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RESPONSE OF *Canavalia ensiformis* (L.) TO INOCULATION WITH DIFFERENT ARBUSCULAR MYCORRHIZAE FUNGI STRAINS IN A FARL SOIL

Respuesta de *Canavalia ensiformis* (L.) A la inoculación con diferentes cepas de hongo micorrízico arbuscular en un suelo FARL

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ABSTRACT. The aim of the study was to evaluate the response of *Canavalia ensiforme* to inoculation of different arbuscular mycorrhizal fungi (AMF) strains in a Ferrallitic Yellow Reddish and Lixiviated soil dedicated to tobacco production. The experiment was carried out using random blocks with five variants and repetitions that include the four strains inoculated *Canavalia ensiforme* with the strains of Rhizo plagus and a control without inoculation. The results demonstrated that *Glomus cubense* strain has the best response for this type of soil. The largest growth and development of canavalia was reached with the application of *Glomus cubense* strains with a dry mass foliar production was 7,3 t ha^{-1} and the highest levels of nutrients such as N, P, K (264,9 kg N ha^{-1}; 37,6 kg P ha^{-1}; 226,7 kg K ha^{-1}) were obtained. The use of inoculated *Canavalia ensiforme* with *Glomus cubense and Rhizophagus intraradices* had a significant response and increased the mycorrhizae functioning in soil used for tobacco production.

Key words: green manure, inoculation, nutrients, soil

RESUMEN. Con el objetivo de evaluar la respuesta de la *Canavalia ensiformis* a la inoculación con diferentes cepas de hongos micorrizícos arbusculares (HMA) en un tipo genético de suelo Ferralítico Amarillento Rojizo Lixiviado (FARL) dedicado al cultivo de tabaco. Se realizó una investigación con un diseño experimental de bloques al azar, con cinco variantes y cuatro repeticiones, que incluyen la canavalia inoculada con las cepas *Rhizophagus intraradices*, *Funneliformis mosseae*, *Glomus cubense* y *Glomus claroideum* y la variante sin inoculación como testigo. Los resultados demostraron que la cepa *Glomus cubense* presentó la mejor respuesta para este tipo de suelo. Se alcanzó el mayor crecimiento y desarrollo de la canavalia por la inoculación con la cepa *Glomus cubense*, con una producción de masa seca foliar de 7,3 t ha^{-1} y se lograron los mayores contenidos de nutrientes NPK (264,9 kg N ha^{-1}; 37,6 kg P ha^{-1}; 226,7 kg K ha^{-1} respectivamente). La utilización de Canavalia inoculada con las cepas *Glomus cubense* y *Rhizophagus intraradices* incrementó el funcionamiento micorrízico en un suelo tabacalero.

Palabras clave: abono verde, inoculación, nutrientes, suelo

INTRODUCTION

The use of green manures favors and increases the microbial activity and diversity of soil microorganisms, such as N-fixing and arbuscular mycorrhizal fungi (AMF) (1). Within them is the *Canavalia ensiformis* (L.) species that provides positive effects on soil fertility, as it increases water retention capacity, reduces washing and nutrient leaching, as well as favors soil microbial activity (2, 3).

The canavalia is a species adapted to the conditions of Cuba due to its vigorous growth, to the contributions of atmospheric N fixed to the soil-plant system, via biological nitrogen fixation (BNF) and by recycling appreciable quantities of P and K[A].

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[A] Pozzi, C. *Estudo de sistemas de uso do solo em rotações de culturas en sistemas agricolas brasileiros: dinâmica de nitrogênio e carbono no sistema solo–planta–atmosfera*. Tesis de Doctorado, Universidad Federal Rural do Rio de Janeiro, 2005, Rio de Janeiro, Brasil, 120 p.

Arbuscular mycorrhizal fungi (AMF) are microorganisms that allow improving the development of the cultures when they interact with the plants, creating symbiosis with each other. They are also present in 95 % of agricultural crops and can increase the processes of absorption and translocation of nutrients in plants (4).

Green manures are management alternatives for soils (5), their association with AMF can condition qualitative and quantitative changes in the populations of these fungi in the soil B (6). Numerous results have been found in Red Ferralitic and Nodal Rhodic Eutric soils that demonstrate the increase of mycorrhizal propagules in the soil when using rotations that include *Canavalia ensiformis* as green manure (3, 7, 8).

In our agricultural system and specifically for these soils, no research has been developed with the species *Canavalia ensiformis* that respond to inoculations with specific strains of AMF. This is why the present work aims to evaluate the response of *Canavalia ensiformis* to inoculation with different AMF strains in a Ferralitic Reddish Yellowish Leached Soil dedicated to tobacco cultivation.

MATERIALS AND METHODS

The climatic conditions presented an ideal behavior for the vegetative development of the canavalia crop, with values above 160 mm of rain fall during this period[B], which favors the vegetative development of this species of legume and corresponds to the contributions of dry biomass and nutrients contributed to the soil.

The research was carried out in a total area of 310,5 m², using an experimental design of random blocks, with five variants and four replications. The variants studied were evaluated during two campaigns with canavalia without inoculation and inoculated with the different strains described below:

Description of treatments

- *Without inoculation (Control)*
- *Rhizophagus intraradices*
- *Funneliformis mosseae*
- *Glomus cubense*
- *Glomus claroideum*

Planting of canavalia was carried out in the second ten of August, by hand; inoculation of the seed was done by coating the different strains of the certified inoculum (30 spores of AMF g inoculum). A homogeneous paste was prepared in a ratio of 1 kg of each inoculum per 10 kg of seed, and with it, the seed was covered until completely covered.

They were then dried in the shade for 5 to 10 minutes and then seeding. At 60 days after germination, the soil was sampled for AMF spores and complete plants, in order to determine the total dry mass, nutrient content and fungal performance variables in the Canavalia roots. For the sampling, three complete plants per linear meter, including the rhizosphere soil and the rootlets of the plants, were selected in the calculation area (central grooves) for each treatment and replication, leaving the first two furrows as edge area.

In the tobacco seasons 2013-2014 and 2014-2015, a study was carried out in areas of the Tobacco Experimental Station, located in Vuelta Abajo; farm "Vivero", San Juan y Martínez municipality, Pinar del Río province. The soil in this area is classified as Ferralitic Reddish Yellowish Leachate (9). Some of its characteristics are presented in Table I.

The behavior of the main variables (mean temperature, relative humidity and rainfall) during the two campaigns that the experiment was developed were taken from the Agrometeorological Station of San Juan y Martínez, being evaluated the periods relative to each of the years in which it took place the field phase.

[B] Martín, G. M. *Manejo de la inoculación micorrízica arbuscular, Canavalia ensiformis y la fertilización nitrogenada en plantas de maíz (Zea mays) cultivadas sobre suelos Ferralíticos Rojos de La Habana*. Tesis de Doctorado, Instituto Nacional de Ciencias Agrícolas (INCA), 2009, La Habana, Cuba, 101 p.

Table I. Some soil characteristics in the experimental area

pH_{KCl}	MO (%)	P_2O_5	K_2O	Ca^{2+}	Mg^{2+}	Na^+	K^+	Nu. Spores AMF 50g soil $^{-1}$
		mg 100g^{-1}			cmol(+) Kg^{-1}			
5,5	1,38	41,70	23,50	3,50	1,40	0,10	0,41	299

Chemical determinations: pH KCl potentiometer, M.O. (10), P Oniani, Cations NH Ac pH 7, No. spores AMF (11)

Production of Aerial Biomass

To determine leaf biomass (t ha^{-1}), the organs of the aerial part of the plants (leaves and stems) included in linear 1 m within each plot were taken. The total fresh mass of each organ was weighed separately on a Sartorius digital METTLER scale (g per plant), from which a fraction was taken and dried in the oven at 70 °C until reaching a constant mass, the dry mass being determined total and extrapolated to ha^{-1}.

Determinations made

The extraction of N, P_2O_5 and K_2O was calculated from the data of the dry mass of the total biomass and the corresponding concentration of each element (percentage of N, P and K), by the formula:

Extraction of N, P_2O_5 and K_2O = [yield of biomass x concentration of chemical compound in each organ]/100

For this it was determined: The concentration of N, P and K in the leaves and stems of the canavalia (%). From the canavalia biomass, a homogeneous sample of leaves and stems was taken, and the total contents of N, P and K were evaluated (12).

Nitrogen (N): wet digestion with H_2SO_4 + Se and colorimetric determination with the Nessler reagent.

Phosphorus (P): wet digestion with H_2SO_4 + Se and determination by the colorimetric method with ammonium molybdate.

Potassium (K): wet digestion with H2SO4 + Se and determination by flame photometry.

AMF spore count: this was done by extracting 50 g of soil (11, 13).

Radical colonization: the evaluation was performed by the method of intercepts by the percentage of mycorrhizal colonization or colonization frequency (14).

Statistical procedures

For the determination of the differences between treatments, the Duncan Multiple Rank comparison was used for p≤0,05 (15). All data were processed with the statistical package Stadistical Package for Social Sciences (SPSS) for Microsoft Windows version 21.0 (16).

RESULTS AND DISCUSSION

Table II shows the behavior of fresh mass, dry mass and nutrient contents of Canavalia in the presence of different AMF strains.

It can be seen that among the evaluated strains, *Glomus cubense* was highlighted with greater production of fresh mass and dry mass, obtaining the best results with significant differences to the rest of the studied strains and the control. The comparison studies of strains carried out in Cuba, although they have demonstrated the existence of a high strain specificity efficient of efficient AMF-type soil (17), have also demonstrated the greater competitiveness of *Glomus cubense* to establish an effective symbiosis, both in the soils where it is recommended (3) as in others where this strain is not the most efficient (18).

The strain *Glomus cubense* has the best response in this type of soil, which is related to investigations in different crops and soil types, including tomatoes, onions and pastures on Red Ferralitic soils and, in general, for soils of medium and high fertility (3, 4, 19, 20).

Species of the genus Glomus have a wide range of functional distribution, predominating in high and medium fertility ecosystems where they are efficient and competitive. The results in Cuba allowed extending these ranges to the conditions of low and very low fertility (4).

Table II. Fresh, dry mass and nutrient extraction (N, P, K) of canavalia in the presence of four strains of AMF

Treatments	Fresh mass (t ha^{-1})	Dry masss (t ha^{-1})	Total absortion of nutrients (kg ha^{-1})		
	Total	Total	N	P	K
Without inoculation (Control)	36,6 b	4,0 d	157,4 c	19,1 c	122,9 d
Rhizophagus intraradices	38,4 b	6,3 b	243,1 a	33,1 ab	181,9 b
Funneliformis mosseae	35,8 b	5,2 c	194,9 b	24,1 bc	153,6 c
Glomus cubense	44,9 a	7,3 a	264,9 a	37,6 a	226,7 a
Glomus claroideum	38,1 b	4,1 d	148,7 c	18,4 c	120,1 d
E.S (+/-)	0,946	0,098	6,400	3,334	4,979
CV (%)	9,55	24,54	23,99	37,16	25,97

Means with different letters in the same column differ from each other according to Duncan test(P<0,05)

The highest aerial dry mass production with this strain reached 7,3 t ha^{-1}, results that correspond to long days and high precipitations (approximately 171 mm) throughout the growing stage, favoring the vegetative increase in a shorter period. However, in spite of obtaining the best results with the strain *Glomus cubense*, it is also important to point out the good behavior in the production of dry mass by the strain Rhizophagus *intraradices* (6,3 t ha^{-1}) for this type of soil.

The results of the dry mass in canavalia in the presence of the strains *Glomus cubense* and *Rhizophagus intraradices* are in correspondence with the reported in the literature on this species as green manure in agricultural systems. Aerial mass production increased favorably with the onset of rains, yielding yields of 5,33 t ha^{-1} of dry mass, in addition to increases in N and P in nutrient absorption. Under the agroclimatic conditions of Cuba this species grows and develops very fast during the spring season, coinciding with the rainiest months (May-October)[D].

It has been shown that green manures in the summer grow faster and accumulate more N, due to the intensity of sunlight (21). As precipitation increases, the growth of the plants used as green manure increases, and the greater production of phytomass increases the nutrient content (22, 23).

Legumes are plants that respond more vigorously to AMF inoculation and a greater association of hyphae for an increase in nutrient transfer (3). This approach ratifies the results shown when evaluating the nutrient content (N, P, K), where differences were observed in the plants inoculated with the different strains compared to the control without inoculation.

The highest amounts of nutrients were found in the canavalia when inoculated with the strain *Glomus cubense*, with N values higher than 264,9 kg ha^{-1} and average values of 37,6 and 226,7 kg ha^{-1} of P And K, respectively, which are in correspondence with the results obtained in the growth of the dry leaf mass (Table II).

Species such as canavalia accumulate much more N than other species of plants and it has been suggested that it is due to the greater aerial phytomass produced by the legumes and the BNF. This process, in conditions conducive to growth, leads to greater aerial and radical development, which implies a greater exploration of the soil and if the availability of elements allows, increases the absorption of these depending on the extraction capacity of the roots (6).

The nutrient uptake indicates the response degree of each species, according to the fungus-host symbiosis, which is why there is compatibility among legume species used as green manure with native AMF and inoculated species (24).

Table III shows the number of AMF spores in the soil and mycorrhizal colonization, after cutting the canavalia plants at 60 days of age.

Table III. Effect of different strains on some fungal functioning variables

Treatments	Nu. Spores en 50 g $soil^{-1}$	Colonization (%)
Without inoculation	255,25 c	36,00 c
Rhizophagus intraradices	325,25 a	56,77 a
Funneliformis mosseae	306,25 b	41,83 b
Glomus cubense	333,00 a	58,45 a
Glomus claroideum	223,50 d	39,17 b
ES (+/-)	3,192	0,675
CV (%)	15,19	20,85

Means with different letters in the same column differ from each other according to Duncan test (P <0.05)

When inoculating canavalia with different species of AMF, the multiplication of AMF spores in the soil after cutting and incorporation of these plants is guaranteed, compared to the number of spores at the beginning of the experiment, which was 299 spores in 50 g of soil dry. The treatment without inoculation at the end of the experiment had less spores than at the beginning. On the other hand, a better behavior was observed in the variables evaluated with the presence of *Glomus cubense* and *Rhizophagus intraradices* strains, showing significant differences with the rest of the strains and the control without inoculation.

The canavalia inoculated with the strains *G. cubense* and *R. intraradices*, has a percentage of colonization of 58 and 56 %, respectively, and greater number of spores per gram of soil, being a relation between the number of AMF spores per gram of soil and the percentage of colonization.

[C]Aguilar, N. *Efecto del abono verde de Canavalia ensiformis (Canavalia) en sistemas agrícolas*. Inst. Centro de Información Documental Agropecuaria (CIDAGRO), Instituto de Investigación Agropecuaria de Panamá (IDIAP), 2005, Panamá, 7 p.

[D]García, M. *Contribución al estudio y utilización de los abonos verdes en cultivos económicos desarrollados sobre un suelo Ferralítico Rojo de La Habana*. Tesis de Doctorado, Instituto Nacional de Ciencias Agrícolas (INCA), 1997, La Habana, Cuba, 98 p.

These results are related to those obtained in canavalia, when finding a percentage of colonization of 55 % and the number of spores per gram of soil, which is greater where canavalia is cultivated, depending on the sampling period and the phenological phase of the plant (3).

Other studies indicate that the percentage of colonization is higher with inoculation of mycorrhizae in relation to the control, concluding that the inoculation of AMF had a positive effect on root colonization (25).

In spite of observing an increase in the number of spores, it was possible to be appreciated that these soils have a high content of native spores and that the use of the canavalia as green fertilizer favors the reproduction of these. AMF naturally exist in soils and green subsistence practices may increase the presence of their infective propagules, which is why canavalia is considered a species of high mycorrhizal response capable of multiplying native soil spores (4)[E] .

The *G. hoi-like* strain, nowadays called *Glomus cubense*, had a greater number of spores than the other AMF strains in canavalia cultivated in Red Ferralitic soil, being this effective not only in growth and nutrition, but also in the reproduction of the mycorrhizal propagules. This is another additional benefit obtained using green fertilizers (3).

Recent studies have reported that canavalia is a plant species, which among other advantages when used as a green manure, has the peculiarity of multiplying the propagules of AMF in the soil, whether native or inoculated and thus favor mycorrhizal colonization of the later crop, thus providing an added value as green manure in the agricultural systems (8).

Studies with green manures and mycorrhizal inoculation in Red Ferralitic Leachate soils, affirm that the increase of AMF spores in these soils is due to the growth of the green manures, being a relation between the dry mass produced and the spores found in the rhizosphere of the Canavalia plants They also emphasize that the reproductive intensity of the propagules is directly related to the differences in the growth of green manures. The explanation for this is that the multiplication of spores is a consequence not only of the mycorrhizal association of these crops, but also of the growth of plants (26).

[E] Filho, P. F. M. *Potencial de rehabilitação do solo de uma área degradada, através da revegetação e do manejo microbiano*. Tese Doutorado, Escola Superior de Agricultura Liuz de Queiroz, 2004, Piracicaba, São Paulo, Brasil, 89 p.

CONCLUSIONS

The highest contributions of fresh mass, dry mass and nutrients (N, P, K) for this type of soil were reached when canavalia was inoculated with the strain *Glomus cubense*. The mycorrhizal function in a Ferrallitic Reddish Yellowish Leachate soil dedicated to the cultivation of tobacco was increased with the use of canavalia inoculated with the strains *Glomus cubense* and *Rhizophagus intraradices*.

BIBLIOGRAPHY

1. N.; Jara, C. y Quintero, J. “Green manure impact on nematodes, arbuscular mycorrhizal and pathogenic fungi in Tropical Soils planted to common beans” [en línea]. En: *XVIII World Congress of Soil Science*, Ed. International Union of Soil Science, Philadelphia, Pennsylvania, USA, 2006, [Consultado: 10 de enero de 2017], Disponible en: <http://www.ldd.go.th/18wcss/techprogram/P15849.HTM>.
2. Torrealba, G. T.; Viera, J. y Bravo, P. “Factores relacionados con la acidez del suelo y su efecto sobre el crecimiento de la *Canavalia ensiformis* (L.) D.C”. *Agronomía Tropical*, vol. 48, no. 1, 1998, pp. 19-32, ISSN 0002-192X.
3. Martín, G. M.; Arias, L. y Rivera, R. “Selección de las cepas de hongos micorrízicos arbusculares (HMA) más efectivas para la *Canavalia ensiformis* cultivada en suelo Ferralítico Rojo”. *Cultivos Tropicales*, vol. 31, no. 1, 2010, pp. 27–31, ISSN 0258-5936.
4. Rivera, E. R. y Fernandez, S. K. *El manejo efectivo de la simbiosis micorrízica, una vía hacia la agricultura sostenible: Estudio de caso El Caribe* [en línea]. Ed. Ediciones INCA, 2003, La Habana, Cuba, 166 p., ISBN 978-959-7023-24-1, [Consultado: 10 de enero de 2017], Disponible en: <https://www.researchgate.net/publication/299979710_El_manejo_efectivo_de_la_simbiosis_micorrizica_una_via_hacia_la_agricultura_sostenible_Estudio_de_caso_El_Caribe>.
5. Prager, M. M.; Sanclemente, R. O. E.; Sánchez, de P. M.; Miller, G. J. y Ángel, S. D. I. “Abonos verdes: Tecnología para el manejo agroecológico de los cultivos”. *Agroecología*, vol. 7, no. 1, 2012, pp. 53-62, ISSN 1989-4686.
6. Espindola, J. A. A.; de Almeida, D. L.; Guerra, J. G. M.; da Silva, E. M. R. y de Souza, F. A. “Influência da adubação verde na colonização micorrízica e na produção da batata-doce”. *Pesquisa Agropecuária Brasileira*, vol. 33, no. 3, 1998, pp. 339–347, ISSN 1678-3921.
7. Martín, G. M.; Costa, R. J. R.; Urquiaga, S. y Rivera, R. A. “Rotación del abono verde *Canavalia ensiformis* con maíz y micorrizas arbusculares en un suelo Nitisol Ródico Éutrico de Cuba”. *Agronomía Tropical*, vol. 57, no. 4, 2007, pp. 313-321, ISSN 0002-192X.
8. Martín, G. M.; Rivera, R.; Arias, L. y Pérez, A. “Respuesta de la *Canavalia ensiformis* a la inoculación micorrízica con *Glomus cubense* (cepa INCAM-4), su efecto de permanencia en el cultivo del maiz”. *Cultivos Tropicales*, vol. 33, no. 2, 2012, pp. 20-28, ISSN 0258-5936.

9. Hernández, J. A.; Pérez, J. M.; Bosch, D.; Rivero, L.; Camacho, E.; Ruíz, J.; Salgado, E. J.; Marsán, R.; Obregón, A.; Torres, J. M.; Gonzáles, J. E.; Orellana, R.; Paneque, J.; Ruiz, J. M.; Mesa, A.; Fuentes, E.; Durán, J. L.; Pena, J.; Cid, G.; Ponce de León, D.; Hernández, M.; Frómeta, E.; Fernández, L.; Garcés, N.; Morales, M.; Suárez, E. y Martínez, E. *Nueva versión de clasificación genética de los suelos de Cuba*. Ed. AGROINFOR, 1999, La Habana, Cuba, 64 p., ISBN 959-246-022-1.
10. Walkley, A. y Black, I. A. "An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method". *Soil science*, vol. 37, no. 1, 1934, pp. 29–38, ISSN 0038-075X, 1538-9243.
11. Gerdemann, J. W. y Nicolson, T. H. "Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting". *Transactions of the British Mycological Society*, vol. 46, no. 2, 1963, pp. 235-244, ISSN 0007-1536, DOI 10.1016/S0007-1536(63)80079-0.
12. Paneque, P. V. M.; Calaña, N. J. M.; Calderón, V. M.; Borges, B. Y.; Hernández, G. T. C. y Caruncho, C. M. *Manual de técnicas analíticas para análisis de suelo, foliar, abonos orgánicos y fertilizantes químicos* [en línea]. Ed. Ediciones INCA, 2010, La Habana, Cuba, 157 p., ISBN 978-959-7023-51-7, [Consultado: 27 de enero de 2016], Disponible en: <http://mst.ama.cu/578/>.
13. Herrera, R. A.; Ferrer, R.; Furrazola, E. y Orozco, M. O. *Estrategia de funcionamiento de las micorrizas (VA) en un bosque tropical. Biodiversidad en Ibero América: Ecosistemas, Evolución y Proceso sociales*. Programa Iberoamericano de Ciencias y Tecnología para el desarrollo. Sub – programa XII, Diversidad Biológica, 1995, Mérida, México, 201 p., ISSN-1576–9526.
14. Giovannetti, M. y Mosse, B. "An Evaluation of Techniques for Measuring Vesicular Arbuscular Mycorrhizal Infection in Roots". *New Phytologist*, vol. 84, no. 3, 1980, pp. 489-500, ISSN 1469-8137, DOI 10.1111/j.1469-8137.1980.tb04556.x.
15. Duncan, D. B. "Multiple Range and Multiple F Tests". *Biometrics*, vol. 11, no. 1, 1 de marzo de 1955, pp. 1-42, ISSN 0006-341X, DOI 10.2307/3001478.
16. IBM Corporation. *IBM SPSS Statistics* [en línea]. versión 21.0, [Windows], Ed. IBM Corporation, 2012, U.S, Disponible en: <http://www.ibm.com>.
17. Herrera-Peraza, R. A.; Hamel, C.; Fernández, F.; Ferrer, R. L. y Furrazola, E. "Soil–strain compatibility: the key to effective use of arbuscular mycorrhizal inoculants?". *Mycorrhiza*, vol. 21, no. 3, 2010, pp. 183-193, ISSN 0940-6360, DOI 10.1007/s00572-010-0322-6.
18. González, P. J.; Arzola, J.; Morgan, O.; Rivera, R.; Plana, R. y Fernández, F. "Manejo de las asociaciones micorrízicas en pastos del género *Brachiaria* cultivados en suelos Ferralítico Rojo y Pardo Mullido". En: *XVI Congreso Científico del INCA*, Ed. Ediciones INCA, La Habana, Cuba, 2008, ISBN 978-959-16-0953-3.
19. Calderón, M. y González, P. J. "Respuesta del pasto guinea (*Panicum maximum*, cv. Likoni) cultivado en suelo Ferralitico Rojo Lixiviado a la inoculacion de hongos micorrizicos arbusculares". *Cultivos Tropicales*, vol. 28, no. 3, 2007, pp. 33-38, ISSN 0258-5936.
20. González, P. J.; Pérez, G.; Medina, N.; Crespo, G.; Ramírez, J. F. y Arzola, J. "Co-inoculation of ryzobium strains and one of arbuscular mycorrhizial fungi (*Glomus cubense*) and its effect on kusdzú (*Pueraria phaseoloides*)". *Cuban Journal of Agricultural Science*, vol. 46, no. 3, 2012, pp. 331-334, ISSN 2079-3480.
21. Cherr, C. M.; Scholberg, J. M. S. y McSorley, R. "Green Manure as Nitrogen Source for Sweet Corn in a Warm–Temperate Environment". *Agronomy Journal*, vol. 98, no. 5, 2006, pp. 1173-1180, ISSN 1435-0645, DOI 10.2134/agronj2005.0036.
22. Perin, A.; Santos, R. H. S.; Urquiaga, S.; Guerra, J. G. M. y Cecon, P. R. "Phytomass yield, nutrients accumulation and biological nitrogen fixation by single and associated green manures". *Pesquisa Agropecuária Brasileira*, vol. 39, no. 1, 2004, pp. 35-40, ISSN 0100-204X, DOI 10.1590/S0100-204X2004000100005.
23. Sodré-Filho, J.; Cardoso, A. N.; Carmona, R. y Carvalho, A. M. de. "Fitomassa e cobertura do solo de culturas de sucessão ao milho na Região do Cerrado". *Pesquisa Agropecuária Brasileira*, vol. 39, no. 4, 2004, pp. 327-334, ISSN 0100-204X, DOI 10.1590/S0100-204X2004000400005.
24. Monzon, A. y Azcón, R. "Relevance of mycorrhizal fungal origin and host plant genotype to inducing growth and nutrient uptake in Medicago species". *Agriculture, Ecosystems & Environment*, vol. 60, no. 1, 1996, pp. 9-15, ISSN 0167-8809, DOI 10.1016/S0167-8809(96)01066-3.
25. Pérez-Luna, Y. del C.; Álvarez-Solís, J. D.; Mendoza-Vega, J.; Pat-Fernández, J. M.; Gómez-Álvarez, R. y Cuevas, L. "Diversidad de hongos micorrícicos arbusculares en maíz con cultivo de cobertura y biofertilizantes en Chiapas, México". *Gayana. Botánica*, vol. 69, no. 1, 2012, pp. 46-56, ISSN 0717-6643, DOI 10.4067/S0717-66432012000100006.
26. Sánchez, E. C.; Rivera, E. R.; Caballero, B. D.; Cupull, S. R.; González, F. C. y Urquiaga, C. S. "Abonos verdes e inoculación micorrízica de posturas de cafeto sobre suelos Ferralíticos Rojos Lixiviados". *Cultivos Tropicales*, vol. 32, no. 3, 2011, pp. 11–17, ISSN 1819-4087.

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