BENEFITS OF JOINT MANAGEMENT OF GREEN MANURE AND MYCORRHIZAL INOCULANTS IN CROP PRODUCTION †

[BENEFICIOS DEL MANEJO CONJUNTO DE ABONOS VERDES E INOCULANTES MICORRIZICOS EN LA PRODUCCION AGRICOLA]

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SUMMARY

Background. Nutrient supply is a decisive component of agricultural systems, but in Cuba only 25 % of cultivated areas can be fertilized, influencing their productivity negatively. From the benefits of the use of mycorrhizal inoculants and green manures and the mycorrhizal dependence of *Canavalia ensiformis*, the hypothesis of integrating both was evaluated. **Objective**. To establish nutrient supply schemes that guarantee high yields with lower amounts of fertilizers, as well as new routes for mycorrhizal inoculation of crops. **Methodology.** The canavalia response to the inoculation of three generalist AMF strains was evaluated in eight edaphic conditions, as well as eight integration experiments of the green fertilizers inoculated with the efficient strain in the nutrient supply schemes of cassava, banana, corn, tobacco, mulberry and king-grass. **Results.** Canavalia responded positively to inoculation, with average increases in biomass and NPK contents of 50 and 70 % respectively, as well as mycorrhizal propagules. The effectiveness of the applied strains depended on the edaphic conditions and presumably the soil pH. The canavalia inoculated with the efficient AMF strain and used as the preceding and/or intercropped green manure was successfully integrated into the nutrient supply systems of the different crops, guaranteeing high yields, effective mycorrhizal function and decreasing between 25 and 75 % the amounts of fertilizers applied to them. **Implications.** The benefits and feasibility of the joint management of green manures and

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mycorrhizal inoculants in agricultural production are demonstrated, as well as being a successful way to achieve effective mycorrhization in crops whose direct inoculation is prohibitive due to the high amounts of biofertilizer or laboriousness. **Conclusion.** The integration of AMF inoculants and green manure increased the benefits of both practices and it is a promising way of managing the arbuscular mycorrhizal symbiosis in sustainable crop production.
Keywords: nutrient supply; canavalia; banana; corn; cassava; tobacco; forage.

RESUMEN

Antecedentes. El suministro de nutrientes es un componente decisivo de los sistemas agrícolas, pero en Cuba solo el 25 % de las áreas cultivadas pueden ser fertilizadas, influyendo negativamente en su productividad. A partir de los beneficios del uso de los inoculantes micorrízicos y de los abonos verdes y la dependencia micorrízica de la *Canavalia ensiformis*, se evaluó la hipótesis de integrar ambos. **Objetivos.** Establecer esquemas de suministro de nutrientes que garanticen rendimientos altos con menores cantidades de fertilizantes, así como nuevas vías para la inoculación micorrízica de los cultivos. **Metodología.** Se evaluó la respuesta de la canavalia a la inoculación de tres cepas generalistas de HMA en ocho condiciones edáficas, así como se ejecutaron ocho experimentos de integración de los abonos verdes inoculados con la cepa eficiente en los esquemas de suministro de nutrientes de yuca, banano, maíz, tabaco, morera y hierba elefante. **Resultados.** La canavalia respondió positivamente a la inoculación, con incrementos promedios en biomasa y contenidos de NPK del 50 y 70 % respectivamente, así como de los propágulos micorrízicos. La efectividad de las cepas aplicadas dependió de las condiciones edáficas y presumiblemente del pH del suelo. La canavalia inoculada con la cepa eficiente de HMA y utilizada como abono verde precedente y/o intercalado se integró exitosamente en los sistemas de suministro de nutrientes de los diferentes cultivos, garantizando rendimientos altos, funcionamiento micorrízico efectivo y disminuyendo entre 25 y 75 % las cantidades aplicadas de fertilizantes a estos. **Implicaciones.** Se demuestran los beneficios y factibilidad del manejo conjunto de abonos verdes e inoculantes micorrízicos en la producción agrícola, siendo además una vía eficaz para alcanzar una micorrizacion efectiva en cultivos cuya inoculación directa es prohibitiva por las altas cantidades de biofertilizante o laboriosidad requeridas. **Conclusión**. La integración de los inoculantes micorrízicos y los abonos verdes incrementa los beneficios de ambas prácticas y es una promisoria vía para el manejo de la simbiosis micorrízica en la producción sostenible de los cultivos.
Palabras clave: nutrientes; canavalia; banano; yuca; maíz; tabaco; forrajes.

INTRODUCTION

The rational supply of nutrients in the farming systems is a necessity to guarantee adequate yields and maintenance of soil fertility (Vanlauwe *et al.,* 2010). In recent years, the importance of establishing technologies has become clear that enhance the biological activity and the use of microorganisms and that allow using smaller amounts of inputs (Le Mire *et al.*, 2016; Schütz *et al.,*2018), its use is still limited and fundamentally restrained to the applications of rhizobia (Lesueur *et al.,* 2016). In parallel, high fertilizer prices have a negative impact on a global scale and in many developing countries low percentages of agricultural areas are fertilized, which causes negative effects on yields and soil fertility. Both causes have driven R + D + I aimed at the use of bioproducts and organic-mineral sources in agricultural production technologies.

There is no doubt in the universality of the mycorrhizal symbiosis and the benefits they bring to plants and the environment associated with the increase in nutrient and water uptake, the effect of bioprotection against some radical and foliar pests, tolerance to heavy metals, as well as other different ecoservices among which are increases in soil aggregates, participation in the C and N cycles and improvements in ecosystem resilience (Jung *et al.,* 2012; Willis *et al.,* 2013; Azcón –Aguilar and Barea, 2015; Lehman *et al.,*2017), being a current challenge the agronomic management of this symbiosis to optimize its benefits in the agroecosystems. (Hamel and Plenchette, 2017; Rillig *et al.,* 2018).

Although positive reports on the use of arbuscular mycorrhizal (AMF) inoculants have increased in recent years and several published metadata make it clear that they are highly effective (Pellegrino *et al.,* 2015; Schütz *et al.,* 2018), there are few publications that allow comprehensive recommendations on management of AMF strains, ways to apply them and integration with cultural practices and fertilizers in agricultural production technologies.

In this sense, in Cuba, from the development of AMF inoculants that are applied by seed coating in small quantities (Fernández *et al*., 2000), the positive results achieved by the inoculation of “generalist” strains in a wide group of crops and edaphic conditions are increased (Rivera *et al*., 2003; González *et al*., 2015; Simó *et al.*, 2017a; Joao *et al.,* 2018). From which, it was established that the effectiveness of the studied strains depended on the edaphic environment of the soil or substrate and with emphasis on the pH at which mycorrhization is developed (Rivera *et al*., 2015), as well as that in the appropriate edaphic condition, the efficient strain established effective mycorrhization with the different mycotrophic crops studied (Rivera *et al.,* 2007).

Among the benefits achieved by the inoculation of efficient strains are the reduction of the requirements of fertilizer, mineral as organic-minerals, to obtain high yields and guarantee an adequate nutritional status an optimal mycorrhizal symbiosis (Rivera *et al.,* 2003; González *et al.,* 2015; Simó *et al.,* 2015; Ruiz *et al.,* 2016 a). In some of the crops these results have been successfully validated at a productive scale in thousands of hectares (Rivera *et al.,* 2020).

In turn, there are many results that support the benefits of using legumes as green manure, related to its capacity to fix N_2, production of important quantities of biomass, contribution to recycling different nutrients, decreasing the requirements of fertilizers for the main crop, and fast soil cover among other (García *et al.,* 2000, Bunch, 2005; Cherr *et al.,* 2006; Mateus and Wutke, 2011; Vargas *et al.,* 2017) and *Canavalia ensiformis* (canavalia) is one of them.

Canavalia is a mycotrophic plant and although its use as a precedent increases the "resident" mycorrhization of the crops in succession (Espíndola *et al.,* 1998), this mycorrhization generally does not reach an effective functioning and does not prevent the positive response to mycorrhizal inoculation (Rivera *et al.,* 2010; Sánchez *et al.,* 2011). Also, some reports (Rivera *et al*., 2003; Ruiz *et al*., 2012), suggest that inoculation of the crops with the AMF efficient strain allows an effective mycorrhization of the first culture in succession (permanence effect), although it depends on the period that mediates between the harvest of the inoculated and the sowing of the successor (Espinosa *et al.,* 2019), as well as probably the strain, the inoculated crop, the type of soil and the resident mycorrhizal community in the soil.

For all of the above, a research program was developed, with the general objective of integrating the joint management of *C. ensiformis* as green manure and the arbuscular mycorrhizal inoculants in the nutrient supply of different economic crops, establishing the bases for them and associated with: 1) greater amounts of biomass and nutrients in *C. ensiformis* inoculated and made available to the crop in association, 2) to be an effective way to achieve efficient mycorrhization of the main crops 3) decrease in the amounts of fertilizers required for high yields and 4) better efficiency in fertilizer use in relation with the simple use of mycorrhizal inoculants. Although this program generated several publications that establish the benefits for one or another crop, some of its objectives require a comprehensive analysis of the information obtained, for example, the basis for recommending AMF strains. In addition, the general evaluation of the main results, as well as the use of different indicators to evaluate the efficiency of the joint application that were not used in these publications, allow establishing its general scope and potential for agriculture, and this publication integrates them and summarize.

MATERIALS AND METHODS

Ethics statement

The research conducted in this document did not involve measurements with humans or animals. The study site is not considered a protected area. For the location / activities, no specific permits were required and the studies did not involve endangered or protected species. Canavalia, corn and tobacco seeds were acquired from a store of agricultural products and for the rest of the crops, vegetative propagules were obtained from experimental areas.

Soils types and experimental localities

The research program was carried out in different locations, crops and types of soils. Two groups of experiments were executed. Table 1 shows the different publications obtained in the program as well as its final report (Rivera *et al*., 2017), which were used to prepare the information presented in this work.

Table 2 shows the provinces, geographical location and types of soils (WRB, 2014) in which these experiments were carried out and the main characteristics of the soils at the beginning of these experiments. They correspond to the broad spectrum of agricultural soils in Cuba, located below 150 meters above sea level. Since low fertility soils associated with pH-H_2O values of 4.7, low contents of OM and elements available in the soil Albic Pisoplintic Plintosol, until soils of the types Calcic Haplic Phaozem and Vertic Phaozem with pH between 7.3 until close to 8 and contents of 40 $cmol_c$ kg^{-1} of interchangeable Ca and high saturation by bases. In general, the contents of mycorrhizal spores were low, with values between 47 and 109 spores in 50 g.

Table 1. Publications obtained in the work program and used to prepare the information presented here and the group of experiments to which they correspond.

Experiment group	Objectives	Publications and Final Program Report
I	Efficient AMF strain selection	Ruiz *et al*., 2009; Bustamante *et al*., 2010; Martin *et al*., 2010, 2015; Tamayo *et al*., 2015; García-Rubido *et al*., 2017; Ojeda *et al*., 2018; Simó *et al*, 2019
	Response of canavalia to inoculation of efficient strains of AMF	Martín *et al.*, 2010, 2015; Tamayo *et al*., 2015; García-Rubido *et al.*, 2017; Simó *et al*., 2017 b; Joao *et al*., 2018; Ojeda *et al.*, 2018; Simó *et al*., 2019.
II	Integration of AMF inoculated canavalia in nutrient supply schemes.	Martín *et al.*, 2009, 2012; Pentón *et al.*, 2016; Joao *et al.*, 2017; Rivera *et al*., 2017; Joao *et al.*, 2018; Simó *et al.*, 2020.

Characteristics of group of experiments I

The first one corresponded to the comparison work of AMF strains for the inoculation of *C. ensiformis* in different types of soils. The used strains belong to the collection of the National Institute of Agricultural Sciences of Cuba (INCA) and corresponded to the following species: *Funneliformis mosseae* (Schüßler and Walker, 2011)/INCAM-2; *Glomus cubense* (Rodríguez *et al.*, 2011) /INCAM-4, DAOM241198/ and *Rhizoglomus irregulare* (Sieverding *et al.,* 2014)/INCAM-11, DAOM711363. The inoculant for each of the strains was formulated solid (Fernández *et al*., 2000) and in all cases the spore content was between 25 and 30 spores g^{-1} of product and undetermined quantities of mycelia and infective roots.

Experimental design and handling of experiments

The treatments consisted in the inoculation of each of the strains and an uninoculated control, with the exception of one experiment in which only two strains were evaluated. They were executed under field conditions with a randomized block design with four repetitions and repeated at two moments, with the exception of those executed on Vertic Phaozem and Eutric Cambisol without carbonates that were in microparcels of 1 m^2 and pots of 2 kg respectively and for these the experimental design corresponded to a completely randomized with 6 repetitions.

The experimental plots were 17.5 m^2 (5 meters x 3.5 meters) and the canavalia was planted in May, 20 cm between plants and 50 cm between rows. For pots and microplots 3 and 10 plants respectively were grown per unit. The inoculation of the AMF strains was carried out by coating the seed, with inoculant quantities of 8 % of the seed weight (Martín *et al.,* 2009). A complete information about each experiments appear in the following articles (Ruiz *et al*., 2009; Bustamante *et al*., 2010; Martin *et al*., 2010, 2015; Tamayo *et al*., 2015; García-Rubido *et al*., 2017; Ojeda *et al*., 2018; Simó *et al*, 2019).

Main evaluations and statistical analysis

After 60 days of sowing, the aerial dry mass was determined and expressed as t ha^{-1} or g $pots^{-1}$ and N, P and K concentrations (Paneque *et al*., 2010) and amounts of N, P and K (kg ha^{-1}) were determined. Likewise the percentage of root colonization (Giovanetti and Mosse, 1980; Rodríguez *et al.*, 2015) and mycorrhizal spores in the first 20 cm of depth of the soil (Gederman and Nicholson, 1963) were evaluated.

ANOVA was performed for the different variables and the means were compared according to Duncan test ($p < 0.05$). With the dry mass (DM) data, the response as an efficiency index of each strain (IE) was calculated:

IE_i = (DM $strain_i$ – DM control) * 100 / DM control, being i each of the three strains.

However, even when working with the same plant species, the experimental conditions were diverse and to compare the effectiveness of the strains of AMF in the different experiments it was necessary to standardize this index, for which the DM response obtained by the strain with the greatest effect as 100 was considered in each experiment and the responses obtained by the other strains were expressed as a percentage of this value. This new index was denominated IER (%) and it was determined in the following way: IER_i (%) = $\underline{IE_i \text{ x } 100}$ / IE greater.

For the rest of the variables the response to inoculation of the efficient strain as an efficiency index (IE) was calculated in a similar way.

Table 2. Types of soils (WRB, 2014), geographical location, province and main soil properties.

Soils, geographical location, province and group of experiments realized	Previous crops	pH H_2O	MO g kg^{-1}	Available P mg kg^{-1}	Ca	Mg (cmol$_c$ kg^{-1})	K	Na	AMF resident spores 50 g^{-1}
1. Vertic Phaozem / 22º 35' N, 80º18' O /Villa Clara (1)		7.8-7.9	20.8	14.0	44.6	3.2	0.49	0.90	60
2. Calcic Haplic Phaozem / 22º 35' N, 80º18' O Villa Clara (1,2)	Various crops fertilized	7.5- 7.9	21.6	14.0	44.8	3.2	0.60	0.42	47
3. Eutric Cambisol (without carbonates) / 20º 09´ N, 76º16´ O/ Santiago de Cuba (1,2)		6.0 -6.2	26.1	11.8	27.0	4.0	0.57	n. d.	n. d.
4. Ferralic, Rhodic, Lixic, Eutric Nitisol / 23º 01'N, 82º 08'O Mayabeque (1,2)		6.5 -7.0	32.5	17.0	9.7	2.2	0.21	0.15	109
5. Ferralic, Rhodic, Lixic, Eutric Nitisol / 22°, 48' 7'' N, 81° 2' 0" O/Matanzas (2)	Mullberry	6.1- 6.5	29-41	3.5-9.2	11.2	3.9	0.12	0.11	n. d.
6. Ferralic, Rhodic, Lixic, Eutric Nitisol / 22º 55' 59" N, 82º 22' 39" O/ Artemisa (2)	Fertilized pastures	6.1	31.7	10.0	8.9	2.0	0.32	0.11	100
7. Rhodic, Alumic Acrisol / 22º 16' 55" N, 83° 49' 19" O /Pinar del Rio (1,2)	Fertilized tobacco	6.0 -6.2	13.6	16.6	4.7	1.9	0.44	0.10	50
8. Dystric Cambisol / 22º 09′ N, 80º 12′ O / Cienfuegos (1)	Pastures	5.2 -5.4	17.8	15.8	n. d.	n. d.	0.06	n. d.	n. d.
9. Albic Pisoplintic Plintosol /22º 39' 33" N, 80º 29' 46" O/ VillaClara (1,2)	Pastures	4.7– 4.9	24.3	7.9- 12.2	3.5	1.2	0.11	0.10	69

(1) and (2): in these soils the experiments of Group I and Group II were executed respectively; n.d.: non-determined. Analytical Techniques used: pH in H_2O in soil: solution relation (1:2.5) by the potentiometric method. Determination of organic matter level by the Walkley-Black method (oxidation of C with $K_2Cr_2O_7$ 0.5 M in H_2SO_4 18 M at 98 %) and estimation with a solution of 0.25 M of ammonium iron sulphate. Extraction of P in soils with pH-H_2O < 7.2 by H_2SO_4 0.05 M; in soils with pH-H_2O > 7.2 extraction with $(NH_4)_2CO_3$ solution with a concentration of 10 g L^{-1}, pH 9.0. Extraction of exchangeable cations with NH_4Ac 1 M and pH 7 in soil: solution relation of 1:5 and shaking for 5 minutes. All techniques according to Paneque *et al.*, (2010).

Characteristics of group of experiments II

Once the most effective strain was obtained for each edaphic condition studied, the second group of experiments was developed. In these experiments was studied, the integration of mycorrhizal inoculants and canavalia used as a green manure inoculated with the nutrient supply schemes for different crops, either as mineral or organic-mineral fertilizers. Table 3 shows some characteristics of these experiments such as soil type, main crop, and management of the canavalia, area of the plots, experimental period and recommended dose of 100 % NPK fertilization for each main crop.

Experimental design

The experimental design in each case was of random blocks with four replications and in a general way the experiments were carried out with several harvest. Within the experimental scheme was included a treatment that corresponded to the optimal dose of mineral fertilization for each crop (100 % NPK), according to the Technical Instructions of each one (IIT, 2012; MINAG, 2004; 2011; 2014), with the exception of banana experiment in which used an equivalent doses of organic mineral fertilizer (100 % FOM) similar to 100 % NPK (Simó *et al.,* 2015) The specific treatments in each experiment, methodologies and fertilizer management used were described in the publications referred to in Table 3. The plots used in each treatment were permanent during the execution of each experiment.

Experimental handling

The previous canavalia was planted at 0.5 x 0.2 meter (100 000 plants ha^{-1}) in the months of May-June with the exception of the cassava experiment that was carried out in September. Sixty days after planting (dap), the plants were chopped with a rotary mower and incorporated in to the soil with a disc plow at 20 cm depth, although in the cassava experiment a second cut was made at 110 dap, before incorporation. In the treatments in which the canavalia was not used, the plots were left fallow and this was cut and incorporated in the same dates as the canavalia.

The economic crops were sowing or planting 30 days after the cutting and incorporation of the canavalia or fallow. The furrows of intercalated canavalia were planted in the streets of the main crops, between 30 and 45 days after the establishment of the plantation. The distribution of the furrows depended on the crops. In bananas they were located 50 cm apart from the rows of the main crop and with a distance between them of 50 cm; in cassava, two furrows of canavalia were located 20 cm apart and 35 cm from the rows of the main crop, and in mulberry, a single row of canavalia in the center of the street. In all cases the distance between canavalia plants was 20 cm.

Inoculation with arbuscular mycorrhizal fungi

The AMF strain was chosen according to the results obtained in the group of experiments I. In canavalia it was carried out via a coating with 8 % inoculant in relation to the seed weight (Martín *et al*., 2009). In the treatments in which the main crops were inoculated, the 10 % seed coat was used for corn (Fernández *et al.*, 2005) and in similar way at 6 % for the king-grass vegetative stakes, covering the tips of the vegetative seeds in the cassava with 13 kg ha^{-1} of inoculant (Ruiz *et al.*, 2010) and for the banana plantation, 20 g were applied to the hole prior to transplanting the vitroplants.

Evaluations and statistical analysis

The evaluations to the canavalia were similar to those already described and for the main crops were carried out in each growth cycle: the yield (t ha^{-1}), the percentage of root colonization and the count AMF spores in the first 20 cm of depth of the soil at the end of cropping time.

As in each experiment, the different doses of fertilizers studied consisted of percentages of the recommended dose (100% NPK) to obtain high yields in each crop (IIT, 2012, MINAG, 2004, 2011, 2014), the estimates of intake efficiency of fertilizers (IEF) for the different treatments were calculated in a similar way to the Agronomic Efficiency (Stewart, 2007), but in relation to the sum of the amounts of macronutrients applied in each treatment. The IEF was calculated as follows:

$$\text{IEF (kg kg}^{-1}\text{)} = \frac{[\text{YST} - \text{YWF}] \times 10^3}{\Sigma\text{kg ha}^{-1}\ \text{N} + \text{P}_2\text{O}_5 + \text{K}_2\text{O}}$$

In where: YST= yield selected treatment (t ha^{-1}); YWF= yield without fertilizer (t ha^{-1}).

Table 3. Main characteristics of the integration experiments of canavalia-AMF in the fertilization schemes of different crops.

Economic crop/cultivated plant name	Soil/ AMF strain inoculated	*C. ensiformis*	Experimental period	Treatments	Fertilization 100 % NPK of the main crop $N-P_2O_5-K_2O$ (kg ha^{-1})
Corn / 'Francisco mejorado' (1)	Ferralic, Rhodic Nitisol/ INCAM-4	Precedent	2007-2008	Split plot design. In the main parcels (fallow, canavalia, canavalia-AMF) in the subplots (corn inoculated or not inoculated)	without fertilizer
Corn / 'Francisco mejorado' (2)	Ferralic, Rhodic Nitisol/ INCAM-4	Precedent	2006-2007	Factorial arrangement 2 x 2, (canavalia inoculated or not), (corn inoculated or not).	all of the treatments received 50 kg ha^{-1} N
Tobacco/ 'Criollo 98' (3)	Rhodic, Acrisol/ INCAM-4	Precedent	2012-2015	Factorial arrangement 3 x 3. (50%, 75 % and 100 %NPK) and (canavalia-AMF, canavalia and without canavalia)	120-60-160
Banana/'FHIA-18' (4)	Calcic Haplic Phaozem / INCAM-11	Precedent and intercropped	2006-2010	Canavalia-AMF with 0, 25%, 50 % and 75 % FOM and other treatments as, 100 % FOM, 75 % FOM, 75 % FOM-AMF, AMF and control without fertilizers	100 % FOM (20 kg $plant^{-1}$ of compost and 10 kg $plant^{-1}$ of sugar cane ash)
Cassava /'CMC-40' (5)	Ferralic, Rhodic Nitisol/ INCAM-4	Precedent and intercropped	2012-2013	Different combinations of canavalia inoculated or not, cassava inoculated or not, all of them receiving 25 % NPK and others treatments only with levels of mineral fertilizer between 0 to 100 % NPK	100 – 40 - 150
Cassava /'CMC-40' (6)	Calcic Haplic Phaozem / INCAM-11	intercropped	2013-2014	Factorial arrangement 2 x 2 x 2 (AMF or not in cassava), (canavalia -AMF intercropped or not), (0 , 25 % NPK) and other treatment with 100 % NPK.	150 – 60 - 200
Mullberry /'Tigreada' (7)	Ferralic, Rhodic Nitisol/ INCAM-4	intercropped	2008-2010	Factorial arrangement 3 x 2 (0, 50 y 100 % NPK) and (canavalia-AMF and without canavalia)	200 – 100 - 100
“king-grass” (*P. purpureum*)/ 'Morado' (3)	Pisoplintic Plintosol/INCAM2	Precedent	2013-2014	Different combinations of canavalia inoculated or not, “king grass” inoculated or not, all of them with 70 % N 50 % PK and other treatments with 100 % NPK and control without fertilizers	200 – 80 - 150
	Ferralic, Rhodic Nitisol/ INCAM-4	Precedent	2014-2015		200 – 50 - 100

AMF strain inoculated in each experiment in function of soil pH-H_2O (Table 2). FOM: organic –mineral fertilizer. A more complete information about each experiment can be found in the following publications: (1) Martín *et al.*, 2012; (2) Martín *et al.*, 2009; (3) Rivera et al., 2017; (4) Simó *et al.*, 2020; (5) Joao *et al.*, 2017; (6) Joao *et al.*, 2018; (7) Pentón *et al.*, 2016.

For each main crop, the IEF obtained when the inoculated canavalia was used in the presence of the optimal dose for this purpose (F1) were compared with that obtained for the 100% NPK treatment (recommended to obtain high yields according to the Technical Instructions). Similarly, the IEF obtained when the inoculation was carried out directly to the main crop in the presence of the optimal dose of fertilizers for such purposes (F2) were compared. For this last evaluation, treatments present in the experiments carried out (Table 3) or results of contiguous experiments carried out with such objectives were used (Ruiz *et al*., 2016, Joao *et al*., 2018).

The statistical analyzes were carried out in correspondence with the experimental designs. For the different variables, the ANOVA were performed and whenever there was significance in these ($p < 0.05$), the means of the treatments were calculated according to Duncan's test at $p < 0.05$.

RESULTS AND DISCUSSION

Response of canavalia to the inoculation of AMF strains in different soils

A significant response of the canavalia to mycorrhizal inoculation was found in all variables evaluated in the different edaphic conditions, with differentiated effects ($p < 0.01$) between the strains. In Figure 1, the results obtained on the production of dry aerial biomass and expressed as IER (%) are presented. The effects differentiated by the inoculation of the strains can be observed and as the strain that originated the greater response, it was not the same in all the edaphic conditions.

In this figure the experiments were ordered according to the increasing pH-H_2O value of the soil, so that in the pH ranges between 4.7-5.4 the strain that gave the greatest response was *F. mosseae* / INCAM -2, while in the experiments that corresponded to the pH range between 6 and 7 the strain that caused the greatest response was *G. cubense* / INCAM-4 and when the soils presented pH between 7.0 to 8.0 the strain with greater effectiveness was then *R. irregulare* / INCAM-11.

Figure 2 summarizes the effects by the inoculation of the better strain in each experiment. The strain that showed the greatest effects in the production of dry biomass in each experiment did it in a similar way in the rest of the variables with significant difference ($p < 0.01$) between the values originated by one or another strain, indicating a relationship between mycorrhizal functioning, nutritional status and biomass production. This strain was called as efficient strain according to the criteria used by Rivera *et al.,* (2007).

The positive effects achieved by the inoculation of the efficient strain on biomass production were remarkable, reaching average increases of 50 % and always greater than 20%. Similarly, the average increases in the amounts of macronutrients (NPK) extracted by canavalia, which were greater than 60%. In the macronutrients concentration, the effects were also significant but more discreet, between 8 and 15% and possibly linked to the remarkable increases obtained in biomass production. The important increases in the amounts of macronutrients extracted by the inoculated canavalia must have a valuable impact on their potential as green manure. Furthermore, the efficient strain generally achieved average colonization percentages of around 60%, which, according to diverse authors, they are indicative of adequate mycorrhizal functioning in different crops (Rivera *et al.,* 2007; Ruiz *et al.,* 2012; González *et al*., 2015).

The significant contribution of effective mycorrhizal functioning to symbiotic fixation in legumes has become clear, related among others by improvements in phosphorus nutrition and other nutrients in legumes (Larimer *et al.,* 2014; Bulgarelli *et al.,* 2017). So the positive effects of both symbiosis explaining the increase in biomass production, concentrations and quantities of nutrients extracted by the AMF inoculated canavalia.

Inoculation of canavalia with the efficient strain markedly increased mycorrhizal spore reproduction with average increments of almost 5 times. (Figure 2 B). In a similar way, reports associated with inoculation of these same strains in forage species in different soils, have shown significant differences in spore production, with the highest values associated with inoculation of the efficient strain (González, 2014) and even linking the permanence effect of AMF inoculation with the number of spores when each cut is made.

Although spores are generally not considered as an indicator of mycorrhizal functioning, some authors such as Helgason and Fitter (2009) and Willis *et al.,* (2013) consider that greater sporulation offers competitive advantages for the species. Furthermore, Quilian *et al*., (2010) associate directly the number of spores produced with the mycorrhizal inoculation potential of the soil.

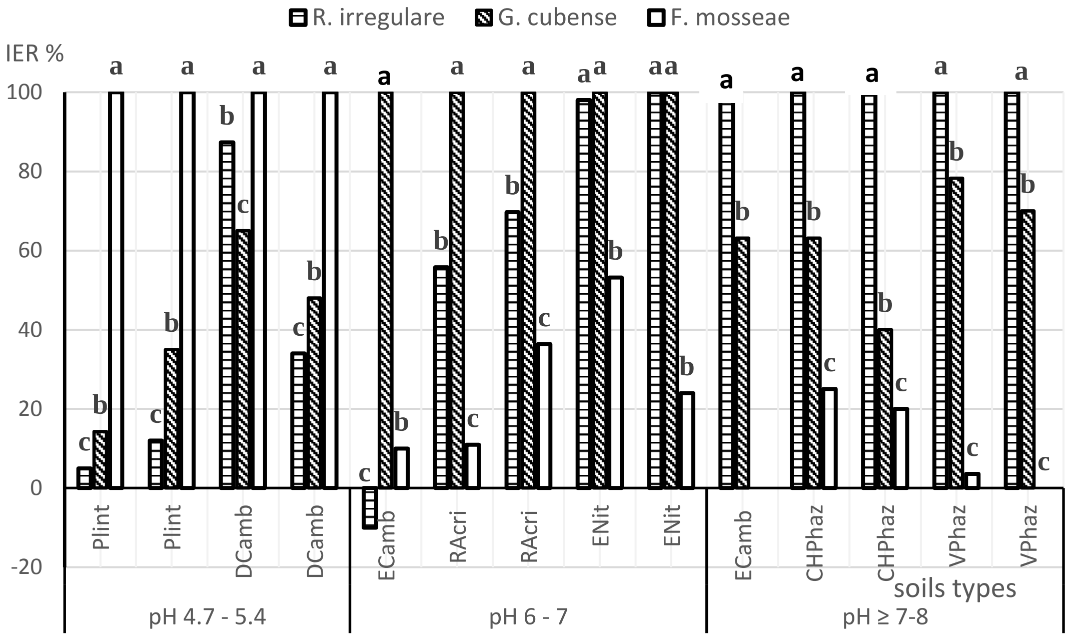

Figure 1. Response of canavalia to the inoculation of each strain and expressed as IER (%) of biomass production in each experiment. Different letters in each experiment indicate significant differences ($p < 0.05$) between the effects caused by the strains in the biomass of the canavalia. pH-H_2O ranges associated to the different soils according to Table 2. **Plint**: Haplic Plinthustult**; DCamb:** Dystric Cambisol; **ECamb**: Eutric Cambisol; **RAcri**: Rhodic Acrisol ; **FNit**: Ferralic, Rhodic, Eutric Nitisol; **CHPhaz:** Calcic Haplic Phaozem; **VPhaz:** Vertic Phaozem.

In recent years there has been an increase in the results that establish the influence of edaphic conditions on the occurrence and diversity of resident AMF species, and a consensus has been reached (Goranson *et al.,* 2008, Oehl *et al.,* 2010; Santos *et al.,* 2011, Sikes *et al.,* 2014; Montiel-Roxas *et al.,* 2017). However, another situation seems to be the conditioning of the effectiveness of the strains inoculated with the edaphic environment and possibly most of the published results have been obtained in Cuba (Rivera *et al.*, 2003; Herrera *et al.*, 2011; González *et al*., 2015). In these reports, the change in effectiveness of the inoculated strains was associated with the type of soil and, more recently, it was linked to the environment or soil condition in which mycorrhization developed and, fundamentally, to the pH-H_2O of the soil or substrate (Rivera *et al.*, 2015). According to these last authors, the concept of edaphic environment integrates not only the type of soil, but also the land use and the soil amendments, which determine the pH of the soil in which the plants are developed. Other authors have also reported the influence of the soil reaction on the effectiveness of inoculated strains (Sano *et al.,* 2002; Ouzounidou *et al*., 2015).

Therefore, the recommendation of efficient strains for canavalia obtained in these experiments, based on edaphic conditions and mainly on soil pH, coincides with that reported for these same strains when inoculated in a wide group of crops and edaphic conditions (Rivera *et al*., 2015). In such a way that the efficient strain condition is specific to the edaphic environment in which the mycorrhization takes place and, at the same time, the efficient strain condition presented a "generalist" performance with the different plant species dependent on the mycorrhization. It should also be taken into account that this change in the effectiveness of the strains evaluated with the soil conditions does not have to be found for all species and strains of AMF, because a group of them may be specific with some plants or types of soils (Opik and Mora, 2012; van der Heidjen *et al.,* 2015).

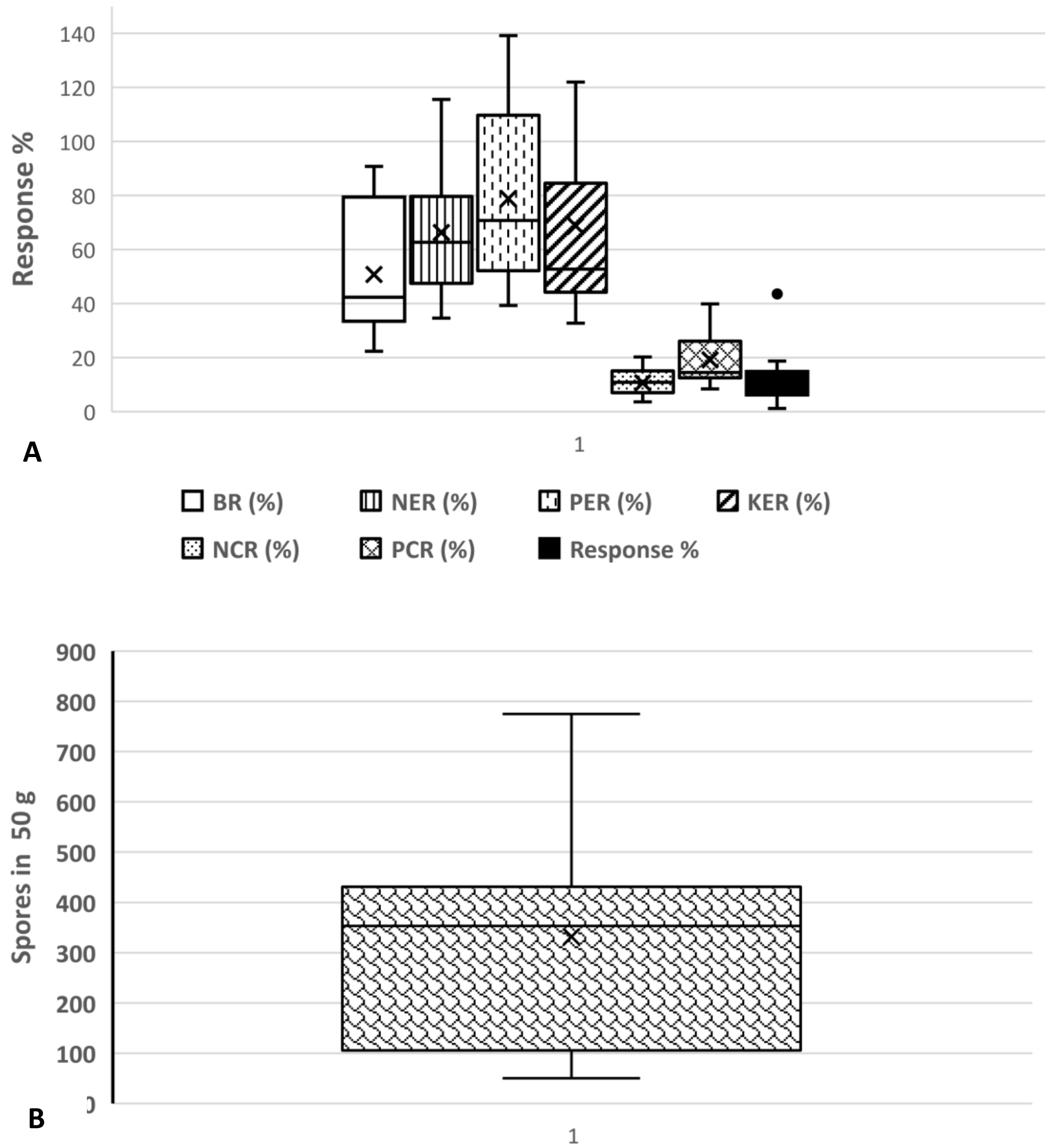

Figure 2. Main results obtained by inoculating canavalia with the efficient AMF strain for each soil condition. A) Responses in aerial biomass (BR%), in the amounts of N, P and K present in it (NER%; PER%; KER%), and in the respective NPK concentrations (NCR%; PCR% and KCR%). B) Response in mycorrhizal spore count (SR %). (x) Average response in each variable, n = 14. The response for each variable in each experiment was significant at least for P <0.01.

Benefits of the efficient AMF inoculation of canavalia in the nutrient supply schemes of different crops.

The canavalia inoculated with the efficient strain and used as the preceding green manure caused positive effects in the various main crops evaluated as: corn, tobacco, banana, tomato and king-grass in different types of soils (Figure 3). In all the crops, significant response (p <0.01) in the yields with average of 50 % were obtained in relation to the treatments that received the same medium or low dose of fertilization (F1) and that did not include inoculated canavalia. Likewise, high yields obtained were similar to those achieved when using the higher fertilization doses (100% NPK) recommended by the Crop Technical Instructions (IIT, 2012; MINAG, 2004, 2011, 2014). Even in tobacco and king grass cultivated in lower fertility soils, Rhodic, Alumic Acrisol and Albic Pisoplintic Plintosol, the yields were higher. Therefore, in all crops a significant decrease was achieved in the amounts of mineral or organic fertilizers necessary to obtain high yields, with an average decrease of 50% and a range between 33 and 75% depending on the crop and type of soil.

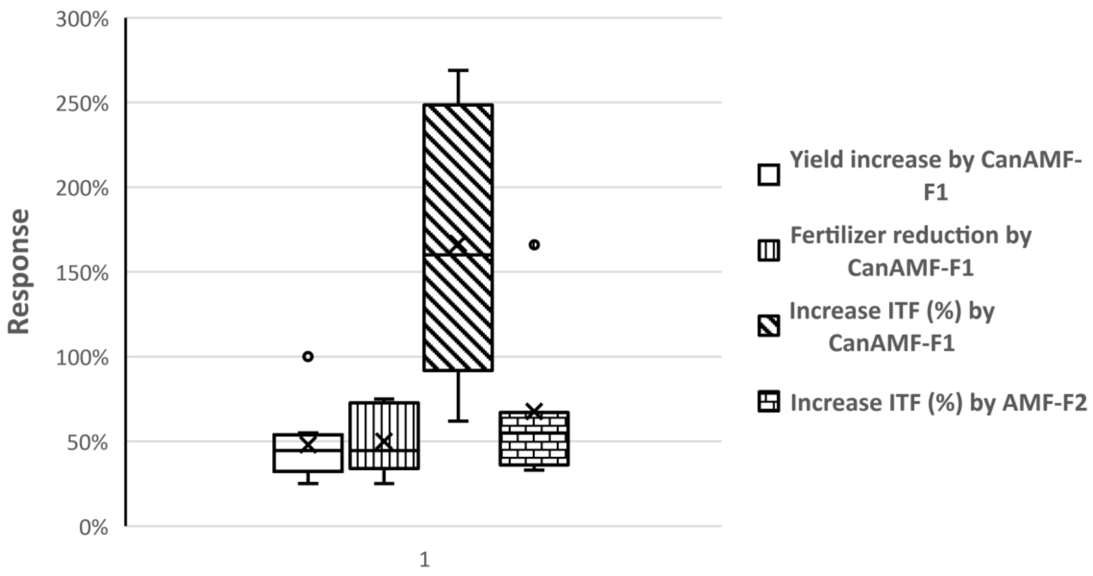

Figure 3. Benefits of the joint management of efficient strains of AMF and canavalia as green manure preceding and/or intercropped (CanAMF) in different crops, in the presence of the optimal doses of fertilizer (NPK) obtained for this management (F1). For each experiment the responses in the increase in yield (%) were calculated in relation to the treatments that received F1 without CanAMF and the reduction of fertilizers (%) were calculated in relation to the 100% treatment of NPK (recommended doses for high yields by the different Crops Technical Instructive). The increase in the efficiency of taking fertilizers (ITF%) were calculated for CanAMF-F1 and for AMF-F2 (crops were AMF inoculated and F2 was the optimal fertilizer doses for this management) and both in relation to the 100% treatment of NPK. The doses of 100% NPK, F2 and F1 were different for each crop, but always 100% NPK > F2> F1. N=8. The response in each experiment was always significant at least for P <0.01.

Likewise, it was also found in all crops that the relationship between the estimates of "intake efficiency of fertilizers" (ITF) and always compared to that obtained for the 100 % NPK treatment were superior when AMF inoculated canavalia was used than when only AMF inoculants were applied (Figure 3). It should be noted that the results found in terms of decreasing amounts of fertilizers, exceed those previously reported by the single use of AMF inoculants that ranged between 25 and 50 % of the doses (Ruiz *et al*., 2012; Simó *et al*., 2015; Ruiz *et al*., 2016 b; Joao *et al*., 2017; Joao *et al*., 2018), all of which supports the benefits obtained with this joint management of canavalia and AMF inoculants.

In addition, the use of inoculated canavalia allowed not only a higher yield of the cultures in succession, but also a satisfactory mycorrhizal functioning in these, with average colonization percentages of around 60% (Figure 4), significantly higher (p <0.01) to those obtained by these cultures in succession to the non-inoculated canavalia. The satisfactory mycorrhizal functioning achieved in the cultures when using the inoculated canavalia and similar to when the cultures were inoculated (Figure 4), demonstrate the existence of the permanence effect of the inoculant applied to the canavalia and corroborate the information previously obtained by Rivera *et al.* , (2010) and Sánchez *et al*., (2011). Although green manure increases the inoculation potential of resident mycorrhizae, this increase generally does not guarantee effective mycorrhizal function in subsequent cultures. Therefore, green manure must be inoculated to enhance its benefits and effectively mycorrhize the crop in succession.

No less important to support the benefits achieved by this joint management in the nutrition of the main crops, are the recent reports that in the presence of organic patches both the lengthening and the number of mycorrhizal hyphae increase, as well as that the microbiota associated with mycorrhizae intensify the decomposition rate of these organic residues (Hodge and Storer, 2015; Thirkell *et al*., 2016; Bukovská *et al.*, 2018). This favors the speed of absorption of nitrogen and other nutrients present in these residues by mycorrhizal plants. Although the inoculated green manure recycles and provides significant amounts of nutrients, the effective functioning of mycorrhizae in the main crop facilitates not only the absorption of these, but also a greater absorption of nutrients from the soil and fertilizers (Yang *et al*., 2014 ; Azcón-Aguilar and Barea, 2015; Cavagnaro *et al*., 2015). The above explains the decrease in the amounts of fertilizers to obtain high yields, as well as the notable increases in ITF of the main crops when inoculated canavalia is used compared to when only use of AMF inoculants.

The effect of the intercropped inoculated canavalia was also very satisfactory, guaranteeing not only high yields of the main crop, but also the fertilizer doses decreased and the ITF of the applied fertilizer was increased (Figure 3). In cassava, its employment improved the results achieved with the use of the inoculated canavalia as precedent or when the cassava was directly inoculated, and in all cases indicated the contribution of the green manure inoculated to the nutrition of the main crop. In the case of the mulberry in which only the intercropped canavalia was inoculated, the increases in yield , as well as the decreases in fertilizers to reach high yields and the highest values of fungal occupation reached (Figure 4) showed that it was an effective way to mycorrhizal the associated main crop. In addition quickly covers the streets, reduces the number of cleaning tasks between 70 and 80 % (Pentón, 2015; Joao *et al*., 2018) and keeps the ground covered especially in the times of high rainfall, therefore is also a favorable handling practice for soil conservation.

The permanence effect of the applied inoculant had been previously reported in crop sequence experiments (Rivera *et al.*, 2003; Marrero *et al.*, 2008) based on the generalist character of the efficient strains with plant species (van der Heidjen *et al*., 2015) and with these results extend to the use of canavalia as a green manure. This effect is important to not only simplify the handling of the inoculant and enhance the benefit of green manure, but also decrease the quantities of inoculants. For example, canavalia only requires 8 to 10 kg ha^{-1} of inoculants, much less than the 22, 26 and 120 kg ha^{-1} that require direct inoculation of cassava, banana and king-grass, respectively; and the high quantities and industriousness that also requires direct application to established crops such as mulberry and fruit plantations (20 to 60 g plant^{-1}).

There are different documents that widely discuss the importance of green manures and aspects to take into account for their use in agricultural production systems, not only focused on family farming, but also as part of sustainable intensification technologies, (Bunch, 2005; Cherr *et al*., 2006; Peoples *et al*., 2019; Royal Society, 2009); however, they do not value or warn about the importance of this joint management of green manures and mycorrhizal inoculants.

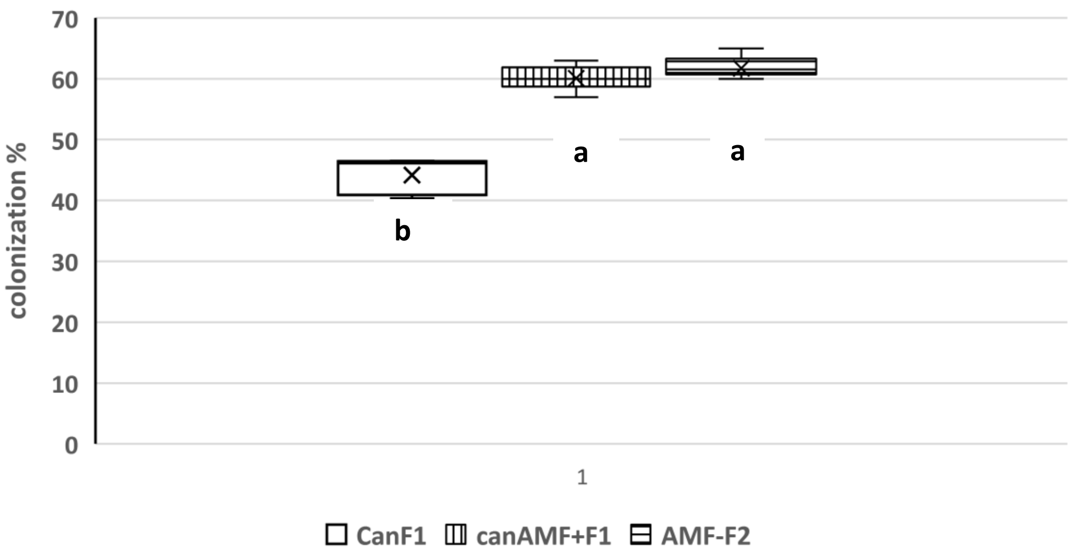

Figure 4. Effect of joint management canavalia - AMF on the percentages of mycorrhizal colonization of the main crops (CanAMF-F1) and compared with the percentages obtained when canavalia is not inoculated (CanF1) and when only the main crop is inoculated with AMF and canavalia is not used as green manure (AMF-F2). F1 and F2 optimal doses to achieve satisfactory mycorrhizal function in the presence or not of inoculated canavalia. F2> F1. N= 8. Letters represent significant differences in mean colonization at the 0.05 significance level.

Vanlauwe *et al*., (2010) define the scope of integrated fertility management systems (ISFM) among whose principles are the joint management of fertilizers and organic fertilizers, use of appropriate germoplasm and integration with cultural practices of proven effectiveness in the location. The positive effects of the joint management of canavalia and mycorrhizal inoculants in the presence of low or medium doses of fertilizers (mineral or organic) in different soils and crops, allow us recommending it as a practice to be included in the ISFM approach, and to pass it to the validation and adaptation stage of these results in the productive conditions of farmers.

CONCLUSIONS

The joint management of AMF inoculants and canavalia used as green manure, either preceding and/or intercalated, not only enhances the benefits obtained individually by the use of them and related, among others, to the decrease in the amounts of fertilizers required to guarantee satisfactory harvests. Furthermore, it becomes an effective way to introduce the AMF efficient strains into agroecosystems, guaranteeing effective mycorrhizal functioning of the mycotrophic economic cultures, with greater impact in those that require such high amounts of AMF inoculants that are not economically feasible and also to ensure mycorrhization with efficient strains in established plantations. This joint management is valid when using AMF strains that have a generalist character with the crops and using them in the edaphic conditions in which they are efficient.

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Compliance with ethical standards. The authors confirm that the research was carried out and managed in accordance with ethical standards.
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Data availability. Data are available with the corresponding author (rrivera@inca.edu.cu) upon reasonable request.

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