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INFLUENCES OF *Canavalia ensiformis* INOCULATED WITH AMF ON SOME MORPHOLOGICAL VARIABLES AND YIELD OF DARK TOBACCO CULTIVATED TO THE SUN

Influencia de *Canavalia ensiformis* micorrizada en algunas variables morfológicas y de rendimiento del tabaco negro cultivado al sol

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ABSTRACT. With the aim to determine the influence of *Canavalia ensiformis* inoculated with the arbuscular mycorrhyzal fungi (AMF) *Glomus cubense*, on some morphological variables and yield of the cultivated black tobacco in the sun, an experiment distributed a random block was carried out. Three variants and four replications, which includes: the handling of canavalia inoculated with AMF, canavalia without inoculation and a control that represents the variant of traditional handling (tobacco - fallow) was establish. In the treatment where canavalia was inoculated with AMF a positive answer expressed on increments in morphological variables, and yield was produce, increasing the agronomic value as successor crop.

Key words: green manure, successor crop, inoculation

RESUMEN. Con el objetivo de determinar la influencia de *Canavalia ensiformis* inoculada con el hongo micorrízico arbuscular (HMA) *Glomus cubense* en algunas variables morfológicas y de rendimiento del tabaco negro cultivado al sol, se realizó un experimento con un diseño de bloques al azar, con tres tratamientos y cuatro repeticiones, que incluyeron: canavalia inoculada con HMA, canavalia sin inocular y el testigo sin canavalia, que representa la variante de manejo tradicional (tabaco – barbecho). En el tratamiento donde se inoculó la canavalia con HMA se encontró una respuesta positiva expresada en incrementos en las variables morfológicas y de rendimiento del cultivo del tabaco, al incrementar su valor agronómico como cultivo sucesor.

Palabras clave: abono verde, cultivo sucesor, inoculación

INTRODUCTION

The elimination of monoculture with the establishment of rotation systems and alternation of crops has been carried out since the 1990s (20th century) in tobacco areas of the province of Pinar del Río, Cuba, where one of the tobacco of better quality worldwide. These practices constitute a strategy for the intensive exploitation of soils, and with their application steady increases have been achieved in agricultural productions and tobacco yields (1).

It is well known that in the medium and long term the yield of the crops in rotation or alternation is greater than in monoculture (2). This is why it is indicated that monoculture can be carried out for a certain number of years (six maximum), after this time, yields decrease by 24 %, due to a drastic decrease in soil fertility and increase in arable plants, among other factors[A, B].

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[A]Lagos, C.; Velazco, R. H. y González, J. U. "Maíz – base de sistemas intensivos de producción en riego en Chile". En: *Diálogo XLIII Maíz: sistemas de producción. Programa 62 cooperativo para el desarrollo tecnológico agropecuario del Cono Sur, Procisur*, Ed. IICA, Montevideo, Uruguay, 1995, p. 188.

[B]Díaz, L.; Tremols, J. A.; Blanco, L.; Sánchez, E. P. y Perera, G. "Sistema de rotación y secuencia de cultivos para los suelos Ferralíticos Rojos dedicados al tabaco". En: *Reunión Nacional de Investigadores y Productores de Tabaco*, Ed. Instituto de Investigaciones del Tabaco, La Habana, Cuba, 1995, pp. 3-4.

The effect of crop rotation and alternation practices with the use of green manures has been described in the literature by different researchers, who have demonstrated their influence on agricultural yield, when they are inserted in crop rotation schemes[B]. Its use brings benefits by improving soil properties and promoting plant nutrition, which directly affects the increase in yields (3).

Other studies have shown that the use of green manures contributes to increase the productivity of crops and their sustainability, being considered fundamental the intercalation of these with crops of economic importance such as tobacco (4); in addition, it has been considered a challenge within sustainable agriculture to manage them with efficient AMF inocula, because they increase the availability of nutrients in the soil and their use by plants (5).

In the tobacco cultivation, there are no studies in which green manures with arbuscular mycorrhizal fungi (AMF) species are combined to improve the productive potential of the crop. This is why the following work was carried out, in order to determine the influence of *Canavalia ensiformis*, used as green fertilizer and inoculated with an efficient strain of arbuscular mycorrhizal fungus (AMF), on some morphological and yield variables Black tobacco cultivated in the sun.

MATERIALS AND METHODS

Site Features

The experiment was carried out in the 2011-2012 and 2012-2013 seasons, in areas of the Tabaco Experimental Station, San Juan y Martínez municipality, Pinar del Río; in a Ferralitic Cuarcític Yellow Soil Typical eutric leachate, according to the New Classification Version of the Soils of Cuba (6).

In the experimental area the soils are characterized by sandy loam texture, slightly acidic pH, low organic matter contents, and poor in exchangeable bases; with high phosphorus and potassium contents (Table I), resulting from the continuous application of mineral fertilizers, made in these soils, according to the technical standards for cultivation.

Description of the experiment

The research was carried out in a total area of 462 m2 with plots of 37.8 m2. An experimental design of Random Blocks was used, with three treatments and four replications. The study was carried out with the following treatments:

- Canavalia with AMF – tobacco
- Canavalia without AMF – tobacco
- Fallow – tobacco

Starting from fallow soil, canavalia was planted in treatments 1 and 2 in the second decade of September. In treatment 1 the seeds were inoculated by the coating method, with an inoculant based on the AMF *Glomus cubense* species. A dose of 5,95 kg ha^{-1} of the product, equivalent to 10 % of the weight of the seeds, was used. First a homogeneous paste was prepared, at a rate of 1 kg of inoculum per 10 kg of seed, then the seeds were covered until completely covered. Finally, they were allowed to dry in the shade for 10 minutes and were sowed, with a distance between plants 30x76 cm of ridge. Sowing and cultivation were done manually. The cutting and incorporation to the soil of the canavalia plants was performed mechanically, between 60-70 days after sowing, using a 682 kg disc harrow, incorporating the crop biomass to a depth of 15-20 cm.

The tobacco cultivar "Criollo 98" was transplanted in the third decade of December. Seedlings obtained from technical seedlings were used. The transplant was done manually, with a 30 cm planting frame between plants by 76 cm of ridge and the cultivation work was carried out according to the Technical Manual for the cultivation of black tobacco in the sun (10). The tobacco harvest was started at 55 days manually, by foliar floors and skewering, extended until 80 days after transplanting, then dried in a natural healing home. The harvest remains were incorporated to the soil and it remained at rest until the rainy season, when soil preparation began again for the next cycle or campaign.

Table I. Some soil characteristics in the experimental area

pH H_2O	P_2O_5	K_2O	OM	Ca^{2+}	Mg^{2+}	Na^+	K^+	S	T
	mg 100 g^{-1}		g kg^{-1}			$cmol_c$ kg^{-1}			
6,4	37,98	29,38	13,6	4,96	1,90	0,11	0,47	7,65	9,75

Chemical determinations: pH (H_2O) potentiometer: soil/solution ratio of 1: 2.5; OM (organic matter) colorimetric method, (7); P_2O_5 - colorimetric method with 0,05 mol L^{-1} sulfuric acid, (8), Cations NH_4Ac at pH 7 (9); S: ability to change bases; T: capacity of cationic change

Measurements and observations in the two campaigns were made by selecting and randomly selecting ten plants in the calculation area in each plot, between 20 and 25 days after the plantation was established. These were carried out at the time of harvest (70-75 days after transplant), in the central soil of the tobacco plant (11).

The measurements and observations made to the tobacco plants were:

- Length and width of the central leave (cm) with graduated precision ruler ± 0,1 mm (11).
- Fresh mass and dry mass of the central leave (g) by the gravimetric method, in precision analytical balance ± 0,1 mg (11).
- Chlorophyll by SPAD-502 (MINOLTA, Spectrum Technologies Inc.)
- Mycorrhizal colonization in roots (%) (12).

At the end of the harvest, it was determined:

- Total yield and yield in classes, according to the Technical Instruction for the Collection and Benefit of Sun-Cultivated Tobacco (13).
- Combustibility, according to the Instruction for the procedure and evaluation of the combustibility of Cuban tobacco (14).

Analysis of variance and multiple comparisons were subjected to one-way ANOVA with a probability of 0,05, using the Tukey's test (15). All data were processed using the statistical package Statistical Package for Social Sciences (SPSS) for Microsoft Windows (16).

RESULTS AND DISCUSSION

Table II shows the behavior of the treatments in the morphological variables of the tobacco crop, reaching the highest values in the variables length, fresh mass and dry mass of the central leaf, whenever canavalia was combined with AMF, with significant increases, regarding to the control treatment in both campaigns evaluated. In the variable width of the central leaf, no differences were observed in the first season between treatments with canavalia and in the second season between inoculated canavalia and control treatment.

The results of the variable length and width of the larger leaf exceed the values reported in the literature (10), when indicating a range of 40 - 45 cm and 20 to 25 cm, respectively, when the tobacco is cultivated in the sun when combinations of tobacco with legumes and grasses are used, the best results are obtained in these variables and the growth and development of tobacco plants is favored (2, 17).

In relation to the variables fresh mass and dry mass of the tobacco, the results are related to those obtained in maize and banana cultivation when canavalia was combined with AMF and incorporated as green manure (18).

Given the deterioration conditions and loss of soil fertility under study (1), these results may suggest the influence of mycorrhizal inoculation, due to the importance of these fungi in nutrient absorption efficiency, which could favor growth of the inoculated culture compared to those that were not (19, 20).

In the contents of chlorophyll in leaves of tobacco, no significant differences were observed between the treatments where canavalia was applied with and without mycorrhizal inoculation, but these did show differences with the control. In the results, it was possible to observe low chlorophyll content in the leaves of tobacco during its growth and development, as the adequate contents of chlorophyll that must be equal to or greater than 40 SPAD are not reached (21-23).

At the same time, the effect of canavalia, whose main characteristic is given by large amounts of N and being a legume that can be associated with N-fixing bacteria, increases its availability for crops in succession and make positive the balance of Nutrient in the soil-plant system.

Table II. Effect of treatments on some morphological variables of black tobacco cultivated in the sun

Treatments	Lenght of the leaf (cm)	Width of blade (cm)	Fresh mass (g)	Dry mass (g)	Chlorophyll (SPAD 502)
Campaign 2011 – 2012					
Can wih AMF – Tobacco	53,3 a	29,9 a	424,15 a	54,00 a	36,0 a
Can without AMF – Tobacco	51,4 b	29,6 a	418,35 b	52,60 b	35,6 a
Fallow – Tobacco	50,8 b	28,1 b	376,65 c	47,85 c	32,0 b
Es (+/-)	0,444	0,363	0,597	0,136	0,732
CV (%)	5,75	8,24	5,44	5,36	10,69
Campaign 2012 – 2013					
Can wih AMF – Tobacco	48,1 a	27,1 a	315,95 a	53,52 a	35,9 a
Can without AMF – Tobacco	45,5 b	24,2 b	283,20 b	52,07 b	37,1 a
Fallow – Tobacco	44,7 b	26,6 a	282,05 c	48,17 c	33,2 b
Es (+/-)	0,507	0,380	0,175	0,190	0,747
CV (%)	7,58	10,44	5,59	4,65	10,35

Means with different letters in the same column differ according to Tukey test (P <0.05) Can: canavalia, AMF: arbuscular mycorrhizal fungus

This effect must be coupled with an adequate management that maximizes the use of this element by plants[C].

The canavalia is a species of legume that accumulates more nitrogen in the leaves than in the stems, the leaves decompose rapidly and its contribution of nitrogen to the system affects the nitrogenous nutrition of successor crops of high demand of N like tobacco (24) . When this legume is inoculated with AMF, when incorporated into crop sequence systems it can increase the transfer of nutrients as P and K due to the mobilizing effect of the AMF and the availability of N, from the symbiotic association for the crops in succession, which means better conditions for the growth and development of these[C] (25, 26).

The results of the yield of the total and upper classes, as well as the combustibility of the leaf in the evaluated campaigns are presented in Table III.

Table III. Evaluation of the variants designed in the performance of black tobacco cultivated in the sun

Treatments	Yield. Superior classes (kg ha^{-1})	Total yield (kg ha^{-1})	Combustibility (s)
Campaign 2011–2012			
Can with AMF – Tobacco	1574,57 a	2360,30 a	30
Can without AMF – Tobacco	1413,90 b	2186,80 b	30
Fallow – Tobacco	1266,65 c	2342,20 ab	30
Es (+/-)	43,172	43,192	
CV (%)	10,77	4,91	
Campaign 2012–2013			
Can with AMF – Tobacco	1192,95 a	2002,80 b	30
Can without AMF – Tobacco	1068,62 b	2242,32 a	29
Fallow – Tabaco	957,95 c	1860,10 c	25
Es (+/-)	33,080	34,513	
CV (%)	10,88	8,61	

Means with different letters in the same column differ from each other, according to Tukey's test (P <0.05). Can: canavalia, AMF: arbuscular mycorrhizal fungus, yld: yield

When incorporating canavalia as green manure in alternation with tobacco, total yields did not show differences with the control in the first season, but in the second one, with values of 2002,80 and 2242,32 kg ha^{-1} when canavalia was used with and without AMF, respectively, compared to 1860,10 kg ha^{-1} obtained in the witness. These results exceed the average yield potential reported in the Technical Manual for the cultivation of black tobacco in the sun, collected in leaves under production conditions (10).

The yields in higher classes were increased in the two campaigns evaluated with the use of canavalia as an ancestor crop, being superior to inoculating the legume with AMF. The upper classes make up the raw material for the twisting of the export cigars, the main objective of the tobacco grown in the sun. The lowest value was obtained in the control variant in the second season, this is related to the results of the variables length, width, green mass and dry mass of the leaf (Table II), which directly influence the agricultural yield of tobacco cultivation (2, 27).

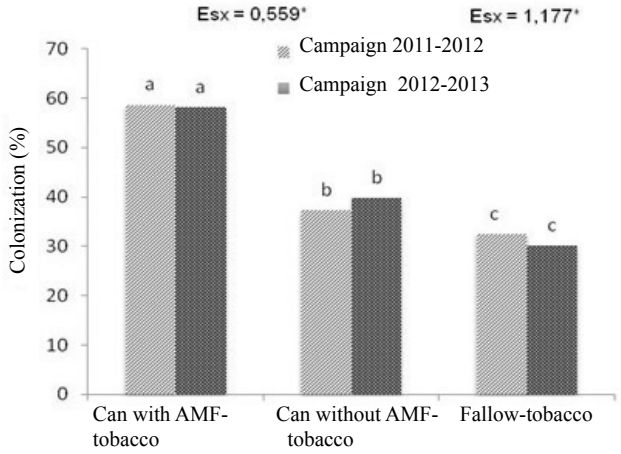
This response can be given by the management system that considers the introduction of canavalia as green manure in the cycle, specifically the joint application of mycorrhizal green manure that contributes considerable amounts of N, essential for the development and tobacco growth, which could influence the increase of total returns and higher classes. There are reports in tobacco cultivation with satisfactory results when alternating with green fertilizers and not with the use of monoculture (2, 3).

Combustibility is an established index and it is valued in excellent (> 20 s), good (15-20 s) and regular (<15 s), according to Technical Instruction for the procedure and evaluation of the combustibility of Cuban tobacco. The combustibility of the leaf was not affected by the different management systems, for all cases it exceeded 20 seconds, reaching the category of excellent according to the established ranges (14). The combustion of tobacco is influenced by a set of chemical and physical properties that are largely related to plant nutrition (28).

The inoculation of the canavalia had a secondary effect on the colonization of the tobacco culture, which could be verified with the results shown in the Figure.

The treatment with canavalia inoculation showed a higher percentage of colonization in tobacco plants as a successor crop, behaving superior to the treatment where no canavalia was inoculated and fallow, which explains the behavior of morphological variables and crop yields.

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



Means with different letters, differ according to Tukey's test (P <0.05). Can: canavalia, AMF: arbuscular mycorrhizal fungus

Figure. Percentage of colonization by the arbuscular mycorrhizal fungus in tobacco plants

When using Canavalia without inoculation there was a percentage of colonization in tobacco that varied between 35-40 %, in what could influence the peculiarity that this green fertilizer has of multiplying the propagules of AMF in the soil, be they native or inoculated and propitiate of this Mycorrhizal colonization of the posterior culture (29). However, in the fallow treatment the results of the colonization percentage of the arbuscular mycorrhizal fungus reached values of 32 %, which corresponded to the existence of at least 55 native spores in the soil used and favored colonization in tobacco as a successor crop to fallow

There are investigations where differences in yields of maize and banana are obtained as successor crops, in the presence of canavalia as green manure, with a higher behavior in the function variable fungal percentage of radical colonization, with respect to the natural fallow vegetation (20, 29).

The results demonstrate how the *Canavalia ensiformis* (L) green manure species was able to respond to the management used in an area dedicated to the production of black tobacco cultivated in the sun, so that there was a positive response in the morphological, performance and colonization by the arbuscular mycorrhizal fungus in tobacco plants as a successor crop, of great economic importance for Cuba and mainly for Pinar del Río province.

CONCLUSIONS

- The use of *Canavalia ensiformis* inoculated with AMF improves the morphological or growth variables of black tobacco cultivated in the sun.
- The use of *Canavalia ensiformis*, as an ancestral crop and incorporated as a green manure, increases the total yields of tobacco and when inoculated with AMF surpasses the yields of this crop in higher classes.
- The use of *Canavalia ensiformis* inoculated with AMF allowed to reach the highest percentage of radical colonization in tobacco as a successor crop.

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