (Abbott and Robson, 1985). Both positive (Medina et al., 1988) and negative (Jensen, 1982) correlations between colonization development and growth responses have been found.

Koomen et al. (1987) suggested the use of multiple rather than single species inocula, but with the appropriate combination of effective species. Therefore, the selection of the effective species from a native population of mycorrhizal fungi for a particular site is one of the first steps towards an evaluation of the contribution of this symbiosis to plant growth. The goal of our study is not to select fungi for large - scale inoculation but

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for in situ management with agricultural practices that preserve or increase the fungal communities. Howeler et al. (1987) consider this can be done as long as the native population contains at least some highly efficient AM fungal species to promote growth.

In Mexico, the use of "criollos" instead of genetically improved varieties is a common practice. "Criollos" can be defined as races and subraces of cultivated maize which have been selected by traditional domestication processes. With the development of agriculture by native indians, maize seeds were hand selected from the best of the last harvest based on their size, shape and color, and they have been selected in the same way since then. The use of "criollos" is associated with traditional low-input farming where machinery and pesticides are not normally used, and fertilizers (always in low amounts) are not consistently applied. Although "criollos" are considered to be domesticated plants, they are expected to differ from newer high-yield varieties in response to soil fertility, climatic conditions, and agricultural practices. Wild plants possess adaptations for growth in nutrient-poor soils and are often less responsive to nutrient input than cultivated species (Chapin et al., 1986). This led Koide et al. (1988) and Bryla and Koide (1990) to hypothesize that mycorrhizal colonization benefits wild plants less than cultivated plants and to explore the possibility that inherent plant traits can determine the response to mycorrhizal colonization. Therefore, it seems important to investigate the functioning of the symbiotic association between traditionally domesticated plants and native arbuscular fungi.

We conducted this experiment to evaluate the effect of different inocula of arbuscular mycorrhizal (AM) fungi on growth response of "criollo" maize under three P fertilization levels. We tested the effectiveness of the whole native population and two single species inocula of AM fungi belonging to the same native population, in order to identify those that produce the greatest plant growth response.

## Materials and methods

### *Soil*

We collected the soil in maize fields located at the footslope of the Malintzin Volcano in Tlaxcala, Mexico. The soil was classified as a Tropofluvent with pH 6.0, 1.2% organic matter, 11 mg $L^{-1}$ of available P (Mehlich II) and 0.131% total N. After sieving through a 5 mm mesh the soil was steam-sterilized for two hours: one hour during the first day and once more 24 hours later. After aeration for one week, a fresh microbial filtrate of a field soil suspension obtained by sieving through a nylon mesh (6 $\mu$m) was added and mixed thoroughly with the steam-sterilized soil. The soil was placed in plastic bags which remained closed for two weeks and were then opened for aeration for one more week.

*Table 1.* List of AM fungal species identified from the native population cultures

| |
|---|
| *Acaulospora bireticulata* Rothwell and Trappe |
| *A. laevis* Gerd. and Trappe |
| *A. mellea* Spain and Schenck |
| *A. spinosa* Walk. and Trappe |
| *A. sp.* nov. (1) |
| *A. sp.* nov. (2) |
| *Gigaspora margarita* Becker and Hall |
| *Glomus etunicatum* Becker and Gerdemann |
| *G. mosseae* (Nicol. and Gerdemann) Gerd. and Trappe |
| *Sclerocystis clavispora* Trappe |
| *S. sinuosa* Gerd. and Bakshi |
| *Scutellospora dipurpurascens* Morton and Koske |
| *S. pellucida* (nicol. and Schenck) Walker and Sanders |
| *S. sp.* nov. |

### *Inocula*

We isolated pure cultures of *Acaulospora bireticulata* Rothwell and Trappe (ABRT) and *Glomus mosseae* (Nicol. and Gerdemann) Gerd. and Trappe (LMSS) from the maize fields where the soil was collected. Native population (NP) inoculum consisted of at least 14 species of AM fungi (Gavito and Varela, 1993) (Table 1). Isolation of the two native species required an initial propagation period using alfalfa (*Medicago sativa* L.) and Bahia grass (*Paspalum notatum* Flugge) as host plants in order to get fresh undamaged spores. Fifty fresh spores of each of the two species provided the inoculum for the single species cultures and 25 g of soil from the propagation cultures were used for the native population cultures. We used a sand-soil mixture as a substrate and alfalfa as the host plant. They grew up during 15 weeks in the greenhouse and then the shoots were cut, the roots were chopped and the soil and roots of each inoculum were thoroughly mixed.

For adequate comparison, we standardized the inocula to produce the same percentage of primary

mycorrhizal colonization using a plant bioassay (Moorman and Reeves, 1979). Dilutions (undiluted, 1:3 and 1:15 v inoculum:v sterilized soil) were made using steam-sterilized field soil as a diluent for the soil-roots inoculum material of each type of inoculum, prepared as explained above. "Criollo" maize was planted and seedlings were harvested after 25 days. Roots were washed and stained (Phillips and Hayman, 1970) and mycorrhizal colonization percentage, as hyphae, arbuscules or vesicles, was determined by the gridline intersect method (Giovanetti and Mosse, 1980). We determined the dilution level at which the three inocula produced the same amount of colonization and adjusted the inoculum density to approximate this level of infection in the effectiveness evaluation (undiluted for ABRT and NP, and 1:3 (v:v) dilution for LMSS).

*Effectiveness evaluation*

A greenhouse factorial experiment was designed with the following treatments:

a) Inoculation. uninoculated control (C) and inoculated with ABRT, LMSS and NP.
b) P fertilization : unfertilized (P1), fertilized with 40 kg P $ha^{-1}$ (P2), and with 80 kg P $ha^{-1}$ (P3).

Infested soil was prepared as explained above with 100 g of ABRT and NP undiluted inoculum, and LMSS inoculum diluted with sterilized sand-soil. We mixed the soil thoroughly to a final weight of 2 kg, placed it into 3 kg capacity black plastic bags and fertilizer was applied as $K_2HPO_4$ in solution. The lower dose corresponded to a level only rarely applied by farmers in the area, since normally they do not use P fertilizer. All the pots received an initial dose of 25 kg N $ha^{-1}$ as ammonium nitrate in solution, and another 50 kg N $ha^{-1}$ one month after planting. Two five-day-old seedlings of "criollo" maize were planted in each pot and thinned to one per pot after one week.

We prepared 20 replicates of each treatment and harvested five replicates at 15, 30, 45 and 60 days after the initiation of the experiment. We measured shoot dry weight and percentage of mycorrhizal infection by the gridline intersect method for all plants.

Data were subjected to regression procedures over time to obtain shoot growth and mycorrhizal colonization rates. These rates (slopes) were subjected to analysis of variance. Inoculation main effect differences on colonization were separated by a Tukey multiple comparisons test. Interactions found in shoot growth rates were analyzed with orthogonal polynomials and contrasts when appropriate. Square root of shoot dry weight and logarithm of mycorrhizal colonization percentage transformations were used to satisfy normal distribution and homogeneity of variances assumptions for the statistical analysis. Results are presented back-transformed, unless otherwise stated. Correlation between shoot growth rates and mycorrhizal colonization rates was determined with Pearson's r test.

*Table 2.* Shoot growth rates and mycorrhizal colonization rates (calculated with back-transformed data) of "criollo" maize for the 12 treatment combinations. Rates followed by the different letters differ significantly within fertilization level at $p<0.05$. Rates followed by an asterisk differ significantly between fertilization level. No interactions were found for colonization rates, inoculation main effect differences are explained in text

| Fertilization | Inoculation | Shoot growth rates ($day^{-1}$) | Colonization rates ($day^{-1}$) |
|---|---|---|---|
| P1 | Control | 0.083 a | 0 |
| | ABRT | 0.117 b | 0.508 |
| | LMSS | 0.199 c | 0.569 |
| | NP | 0.137 b | 0.329 |
| P2 | Control | 0.120 a | 0 |
| | ABRT | 0.134 a | 0.274 |
| | LMSS | 0.223 b | 0.309 |
| | NP | 0.113 a | 0.266 |
| P3 | Control | 0.205 a* | 0 |
| | ABRT | 0.163 b | 0.546 |
| | LMSS | 0.216 a | 0.451 |
| | NP | 0.147 b | 0.636 |

## Results

*Effect of inoculation and fertilization on shoot growth rates*

Main effects and interactions were statistically significant at $p<0.05$. At P1, the non-mycorrhizal control showed the lowest growth rate of all inoculation treatments (Table 2). The ABRT and NP treatments resulted in similar rates and were higher than the control but lower than the LMSS rate. At P2, the control, ABRT and NP showed similar growth rates which were lower than LMSS rate. At P3, the control and LMSS rates were similar and significantly higher than the rates for ABRT and NP. Growth rates for ABRT, NP and LMSS did not respond to P fertilization, whereas the rates for

the control increased linearly with fertilization, indicating that the available P level in the soil was limiting 'criollo' maize growth.

*Effect of inoculation and fertilization on mycorrhizal colonization rates*

The inoculation main effect was significant at $p<0.05$. No fertilization main effect or interactions were detected, probably due to high variation in the colonization data. The NP colonization rate (relative colonization rate=0.028 $day^{-1}$) was higher than the ABRT treatment colonization rate (RCR=0.020) but similar to the LMSS rate (RCR=0.023). The LMSS and ABRT colonization rates were similar.

We found no correlation between mycorrhizal colonization rates and shoot growth rates.

## Discussion

Maize is considered to have a low dependence on mycorrhizae (Howeler et al., 1987). A lack of growth response of inoculated maize is well documented for fertilized soils (Kothari et al., 1990; Simpson and Daft, 1990). Nevertheless, for low fertility soils, differences between inoculated and non-inoculated plants have been found (Hetrick et al., 1987). The present results indicate that "criollo" maize growing in unfertilized soil responded positively to inoculation although the soil presented a medium level of available P. At the low P fertilization level, "criollo" was still responsive to inoculation with *Glomus mosseae* but not to inoculation with *Acaulospora bireticulata* or the native population.

The low efficiency for ABRT reported in this case is a particularly interesting result in this case when one considers that mycorrhizae researchers have observed (unfortunately without sufficient supporting published data) that many species of the genus *Acaulospora* seem to be less efficient in promoting growth than *Glomus* species. To date, most of *Acaulospora* species that have been described are from tropical and subtropical regions while most of the *Glomus* species have been described from temperate regions (Allen, 1991). In our study site, we have found 8 species belonging to this genus, including *A. delicata* and *A. splendida* (Estrada-Torres et al., 1992), and only 3 species of *Glomus*. Evaluations of the effectiveness of native species in tropical soils are very scarce. Howeler et al. (1987), reported that only 41% of their *Acaulospora* native isolates were effective in promoting cassava growth in the greenhouse, while 92% of their *Glomus* isolates were effective. Sieverding (1991), provided effectiveness evaluations of many isolates from Colombia of the same two genus on different plant hosts that showed similar results, despite a larger variation. Also, their results do not show a relation between dry matter production and root colonization, in accordance with our results and the observation by Wilson and Tommerup (1993) that the most infective fungus is not necessarily the most effective. Effectiveness may be related to the ability of some fungal species to develop an extensive mycelial network that increases P uptake. However, the techniques available to measure mycorrhizal development are deficient because they only include intraradical structures and measurements of extraradical mycelium have a difficulty distinguishing mycorrhizal and non-mycorrhizal hyphae. We will be able to test more accurately the relationship between mycorrhizal colonization and plant growth when we have better procedures to measure mycorrhizal development. Nevertheless, additional measurements, such as shoot P, that provide information about the ability of the inocula to increase P in the plant are desirable for a more complete evaluation of AM fungi inocula.

The possibility that ineffective species are abundant in the tropical regions, which we take cautiously because it obviously needs further study, opens an interesting question. The results obtained up to date show that the question is worth exploring.

The native population, which includes more than 14 arbuscular mycorrhizal species, represents the sum of all possible interactions in a mixture of different infective, physiological and competitive abilities. As stated by Wilson and Trinick (1983), in mixed inoculum, the proportion of each fungus influences colonization directly because of competition for nutrients inside the roots. We attempted a fair comparison by using inoculum that was of the same age in a proportion that would produce the same percentage of primary colonization. In this way, we observed the performance of each kind of inoculum after the same amount of primary colonization was achieved. Since we had to dilute LMSS inoculum to equate primary colonization, we can consider this inoculum to be the most effective at initiating colonization but, from the results of the experiment, NP was more effective in propagating in the roots. Another observation is that we inoculated NP as it was in the field, we only propagated it in the greenhouse to obtain inoculum of uniform age and we do not know

what species were actually colonizing the roots from that inoculum.

The native population of VAM fungi acting together and ABRT seemed to be less effective at increasing shoot dry weight in maize relative to the LMSS single species inoculum. Therefore, selection of effective fungal species would seem important. In these fields where P fertilization is not likely to occur because of the high costs of fertilizers, attention has to be paid to the benefits obtained from mycorrhizal associations at the local soil fertility level. LMSS inoculated plants showed the highest growth rates at the original soil P level and the low fertilization rate. Although results from pot experiments cannot be used easily to predict the behaviour of the endophytes in the field, our results show that LMSS is an effective species whose properties should be tested under field conditions.

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## References

Abbott L K and Robson A D 1981 Infectivity an effectiveness of vesicular arbuscular mycorrhizal fungi: effect of inoculum type. Aust. J. Agric. Res. 32, 631–639.

Abbott L K and Robson A D 1982 The role of vesicular arbuscular mycorrhizal fungi in agriculture and the selection of fungi for inoculation. Aust. J. Agric. Res. 33, 389–408.

Abbott L K and Robson A D 1985 The effect of VA mycorrhizae on plant growth. *In* VA Mycorrhizae. Eds. C L L Powell and D J Bagyaraj. pp 113–130. CRC Press, Boca Raton.

Allen M F 1991 The ecology of mycorrhizae. Cambridge University Press, Cambridge. 184p.

Bethlenfalvay G J, Franson R L, Brown M S and Mihara K L 1989 The *Glycine-Glomus-Bradyrhizobium* symbiosis. IX. Nutritional, morphological and physiological responses of nodulated soybean to geographic isolates of the mycorrhizal fungus *Glomus mosseae*. Physiol. Plant. 76, 226–232.

Bryla D R and Koide R T 1990 Role of mycorrhizal infection in the growth and reproduction of wild cultivated plants. II. Eight wild accessions and two cultivars of *Lycopersicum esculentum* Mill. Oecologia 84, 82–92.

Chapin F S III, Vitousek P M and Van Cleve K 1986 The nature of nutrient limitation in plant communities. Am. Nat. 127, 48–58.

Dodd J, Krikun J and Haas J 1983 Relative effectiveness of indigenous populations of vesicular arbuscular mycorrhizal fungi from four sites in the Negev. Isr. J. Bot. 32, 10–21.

Estrada-Torres A, Varela L, Hernandez-Cuevas L and Gavito M E 1992 Algunos hongos micorrizicos arbusculares del Estado de Tlaxcala, Mexico. Rev. Mexicana Micologia 8, 85–110.

Gavito M E and Varela L 1993 Seasonal dynamics of mycorrhizal associations in maize fields under low input agriculture. Agric. Ecosys. Enviro. 45, 275–282.

Giovanetti M and Mosse B 1980 An evaluation of techniques, for measuring vesicular-arbuscular myccorrhizal infection in roots. New Phytol. 84, 489–500.

Harley J L and Smith S E 1983 Mycorrhizal Symbiosis. Academic Press, London. 483p.

Hetrick B A D, Gerschefske K D and Thompson G 1987 Effects of drought stress on growth response in corn, sudan grass and big bluestem to *Glomus etunicatum*. New Phytol. 105, 405–410.

Howeler R H, Sieverding E and Saif S 1987 Practical aspects of mycorrhizal technology in some tropical crops and pastures. Plant and Soil 100, 249–283.

Jensen A 1982 Influence of four vesicular-arbuscular mycorrhizal fungi on nutrient uptake and growth in barley (*Hordeum vulgare*). New Phytol. 90, 45–50.

Koide R, Mingguang L, Lewis J and Irby C 1988 Role of mycorrhizal infection in the growth and reproduction of wild vs. cultivated plants, I. Wild vs. cultivated oats. Oecologia 77, 537–543.

Koomen I, Grace C and Hayman D S 1987 Effectiveness of single and multiple mycorrhizal inocula on growth of clover and strawberry plants at two soil pHs. Soil Biol. Biochem. 19, 539–544.

Kothari S K, Marschner H and George E 1990 Effect of VA mycorrhizal fungi and rhizosphere microorganisms and root and shoot morphology, growth and water relations in maize. New Phytol. 116, 303–311.

Lambert D H, Cole H and Baker D E 1980 Adaptation of vesicular-arbuscular mycorrhizae to edaphic factors. New Phytol. 85, 513–520.

Medina O A, Sylvia D M and Kretschmer A E 1988 Response of siratro to vesicular arbuscular mycorrhizal fungi in amended soil. Soil Sci. Soc. Am. J. 52, 416–419.

Moorman T and Reeves F B 1979 The role of endomycorrhizae in revegetation practices in the semi-arid west. II. A bioassay to determine the effect of land disturbance on endomycorrhizal populations. Am. J. Bot. 66, 14–18.

Phillips J M and Hayman D S 1970 Improved procedures to clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55, 158–160.

Sieverding E 1991 Vesicular-Arbuscular Mycorrhiza Management in Tropical Agroecosystems. Technical Cooperation, Federal Republic of Germany, Eschborn. 371p.

Simpson D and Daft M J 1990 Interactions between water-stress and different mycorrhizal inocula on plant growth and mycorrhizal development in maize and sorghum. Plant and Soil 121, 179–186.

Wilson J M and Tommerup I C 1992 Interactions between fungal symbionts. VA mycorrhizae. *In* Mycorrhizal Functioning. An integrative Plant-Fungal Process. Ed. M F Allen. pp 199–248. Chapman and Hall, New York.

Wilson J M and Trinick M J 1983 Infection development and interactions between vesicular-arbuscular mycorrhizal fungi. New Phytol. 93, 543–553.

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