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# INTEGRATED NUTRITION SYSTEM WITH AMF, GREEN MANURE AND MINERAL FERTILIZER IN *Manihot esculenta* Crantz

## Sistema integral de nutrición con HMA, abonos verdes y fertilizantes minerales en *Manihot esculenta Crantz*

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Abstract. In recent years the positive results of mycorrhizal inoculant application in different crops and their integration with the mineral fertilization schemes have increased. In order to establish an integrated plant nutrition system for the cultivation of Manihot esculenta Crantz, including also green manure and low doses of mineral fertilizer, two field experiments were carried out on Ferralitic Red Lixiviated Soils. In the first one, five levels of mineral fertilizer were studied, in the presence or not of the inoculation with the arbuscular mycorrhizal fungus Glomus cubense/ INCAM-4, in a random block design with factorial arrangement and four replicates. In the second, Canavalia ensiformis was introduced as a green manure, preceded and intercalated, and 12 treatments were studied, consisting of mycorrhizal inoculation or not of canavalia and cassava in the presence of a fixed fertilization fund of NPK corresponding to 25 % of fertilization recommended for cassava. Treatments with four doses of fertilizers (0, 25, 50 and 100 % NPK) were also included in a randomized block design with four replicates. The inoculation decreased the fertilizer quantities by 33 % in order to obtain high yields and increased its agronomic efficiency by 67 %, with the crop showing a good mycorrhizal function. The inclusion of the previous and intercalated inoculated canavalia decreased in another 33 % plus the amounts of mineral fertilizer, with a positive response to the intercalation in the yield (P <0,05 %) and doubling the agronomic efficiency of fertilizers.

*Key words*: *Canavalia ensiformis,* fertilization efficiency, red ferralitic, AMF, yucca

**RESUMEN**. En los últimos años se incrementan los resultados positivos de la aplicación de los inoculantes micorrízicos en diferentes cultivos y su integración con los esquemas de fertilización mineral. Con el objetivo de establecer un sistema integral de sistema integral de suministro de nutrientes para el cultivo de *Manihot esculenta* Crantz, incluyendo además abonos verdes y bajas dosis de fertilizantes minerales, se ejecutaron dos experimentos de campo en suelos Ferralíticos Rojos Lixiviados. En el primero se estudiaron cinco niveles de fertilizantes minerales, en presencia o no de la inoculación con la cepa de hongo micorrízico arbuscular *Glomus cubense*/ INCAM-4, en un diseño de bloques al azar con arreglo factorial y cuatro réplicas. En el segundo se incorporó la *Canavalia ensiformis* como abono verde precedente e intercalado y se estudiaron 12 tratamientos conformados por la inoculación micorrízica o no, de la canavalia y de la yuca en presencia de un fondo fijo de fertilización de NPK correspondiente al 25 % de la fertilización recomendada para el cultivo. Se incluyeron además tratamientos con cuatro dosis de fertilizantes (0, 25, 50 y 100 % NPK), en un diseño de bloques al azar con cuatro réplicas. La inoculación disminuyó en 33 % las cantidades de fertilizantes para obtener altos rendimientos e incrementó en 67 % la eficiencia agronómica de este, presentando el cultivo un buen funcionamiento micorrízico. La inclusión de la canavalia inoculada precedente e intercalada disminuyó en otro 33 % más las cantidades de fertilizantes minerales, con una respuesta positiva al intercalamiento en el rendimiento (P<0,05 %) y duplicando la eficiencia agronómica de los fertilizantes.

*Palabras clave*: *Canavalia ensiformis*, eficiencia de la fertilización, ferralítico rojo, HMA, yuca

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## INTRODUCTION

Cassava is the fourth most important food after rice, wheat and corn, forming part of the diet of more than 1 billion people (1) and in 2015, 270 tons of cassava were freshly produced on a fresh basis which approximately 65 % were used for human food (2) and the rest for animal feed and as an efficient source of ethanol (3).

Although it is a rustic crop that can also be cultivated under conditions of acid and infertile soils to guarantee high yields, in the order of 40 to 60 t $ha^{-1}$ $yr^{-1}$, considerable quantities of fertilizers or other sources of nutrients are required (3). In Cuba, doses of 140 kg $ha^{-1}$ of N, 50 to 60 kg $ha^{-1}$ of $P_2O_5$ and 160 to 200 kg ha-1 of $K_2O$ are recommended for yields of 35 t $ha^{-1}$, depending on the type of soil (4); although, due to the country's financial capacity, only 20 % of the planted area is being fertilized, contributing to the low average yields obtained of 6,34 t $ha^{-1}$ (5) and to the gradual decrease in soil fertility .

This crop has a high dependence on mycorrhization (6) and at the end of the last century, several investigations began in the country to integrate the benefits of an effective mycorrhizal functioning, associated with increases in the absorption of nutrients (7-8), within the schemes of fertilizer supply to crops (9,10). Results are available for this crop in soft carbonate Brown soils (11), but in the case of Red Ferralitic soils, although there is a recommendation that the arbuscular mycorrhizal fungus (AMF) strain be used (12), there are no published results the quantities of fertilizers required for inoculated cassava to ensure high yields and satisfactory mycorrhizal functioning.

In parallel, work has been carried out on other crops, using Canavalia ensiformis inoculated with efficient AMF strains, as a previous green manure, to enhance not only the production of biomass and recycling of nutrients for the subsequent economic crop nutrition, but also as a via to mycorrhize this (13,14). In the cultivation of cassava, although there is information on the satisfactory use of green manure species as precedents (3), there is no published information that integrates the management of these green manures with mycorrhizal inoculation and its relationship with the supply schemes of nutrients for this crop.

For all the above, this work is developed with the objectives of evaluating the feasibility and benefits of the use of *C. ensiformis*, both precedent and intercalated, and mycorrhizal inoculants in the schemes of nutrient supply in the cassava crop, to reduce the amounts of fertilizers and make more efficient use of these, guarantee high yields and lay the foundations for the implementation of new sustainable technologies for cultivation.

## MATERIALS AND METHODS

The experiments were carried out in Ferralitic Red Leached soil (15), also classified as ferritic, rhodic, lithic and eutric Nitisol (16), in the areas of the National Institute of Agricultural Sciences (INCA), located in San José de las Lajas, province Mayabeque, at 23o 01 'North latitude and 82° 08' West longitude and 120 m a,s.l The soil presented a neutral pH, with high Ca contents and interchangeable Mg media, of the order of 11,9 and 2,6 cmolc $kg^{-1}$ respectively, which are typical for these soils (Table I). The content of organic matter, although it was medium, indicated a good state of conservation for a leached Ferralitic Red soil (17) with continuous culture (Table II). The available phosphorus content was high and related to continued previous applications of mineral fertilizers. The resident mycorrhizal spores were low and possibly associated with previous applications of fertilizers and the high availability of phosphorus in the soil.

**Table I. Some chemical characteristics of the soils at the beginning of the experiments (*) and number of resident AMF spores (0-20 cm depth)**

| pH $H_2O$ | MO g $kg^{-1}$ | P (mg $kg^{-1}$) | $Na^+$ | $K^+$ | $Ca^{2+}$ | $Mg^+$ | Quantity Spores 50 $g^{-1}$ soil |
|---|---|---|---|---|---|---|---|
| | | | (cmol$_c$ $kg^{-1}$) | | | | |
| 6,6 | 32,0 | 341,2 | 0,12 | 0,25 | 11,9 | 2,4 | 70 |

*Average values of six samples

### Weather conditions

The experimental years were characterized by annual rainfall higher than the average of the last 25 years (Figure 1), with records of the order of 1700 mm per year in both years and with monthly averages in the rainy period between 218 and 250 mm, which they are suitable for the cassava cultivation (18); nevertheless, the year 2012 presented very low values of rainfall in November and December and that continued to be very low in January and February 2013 (Figure 1). The temperature regime was relatively similar in the two years and with values close to the annual historical average of 24,2 °C, being representative of this zone located in the western region of Cuba.

### Conducted experiments

Two experiments were conducted. The first one studied the response of *Manihot esculenta* Crantz to the inoculation of the *Glomus cubense* / INCAM-4 strain (19), an efficient strain for this edaphic condition (12), combined with different doses of NPK fertilizer in a random block design with a factorial arrangement of 2x5, with four replicas.

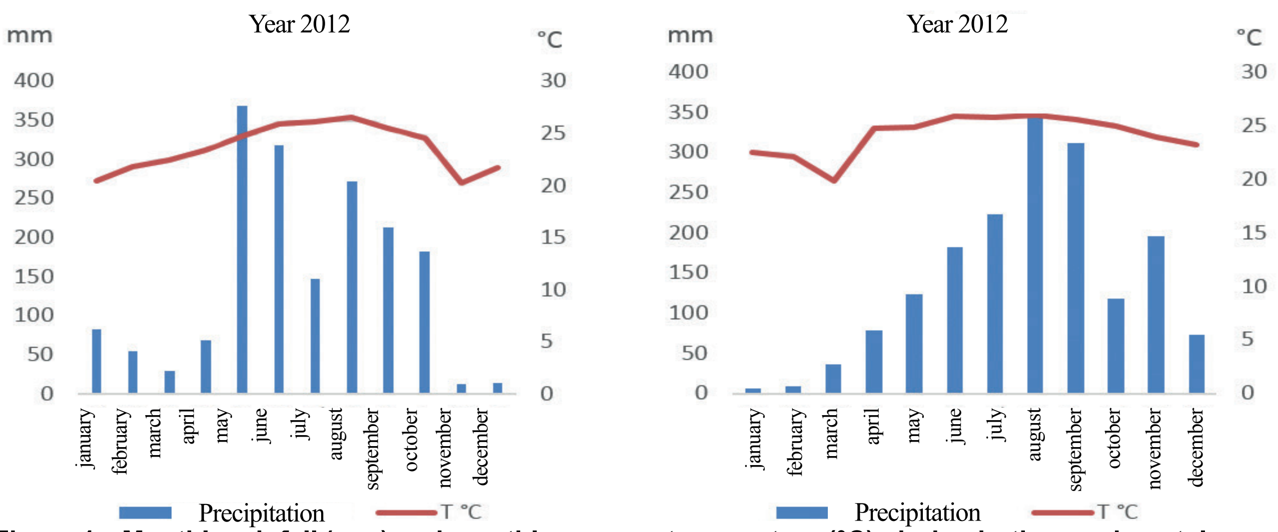



**Figure 1. Monthly rainfall (mm) and monthly average temperature (°C), during both experimental years. Data coming from Station # 78374 of the Institute of Meteorology of Cuba, located 1 km from the experimental area**

The factor inoculation had two levels, with and without. The levels of the factor fertilization were: 0, 25, 50, 75 and 100 % of the NPK dose recommended by the Technical Instructions of the crop (4). The clone used was CMC-40, with a planting frame of 0,90 x 1,2 m. The plots had 32 plants, of which 12 were evaluated. The inoculation of the cassava was done by coating the tip of the cuttings (20), with an amount of 13 kg ha$^{-1}$ of inoculant. The experiment was planted on February 18$^{th}$, 2012 and the harvest was carried out 10 months after planting.

In the second experiment, the response of *M. esculenta*, clone CMC-40, to different treatments formed by the use of *C. ensiformis* as a preceding green intercropped fertilizer and the inoculation with the *G. cubense* / INCAM-4 strain were studied of the canavalia as of the cassava and always in the presence of a fixed fund of 25 % NPK. Four treatments were included, which consisted in the application of 0, 25, 50 and 100 % of the NPK fertilization dose recommended for high yields (4), for a total of 12 treatments, in a randomized block design with four replications.

The experiment was developed in two stages; the first one corresponded to the sowing of the previous green manure and the second one to the planting of the cassava in presence or not of the intercalation of the canavalia. The treatments studied are presented in Table II. The plots were 3,6 meters wide and 11 meters long.

The previous canavalia was planted on September 10$^{th}$, 2012, with a planting frame of 20 cm between plants and 45 cm between streets. The inoculation of the canavalia was carried out by coating the seeds and applying 10 kg ha$^{-1}$ of inoculant (13).

At 65 days of sowing (das) a first cut was given at 15 cm in height, the second at 125 (das) and it was incorporated into the soil up to 15 cm deep with a disk plow. The canavalia was not watered or fertilized. The treatments that did not include the previous canavalia were left in fallow (Table II) this was eliminated on January 15 and proceeded in a similar way to how the canavalia was incorporated.

The clone of cassava CMC-40 was planted in the same plots on February 5$^{th}$, 2013, according to the treatments studied (Table II), with a planting frame of 0,9 x 1,1 m, using 14 plants of calculation. The inoculation of cassava was done in a similar way to that carried out in the first experiment. 45 days after the cassava was planted, an earthing up (4) was made, and immediately afterwards and according to the treatments, a double furrow of canavalia was inserted in the center of each street, separated by 20 cm between each other and 35 cm from each row of cassava. The canavalia was inoculated according to the treatments (Table II) and was handled in a similar way to the previous one, wrapping the vegetal material of each cut on the cassava rows. The cassava harvest was carried out manually in each plot nine months after planting.

In both experiments, weekly irrigations were carried out until the total germination of cassava cuttings was guaranteed and continued every 10 days until the start of the rainy season. The rest of the cultural attentions were executed in accordance with the Technical Instructions (4).

*Characteristics of the inoculant*. The inoculant was made up of propagules of the *G. cubense*/INCAM-4 strain, belonging to the collection of the National Institute of Agricultural Sciences of Cuba. In all cases, the inoculant had at least 25 g$^{-1}$ spore.

**Table II. Characteristics of the treatments studied in experiment 2**

| Previous stage | Stage of cassava cultivation | | |
|---|---|---|---|
| | Main crop | Intercropping | Dose of mineral fertilizer applied (% NPK) |
| $Canavalia_p$MA | CassavaMA | $Canavalia_i$MA | 25 |
| $Canavalia_p$MA | CassavaMA | | 25 |
| $Canavalia_p$MA | Cassava | $Canavalia_i$MA | 25 |
| $Canavalia_p$MA | Cassava | | 25 |
| $Canavalia_p$ | CassavaMA | $Canavalia_i$MA | 25 |
| $Canavalia_p$ | CassavaMA | | 25 |
| $Canavalia_p$ | Cassava | $Canavalia_i$ | 25 |
| $Canavalia_p$ | Cassava | | 25 |
| Fallow | Cassava | | |
| Fallow | Cassava | | 25 |
| Fallow | Cassava | | 50 |
| Fallow | Cassava | | 100 |

Subscripts p, i: precedent and interspersed respectively MA: inoculation with the strain of G. cubense / INCAM-4 100% NPK: 100, 40 and 150 kg ha-1 of N, P2O5 and K2O respectively (4)

*Fertilization*. In both experiments fertilization was carried out 60 days after the cassava was planted and according to the treatments. The amounts of fertilizers equivalent to 100% NPK were 100, 40 and 150 kg $ha^{-1}$ of N, $P_2O_5$ and $K_2O$ respectively (4). The complete formula (9-13-17), urea (46-0-0) and potassium chloride (0-0-60) were used as carriers.

## Methodologies used in evaluations

### Chemical analysis of Soils

Composite samples between 0 and 20 cm deep were taken at the beginning of the experiments, throughout the experimental area. The determinations made were: pH $H_2O$ in ratio to soil: solution of 1:2,5; organic matter by the Walkley and Black method; P assimilable (mg $kg^{-1}$) by extraction with 0,1 N $H_2SO_4$; interchangeable cations (cmolc $kg^{-1}$), by extraction with 1 mol $NH_4Ac$ $L^{-1}$ at pH 7 (21).

*Mycorrhizal spore count*. It was carried out in the initial soil samples. The wet sieving and decanting method was followed (22).

*Mycorrhizal colonization and visual density*. They were always determined by plot, both in the canavalia and in the cultivation of cassava. Approximately 200 mg of rootlets that were dried at 70 ° C were weighed to be dyed (23). The evaluation of the colonization was carried out by the intercepts method, (24) and the visual density by the method proposed by Trouvelot (25).

*Determination of the biomass of green fertilizers.* It was done in each court. In the previous canavalia the plants that were in 1,0 $m^2$ in the center of each plot were taken and in the case of the intercalated ones that were in a linear meter. The plants were separated into leaves and stems and fresh mass and dry mass were determined. The biomass was expressed on a dry basis in t $ha^{-1}$.

*Concentration of N, P and K (g $kg^{-1}$).* They were determined from a wet digestion with $H_2SO^4$ + Se. The Nessler method was used to determine N, the aminonaphthol sulphonic reagent for P and flame photometry for K (21).

*Contents of N, P and K in green fertilizers*. The extraction of N, P and K, was calculated from the data of the dry mass of the aerial part and its corresponding concentration of each element (g $kg^{-1}$). It was expressed in kg $ha^{-1}$ of N, $P_2O_5$ and $K_2O$.

*Commercial root yield* (t $ha^{-1}$). The experiments were harvested manually and quantified separately in commercial and non-commercial roots in each plot. The results were expressed in t ha-1 of commercial roots, according to the plantation frame used.

*Agronomic efficiency (kg $kg^{-1}$).* The agronomic efficiency (EA) of the mineral fertilization (NPK) in the different treatments was calculated from the yields of the treatments (Yield Treatment) in question and the non-fertilized control (Yield Control) according to the following formula:

$$EA = \frac{[\text{Rdto Tratamiento } (t\ ha^{-1}) - \text{Rdto Control } (t\ ha^{-1})] \times 1000}{\text{Fertilizante aplicado } (kg\ ha^{-1})}$$

As in both experiments, the fertilization levels studied consisted of percentages of the fertilizer recommendation NPK (4), for the estimation of the EA, the kg $ha^{-1}$ of applied fertilizer were calculated by adding the amounts of the three primary macronutrients added in each treatment.

In the first experiment, the Agronomic Efficiency of the fertilizer applications in the inoculated treatments was also calculated, but in relation to the yield of the inoculated treatment that did not receive fertilizers and in this way to establish the Agronomic Efficiency of the fertilizer in the presence of the inoculated culture. In the second experiment besides calculating the EA of the joint treatment, the participation of the different components was estimated (1.fertilization, 2. combined use of the preceding canavalia and the arbuscular mycorrhizal inoculation (MA) and 3. the intercalation of the canavalia inoculated), in said Agronomic Efficiency in the following way.

- Participation of the combined use of the canavalia$_p$ and the MA inoculation in the EA = [average of the differences between the yields of the treatments with canavalia$_p$ and MA (without intercalation) in relation to the performance of the treatment 25 % NPK] x1000 and divided by the amount of fertilizer applied in the treatment 25 % NPK.
- Participation of the inoculated canavalia intercalated in the EA (EA Can$_i$MA) = (average of the differences in yield between the homologous treatments with and without canavalia$_i$MA) x 1000 and divided by the amount of fertilizer applied in the treatment 25 % NPK.

### Statistical processing

The assumptions of normality and homogeneity of variance were verified in each case by the Kolmogorov-Smirnov and Levene tests (26,27). The variance analysis of double classification in the different variables was carried out in each experiment and according to the design used. The differences between treatments were established by the Duncan test at P≤0,05 (28). In experiment 2, the effect of the MA inoculation on the preceding canavalia and on the intercalated canavalia was established by a t test (P <0.01).

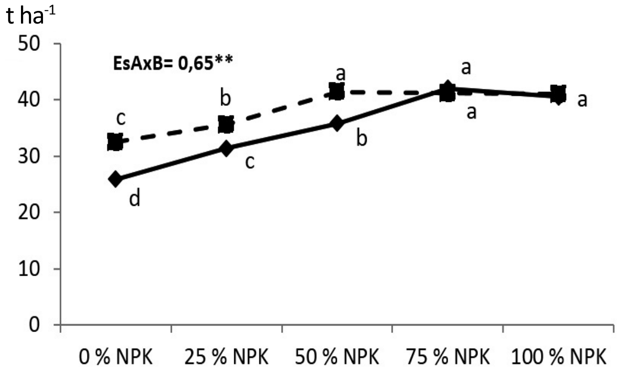
## RESULTS AND DISCUSSION

### Integration of inoculation with efficient strains of AMF and doses of fertilizers in the cassava crop

The cultivation of cassava in Ferralitic Red Leached soil responded significantly to the factors mineral fertilization (NPK) and mycorrhizal inoculation, being also significant the interaction between both (P <0,05). So, in these soil conditions and with the application of only 75 % of the NPK fertilizer dose recommended in the Instructions (4) and corresponding to 75-30-112,5 kg ha$^{-1}$ of N, $P_2O_5$ and $K_2O$ respectively, it was sufficient to reach the highest yields of the order of 40 t ha$^{-1}$ of edible roots (Figure 2) and similar to those obtained with the recommended NPK fertilizer dose (100 % NPK).

In the presence of the *G. cubense*/INCAM-4 strain inoculation, the needs of mineral fertilizers decreased and then only applying 50 % of the recommended dose (4) was sufficient to obtain high yields similar to those obtained in the best treatment that only received mineral fertilizers (Figure 2) The decrease in the quantities of fertilizers necessary to guarantee high yields, by virtue of the of efficient strain inoculation was therefore 33 % under these conditions.

Figure 3 shows the variations of total colonization and visual density when the different factors under study were combined. The inoculation with the *G. cubense* strain originated significant interaction terms (P <0,05), with values always higher in both indicators when compared with the non-inoculated counterparts. Initially in the inoculated treatments an increasing increase was found with the application of fertilizer doses and the maximum values were found with the application of 50 %, which differed significantly from those obtained with the other treatments inoculated. The increase in fertilization above this dose caused a decrease in both indicators of mycorrhizal functioning.

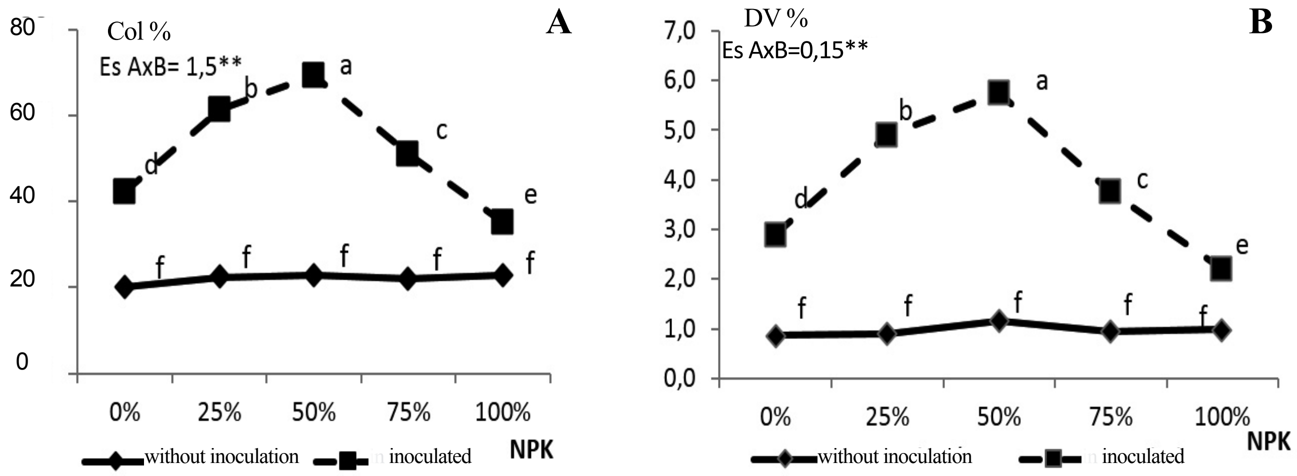



Different letters lead to significant differences at P <0,05 according to the Duncan test. Dose of 100 % NPK equals 100, 40 and 150 kg ha-1 of N, $P_2O_5$ and $K_2O$ respectively

**Figure 2. Experiment 1: Effect of the inoculation of the G. cubense strain (INCAM-4) on the amounts of mineral fertilizer necessary to guarantee high yields in the cassava crop**

It should be noted that the results obtained in the colonization percentages of the best treatment, close to 70 %, were indicative of good performance, based on the results reported in the cassava crop (11,20) and in several other crops. (10,11). Likewise, the values of visual density were also high, although there were no previous reports of this indicator in the cultivation of cassava in Cuba. In *Brachiaria decumbens*, values between 5 and 7 % of visual density associated with effective mycorrhizal function were also reported (10). The results obtained allow relating the positive effects of the inoculation in the decrease of the quantities of fertilizers necessary to guarantee high yields (Figure 2), with the greater mycorrhizal functioning obtained by the inoculated cassava plants.

Although internationally positive results are increased by the inoculation of strains of AMF in different crops (29-32); however, there is a scarcity of work done internationally that integrates inoculation with fertilization schemes.




Different letters lead to significant differences at P <0.05 according to the Duncan test. Dose of 100% NPK equals 100, 40 and 150 kg ha-1 of N, P2O5 and K2O respectively

**Figure 3. Experiment 1: Effect of the inoculation of the G. cubense strain (INCAM-4) and of mineral fertilizer doses on: (A) colonization percentages and (B) visual density (DV) in the culture of the cassava (Clone CMC-40) in Ferrallitic Red Leached soils**

The decrease in fertilizer needs associated with the inoculation of efficient strains of AMF, guaranteeing high yields with satisfactory mycorrhizal functioning and nutritional status, has been one of the most commonly found benefits with the application of these inoculants in Cuba, in different crops such as sweet potatoes, yams, bananas, brachiaries, tomatoes, among others (10,11,33,34). In the specific case of cassava cultivation, the published results correspond to those obtained in carbonated soft Brown soils (11,20), reporting that in the presence of AMF inoculation it was only necessary to apply 25 % of the dose of NPK recommended to obtain high yields (4), although dependent on the crop and the availability of nutrients in the soil.

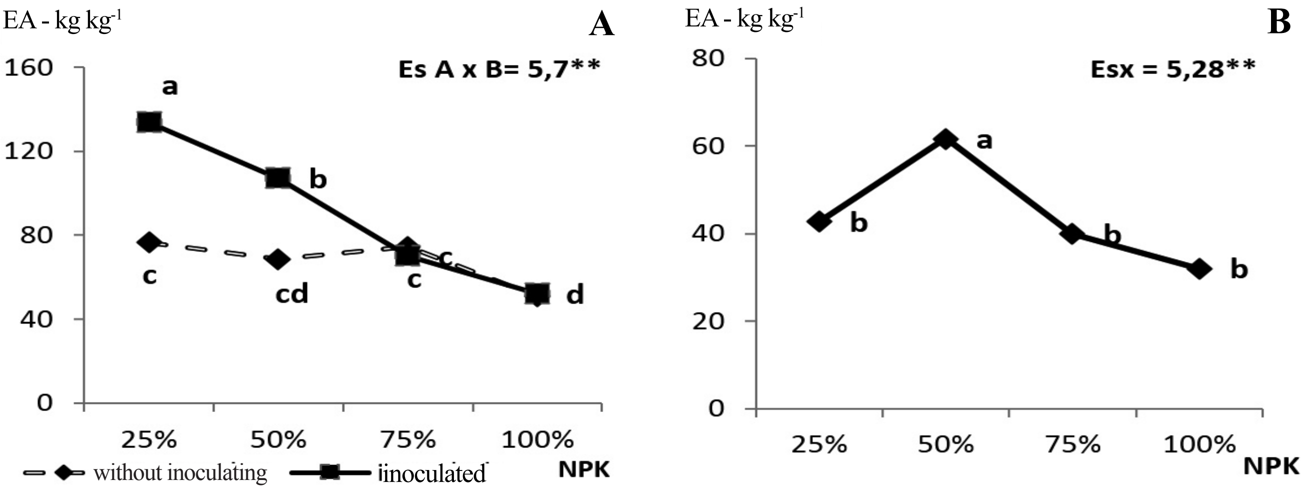
The results obtained also corroborated the proposals that plants inoculated with efficient strains of AMF require an adequate supply of nutrients to guarantee both the growth and yield of the crop, as well as optimal mycorrhizal functioning (10) and that in this case was obtained with the dose of 50 % NPK, which corresponds to a suboptimal supply of nutrients for the non-inoculated culture (Figure 2) and coincides with the general criteria that an effective mycorrhizal functioning is established under conditions of sub-optimal nutrient supplies (7, 35).

In the treatments that only received mineral fertilization (Figure 4A), the values of the agronomic efficiency (EA) of the fertilizer applied were maintained with similar values up to the dose of 75 % NPK, from which they decreased and associated with that was found a linear response to fertilization up to a dose of 75 % NPK and without increases in yield due to the addition of higher doses (Figure 2). The inoculation of the efficient AMF strain significantly increased (P <0,05) the agronomic efficiency of the applied fertilizer (Figure 4A) in the doses of 25 and 50 % NPK and for higher doses were similar to those found with mineral fertilization and indicative also that already above the dose of 50 % NPK the effect of the inoculation was disappearing (Figures 2 and 3).

The higher value of the EA in the inoculated treatment that received the dose of 25% NPK, although it was suggested that in the presence of the lowest doses of fertilizers the inoculated plants manage to more efficiently convert the fertilizer into yield, does not mean that with this dose the highest mycorrhizal functioning and therefore the highest yields due to inoculation were achieved, which were achieved in the inoculated treatment that received 50 % of the NPK fertilization (Figures 2 and 3).

This behavior, found with the application of the 25 % NPK dose, is greatly influenced by the increase in yield achieved when the crop is inoculated in the absence of fertilization (Figure 2).

When the EA was calculated in relation to the yield of the inoculated and unfertilized control (Figure 4B), the previously mentioned effect was eliminated and then the maximum EA values are obtained precisely with the dose that guaranteed the highest yields and mycorrhizal functioning, indicating that in the conditions of greater mycorrhizal functioning, greater efficiency of the applied fertilizer was achieved.




In Figure A were calculated in relation to the average yield of the non-inoculated treatment and without fertilization and in the B with respect to the inoculated treatment and without fertilization. Different letters lead to significant differences at P <0,05 according to the Duncan test

**Figure 4. Experiment 1: Effect of inoculation with the strain of G. cubense / INCAM-4 and fertilization in Agronomic Efficiency (EA)**

## Integration of the inoculation with the strain of *G. cubense* (INCAM-4), *C. ensiformis* precedent and intercalated and dose of mineral Fertilizer

### Effect of inoculation with *G. cubense* in the canavalia

The inoculation of *G. cubense* significantly increased (P> 0,05) the biomass production of *C. ensiformis* used as a preceding green manure (Table III), in any of the two growth periods, with average increases of 20 %.

In the first cut it presented an adequate behavior for the climatic epoch according to the results obtained by several authors (13,14) and associated with the favorable rainfall regime in that period (Figure 1); nevertheless, the production of biomass for the second cut, framed in a period of growth of scarce rainfall and lower amounts of light hours, was much smaller and of the order 40 % of that obtained in the first cut.

The inoculation significantly increased the contents of the three macronutrients in the canavalia (Table III), oscillating between 6 and 14 %, depending on the macronutrient in question, as well as the amounts of extracted macronutrients increased between 33 to 37 %, indicating notable effects by inoculation. A similar behavior was obtained by inoculating canavalia with the strain of G. cubense / INCAM-4 in similar soils (13) and with the efficient strain of *R. intraradices*/INCAM-11 in soft carbonated Brown soils (14), with increases of 20 to 30 % in biomass and 30 to 40 % in the amounts of nutrients extracted.

Correspondingly, the inoculation increased the percentage of colonization and the visual density in the canavalia (Table IV), reaching the inoculated canavalia at 60 days of sowing (das) values close to 60 % of colonization and DV values of 5,0 % that according to previous results obtained in canavalia, (13,14), were indicative of satisfactory mycorrhizal functioning. Mycorrhizal spores increased significantly when inoculating the canavalia (Table IV), although with increases lower than those found by other authors in this same type of soil (13); nevertheless, they prove that this practice was an effective way to increase the population of mycorrhizal propagules and face the subsequent cultivation with a high potential for inoculation in the soil (16).

## Effect of previous and interspersed *C. ensiformis* and its combination with the inoculation of *G. cubense* and dose of fertilizers in the cassava crop

In the treatments that only received mineral fertilizers, a significant and growing response was found to the doses of applied fertilizers (Figure 5).

The highest yields and of the order of 42 t $ha^{-1}$ were achieved with the 100 % NPK dose, with significant differences (P <0,05) to those obtained with the rest of the mineral fertilization treatments studied.

**Table III. Experiment 2. Inoculation effect of G. cubense/INCAM-4 strains in different growth indicators and nutritional contents of *C. ensiformis* used as precedent crop**

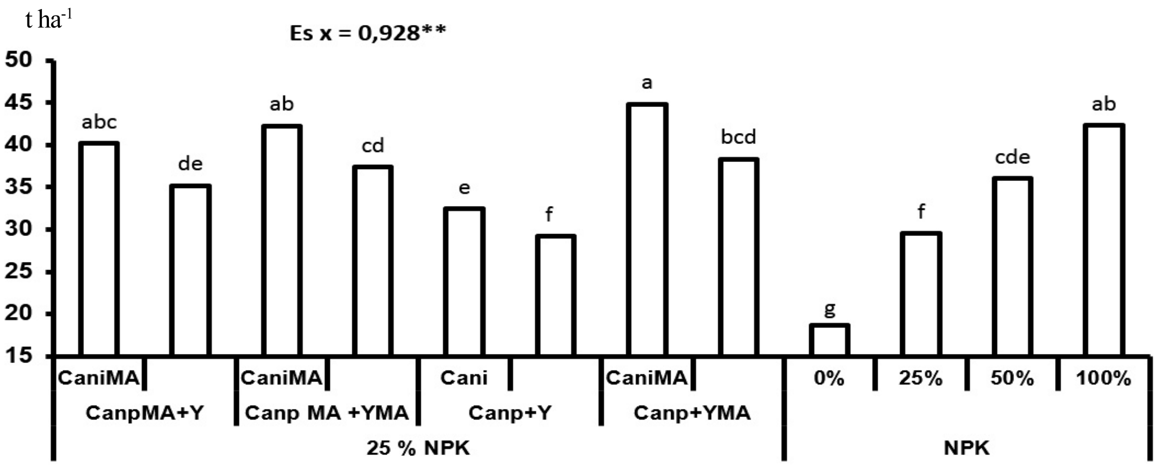
| | MS 1er Corte | MS 2do Corte | N(1) | P | K | N(2) | $P_2O_5$ | $K_2O$ |
|---|---|---|---|---|---|---|---|---|
| | t ha$^{-1}$ | | g kg$^{-1}$ | | | kg ha$^{-1}$ | | |
| Canavalia -MA | 5,44 a | 2,23 a | 27,9 a | 2,28 | 21,8 a | 214,1 a | 40,0 a | 200,6 a |
| Canavalia | 4,44 b | 1,83 b | 25,3 b | 2,0 | 20,0 b | 158,6 b | 28,7 b | 150,6 b |
| Es X | 0,22** | 0,07** | 0,05** | 0,03 | 0,04** | 9,11** | 1,79** | 6,67** |

(1) The nutrient contents are averages of the two growth cycles, while the amounts of nutrients (2) were those accumulated in the two cuts. Different letters in each column imply significant differences at P<0.01

**Table IV. Experiment 2. Inoculation effect with G. cubense/INCAM-4 strain in different indicators del funcionamiento micorrízico de C. ensiformis a los 60 dds**

| | Colonization % | Visual density % | Nu. AMF spores in 50 g |
|---|---|---|---|
| *C. ensiformis* + MA | 59,0 a | 5,01 a | 188,3 a |
| *C. ensiformis* | 42,0 b | 3,54 b | 116,5 b |
| Es x | 1,6 ** | 0,18 ** | 11,68** |

MA: inoculación con *G. cubense*/INCAM-4. ** significación a P<0,01. Letras diferentes en cada columna implican diferencias significativas a P < 0,01




Different letters lead to significant differences at P <0.05. CanpMA: previous inoculated canavalia. Canp: previous canavalia not inoculated. YMA: inoculation of cassava in the plantation. Y: cassava planting without inoculation. CaniMA: intercalated inoculated canavalia. Cani: canavalia intercalated without inoculating

**Figure 5. Experiment 2. Effects of the use of C. ensiformis, inoculation with G. cubense/INCAM-4 and mineral fertilization on the yield of commercial roots of the clone CMC-40, in Ferralitic Red Leached soil**

In the treatments with previous canavalia, a significant response was obtained in the yield of the cassava to the mycorrhizal inoculation; either by inoculating the canavalia or the cassava crop, in such a way that in treatments that were not inoculated the yields of the cassava were always significantly lower. The use of intercropped green fertilizer, inoculated or not, also presented a significant response (P <0,05) and its presence always increased cassava crop yields when compared with the homologous treatments in which intercropping was not present; however, the effects were greater when the intercropped canavalia was inoculated (t = 3,8**).

The application of previous and intercalated canavalia and the inoculation with *G. cubense* guaranteed high yields (45 t ha$^{-1}$) needing only 25 % of the mineral fertilization NPK (4) and with yields similar to those obtained by fertilizing with the doses of 100 % NPK, overcoming the effects found with the simple application of the mycorrhizal inoculant (Figure 2).

In these treatments, not only the highest yields were reached (Figure 5), but the EA at least doubled when compared with the EA obtained when only mineral fertilizers are applied (Table V).

The specific contribution of each of these practices in the EA, reflected that the joint application of previous and intercalated canavalia and the inoculation of HMA presented a higher contribution in the EA than the mineral fertilization, which together with the higher yields achieved make clear the importance of this joint application in the presence of low doses of mineral fertilizers for the design of crop technologies.

It is noteworthy that the intercalation of the green manure led to a considerable production of biomass (Table VI), which was higher when it was inoculated. The canavalia not only quickly covered the streets and in this way controlled the undesirable vegetation; rather, that this biomass with a low C/N ratio when cut and protected the cassava furrow, due to its rapid decomposition (36), must make available to the crop important quantities of macronutrients. In addition, by increasing the mycorrhizal propagules, it increased the inoculation potential on the cassava plantation and could collaborate in maintaining an adequate mycorrhizal functioning in this crop and its benefits, in a similar way as reported for *Morus alba* plantations interspersed with canavalia inoculated with this strain, in this same type of soil (37).

In Nigeria, several works have been carried out combining the application of mycorrhizal inoculants and the intercalation of cassava in plantations of different tree species such as *Leucaena leucocephala*, *Gliricidia sepium* and *Senna siamea* in systems called "alley crops", with significant increases in cassava yields (38,39); nevertheless, the high quantities of inoculants used in these experiments of 100 to 200 kg ha$^{-1}$, seem to prevent the massive use of these results.

In Cuba, in experiments conducted on bananas, it was found that the use of mycorrhizal inoculants decreased by 25 % the amounts of fertilizers to guarantee high yields (33) and that the additional presence of *C. ensiformis* inoculated with AMF and used as precedent and intercalated, these quantities decreased even more between 25 and 50 %, depending on the crop cycle, maintaining also positive balances of the macronutrients in the first three harvest cycles (14). Similar results of integration of green fertilizers and mycorrhizal inoculants in the nutrient supply schemes, guaranteeing high yields and lower amounts of mineral fertilizers and overcoming the effect of using only green fertilizers, have been reported in corn and tobacco (13, 40) and support the possible inclusion of this practice of joint application in the concept of integrated soil fertility management "ISFM" (41).

**Table V. Experiment 2. Contribution of different practices to the agronomic efficiency (EA) of fertilization in cassava cultivation**

| Treatments | Yield | EA | EA per component kg kg$^{-1}$ | | | |
|---|---|---|---|---|---|---|
| | t ha$^{-1}$ | kg kg$^{-1}$ | 25 % NPK | $Can_p$MA | $Can_i$MA | $Can_{p+i}$MA |
| 25 % NPK + $Can_p$MA + $Can_i$MA | 42,45 | 306,84 | | | 67,6 | 166,97 |
| 25 % NPK + $Can_p$ and MA | 37,21 | 239,22 | | 99,4 | | |
| 25 % NPK | 29,51 | 139,87 | 139,87 | | | |
| Control 0 % NPK | 18,67 | | | | | |

$Can_p$ and MA: includes the treatments with previous canavalia and that receive inoculation either in the canavalia and/or in the cassava and that the canavalia was not interspersed with Canp+iMA: it includes the different treatments with intercalated inoculated canavalia
Canp+iMA: it includes the treatments with canavalia precedent and interspersed and mycorrhizal inoculation

**Table VI. Experiment 2: Effect of the inoculation of the strain of G. cubense / INCAM-4 in the biomass and extractions of N, P2O5 and K2O of *C. ensiformis* intercalated**

| | MS tha$^{-1}$ | | | N$^{(1)}$ | $P_2O_5$ | $K_2O$ |
|---|---|---|---|---|---|---|
| | 1$^{er}$ Corte | 2$^{do}$ Corte | Total | | kg ha$^{-1}$ | |
| $Canavalia_i$ + MA | 2,0 a | 1,4 a | 3,5 a | 96,6 a | 18,1 a | 90,5 a |
| $Canavalia_i$ | 1,5 b | 1,0 b | 2,5 b | 63,2 b | 11,5 b | 60 b |
| Es X | 0,08** | 0,05** | 0,12** | 4,0 ** | 0,85** | 4,2** |

Canavaliai: intercalated canavalia; MA mycorrhizal inoculation. (1) the extractions correspond to the accumulated in the two cuts. Different letters, significant differences P<0.01

The positive influence of mycorrhization on the absorption of N supplied in organic forms has been related both through increases in the processes of mineralization of plant residues, by the greater number of associated microorganisms, and the greater growth of hyphae in these conditions and increases in absorption capacity (7,42,43).

A positive effect of mycorrhizal inoculation of the preceding canavalia was also found as a way to mycorrhize cassava, obtaining significantly higher yields of the crop in the treatments that included previous inoculated canavalia and compared with the non-inoculated counterparts (Figure 5). However, the possible benefits of the previous canavalia inoculated on the yield of cassava and associated with the higher biomass and nutrient recycling were not found in this experiment, since there were no differences between the yields of the treatments with inoculated or not , provided that the cassava was inoculated (Figure 5); as well as the performance of the treatment with previous unvaccinated canavalia and with 25 % NPK did not differ from that obtained in the treatment that only received 25 % of the fertilization. This must be related to the use of two periods of growth of the previous canavalia and the fact that the second growth period was characterized by very low rainfall (Figure 1) and strongly affected the biomass production of this green manure (Table I).

Starting from the positive effects of the use of the previous inoculated canavalia as a way to mycorrhize the main crop reported for corn, banana and tobacco (13,14,40) and the high mycorrhizal dependence of cassava (6), it can be considered that the use of the inoculated canavalia as a precedent for cassava cultivation must be successful, but considering a single cycle of growth of green manure, which can range from 60 to 120 das, adapting to the date of cassava planting and optimizing the synchrony between the decomposition of green manure and the growth rate of the crop.

## CONCLUSIONS

The inoculation of *M. esculenta* Crantz with the G. cubense / INCAM-4 strain in Ferralitic Red Leachate soils and integrated with the mineral fertilization is effective, with decreases in 33 % of the amounts of fertilizers recommended to obtain high yields and increases in 67 % of EA of fertilizers. The additional inclusion of *C. ensiformis* inoculated as a previous and intercropped crop, maintains an adequate mycorrhizal functioning of the cassava and high yields of the crop, decreasing in another 33 % the amounts of mineral fertilizers and doubling the EA of these. The results obtained can be the basis of an integral nutrient supply system in the cassava crop that guarantees high yields, effective mycorrhizal functioning and lower fertilizer needs.

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