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Influence of several arbuscular mycorrhizal fungi alone or combined on the growth of three varieties of tomato (*Solanum lycopersicum* L.)

Influencia de varios hongos micorrizógenos arbusculares solos o combinados sobre el crecimiento de tres variedades de tomate (*Solanum lycopersicum* L.)

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Palabras clave: agresividad, efectividad, mezcla de inóculos, pH, tomate

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ABSTRACT

The effect of the inoculation of eight different VAM fungi single or in combination of two or three isovolumetrical inocula were tested on the growth and mycorrhizal characteristics of three different varieties of tomato. Plants grew on a Yellowish Red Ferralithic during eight weeks. Plants were fertilized weekly since the fourth week. Final height, stem neck diameter, number of flowers, shoot, root, rootlets and total plant dry weights, percentage VAM colonization and total length of colonized roots were analyzed. Effectiveness categories were also determined for all the analyzed characters. Among the single inocula tested *Rhizoglomus intraradices*, *Diversispora spurca* and *R. fasciculatum* were the best for all three varieties in our experimental conditions. Double and triple inocula produced an increase in growth statistically lower or similar than that produced by pure inocula when plants were inoculated with the effective and aggressive fungi *R. fasciculatum* or *R. manihotis*. Triple inoculation produced always an increase in growth statistically similar or higher than double inoculations. These results suggest that the use of multiple inocula may be desirable but care should be taken about the species involved and the proper pH. The use of multiple inocula could be more convenient that the pure strains inoculation, but it should know the strains that are going to be mixture with regard to effectiveness, aggressiveness and proper pH.

RESUMEN

Se ensayó el efecto de ocho cepas de hongos micorrizógenos arbusculares y las combinaciones dobles y triples, sobre el crecimiento y la micorrización de tres variedades de tomate, durante ocho semanas en suelo Ferralítico Rojo Amarillento. Se evaluaron la altura final de las plantas, diámetro del cuello, número de flores, pesos secos de follaje, raíz principal, raicillas y total, así como el porcentaje de colonización micorrízica y la longitud total de raicillas colonizadas. Se determinaron las categorías de efectividad para todas las características medidas. En los tratamientos de cepas puras ensayadas *Rhizoglomus intraradices*, *Diversispora spurca* y *R. fasciculatum* resultaron las mejores para todas las variedades de tomate ensayadas. Los inóculos dobles y triple produjeron un incremento del crecimiento estadísticamente inferior o igual al de los inóculos puros involucrados, cuando las plantas fueron inoculadas con uno de los hongos efectivos y agresivos *R. fasciculatum* o *R. manihotis*. La inoculación triple siempre produjo un incremento del crecimiento estadísticamente similar o mayor que las mezclas dobles. El análisis de los resultados para las inoculaciones múltiples y sencillas sugirió que mientras menos efectiva sea una cepa, más conveniente será su combinación con cepas de efectividad mayor, siempre que todas se desarrollen en un pH adecuado. El uso de inóculos múltiples puede ser más conveniente que la inoculación de cepas puras, pero se debe conocer las cepas que se mezclan en cuanto a efectividad, agresividad y pH apropiado.

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INTRODUCTION

The symbiosis known as "mycorrhiza" has attracted the interest of many research groups for the last fifty years, mainly because of its potential as biological fertilizer (Chu *et al*., 2016; Ferrazzano and Williamson, 2013; Kohler, *et al*., 2015; Püschel *et al*., 2014). At present, any economical or ecological study dealing with plant communities should not neglect them.

Data on effectiveness and usefulness at different situations of only some all described fungi are scarce and too spread in the literature. A good and accessible compilation is being made mainly for taxonomy and reported through INVAM (2012). Somewhat similar or addendum to this one is being needed for all data leading to the correct management of VAM strains in sustainable agriculture.

Tomato is an important crop everywhere. Differences in behavior between cultivars of the same crop in relation to mycorrhizae have been established (Azcón and Ocampo, 1981; Estaún *et al*., 2010). The knowledge of the potential for colonization, the effectiveness of VAM fungal species occurring in the soil to be planted, and the selection of effective VAM species for each crop and conditions have been recommended (Cavagnaro *et al*., 2006; Cuenca *et al*., 2007; Gianinazzi-Pearson *et al*., 1985; Pellegrino and Bedini, 2014; Ryan and Graham, 2002; Sieverding, 1991).

In a study with clover and strawberry at two different soil pH Koomen *et al.* (1987) concluded that mixed inocula should be preferred for field inoculation, like has been demonstrated also at greenhouse conditions by Ley-Rivas *et al.* (2011). Sieverding (1991) mixed *Glomus manihotis, G. occultum* and *Entrophospora colombiana* in 1:1 or 1:1:1 ratio to inoculate cassava. He concluded that in general, when several highly effective fungi are present in the inoculum, a growth response should be expected in any case. Edathil *et al.* (1996) mixed equal weights of four species in 15 combinations containing one to four fungi and concluded that multiple VAM fungal inocula may result in better plant growth response than with a single inoculation.

The aim of this work was to assess the effect of several VAM fungal species on the growth of three different varieties of tomato and to test the effect of combined inocula by mixing two or three cultotypes of VAM fungi.

MATERIALS AND METHODS

Soil

The soil used in this work was collected from the border of a field at the Institute of Ecology and Systematic. It had been unlabored for more than 30 years, and it was an Acrisol classified as clayey and deep Yellowish-Red Ferralithic with iron concretions laying on chalky rock, with pH=7.7 and 6.8 (H_2O and KCl, respectively), P=12 μg/g (Olsen), N=0.2%, K=526.5 μg/g, Na=20.93 μg/g, and organic matter=1.48%. Soil was mixed with washed quartzy sand (3:1; v/v) and fumigated with methyl bromide. After proper aeration, the mixture was used to fill 1.3 L pots.

VAM cultotypes and inoculation procedures

Eight pure cultotypes were used:

FAS: *Rhizoglomus fasciculatum*, originally from Turin, Italy, LPA-7 strain, kindly supplied in 1986 by Dra. Vivienne Pearson, from the Station of Genetics and Improvement of Plants, INRA, Dijón, France, and maintained for several years in our collection in pot cultures with sorghum.

MAN: *Rhizoglomus manihotis*, pure culture kindly supplied by Dr. Ewald Sieverding (CIAT, Colombia) and reproduced in our collection.

MOS: *Funneliformis mosseae*, originally from UK, and obtained in 1990 from the collection of Dr. Sergio Palacios, UNAM, México. Reisolated and maintained in our collection since then.

INT: *Rhizoglomus intraradices*, isolated from the same culture than MOS. Identified in October 1995 at INVAM by Drs. Joseph Morton and Steve Bentivenga.

SCR*: Acaulospora scrobiculata*, imported in 1986 from CIAT, Colombia and reproduced in our collection.

OCC: *Paraglomus occultum*, also imported in 1986 from CIAT, Colombia and reproduced in our collection.

CAL*: Funneliformis caledonium*, originally from UK, reisolated from the original culture of MOS, UNAM, Mexico.

SPU: *Diversispora spurca*, isolated in 1990 from a Mountain Red Ferralithic soil from La Felicidad, Topes de Collantes, Villa Clara, Cuba with pH=4.9-5.4 and maintained since then in our collection. Deposited and Classified by Dr. Joseph Morton at INVAM in Oct. 1995.

In addition to the plants inoculated with the pure cultotypes, uninoculated controls and mixtures of FAS+MAN, FAS+MOS, MAN+MOS, and FAS+MAN+MOS were established as treatments. All fungal mixtures were prepared by using equal volumes of the pure soil inocula containing homogenized spores, mycelium and colonized root fragments.

All pure cultures were checked for purity. Many spores were seen in each one in addition to other infecting structures, but counts were not made because previously dealing with Most Probable Number (MPN) (Porter, 1979) determinations in fourteen different natural forest soils, thirteen places showed colonization in all plants for the 4^{-1} (1/4) dilution level. As pure cultures have usually higher potentials than natural soils, all eight cultotypes were assumed to be strong enough even after diluting to 1/2 or 1/3 in the mixtures. By the other hand, unpublished data showed that the counting of spores of commercial inocula were higher after some time of storage for some but not all species, making counting of spores and MPN values probably doubtful after six weeks.

Inoculation was done for all except control treatments placing 10 ml of pure or mixed inocula in the planting hole. One point five ml of the microbial leaching was added to each pot. Uninoculated control pots only received the microbial leaching. For the preparation of the leaching, 100 ml of isovolumetric mixture of all eight dry cultotypes and 100 ml of the soil used for the experiment were suspended in 350 ml of sterile distilled water, soaked during 30 min. and filtered through a Watman No. 1 filter paper.

Plant material
Three varieties of tomato were used in the experiment: CV. Manaluce, with optimal culture period in October-November; CV. Roma, cultivable throughout all the year; and CV. HC-7880, genetically improved variety for production out of season. Three or four seeds of the corresponding variety of tomato were seeded and plants were thinned to one per pot after establishment.

Experimental Design
Factorial design in random blocks was applied for 39 treatments with three replicates for each one. The trial was maintained for eight weeks in the greenhouse and carefully watered daily. Fifteen ml per pot of Long Ashton solution (Briceño and Pacheco, 1984) -with P reduced to 5 µg/g- were weekly applied since fourth week after sowing.

Measurements
Final plant height, stem neck diameter and number of flowers were measured at harvest. Shoot dry weight, root dry weight (main and lateral thicker than 2 mm roots) and rootlets dry weight (terminal roots thinner than 2 mm with root hairs) were determined after drying plant material. AM colonization (Giovannetti and Mosse, 1980) and total length of colonized rootlets were both estimated after staining (Phillips and Hayman, 1970) aliquots of known weight. Data were submitted to Analysis of Variance and Multiple Range Duncan´s Test. Effectiveness of each treatment was characterized in four classes using the values of non-mycorrhizal controls and the trial mean as standards according to Sieverding (1991). Least Significant Differences (P=0.01) to the trial averages were calculated for each parameter.

RESULTS

All control plants died before the first addition of nutrient solution. No differences were achieved in general for most of the development parameters in the three crop varieties and the (factorial) analysis of variance gave no interactions fungus-tomato variety (Table 1). In consequence, the analyses were performed separately for each tomato variety.

Tomato CV. Manaluce produced fewer flowers than the other two varieties (Tables 2, 3 and 4). Otherwise, the performance of all three tomato cultivars assayed was very similar. Statistical differences were not found for the number of flowers of variety Manaluce (Table 2). The other two varieties produced higher numbers of flowers (Tables 3 and 4).

Single inocula of FAS, INT and SPU were effective for all growth and mycorrhizal parameters in all three tomato varieties for the conditions assayed (Tables 2, 3 and 4). MAN resulted slightly more effective than FAS for the variety HC-7880 but the contrary happened for the varieties Manaluce and Roma, so they both were considered as very effective VAM fungal species for tomato in this conditions. MOS gave values statistically lower than FAS and MAN for near all parameters and varieties.

Compared to single FAS or MAN inocula, double and triple ones resulted in general for all parameters in values statistically equal or lower than they did. Besides, double and even triple inocula still were moderately or highly effective (Tables 2, 3 and 4).

On the contrary, the combination of inoculum of MOS with any of those faster ones (FAS or MAN) produced values in general higher than the single MOS inoculum. Nevertheless, the double inoculation with both fast infecting fungi showed a clear negative effect when together for all parameters in all three varieties, with values always significatively lower or equal.

Table 1. Statistical significance of all studied variables, after factorial analysis of variance. Significance level. ns: no significant; significant to **: $P < 0.05$; significant to ***: $P < 0.001$.
Tabla 1. Significación estadística de todas las variables estudiadas, después del análisis de varianza factorial. Nivel de significación: ns: no significativo; *: significativo a $P < 0.05$; ***: significativo a $P < 0.001$.

Dependent variable	Fungi (A)	Variety (B)	Interaction (A x B)
Final Height	***	***	ns
Stem Diameter	***	ns	ns
Number of flowers	**	***	*
Shoot Dry Weight	***	ns	ns
Root Dry Weight	***	ns	ns
Rootlets Dry Weight	***	ns	ns
Plant Dry Weight	***	ns	ns
Percentage of AM Colonization	***	ns	ns
Total Colonized Root Lenght	***	ns	ns

Triple inoculum behaved in general similarly to the double ones compared both to single inocula. Compared to double inocula, the inoculation of triple ones resulted in values statistically equal to higher for most growing parameters in all three varieties.

A highly significative correlation was found (P=0.01) between whole plants dry weight and total colonized rootlets length (y=0.16 + 0.87x; r=0.74 for n=101).

DISCUSSION

The fact that all control plants died before the first application of nutrient solution pointed out the high mycorrhizal dependency of all three varieties of tomato for nutrition, at least under the established experimental conditions. Siqueira and Franco (1988) and Poulton *et al*. (2002) also found tomato highly dependent on mycorrhizae for normal growth development. Araújo *et al.* (1996) also began periodic fertilization 16 days after the starting of the experiment, but they in addition, improved the substrate by dry mixing basal fertilizer before inoculation and transplant.

The minimized production of flowers for CV Manaluce compared to the other two varieties and the absence of statistical differences between treatments was probably due to its cultivation out of season.

The inocula of SCR, OCC and CAL did not produce normal growths as they "worked" at improper pH conditions according to sparse literature. The fact that FAS gave higher values than MAN of most parameters and this last higher than MOS confirmed the order of effectiveness for these three species in tomato cultivated in a soil with high pH. The order of aggressiveness was assumed according to the experience that MOS is a very slow infecting fungus (although generally very effective) and the other two fungi (FAS and MAN) are very fast infecting in addition to be also very effective fungi.

We understand as more "aggressive" the fungus that infects first, and not as Wilson (1984a,b) who consider an aggressive fungus that which can maintain its infectivity from a mixed inoculum at a similar level as when single, being the "infectivity" considered by this author to be the maximum level of root length colonized. So, his infectivity concept is called by us fungal "occupancy", and is not a subject of this paper. Anyway, results of Wilson (1984b) were similar to ours as interactions between pairs of fungi varied according to the fungi involved.

Although Koomen *et al.* (1987) recommended the use of multiple inocula, they obtained in their experiment that inoculum of E_3 at pH 4.8 produced more growth of the host (clover) than the double inoculum E_3 + *G. mosseae*. This could have happened because *G. mosseae* was working at an improper pH according to Hayman and Tavares (1985) who stated that proper pH for this species was 6-7.

Sieverding (1991) working in cassava with species highly effective (*G. manihotis*), moderately effective (*Entrophospora colombiana*) and a non effective one (*G. occultum*) found that mixtures of them resulted in production values higher than the trial average even when the inoculum contained 50% of the non effective species or of the moderately effective one. He concluded for

Table 2. Means and effectiveness classes (as proposed by Sieverding, 1991*; between parantheses) of the different pure and mixed inocula for tomato cv. Manaluce. Data was transformed as arcsen of percent square root for statistics.

Tabla 2. Valores promedios y clases de efectividad (según lo propuesto por Sieverding, 1991*; entre paréntesis) de los diferentes inóculos puros o mezclados para tomate cv. Manaluce. Datos transformados para el análisis estadístico como arcoseno del porcentaje de la raíz cuadrada.

Treatment	Final height (cm)	Stem diameter (mm)	No. of flowers	Shoot dry weight (g)	Root dry weight >2 mm (g)	Root dry weight <2 mm (g)	Plant dry weight (g)	% of AM colonization	Total colonized root lenght (m)
CONTROL NM	0.0 e	0.0 g	0.0 a	0.0 d	0.00 e	0.00 e	0.00 d	0.0 c	0.00 d
FAS	53.3 ab (3)	5.3 abc (3)	3.0 a (0)	4.6 a (3)	0.25 ab (3)	1.35 a (3)	6.21 a (3)	37.8 ab (1)	5.35 ab (3)
MAN	55.3 a (3)	4.7 bcd (2)	0.7 a (0)	4.1 a (3)	0.19 bc (2)	0.96 b (3)	5.21 a (3)	50.0 ab (2)	2.46 bcd (0)
MOS	28.0 d (1)	4.0 d (2)	0.0 a (0)	1.3 c (1)	0.10 d (1)	0.32 de (0)	1.73 c (1)	48.9 ab (2)	1.88 cd (0)
INT	56.0 a (3)	5.7 a (3)	2.3 a (0)	4.2 a (3)	0.24 ab (3)	1.99 b (3)	5.43 a (3)	29.1 ab (1)	6.11 a (3)
SCR	7.3 e (0)	2.6 e (1)	0.0 a (0)	0.1 d (0)	0.005 e (0)	0.007 e (0)	0.09 d (0)	39.3 ab (1)	0.05 d (0)
OCC	4.2 e (0)	2.5 f (1)	0.0 a (0)	0.1 d (0)	0.002 e (0)	0.005 e (0)	0.04 d (0)	16.7 c (0)	0.02 d (0)
CAL	27.7 d (1)	4.4 cd (2)	0.0 a (0)	1.2 c (0)	0.09 d (1)	0.28 de (0)	1.61 c (1)	44.9 ab (2)	1.15 cd (0)
SPU	53.0 ab (3)	5.5 ab (3)	1.3 a (0)	4.9 a (3)	0.26 a (3)	1.06 ab (3)	6.27 a (3)	54.3 ab (2)	5.35 ab (3)
FASt+MAN	36.3 cd (2)	4.3 d (2)	0.0 a (0)	2.1 bc (1)	0.14 cd (2)	0.61 cd (1)	2.87 b (1)	69.6 a (3)	3.32 abc (0)
FASt+MOS	38.5 c (2)	4.8 abcd (3)	0.0 a (0)	2.5 b (2)	0.20 abc (3)	0.57cd (1)	3.29 b (2)	56.0 ab (2)	3.08 bc (0)
MAN+MOS	41.7 c (2)	4.2 d (2)	5.0 a (0)	2.2 b (1)	0.11 d (1)	0.54 cd (1)	2.86 b (1)	57.8 ab (2)	3.09 bc (0)
FASt+MAN+MOS	45.3 bc (3)	4.3 d (2)	0.0 a (0)	2.9 b (2)	0.14 cd (2)	0.75 bc (2)	3.76 b (2)	56.3 ab (2)	2.62 bcd (0)
Standard Error	3.07	0.29	1.47	0.29	0.02	0.11	5.67	0.91	0.38
Test Mean	34.4	4.0	0.9	2.3	0.13	0.65	3.03	43.1	3.03

Means with common letters are not statistically different (P=0, 05) after Duncan's Test.
* (0): Non effective; (1): Slightly effective; (2): Moderately effective; (3): Highly effective.
Medias con letras comunes no son estadísticamente diferentes (P=0, 05) de acuerdo con el Test de Duncan.
* (0): No efectiva; (1): Ligeramente efectiva; (2): Moderadamente efectiva; (3): Altamente efectiva.

Table 3. Means and effectiveness classes (as proposed by Sieverding, 1991*; between parantheses) of the different pure and mixed inocula for tomato cv. Roma.

Tabla 3. Valores promedio y clases de efectividad (según lo propuesto por Sieverding, 1991*; entre paréntesis) de los diferentes inóculos puros o mezclados para tomate cv. Roma

Treatment	Final height (cm)	Stem diameter (mm)	No. of flowers	Shoot dry weight (g)	Root dry weight >2 mm (g)	Root dry weight <2 mm (g)	Plant dry weight (g)	% of AM colonization **	Total colonized root lenght (m)
NM CONTROL	0.0	0.0 d	0.0 b	0.0 h	0.00 e	0.0	0.00 f	0.0 d	0.00 e
FAS	50.7 a (3)	5.3 ab (2)	8.0 a (3)	4.3 ab (3)	0.27 a (3)	1.34 a (3)	5.95 a (3)	38.6 a (1)	6.36 a (3)
MAN	51.0 a (3)	4.9 ab (2)	8.0 a (3)	3.7 bc (3)	0.19 b (2)	0.88bc(2)	4.72 b (3)	37.1 a (1)	3.12 cd (0)
MOS	30.0 cd (1)	4.3 b (2)	0.0 b (0)	1.5 fg (1)	0.07 de (0)	0.40de(0)	1.95 e (1)	37.4 a (1)	1.59 de (0)
INT	49.3 a (3)	6.0 a (3)	6.3 a (2)	4.4 a (3)	0.26 a (3)	1.37a (3)	6.06 a (3)	42.9 a (2)	6.12 ab (3)
SCR	5.7 e (0)	2.8 c (1)	0.0 b (0)	0.04 h (0)	0.003 e (0)	0.006f (0)	0.21 f (0)	29.6 ab (1)	0.04 e (0)
OCC	3.7 e (0)	3.0 cd (1)	0.0 b (0)	0.1 h (0)	0.002 e (0)	0.003f (0)	0.06 f (0)	14.3 bc (0)	0.03 e (0)
CAL	24.0 d (1)	4.2 b (2)	0.0 b (0)	1.1 g (1)	0.07 de (0)	0.26ef (0)	1.45 e (1)	54.7 ab (2)	1.45 de (0)
SPU	48.5 a (3)	6.2 a (3)	6.0 a (2)	4.7 a (3)	0.27 a (3)	1.10ab(3)	6.04 a (3)	46.9 a (2)	5.14 abc (2)
FASt+MAN	36.7 bc (2)	4.3 b (2)	2.0 b (0)	2.5 de (2)	0.12 cd (1)	0.73cd(2)	3.35 cd (2)	52.6 a (2)	3.40 bcd (0)
FASt+MOS	36.2 bc (2)	4.3 b (2)	1.7 b (0)	1.9 ef (1)	0.12 cd (1)	0.47 de(1)	2.47 de (1)	50.7 a (2)	1.90 de (0)
MAN+MOS	48.3 a (3)	5.0 ab (2)	7.3 a (3)	2.9 cd (2)	0.15 bc (2)	0.69 cd(2)	3.78 bc (2)	56.5 a (2)	4.93 abc (2)
FASt+MAN+MOS	45.0 ab (3)	4.5 b (2)	6.3 a (2)	3.4 c (3)	0.16 bc (3)	0.96 bc(3)	4.48 b (3)	53.2 a (2)	6.35 a (3)
Standard Error	3.11	0.44	1.32	0.24	0.02	0.11	0.34	6.69	0.88
Test Mean	33.0	4.2	3.5	2.3	0.13	0.63	3.12	39.6	3.11

Table 4. Means and effectiveness classes (as proposed by Sieverding, 1991*; between parantheses) of the different pure and mixed inocula for tomato cv. HC-7880.

Tabla 4. Valores promedio y clases de efectividad (según lo propuesto por Sieverding, 1991*; entre paréntesis) de los diferentes inóculos puros o mezclados para tomate cv. HC-7880.

Treatment	Final height (cm)	Stem diameter (mm)	No. of flow-ers	Shoot dry weight (g)	Root dry weight >2 mm(g)	Root dry weight <2 mm (g)	Plant dry weight (g)	% of AM Colonization	Total colonized root lenght (m)
NM CONTROL	0.0h	0.0d	0.0d	0.0f	0.00e	0.00f	0.00g	0.0c	0.00e
FAS	41.7abc (3)	5.0ab (2)	6.7bc (0)	3.9ab (3)	0.29ab (3)	1.18ab (3)	5.39ab (3)	44.1ab (2)	4.06abc (2)
MAN	44.7ab (3)	5.2ab (3)	13.7a (3)	3.5bc (3)	0.23bc (3)	1.12abc (3)	4.84bc (3)	58.1ab (3)	4.60abc (2)
MOS	29.3ef (2)	4.5b (2)	3.7cd (0)	1.2e (0)	0.08de (0)	0.37ef (0)	1.69f (1)	43.2ab (2)	1.64cde (0)
INT	47.0a (3)	5.8a (3)	8.3abc (2)	4.7a (3)	0.32a (3)	1.45a (3)	6.44a (3)	40.2ab (1)	5.07ab (2)
SCR	8.2g (0)	2.7c (1)	0.0d (0)	0.1f (0)	0.007e (0)	0.02f (0)	0.15g (0)	32.1b (1)	0.14e (0)
OCC	3.3gh (0)	3.0d (1)	0.0d (0)	0.1f (0)	0.003e (0)	0.009f (0)	0.07g (0)	12.2c (0)	0.04e (0)
CAL	26.0f (1)	4.6ab (2)	2.7cd (0)	1.2e (0)	0.11d (1)	0.35ef (0)	1.67f (1)	32.3b (1)	0.67de (0)
SPU	38.0bcd (3)	5.3ab (3)	12.0ab (3)	3.6bc (3)	0.24b (3)	1.00bc (2)	4.79bcd (3)	46.3ab (2)	4.76abc (2)
FASt+MAN	35.5cde (3)	4.2b (2)	6.0bcd (0)	2.3e (2)	0.16cd (2)	0.71cde (2)	3.17e (2)	51.5ab (2)	3.55abcd (0)
FASt+MOS	30.2def (2)	4.5b (2)	4.7cd (0)	1.9de (1)	0.14d (2)	0.51de (0)	2.56ef (1)	53.6ab (2)	2.07bcde (0)
MAN+MOS	42.7abc (3)	4.8ab (2)	5.7bcd (0)	2.8cd (2)	0.16cd (2)	0.83bcd (2)	3.74cde (2)	66.2a (3)	4.95abc (2)
FASt+MAN+MOS	39.0abc (3)	4.3b (2)	6.3bcd (0)	2.6cd (2)	0.12d (1)	0.94bcd (2)	3.64de (2)	66.6a (3)	5.71a (3)
Standard Error	2.56	0.38	1.98	0.33	0.03	0.14	0.38	5.86	1.00
Test Mean	29.7	4.1	5.4	2.1	0.14	0.65	2.93	42.0	2.87

cassava that when the inoculum contains the highly effective and competitive fungus *G. manihotis*, it is apparently not important whether the inoculum is clean (only MAN) or contains up to 50% of other fungal species. He also pointed out that for other crops and conditions, any other of the fungi could be the most effective, and that if several highly effective fungi are present in the inoculum, a growth response should be expected in any case.

Edathil *et al.* (1996) found that quadruple inoculum promoted markedly better shoot length and biomass than any other lower level combination. They also found a negative interaction *between G. fasciculatum* and *G. aggregatum*. This negative effect of two separately highly effective and aggressive fungi oppose to the conclusion of Sieverding (1991) mentioned above, but agree with our results (FAS + MAN) as showed before. Both fungi **id**. *G. aggregatum* in Edathil *et al.* (1996) and *G. manihotis* in our experiment developed at improper pH values (7.2 and 7.7, respectively) leading probably to some parasitic behavior.

Results dealing with mixing inocula are still inconsistent. Our experiment agrees partially with those cited above. The present work suggests that multiple inocula could be convenient or better adapted to a variety of conditions than single inocula. Anyway, attention should be given to the effectivity and agressivity of the species or strains to be combined, and the appropriated pH for them.

Some research groups are leading basic investigations for the understanding of many different aspects of the symbiosis (see the revision by Smith and Read, 2008). Other groups are doing more practical research dealing with the application of the accumulated knowledge. For instance, Mendoza and Oliveira (1996; cited by Siqueira 1996) concluded for Brazil that obtained progresses are not proportional to the applied efforts and most researches are not well directed to a goal and still without continuity. Anyway, until all those investigations conclude, and mechanisms involved in behaviors as those reported here are known, the only practical way for applying VAM in agriculture is through the development of ecotechnologies which include the vegetative mass reproduction of VAM fungi and their use alone or combined.

Most commercial products include more than one fungus in their formulations. In terms of fungal content, the tendency is to introduce a mix of several AMF in commercial inoculums (Dalpé and Monreal, 2004). As it is recommended for single inoculations, mixtures of inocula should be assayed for each culture and situation before large-scale applications of VAM fungi are stated.

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