THE INTEGRATION OF AMF INOCULANTS, GREEN MANURE AND ORGANO-MINERAL FERTILIZATION, IN BANANA PLANTATIONS ON CALCIC HAPLIC PHAEOZEMS †

[LA INTEGRACION DE INOCULANTES MICORRÍZICOS, ABONOS VERDES Y ABONAMIENTO ORGÁNICO- MINERAL EN PLANTACIONES DE BANANO EN SUELOS PARDOS]

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SUMMARY

Background. Banana plants require large amounts of nutrients but fertilizer price is restrictive for many farmers, so it is important to develop technologies that support the productivity of banana crops relying on local nutrient sources by enhancing soil biological activity. **Objective.** To evaluate the feasibility of integrating the management of mycorrhizal inoculants, green manure and organo-mineral fertilization in banana. **Methodology.** Different treatments with compost and sugar cane straw ash as organo-mineral fertilizers, jack bean (*Canavalia ensiformis*) as preceding and intercropped green manure, and inoculation with *Rhizoglomus irregulare* (INCAM-11), were evaluated in banana cv. 'FHIA-18'. The experiment was planted on Calcic Haplic Phaeozems and conducted during the cycles of planting, first ratoon, and second ratoon. The experiment had a randomized complete block design with four replicates. **Results**. Inoculation at transplanting with *R. irregulare (INCAM-11)* remained effective during the three production cycles evaluated. The mycorrhizal inoculation of *C. ensiformis* not only increased the biomass and the quantity of recycled nutrients, but also resulted in the production of high quantities of mycorrhizal propagules in the soil. **Implications.** The feasibility of integrating the management of efficient arbuscular mycorrhizal fungi (AMF) strain, green manure and low rates of mineral-organic fertilizers to obtain high banana yields, an adequate plant nutritional status and a satisfactory mycorrhizal functioning was shown. **Conclusion.** The integration of efficient AMF strains 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: arbuscular mycorrhizae; *Canavalia ensiformis*; foliar analysis; banana; organic fertilizer.

RESUMEN

Antecedentes. El banano requiere de grandes cantidades de nutrientes, pero el precio de los fertilizantes es prohibitivo para muchos de los pequeños productores y por tanto se requiere de desarrollar tecnologías basadas en fuentes locales de nutrientes y potenciando la actividad biológica en el sistema. **Objetivo.** Evaluar la factibilidad de integrar el manejo de inoculantes micorrícicos, abono verde y fertilización organo-mineral en banano. **Metodología.** Se evaluaron en banano cv FHIA 18 sobre suelo Pardo mullido carbonatado, diferentes tratamientos integrando compost y ceniza de caña de azúcar como fertilizantes orgánico-mineral, *Canavalia ensiformis* como abono verde precedente e intercalado y la inoculación con *Rhizoglomus irregulare* (INCAM-11) durante los ciclos de planta madre, vástago-1 y vástago-2. Se utilizó un diseño de bloques al azar con cuatro repeticiones. **Resultados.** La inoculación en el trasplante con *R. irregulare* mantuvo su efectividad en los tres ciclos productivos. La inoculación micorrízica de *C. ensiformis* aumentó la biomasa y la cantidad de nutrientes reciclados, y originó la producción de grandes cantidades de propágulos micorrícicos en el suelo en la plantación de banano. **Implicaciones.** Los resultados demostraron la factibilidad de integrar los inoculantes micorrízicos y los abonos verdes con dosis bajas de fertilización órgano-mineral, para garantizar altos rendimientos, estado nutrimental adecuado y un funcionamiento micorrízico satisfactorio. **Conclusiones.** La integración de cepas de AMF eficientes y abono verde aumentó los beneficios de ambas prácticas y es una forma prometedora de manejar la simbiosis micorrízica arbuscular en la producción sostenible de cultivos.

Palabras claves: micorrizas arbusculares; abonos verdes; abonos orgánicos; banano; análisis foliar.

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INTRODUCTION

Banana (*Musa spp.*) is an important food crop averaging yields of 20.2 t ha^{-1} globally (FAOSTAT, 2017). In Cuba a little more than 30 000 ha are cultivated $year^{-1}$ with yields that not exceeding 11 t ha^{-1} (ONEI, 2016). Banana plants require large amounts of soil nutrients, due to their high productivity. Potassium and nitrogen are the elements that influence yield most, hence most banana plantations in Central America are on soils with high cation exchange capacity (Álvarez, 2011). In Cuba, the technical guideline for banana production, (MINAG, 2011) recommends N rates of 420 kg ha^{-1} and 1 000 kg ha^{-1} of K_2O per production cycle. The high cost of fertilizers is restrictive. The use of organic fertilizers and non-conventional mineral sources of nutrients with high effectiveness can reduce or replace costly chemical inputs in agro-ecosystems, including in banana plantations (Ndukwe *et al*., 2011; Roobroeck *et al*., 2016). For Cuba's conditions, the recommended scheme of organo-mineral fertilization is the application of 28 t ha^{-1} of compost (65 % humidity) and 14 t ha^{-1} of sugar cane straw ash (MINAG, 2011). However, the relatively low availability of these resources allows their application in only a few banana – producing areas of the country, so the nutrient supply to banana plantation often limits yield.

It is a universally accepted that mycorrhizae improve plant nutrition and development, among other benefits (Yang *et al*., 2014; Priyadharsini and Muthukumar, 2015; Van der Heidjen *et al*., 2015; Bhandari and Garg, 2017) and satisfactory results have been obtained with the inoculation of several crops, including tropical roots, beans, corn, tobacco and pastures, with efficient strains of arbuscular mycorrhizal fungi (AMF) selected for their adaptation to edaphic conditions (Rivera *et al*., 2007; Cruz *et al.*, 2014; González *et al*., 2015). Banana, a mycotrophic crop (Declerck *et al*., 1995), has not been the exception and showed positive effects of AMF application during acclimatization, with increments in growing between 25 to 30 % (Jaizme Vega *et al.*, 1997; Gaidashova *et al*., 2013; Simó *et al*., 2017). Inoculation also improves the survival at transplanting up to 100 % (Kavoo-Mwangia *et al*., 2013; Simó *et al*., 2017) and protects host plants from root pathogens (Jaizme Vega *et al.*, 1997; Gañán, *et al.*, 2011), depending of soil type, banana cultivar, and AMF strain effectiveness. At planting stage, in our conditions, inoculation of banana with *R. irregulare* has resulted in high yields and reduced fertilizer requirements by 25 % during three production cycles (Simó *et al*., 2015; Ruiz *et al*, 2016). However, the recommended rates of organo-mineral fertilizers still remain high.

Likewise, green manure legumes used as previous crop or intercropped, provide nutritional benefits to the main crop. They contribute nitrogen via biological fixation (BNF), and also recycle nutrients, increase soil biological activity and protect the soil (Ambrosano *et al*., 2013; Solti *et al*., 2016). Although a previous green manure crops can increase resident mycorrhizal populations and, hence, increase the level of mycorrhization of following crops (Espindola *et al*., 1998), different studies in our conditions have shown that such a legacy effect might not be the most effective way to manage AMF and does not prevent crop responses to inoculation with efficient AMF isolates (Sánchez *et al*., 2009; Martín *et al*, 2012).

Jack bean (*Canavalia ensiformis* (L.) D.C.) is a leguminous cover crop with vigorous growth that establishes a mutual symbiosis with rhizobia and fix atmospheric nitrogen. It has a good dry mass production, and nutrient extraction capacity, and a leaves/shoots ratio favoring its decomposition (Garcia *et al*., 2000). The inoculation of a preceding green manure crop of *C. ensiformis* with *Glomus cubense/* INCAM-4, an AMF strain that is efficient on Rodic Ferralic Nitisol soils, has increased biomass production and nutrients extraction by this crop, and the multiplication of AMF propagules by *C. ensiformis* has positively impacted the following crop of corn (Martín and Rivera, 2015). Despite that green manure contribute nutrients to the main crop (Garcia *et al*., 2000; Ambrosano *et al*, 2013), AMF stimulate the mineralization of plant residues (Hodge *et al*., 2001), legumes fix nitrogen (Larimer et al., 2014; Bulgarelli *et al*., 2017) and organic material patches stimulate mycorrhizal hyphal proliferation (Hodge and Fitter, 2010), there is no published information on the integration of mycorrhizal inoculants and green manure in banana production. For all the above, our research evaluated the feasibility of integrating the management of mycorrhizal inoculants, green manure and organo-mineral fertilization in banana. We used a productive Calcic Haplic Phaeozems soils in order to guarantee banana high yields and an adequate nutritional status through an effective mycorrhizal functioning without applications of synthetic fertilizer and with lower quantities of organic mineral manure

MATERIALS AND METHODS

Ethics statement

The research conducted herein did not involve measurements with humans or animals. The study site is not considered a protected area. For location/activities, no specific permissions were required and the studies did not involve endangered or protected species. The seeds of *Canavalia ensiformis* used were obtained from a store of agricultural

products at Villa Clara, Cuba. The banana vitroplants was obtained in specialized factory at Santo Domingo, VillaClara, Cuba.

Study area and characteristics of the place

The experiment was conducted on a Calcic Haplic Phaeozems soils (WRB, 2014) in the experimental field of the Research Institute on Tropical Root Crops (INIVIT), Santo Domingo, Villa Clara province, Cuba, 22º 35' N, 80º18' W, 40 m.a.s.l., from April 2006 to October 2008. The attributes of the soil (Table 1) were typical of this soil type in particular a slightly basic pH and high contents of Ca and Mg (Ruiz *et al*., 2012; Simó *et al*., 2015). However, soil organic matter content was low, probably due to its history of intensive production of tropical roots, banana and plantain. The initial number of AMF spores was low (<1 in g of soil) and similar to that of other soils of this type at the same location and cultivated with similar crops and management (Ruiz *et al*., 2012; Espinosa et al., 2015, 2016), probably due to the high rates of mineral fertilizers (NPK) applied to the crops at this location in the last 25 years or more.

The annual rainfall and average temperature during the experimental period were generally representative of the climate in this region of Cuba. Year 2006 was dryer, with a total of 1093 mm of rain, and the other years were rainy with cumulative amounts of 1509 and 1698 mm, respectively, which is higher than the annual average of 25 years of 1349 mm. However, even in the least rainy year, about 80 % of the rainfall occurred in the May-October period. The average annual temperatures are very similar in different years, ranging between 24.2 and 24.4^{0}C and similar to the long-term average of 24.3^{0}C.

Table 1. Characteristics of the top 0-20 cm layer of the Calcic Haplic Phaeozems soil with their confidence interval (α = 0.05, n = 4)

pH		MO	P_2O_5	Ca	Mg	K	# of spores
KCl	H_2O	(%)	(mg100 g^{-1})		($cmol_c kg^{-1}$)		(spores in 50 g)
6.81	7.85	2.10	3.19	44.94	3.24	0.40	47.50
±0.32	±0.26	±0.28	±0.31	±2.89	±0.27	±0.21	±15.22

Analytical Techniques used: pH in H_2O and KCl 1 M, 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 with $(NH_4)_2CO_3$ solution with a concentration of 10 g L^{-1}, pH 9.0 and titration with HCl 0.05 M and methyl orange indicator. 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 the "Handbook of Analytical Techniques for soil and foliar analyses, organic manure and chemical fertilizers" (Paneque *et al*., 2010).

Table 2. Treatments applied in a randomized complete block design, on a Calcic Haplic Phaeozems of the Province of Villa Clara, Cuba.

Nu.	Previous crop	Banana (Main crop)
1	Bare soil	Control
2	Bare soil	AMF
3	C. ensiformis (Ce)	Ce
4	Ce + AMF	Ce + AMF
5	Ce + AMF	Ce + AMF + 25 % OMF
6	Ce + AMF	Ce + AMF + 50 % OMF
7	Ce + AMF	Ce + AMF + 75 % OMF
8	Bare soil	100 % OMF
9	Bare soil	75 % OMF + AMF
10	Bare soil	75 % OMF

Legend: 100 % OMF = 20 and 10 kg plant^{-1} cycle^{-1} of compost and ash, respectively (MINAG, 2011); 25 % OMF = 5 and 2.5 kg plant^{-1} cycle^{-1} compost and ash, respectively; 50 % OMF = 10 and 5 kg plant^{-1} cycle^{-1} compost and ash, respectively; 75 % OMF = 15 and 7.5 kg plant^{-1} cycle^{-1} compost and ash, respectively; Ce = *Canavalia ensiformis* as previous crop and intercrop respectively; AMF = *R. irregulare* / INCAM-11 in *C. ensiformis* inoculated at seeding so much in previous and intercrop and in banana plants inoculated in the nursery (acclimatization stage) and at the time of transplanting to the field.

Experimental design

The trial was done in two stages. The *C. ensiformis* (CE) green manure crop was first planted or the soil was left bare and secondly, banana was cropped in the same plots. A randomized complete block design with four replicates was used. The treatments of this trial are shown in Table 2.

Experimental management

Planting and management of previous C. ensiformis in banana plantation. *C. ensiformis* was planted on April 24th, 2006 in designated plots. There was 0.5 m between rows and 0.2 m between plants (100 000 plants ha^{-1}). Plots were 12 m x 12 m (144 m^2). Sixty days after planting (dap), the plants were chopped with a rotary mower and incorporated in to the soil with a disc plow. Non planted plots were kept weed free in this period by manual control of weeds.

Banana plantation. Banana was planted manually in all the plots 30 days after cutting the green manure crop. Plant spatial distribution was 4 m and 2 m between rows (double row) and 2.4 m between plants, equivalent to 1 388 plants ha^{-1}. Each experimental plot had an area of 144 m^2, and a total of 20 plants. Yield was calculated on 43.2 m^2 (i.e. six plants). Three productive cycles were evaluated in the experiments: plant crop (PC) in the period May – June 2007, first ratoon crop (FR) in the period November -December 2007 and second ratoon crop (SR) in the period May-June 2008. Banana set was made by a carrier and the best banana sword sucker, according to the methodology described by the Technical Guide for Banana Crop (MINAG, 2011). The irrigation was applied by spraying with a frequency of three monthly irrigations of 350 m^3 during the months of November to February and four monthly irrigations during the months of March to May, until the spring rains began. During the rainy season, was irrigated only in the absence of rain for more than 10 days.

Planting and management of intercropped C. ensiformis in banana plantation. The intercrop was seeded 30 days after transplanting (dat) banana plants. *Canavalia ensiformis* was planted in seven rows where tree spacing was 4 m and in three rows where tree spacing was 2 m (Figure 1). There was 0.5 m between rows of *C. ensiformis* and 0.2 m between plants (66 000 plants ha^{-1}). *Canavalia ensiformis* plants were always separated by 0.5 m from a banana rows. *Canavalia ensiformis* was cut 60 and 120 days after sowing with a slashing knife at the height of 12 to 15 cm from the soil. The cut material was mulched over the banana rows.

Inoculation with arbuscular mycorrhizal fungi. The strain *Rhizoglomus irregulare (*Sieverding *et al*., 2014*)*(INCAM-11, DAOM711363) was used as it is effective in Calcic Haplic Phaeozems (Rivera *et al.*, 2015). The formulation of the inoculant was a solid preparation in a mineral substrate (Fernández *et al*., 2000) containing 30 spores g^{-1} and undetermined quantities of mycelia and infective roots. Inoculation of *C. ensiformis* was done by seed coating with inoculant amounts equivalent to 8 % of seed weight (Martín *et al*., 2012). In banana, inoculation consisted in the application of 10 g $plant^{-1}$ of inoculant at the beginning of the acclimatization stage of the *in vitro* plants in nursery (Simo *et al.*, 2017) and of 20 g $plant^{-1}$ at the time of transplanting. The inoculant was placed at the bottom of the hole, always below the roots and in contact with them.

Nutrient supply in banana. The organo-mineral fertilizer (OMF) was a mixture of compost containing 19.5, 3.1 and 9.9 g kg^{-1} of N, P and K, respectively, and sugar-cane straw ash with average contents of 6.6 and 42.8 g kg^{-1} of P and K. The rate of 100 % OMF (MINAG, 2011) consisted in the application of 20 and 10 kg $plant^{-1}$ of compost and ash respectively, per growth cycle. This was equivalent to 191, 281 and 835 kg ha^{-1} of N, P_2O_5 and K_2O, respectively. In the PC cycle, the amount of fertilizing materials was applied at two times. Half was placed at the bottom of the row at planting, and half was placed in circles around the plant 90 days later. In the cycles FR and SR, fertilization was also split. Half the amount was placed in a half circle in front of the pseudo stem, when 80 – 90% of the plants of the previous cycle were harvested and the second application was made 60 days later. The management of the banana plantations followed the recommendation of the Technical Instruction Guidelines (MINAG, 2011)

Evaluations

Biomass and quantities of nutrients in C. ensiformis. The biomass production and nutrient content of *C. ensiformis* were determined in all cuts by harvesting the central 1 m^2 area of each plot. The fresh mass (FM) of the aerial tissue (g m^{-2}) was determined with a technical scale (±0.1 g) by weighing. Dry mass (DM) was determined after drying in a forced air drying oven at 70 ºC until constant mass. Biomasses were expressed in t ha^{-1} taking into account the size of the area harvested. Aerial tissue N, P, K concentrations (g kg^{-1}) were determined by doing a humid digestion with H_2SO_4 + Se and using the Nessler method for N, sulphonic-aminonaphtol for P and flame photometry for K, according to the "Manual of analytic techniques

for soil, foliar, organic manure and chemical fertilizers analysis" (Paneque et al., 2010). Plant extraction of N, P_2O_5 and K_2O (kg ha^{-1}) were calculated from the concentrations of N, P and K in the aerial dry tissue of the plant and dry mass.

AMF spores. The number of spores in 50 g^{-1} of soil, was determined seven times: at the beginning of the trials, when precedent *C. ensiformis* was incorporated, i.e. just before transplanting banana, and immediately after each intercropped *C. ensiformis* cut, as well as at flowering in each banana cycle. Soil samples were always taken at 0-20 cm depth. For initial counting of AMF spores, eight samples of soil from all the experimental area were taken. After experimental setup, each plot was sampled. In each cutting of *C. ensiformis*, used as previous or intercropped crop, soil samples composed of five subsamples/plot of rhizospheric soil were taken too. In banana the samples were done at the flowering stage in all phases of the growth cycles (i.e. PC, FR and SR), soil samples were taken at eight locations per plant (15 – 30 – 45 – 60 – 75 – 90 – 105 – 120 cm), along a spiral around the pseudostem of four plant as shown in Figure 1, so that the sample from each plot was made up of 32 subsamples. Spore extraction followed the protocol of Herrera *et al.* (1998) which is an adaptation of the initial protocol of Gerdemann and Nicholson (1963). Extracted spores were washed with distilled water and placed in Petri dishes. Spores were counted under a stereoscopic microscope at 70 x (Stemi 2000-C).

Total mycorrhizae colonization percentage. The percentage of mycorrhizal root colonization of previous crops and intercrops of *C. ensiformis* and of banana was determined on the finest roots, taken in each plot, at the time of biomass determination. Roots of *C. ensiformis* were collected in the 1 m^2 area used for sampling the aboveground plant biomass. Roots from four banana plants were taken from the soil sampling done at each cycle. Approximately 200 mg of roots per sample were used. Root samples were dried in a forced air drying oven at 70 ºC until constant mass, and stained as per the methodology of Rodriguez *et al* (2015). The evaluation of the percentage of root colonization was done on a stereoscopic microscope at 70x (Stemi 2000-C) using the line intercept method developed by Giovanetti and Mosse (1980).

Foliar concentrations of N, P and K in banana. Banana leaves for leaf tissue analysis was sampled at the flowering stage of each crop cycle. The third youngest leaf was chosen in six plants in each plot and a band of 10 cm was taken from each semilimb at the leaf limb center (Garcia *et al.*, 1979). The leaf samples from each plot were dried in forced air oven at 70 ºC, and ground. The concentrations of N, P and K (g kg^{-1}) in leaf tissue were determined after wet digestion (H_2SO_4 + Se), as described for *C. ensiformis* aerial tissue samples. The criteria for the interpretation of the foliar contents established by Garcia et al., (1979) were used to assess the nutritional status of banana plants.

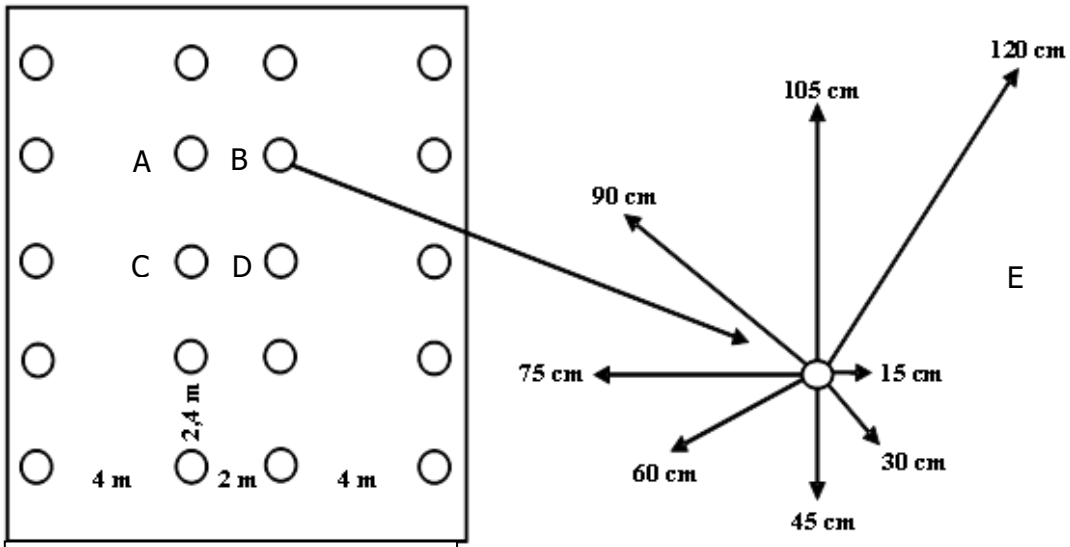

Figure 1. Graphical representation of the spatial distribution of banana plants in the plots (4.0 m x 2.0 m x 2.4 m) and the sampling strategy per plant used for the determination of spores abundance and mycorrhizal colonization in the top 0-20 cm soil layer. In each plot 4 plants in similar position as A, B, C and D were sampled at eight locations per plant (E).

Agricultural yield in banana (t ha^{-1}). Banana yield was determined by weighing the fruit clusters of six plants per plot at each growth cycle. The yield of each growth cycle and total yield was expressed as t ha^{-1}.

Statistical analysis

Assumptions of normality and homogeneity of variance were checked with the Kolmogorov-Smirnov and Levene tests on all data. The effect of AMF inoculation on *C. ensiformis* was assessed by student t test for each of the variables measured. Confidence intervals (1-α = 5 %) were used to analyze soil properties and spore abundance at the onset of the experiment. In the second stage of the experiment and in each banana production cycle, mixed model ANOVA with random effect assigned to block were done for each variable measured. The significance of differences between treatment means were assessed using Tukey's multiple comparison test following significant ANOVA. The relationships between leaf N and K concentrations and yield were analyzed by linear regression. The significance level used for rejecting the null hypothesis was set at 0.05 in all analyses. The Statistical Package SPSS was used for all statistical analysis.

RESULTS

Inoculation effect on *C. ensiformis* dry mass, associated fungal variables and nutrient extraction

Significant positive effects of inoculation with the efficient strain of *R. irregulare* were observed on dry mass production, root colonization, mycorrhizal spores production, and nutrient concentrations of *C. ensiformis*, at all planting stages (Table 3).

Increase in *C. ensiformis* biomass production ranged from 25 to 41 % in the different cuts. Inoculated previous *C. ensiformis* crop produced 6 t ha^{-1} of dry mass and total inoculated *C. ensiformis* (previous plus intercropped) reached 10 t ha^{-1}, a productivity well above that of non-inoculated *C. ensiformis*. An effect of inoculation was also recorded on the indicators of mycorrhizal functioning. The root colonization level of inoculated plants ranged from 60 to 68 %. That is four times higher than the level of colonization of non-inoculated *C. ensiformis*. Spores counts were nearly five times lower in non-inoculated than inoculated *C. ensiformis.*

The effects of inoculation with *R. irregulare*/INCAM-11 on the N, P and K concentrations of *C. ensiformis* tissue were less intense. Nitrogen and K showed significant effects ($p \leq 0.05$) in all cycles and their increase ranged from 9 to 11 % for K and 11 to 15 % for N. Phosphorus concentration was not influenced by inoculation.

Effects of inoculation with *R. irregulare*/INCAM-11, *C. ensiformis* and application of organo-mineral sources (OMF) on the yield of banana

Banana plants significantly responded to organo-mineral fertilization (OMF) in all cycles and produced high yields with 100 % OMF (Figure 2). The application of *R. irregulare* in the absence of complementary OMF rates did not produce positive effects on banana yield, however, in the presence of 75 % OMF, it reached similar yields to those achieved with 100 % OMF. Furthermore, the previous incorporation and mulching with inoculated *C. ensiformis* allowed a greater reduction of OMF requirements for similar yields. A complement of only 25 % of the OMF recommended was required in the PC and FR cycles. The OMF requirements for plots receiving inoculated *C. ensiformis* increased to 50 % in the last cycle evaluated (SR). The treatments that received *C. ensiformis* without OFM (CE and CE+AMF) had similarly low yields that were only slightly higher than the control and the AMF treatment.

Effect of inoculation with *R. irregulare/* INCAM-11, *C. ensiformis* and application of organo-mineral sources (OM) on the banana´s fungal variables

The percentages of mycorrhizal colonization of banana root and spore densities in plantation soil were significantly higher ($p \leq 0.05$) in inoculated than uninoculated plots (Table 4). The inoculated banana receiving complementary OMF rates, reached the highest mycorrhizal colonization percentages, around 60 %, in the PC and FR cycles. In the cycle SR values ranging from 51 to 57 % were found in inoculated treatments receiving OMF. The treatment with inoculated *C. ensiformis* receiving 25 % of the OMF in cycle SR, exhibited a colonization percentage significantly lower than the rest of inoculated treatments that received OMF.

The highest spore counts were obtained with inoculated *C. ensiformis* and OMF application. Spore counts in these treatments ranged from 900 to 1500 spores in 50 g of soil, depending on the cycle. Similar to observations made on banana yield and colonization percentage, the combined use of inoculated *C. ensiformis* and 25% OMF was less effective in the SR cycle than in PC and FR, although it produced higher spore counts than treatments excluding inoculated *C. ensiformis.* In SR, the highest spore counts were achieved using more than 25% OMF in combination with inoculated *C. ensiformis.*

Table 3. Effect of inoculation with *R. irregulare*/INCAM-11 on the dry mass (DM) production (t ha^{-1}) and N, P and K concentration (g kg^{-1}) of the aerial part of *C. ensiformis*, root colonization and spore count.

	DM	N	P	K	Root colonization	AMF spore count
	(t ha^{-1})		(g kg^{-1})		(%)	(spores in 50 g of soil)
Previous crop						
Ce + AMF	6.11 a	27.93 a	2.27	21.80 a	67.88 a	536. a
Ce	4.57 b	25.27 b	1.99	19.98 b	16.25 b	106 b
Es $\bar{x}$	0.24 **	0.42**	0.10 ns	0.12**	0.93 **	20 **
Intercropped (harvest 1)						
Ce + AMF	2.34 a	25.42 a	2.28	18.47 a	63.38 a	385 a
Ce	1.87 b	22.79 b	2.05	16.79 b	15.25 b	89 b
Es $\bar{x}$	0.12**	0.38**	0.12 ns	0.30**	1.10 **	18**
Intercropped (harvest 2)						
Ce + AMF	2.02 a	23.95 a	2.21	17.15 a	60.63 a	313 a
Ce	1.43 b	20.80 b	1.93	15.47 b	13.50 b	70 b
Es $\bar{x}$	0.11**	0.39**	0.14 ns	0,32**	1.25**	19 **

Ce + AMF = *C. ensiformis* inoculated with *R. irregulare/INCAM-11*, Ce = non-inoculated *C. ensiformis*. Different letters in a column indicate significant differences ($p \leq 0.05$) among treatment means according student t test.

In absence of *C. ensiformis*, spore counts were lower ($p < 0.05$), and the inoculation treatment that received 75 % of OMF without *C. ensiformis* produced lower spore counts ($p<0.05$) than inoculation treatments with *C. ensiformis* and OMF, however all these treatments (except *C. ensiformis* with 25 % OMF in SR), produced similar responses on yields and colonization percentages. In general, the lowest spore counts were found in the PC cycle and the highest ones in the cycle FR.

Effect of inoculation with *R. irregulare*/INCAM-11, *C. ensiformis* and application of organo-mineral fertilizer (OMF) on foliar nutrient concentrations in banana

Significant differences were found among treatments for N and K contents, in all three banana production cycles (Table 5). The highest N concentrations were recorded in the control and only AMF treatments, which were the lowest yielding treatments (Figure 2). These N concentrations were significantly higher ($p \leq 0.05$) than those found in the treatments combining *C. ensiformis*, AMF, and OMF, or the 100 % OMF treatments, which were the highest yielding treatments. There were no treatment effects on banana leaf P concentration in any of the three growth cycles monitored.

A marked effect on the foliar K concentration was found in all three cycles (Table 5). The highest K concentrations were seen in the treatments combining *C. ensiformis*, AMF, and OMF, or the 100 % OMF treatments. However, it is noteworthy that the 25 % OMF treatments in inoculated *C. ensiformis* was again less effective in increasing banana leaf K in the last production cycle. A positive relationship ($R^2 = 0.88$**) was found between leaf K concentration and yield in the three harvest cycles (Figure 3), so the treatments with more yield ever presented leaf K concentrations associated with satisfactory levels (33 – 37 g kg^{-1}), and lower yields were related with leaf K deficient levels (< 33 g kg^{-1}). Unlike potassium, leaf nitrogen level was highly negatively related with yield in each cycle, with R^2 ranging 0.73** to 0.93** (not shown data). In general, the banana production cycle did not influence the concentrations of the different elements in banana.

DISCUSSION

The positive effect of inoculation with *R. irregulare*/INCAM-11 on the biomass, mycorrhizal functioning and nutrient content of *C. ensiformis*, can be explained by the establishment of an effective mycorrhizal symbiosis enhancing nutrient uptake (Yang *et al*., 2014; Van der Heidjen *et al*., 2015; Berruti *et al.*, 2016) with impact on plant biomass production. Such effect has been reported by other authors using efficient AMF strains in different crops as tropical roots, vegetables, pastures, banana and others crops (Rivera *et al*., 2007, Ruiz *et al*., 2012, González *et al*., 2015; Ruiz *et al.*, 2016) and also in *C. ensiformis* (Garcia *et al*., 2017 b; Martín *et al*., 2015). In legume, positive effects of inoculation with AMF were also reported on biological nitrogen fixation (Larimer *et al*., 2014; Bulgarelli *et al*., 2017).

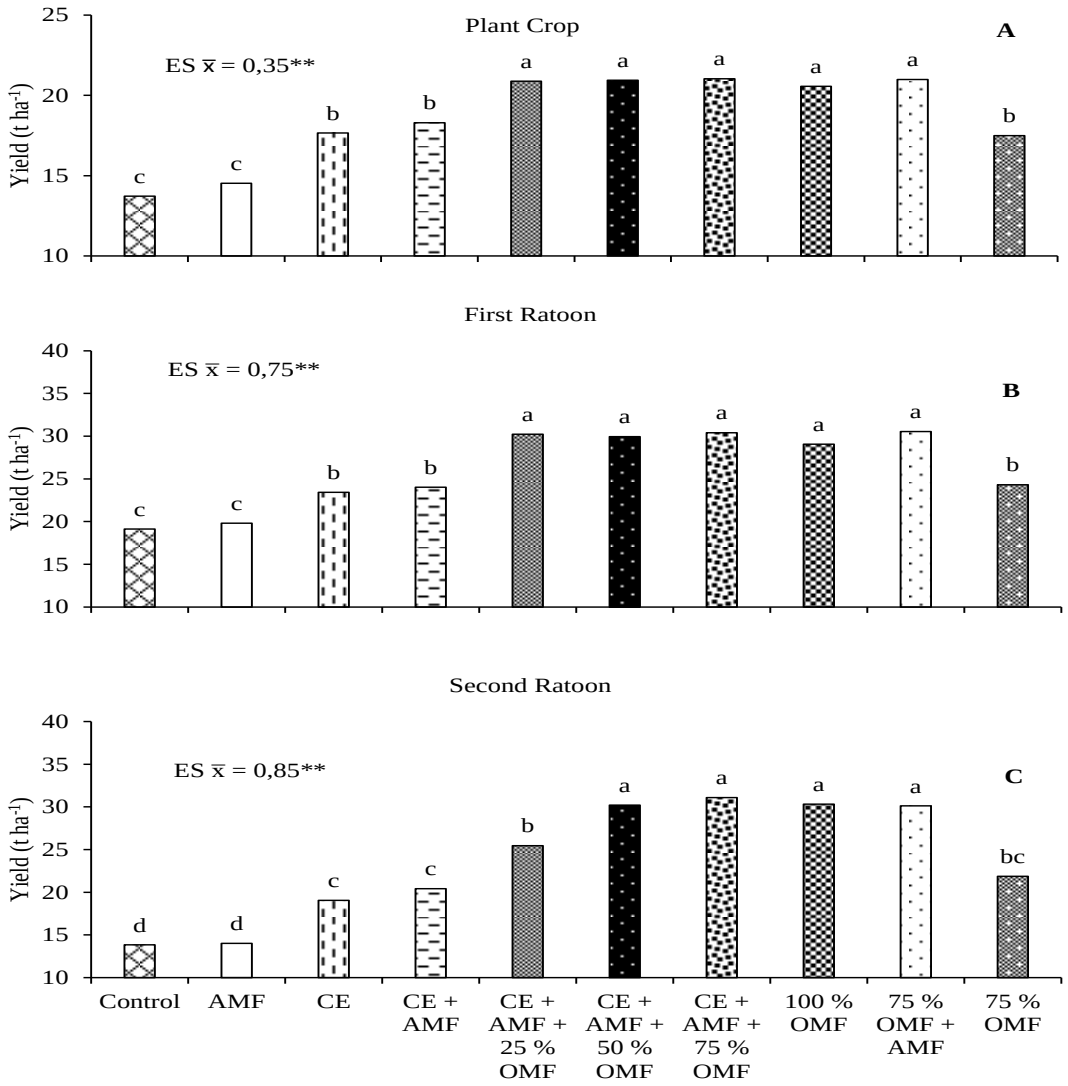

Figure 2. Effect of inoculation with *R. irregulare*/INCAM-11, green manure, and rates of compost and ash in each production cycle on the yield of banana cv. 'FHIA-18' on Calcaric Haplic Phaeozem. Different letters in a cycle indicate significant differences ($p \leq 0,05$) according to Tukey's Test. (Legend: Ce = *C. ensiformis* precedent and intercropped; AMF = inoculated *C. ensiformis* and inoculated banana plants at acclimatization and transplantation to the field; 100 % OMF = 20 and 10 kg plant^{-1} cycle^{-1} of compost and ash; 25 % OMF = 5 and 2,5 kg plant^{-1} cycle^{-1} of compost and ash; 50 % OMF = 10 and 5 kg plant^{-1} cycle^{-1} of compost and ash; 75 % OMF = 15 and 7,5 kg plant^{-1} cycle^{-1} of compost and ash).

In addition to the large biomass production and nutrient extraction by *C. ensiformis*, inoculation with *R. irregulare*/INCAM-11 in a Calcic Haplic Phaeozems led to a large increase in AMF spore production, presumably due the sporulation of the inoculated strain. These results are similar to those found when *C. ensiformis* was inoculated with *Glomus cubense*/INCAM-4, on a Rodic Ferralic Nitisol soil, and with *Funneliformis mosseae*/INCAM-2 on Albic Pisoplintic Plinthosol (Martín *et al.*, 2015). All this indicates that AMF management with inoculants can be successful under different edaphic conditions provided *C. ensiformis* is inoculated with the efficient AMF strain recommended for the edaphic conditions (Rivera *et al*., 2015).

Canavalia ensiformis increased slightly the sporulation of resident AMF, but this increase was insufficient for efficient formation mycorrhizas in banana, as indicated by the large difference between the root colonization percentages of inoculated and non-inoculated banana in the *C. ensiformis* treatments. The need for inoculation could be related to an initial amount of resident propagules that was too low to be

useful. Similar results have been reported with *C. ensiformis* in different types of soils and crops (Martín et al., 2012; Rivera et al., 2010; Sánchez et al., 2009) confirming the need to inoculate *C. ensiformis* or the main crop with efficient AMF strains to insure an efficient mycorrhization of the main crop.

Table 4. Effect of inoculation with *R. irregulare*/INCAM-11, green manure, and rates of compost and ash in each banana production cycle on the percentage of mycorrhizal root colonization and the number of spores of HMA at 0-20 cm depth in Calcic Haplic Phaeozem.

Treatment	Root AMF colonization			AMF spores		
	PC	FR	SR	PC	FR	SR
	(%)			(spores in 50 g of soil)		
Control	13.50 c	16.50 c	9.50 c	48 d	38 e	31 d
AMF	51.75 b	54.50 b	49.25 b	162 d	229 d	99 d
Ce	13.25 c	14.50 c	11.00 c	115 d	108 de	74 d
Ce + AMF	55.00 b	57.25 b	50.00 b	830 b	1229 b	499 c
Ce + AMF+25 % OMF	60.00 a	61.75 a	51.00 b	894 ab	1369 ab	810 b
Ce + AMF+ 50 % OMF	60.00 a	61.75 a	56.25 a	991 ab	1494 a	1145 a
Ce + AMF+ 75 % OMF	60.00 a	61.50 a	56.50 a	1071 a	1527 a	1256 a
100 % OMF	13.75 c	15.75 c	11.50 c	66 d	60 de	58 d
75 % OMF + AMF	61.25 a	63.25 a	57.50 a	368 c	424 c	333 c
75 % OMF	12.75 c	15.25 c	10.50 c	54 d	51 e	47 d
Es $\bar{x}$	0.66**	0.76**	0.58**	45 **	44 **	42 **

-** ANOVA is significant at p<0,01. Different letters in a column indicate significant differences ($p \leq 0,05$) among treatment means according to Tukey's test.

PC = plant crop; FR = first ratoon crop and SR= second ratoon crop; Ce = precedent and intercropped *C. ensiformis* ; AMF = inoculated *C. ensiformis* (precedent and intercropped) and/ or inoculated banana plants at acclimatization and transplantation to the field; 100 % OMF = 20 and 10 kg plant^{-1} cycle^{-1} of compost and ash; 25 % OMF = 5 and 2.5 kg plant^{-1} cycle^{-1} of compost and ash; 50 % OMF = 10 and 5 kg plant^{-1} cycle^{-1} of compost and ash; 75 % OMF = 15 and 7.5 kg plant^{-1} cycle^{-1} of compost and ash. Es $\bar{x}$: standard error of means.

Table 5. Effect of inoculation with *R. irregulare*/INCAM-11, green manure, and rates of compost and ash in each banana production cycle on the foliar concentration of N, P, K (g kg^{-1}) of banana cv. 'FHIA-18' at the flowering stage, in Calcic Haplic Phaeozem.

Treatments	PC			FR			SR		
	N	P	K	N	P	K	N	P	K
	(g kg^{-1})								
Control	32.3 ab	2.1	22.9 c	30.5 a	2.2	24.1 d	29.3 a	2.1	22.6 c
AMF	32.9 a	2.0	22.1 c	30.0 a	2.2	24.6 d	28.9 a	2.0	22.0 c
Ce	29.0 cd	1.9	28.9 b	26.6 bc	2.1	29.3 bc	27.4 ab	2.2	26.9 b
Ce + AMF	30.1 abc	2.1	28.1 b	27.9 ab	2.1	29.0 c	26.1 bc	2.2	27.0 b
Ce+AMF + 25 % OMF	25.1 e	2.1	33.3 a	26.1 bc	2.2	34.6 a	25.6 bc	2.1	30.4 b
Ce+AMF + 50 % OMF	26.2 de	2.0	34.0 a	24.8 bc	2.2	35.6 a	24.2 c	2.2	36.3 a
Ce+AMF + 75 % OMF	25.7 e	2.1	34.8 a	24.4 c	2.1	35.8 a	23.8 c	2.2	36.5 a
100 % OMF	27.0 de	2.0	33.6 a	25.3 bc	2.1	35,1 a	24.3 c	2.1	34.8 a
75 % OMF+ AMF	26.3 de	2.0	33.5 a	25.3 bc	2.2	34.8 a	23.9 c	2.1	35.3 a
75 % OMF	29.4 bc	2.0	27.9 b	27.5 bc	2.1	30.6 b	26.4 bc	2.0	29.0 b
Es $\bar{x}$	0.62**	0.08 ns	0.72**	0.64*	0.10 ns	0.34**	0.64**	0.11 ns	0.82**

** ANOVA is significant at p<0.01. Different letters in a column indicate significant differences ($p \leq 0,05$) among treatment means according to Tukey's test. ns: non-significant

PC = plant crop; FR = first ratoon crop and SR= second ratoon crop; Ce = precedent and intercropped *C. ensiformis* ; AMF = inoculated *C. ensiformis* (precedent and intercropped) and / or inoculated banana plants at acclimatization and transplantation to the field; 100 % OMF = 20 and 10 kg plant^{-1} cycle^{-1} of compost and ash; 25 % OMF = 5 and 2.5 kg plant^{-1} cycle^{-1} of compost and ash; 50 % OMF = 10 and 5 kg plant^{-1} cycle^{-1} of compost and ash; 75 % OMF = 15 and 7.5 kg plant^{-1} cycle^{-1} of compost and ash. Es $\bar{x}$: standard error of means.

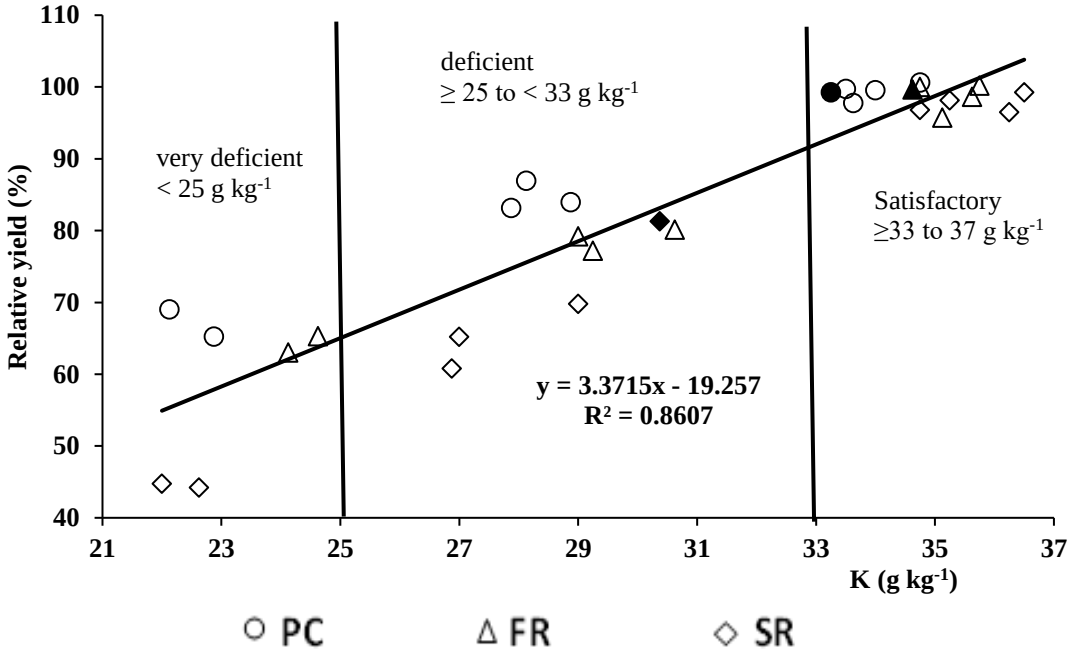

Figure 3. Relationship between the concentration of K in banana leaf and relative yield (%) of banana in the cycles (PC) plant crop ; (FR) first ratoon crop and (SR) second ratoon crop of banana cv. 'FHIA-18' and the associated nutritional status (Garcia et al., 1979). The relative yield (%) was calculated for each treatment in relation with the maximum yield obtained in each cycle. Black dotted symbols represent the treatment Ce + AMF + 25 % OMF, observe the fall of K concentration in this treatment in second ratoon crop.

FHIA – 18 banana requires large amounts of fertilizers for adequate nutrition and to achieve high yields in these soils (Ruiz *et al*., 2016). It has been shown that mineral and organo-mineral fertilizers can be similarly effective nutrient sources in banana FHIA – 18, while inoculation with efficient AMF strain can reduce the requirements of the crop to 75 % of the current recommended rate of organo – mineral fertilizer (Simó *et al*., 2015) or to 50 – 66 % of mineral fertilizer recommendation (Ruiz *et al.*, 2016) due to the formation of mycorrhiza taking up the nutrient highly efficiently. Integrating the use of green manure and AMF inoculants was successful in reducing further the OMF requirements of banana. In this case, the recycling of the nutrients contained in inoculated *C. ensiformis* and the efficient mycorrhiza function of banana, reduced the compost and ash requirements of the crop to 25% of the OMF recommendation (MINAG, 2011) in the first two production cycles, and to 50 % of the recommendation in the third production cycle of the banana crop.

The additional reduction of fertilizer rates provided by using inoculated *C. ensiformis* green manure crop, can be attributable not only to the considerable contribution of N and K of *C. ensiformis* residues, especially after inoculation, and to the positive effects of green manure on soil biological activity (Soti *et al*., 2016), but also to soil properties enhancing the function and effects of the mycorrhiza introduced in the crop (Dodd and Ruiz - Lozano, 2012). Moreover, some reports (Hodge and Storer, 2015) have related the positive effect of mycorrhiza growing in organic patches through a stimulation of the process of plant residues mineralization, and also favour hyphal development (Bukovská *et al*., 2018; Hodge and Fitter, 2010) .

The percentages of mycorrhizal colonization found in inoculated fertilized plots were close to 60 % and associated with the highest yields. This shows the high potential of mycorrhizal inoculation that can be achieved with nutrients supply allowing effective mycorrhizal functioning (Rivera *et al*., 2007). The lower nutrient supply rate in inoculated treatments deprived of organic-mineral fertilizers, led to reduce mycorrhizal functioning, faulty nutritional status and lower yields of banana.

The similarity in the effects of combining 75 % OMF and inoculation, and of combining *C. ensiformis*, mycorrhizal inoculation and OMF on root colonization, yields and nutritional status, indicated an effective mycorrhizal functioning of the introduced AMF strain all through the three cycles of production, although the inoculation was done at beginning of the

first cycle. The persistence of the initial inoculation of the efficient strain AMF through three banana production cycles may be attributable to the coexistence of first ratoon crop root growth with the active development and satisfactory mycorrhizal functioning of the plant crop cycle and in a similar way the second ratoon crop root growth with the satisfactory mycorrhizal functioning of the first ratoon cycle, ever in presence of appropriate cultural practices and irrigation. So, a satisfactory mycorrhizal functioning was carried over between the cycles.

The production of AMF spores in the rhizosphere of banana responded very differently in the treatments combining inoculation of *C. ensiformis* and OMF rates, and in other treatments without inoculated *C. ensiformis*. The observations of treatments leading to similarly high yields, optimum root colonization percentages and nutritional contents (i.e., 75% OMF and banana inoculation, and of combining *C. ensiformis*, mycorrhizal inoculation and OMF) but with very different AMF spores abundance triggers questions. Is mycorrhizal functioning similar under these treatments? Is high spores abundance in the treatments with *C. ensiformis* inoculation indicative of a large extraradical AMF functional structures, necessaries for the quick capture of the nutrients released by the green manure?

Cheng *et al*., (2016) found that soil organic patches trigger root and mycorrhizal fungal proliferation. Hodge and Fitter, (2010), Thirkell *et al.* (2016) and Bukovská *et al.* (2018) also reported increments in hyphal growth, increase in the activity of microorganisms that break down this organic material (Hodge *et al* 2001; Hodge and Stokes, 2015), and enhanced total plant N and other nutrients and a substantial increase in plant biomass with AMF inoculation in presence of fertilization with organic matter. The combination of inoculated green manures and OMF could have stimulated the proliferation of mycorrhized roots and extraradical hyphae to achieve a satisfactory banana nutrient uptakes, keeping the colonization percentage similar. The production of spores in addition to being a mechanism of competition of the fungus, is also a consequence of an effective mycorrhizal functioning (Willys et al., 2013) and to the extent that when there is greater proliferation of hyphae, there is greater production of spores (Quilliam et al ., 2010). Higher spore counts would result from such mechanism and suggest that in fact the treatments with inoculated canavalia and OMF showed a greater mycorrhizal functioning, although in this case the colonization percentages do not show these differences.

Leaf analysis results confirmed the importance of potassium for high yields in banana (García *et al.*, 1979; Ruiz *et al*., 2016), and leaf analysis appears as meaningful tool to evaluate not only the fertilizer supply, but also the OMF supply for inoculated banana. Leaf K concentrations associated with different nutritional status and yield levels were similar to those previously found in the country (Garcia *et al.*, 1979). Therefore, the significant reduction in the K concentrations of banana leaves in plots with inoculated *C. ensiformis* and 25 % of recommended OMF rate, from a satisfactory 35 g kg^{-1} in the PC and FR cycles to a deficient 30 g kg^{-1} in cycle SR, seem to explain why this treatment led to reduced yield in the last cycle of banana production and indicated that the potassium supply is was insufficient at this fertilization rate in cycle SR.

The potassium and nitrogen status of banana responded differently. The highest N concentrations were associated with lower yields and vice versa, which suggests the existence of a possible dilution effect in high yielding treatments. Thus, N was seemingly not the element limiting yield in this conditions. Similarly, high leaf N concentrations were reported in banana plantations with K deficiency (Rodríguez, 1980). The absence of treatment effect on leaf P concentration concurs with reports (Castillo *et al*., 2011; Hoffmann *et al*., 2010) of little or no response of banana to phosphorus fertilization.

Inoculation with *R. irregulare*/INCAM-11 in the presence of mean rates of organo-mineral fertilizers insured adequate foliar potassium levels, similar to those reached with higher rates of fertilizers in the absence of inoculation, which suggests a positive effect of inoculation on the uptake of potassium. Similar effects of increased potassium concentration in response to the inoculation with efficient AMF strains have been found in different crops including maize, tropical roots, coffee and *Uroclhoa* species (Liu *et al.*, 2002; González *et al.*, 2015; Rivera *et al*., 2007). The direct involvement of AMF in K uptake was recently reviewed (Garcia and Zimmermann, 2014; Berruti *et al*., 2016). Plant potassium nutrition is improved by AMF, especially under potassium limiting conditions.

CONCLUSIONS

Results showed the feasibility and benefits of integrating the effective management of efficient AMF strains according to edaphic conditions, green manure, and fertilization with organo-mineral amendments in the production of banana cv. 'FHIA-18' on Calcic Haplic Phaeozems soils. Combining these strategies led to high yield production, fertilizer efficiency, percentages of mycorrhizal colonization, AMF spore production, and adequate plant nutrient contents, and allowed to reduce by at least 50% the requirements for organo-mineral fertilizers of the banana crops.

The *R. irregulare* strain inoculated on the crops kept its effectiveness throughout the three banana production cycles monitored. Inoculation of the previous crop and intercropped of *C. ensiformis* with *R. irregulare*/INCAM-11 not only increased green manure biomass and the quantities of recycled nutrients, but was also an adequate method to increase the numbers of mycorrhizal propagules in the soil of the banana plantation.

Foliar concentration of potassium was directly related with yield and mycorrhizal functioning. Leaf analysis should be integrated in the effective management of mycorrhizal inoculants.

Inoculation of green manure crops with effective AMF strains increased the benefits of both practices and is a promising strategy for the management of arbuscular mycorrhizal symbioses in sustainable crop production.

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