Effect of chemical and biological fertilization on the morphoagronomic yield of Morus alba

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ABSTRACT

The effect of nitrogen fertilization, the vesicular arbuscular mycorrhiza (VAM) *Glomus fasciculatum* and the brassinosteroid phytohormone Biocep-6, on the morphoagronomic characteristics of *Morus alba* var. Acorazonada, was evaluated. For such purpose three fertilization rate (0, 150 and 300 kg/N/ha/year), two with –and without- Ecomic® and two with –and without- Biocep-6 were used, which originated a total of 12 treatments. Five plants were randomly selected and the variables height, number of primary branches and dry matter yield of: total biomass (DMYTB), edible biomass (DMYEB), leaves (DMYL) and fresh stems (DMYFS), were studied in them. A direct relation was observed between height and yield, as well as the effect of Biocep-6 on the number of primary branches. The DMYTB was 18,25; 17,24; 17,56; 18,43; 17,04; 17,22 and 18,31 t DM/ha/year in T4, T5, T6, T8, T9, T11 and T12, respectively. The DMYEB, DMYL and DMYFS behaved differently, according to each treatment. The synergy between biological and chemical fertilizers was concluded to be remarkable for all the morphoagronomic variables. The treatments Ecomic® + Biocep-6 and 150 kg N/ha/year + Ecomic® + Biocep-6 were significantly higher for all the variables. The total and edible dry matter yields obtained with the biological and mineral fertilization were within the range reported by other authors. The combined use of Ecomic® + Biocep-6 in order to obtain good agricultural yields in *M. alba*, as well as continuing the studies of bromatological composition to determine the effect of the combinations of chemical and biological fertilizers, is recommended.

Key words: mycorrhiza, *Morus alba*, plant growth substances

INTRODUCTION

Mulberry (*Morus alba*) is a plant that originated in Asia (Datta, 2002) and is distributed in several regions of the world. Among its main characteristics the excellent palatability, good bromatological composition, high dry matter yields per hectare and high adaptation to different environmental conditions stand out; however, one of its limitations is stated to be the fact that it is a nutrient-extracting plant, for which it is considered very demanding on fertilization (Martín, 2004).

In this sense, it has been proven to respond efficiently to chemical fertilization when rates of 300 kg of N/ha/year were applied (Martín, 2004). Nevertheless, the negative effects caused by chemicals on the environment deterioration are known, and at present other alternatives are being used throughout the world in order to preserve the environment and mankind.

A choice that can make the productivity of this crop sustainable is the use of biological fertilizers, which constitute an ecological source that allows to safeguard the physical and chemical characteristics of soils, without degrading the environment or affecting human and animal health.

Biofertilization is a technology related to the inclusion of microorganisms in the seeds (inoculation), among which vesicular arbuscular mycorrhizae (VAM) are found. These microorganisms contribute nitrogen and phosphorus to the plants, and they also have other important functions: more abundant root development and protective effect against fungal diseases of the root (Gabriel, 2009).

Another alternative used in the search for sustainable solutions for the environment has been biological stimulators –such as brassinosteroid analogues-, which are compounds with sufficient capacity to participate in the main metabolic processes of plants (Zullo and Adam, 2002). They also promote different effects on plant growth and development, such as: stimulating cell elongation and division, and increasing leaf surface, plant biomass and the yield of different crops (Mariña, Rosabal, Nieto and Castillo, 2002).

Taking these premises into consideration, the objective of the study was to evaluate the effect of chemical nitrogen fertilization, the VAM *Glomus fasciculatum* and the brassinosteroid phytohormone Biocep-6 on the morphoagronomic characteristics of *M. alba* var. Acorazonada.

MATERIALS AND METHODS

Climate and soil. The trial was conducted at the Experimental Station of Pastures and Forages (EEPF) "Indio Hatuey", located in the Perico Municipality, Matanzas province, in the period between August, 2006, and September, 2008. During this period 3 603,6 mm of rainfall were recorded and the mean temperature was 28,0 ºC. The soil showed plain topography and it was classified as lixiviated Ferralitic Red, according to Hernández *et al*. (1999).

Design and treatments. The design was randomized blocks and three rates of chemical combined nitrogen fertilization (0, 150 and 300 kg of N/ha), wilh two of VAM *G. fasciculatum* (with –and without- Ecomic®) and two of brassinosteroids (with –and without- Biocep-6) were studied, which originated a total of 12 treatments (described below), four replications and 48 plots measuring 6,0 x 3,5 m, with a net area of 5,0 x 2,5m.

- Treatment 1 (T1): without chemical fertilizer, without Ecomic® and without Biocep-6 (control)
- Treatment 2 (T2): without chemical fertilizer, without Ecomic® and with Biocep-6
- Treatment 3 (T3): without chemical fertilizer, with Ecomic® and without Biocep-6
- Treatment 4 (T4): without chemical fertilizer, with Ecomic® and with Biocep-6
- Treatment 5 (T5): with 150 kg N/ha/year, without Ecomic® and without Biocep-6
- Treatment 6 (T6): with 150 kg N/ha/year, without Ecomic® and with Biocep-6
- Treatment 7 (T7): with 150 kg N/ha/year, with Ecomic® and without Biocep-6
- Treatment 8 (T8): with 150 kg N/ha/year, with Ecomic® and with Biocep-6
- Treatment 9 (T9): with 300 kg N/ha/year, without Ecomic® and without Biocep-6
- Treatment 10 (T10): with 300 kg N/ha/year, without Ecomic® and with Biocep-6
- Treatment 11 (T11): with 300 kg N/ha/year, with Ecomic® and without Biocep-6
- Treatment 12 (T12): with 300 kg N/ha/year, with Ecomic® and with Biocep-6

Experimental procedure. Propagules from six-month-old branches of the Acorazonada variety (from the seed bank of the EEPF "Indio Hatuey") were used for planting. The cuttings had an average length between 20 and 30 cm, and a diameter from 8 to 10 mm. They were planted at a distance of 0,50 m between rows and at 1,0 m of separation from one double row to the other; the distance between plants was 0,40 m.

The propagules of the treatments with Ecomic® were previously inoculated. For such purpose a mixture of 1 kg of the biofertilizer was prepared in 600 mL of H_2O and the third of each propagule was submerged during 20 minutes (Manual de Instructivo Técnico del EcoMic®, 2003).

The crop was considered established when the plants reached between 1,0 and 1,5 m of height, around 10 months after the planting date. Afterwards, a homogenization cutting was performed in each plot and a cutting frequency was fixed every 90 days (Martín, 2004).

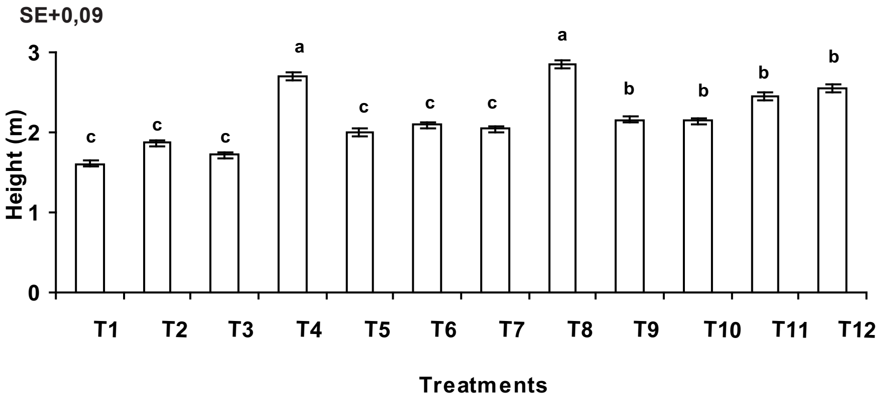
Regarding the plots with Biocep-6, a first application was made –on the leaves- two months after the var. Acorazonada was planted; it was also applied 20 days after each cutting of the rainy season (June and September), in order to guarantee the emission of new shoots. A dose of 15 mL of the product in 16 L of H_2O was used for each plot; this mixture was well agitated before being applied.

Measurements. The evaluated variables were: plant height, number of primary branches and dry matter yield of total biomass (DMYTB), edible biomass (DMYEB), leaves (DMYL) and fresh stems (DMYFS), expressed in tons of DM/ha/year.

Statistical analysis. The data were processed through a variance analysis (ANOVA) corresponding to the statistical pack Infostat, free version . The means were compared through Duncan´s multiple range test, for a significance level of $P<0,05$ (Duncan, 1955). In addition, a correlation analysis was performed to find the interrelation among the variables height, DMYTB and DMYEB.

RESULTS AND DISCUSSION

Figure 1 shows the effect of each treatment on plant height. The best result was obtained with the application of Ecomic® + Biocep-6 (T4) and 150 kg N/ha/year + Ecomic® + Biocep-6 (T8); they



a, b ,c: bars with different letters differ at P<0,05 (Duncan, 1955).

Fig. 1. Effect of the treatments on plant height.

did not differ from each other and showed higher means than the control (T1).

This aspect is very important if the relation between height and yield is taken into consideration, as long as the characteristics of the species and the environmental conditions are similar, as in this study (table 1). This shows that mulberry has a similar performance to that of other trees such as *Leucaena leucocephala* (Wencomo, 2008), *Albizia lebbeck* (Francisco, 2002) and *Erytrina poeppigiana* (Torres, Chacón, Arriojas and Armas, 2000).

Table 1. Correlation matrix of the variables.

Indicator	DMYTB	DMYEB	Height
DMYTB	-		
DMYEB	0,96*	-	
Height	0,78*	0,67*	-

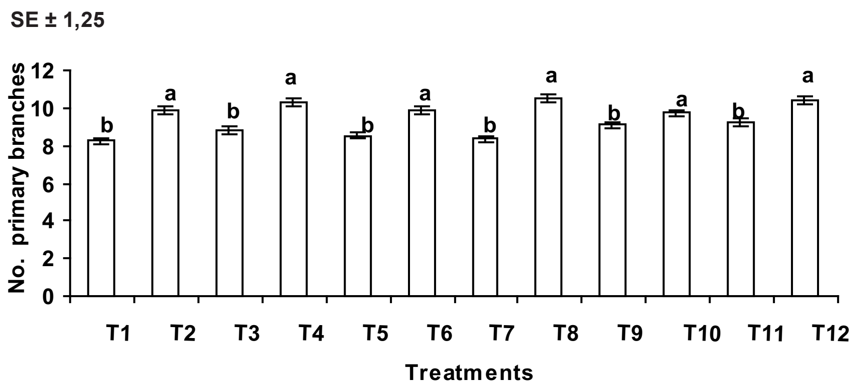
*The correlation is significant at the level of 0,05

Regarding the number of primary branches, T2, T4, T6, T8, T10 and T12 were determined to be the ones with higher influence; there were no significant differences (P≤0,05) among them, but they were found with regards to the others (fig. 2). It must be emphasized that Biocep-6 was part of these treatments, which was determinant in this variable. According to Pecina *et al.* (2005), these hormones have an important role in the formation of new shoots and in cell elongation, for which the performance is logical if it is also taken into consideration that the application was on the leaves.

Brassinosteroids regulate different physiological processes, vegetative as well as reproductive, according to Coll and Coll (2012). Due to their characteristics, these compounds are very useful to improve crop yields when exogenously applied at different times of the life cycle. These authors confirmed the report by Pecina *et al.* (2005), regarding the fact that these products modulate the physiological processes, which has incidence on the stimulation of growth in length and diameter, and they also influence flowering increase and homogenization, the advance of harvests and the increase of fruit quantity and quality. They act more markedly when the crops are subject to different conditions of environmental (extreme temperatures, water excess or lack, salinity, etc.) as well as biotic stress which activates molecular mechanisms that induce tolerance.

On the other hand, the result obtained in the plants to which Biocep-6 was applied differed from the one found for Ecomic®, that is, all the plants which received the latter did not reach the highest number of primary branches, for which it was proven that for this variable the hormones and mycorrhizae are not necessarily in symbiosis, as occurs for others which will not be described below.

These results corroborate the report by Pentón *et al.* (2011), who when inoculating mulberry cuttings with VAM and comparing them to a control (without inoculum) found that the number of primary branches did not differ among the treatments, and the values were two or three branches as average. Nevertheless, the response to biofertilization in the green weight of rootlets and roots was remarkable, which coincides with the report by Riera (2002). These authors determined the effect exerted by the VAM on the root development, aspect which should be emphasized because the biofertilizers used had a particular effect on the different plant organs (roots and leaves).



a,b: bars with different letters differ at P<0,05 (Duncan, 1955)

Fig. 2 Effect of each treatment on the number of primary branches of the plants.

Table 2 shows the effect of each treatment on the evaluated yield variables, for which significant differences were found (P≤0,05). The DMYTB was 18,25; 17,24; 17,56; 18,43; 17,04; 17,22 and 18,31 DM/ha/year in T4, T5, T6, T8, T9, T11 and T12, respectively. These values are within the range observed by Elizondo (2007), who obtained total dry matter yields between 17 and 21 t DM/ha/year, when evaluating the effect of chemical and organic fertilizers on the biomass production and quality of mulberry.

Rodríguez, Quiñonez and Arias (1992) also reported from 2 to 26 t DM/ha/year in whole plants harvested at 30 cm above the soil level, with pruning intervals of six to twelve weeks and fertilization rates from 0 to 80 kg N/ha/year. On the other hand, when using a dose of 150 kg N/ha/year with different planting distances (0,60-1,20 m) and cutting frequencies (56-112 days), Boschini, Dormond and Castro (1999) determined that the average annual dry matter production was 19,7; 9,9 and 9,8 t/ha/year for the whole plant, stems and leaves, respectively. The DMYEB was higher in T4, T5, T6, T7, T8, T9, T10, T11 and T12, which differed from the other treatments (P≤0,05).

The treatments of higher effect on the edible biomass yield were those composed by two fertilizers or more, chemical as well as biological. All seems to indicate that there was synergy among the microorganisms of Ecomic®, the active substances stimulated by Biocep-6 and the minerals extracted by the plant through the chemical fertilizer, which was translated into higher agricultural yield of the crop. Similar effects were found by Veeraswamy, Padmavathi and Venkataswarlu (1992) in the yield of sorghum, when determining the highest productions with the interaction of mycorrhizal fungi and brassinosteroids. With these studies the possible symbiosis between microorganisms and the active substances stimulated by the phytohormone in the plant was proven.

In this sense, one of the ways which may be used to improve soil fertility and stimulate plant nutrition is to increase the population of microorganisms that help in this process, from their inoculation. The group of these substances, assimilated through the roots allows each of them to act at the moment the crop requires it, because one of its functions is to stimulate the development of roots or of the whole plant, which represents an increase in yields (Treto, García, Martínez and Febles, 2001).

On the other hand, mulberry is highly demanding on chemical fertilization (Martín, 2004), to which it responds efficiently because it reaches high DM yields and high CP contents. If the plant is also stimulated by hormonal substances and microorganisms which allow it to extract and make better and higher use of minerals, it should be assumed that the response will be higher in these treatments.

The DMYL and DMYFS varied in each treatment. The highest leaf yield was obtained in T4, T5, T6, T7, T8, T9 and T10, without significant differences among them; however, they differed from T1, T2, T3, T11 and T12 (p≤0,05) and showed means higher than the control. The yield of fresh stems was higher in T2, T3, T4, T6, T7, T8, T9, T10, T11 and T12, which differed from T1 and T5.

The range in which the means of both variables oscillated, contrasts with the report by Elizondo (2007) in the humid tropic of Costa Rica, who obtained values between 12 and 15 t DM/ha/year in DMYL and between 8 and 11 t DM/ha/year in the DMYFS.

The response obtained in the study could have been determined by the specific environmental conditions of this period, in which there were high temperatures and little rainfall; in addition, the crop did not receive irrigation.

With regards to the analysis of total biomass, in which the ranges obtained in this study were compared with the ones reported by Elizondo (2007), it is important to emphasize that it is composed by the leaves, fresh stems and woody stems, and the last ones were not evaluated, for which it is estimated that they could have had a significant effect on the weight of such variable.

Table 2. Effect of the fertilization treatments on the agronomic indicators (t DM/ha/year).

Treatment	DMYTB	DMYEB	DMYL	DMYFS
T1	14,01^{c}	7,12^{b}	4,82^{c}	1,09^{b}
T2	15,16^{b}	7,22^{b}	5,24^{c}	1,10^{ab}
T3	14,78^{c}	7,19^{b}	5,03^{c}	1,12^{ab}
T4	18,25^{a}	9,10^{a}	7,84^{a}	1,14^{ab}
T5	17,24^{ab}	9,00^{a}	7,62^{a}	1,09^{b}
T6	17,56^{a}	8,14^{a}	7,06^{ab}	1,10^{ab}
T7	14,35^{c}	8,52^{a}	8,00^{a}	1,13^{ab}
T8	18,43^{a}	9,08^{a}	7,92^{a}	1,12^{ab}
T9	17,04^{ab}	8,36^{a}	7,58^{a}	1,13^{ab}
T10	16,66^{b}	8,00^{ab}	7,84^{a}	1,17^{a}
T11	17,22^{ab}	8,42^{a}	6,06^{b}	1,10^{ab}
T12	18,31^{a}	8,89^{a}	6,63^{b}	1,13^{ab}
SE(±)	1,66	1,92	0,97	0,006

a, b, c: values with different superscripts in each row differ at P<0,05 (Duncan, 1955).

In general, if it is considered that in the morphoagronomic variables no differences were found between the treatment with Ecomic® + Biocep-6 (T4) and the one containing 150 kg N/ha/year + Ecomic® + Biocep-6 (T8), it is logical to assume that with the adequate use of T4 the production costs would be lower, there would be no negative environmental effect and the dry matter productions would be acceptable and similar to the ones obtained using chemical products. Thus, the results of this study corroborated those obtained by such authors as Espinosa and Benavides (1998); Boschini (1999); Martín (2004) and Elizondo (2007), about the importance of fertilizing this crop. In addition, knowing the adequate management of this practice allows to reach high biomass yields, which could substitute commercial concentrates and be used as animal feed.

CONCLUSIONS

The effect of the synergy between the biological and chemical fertilizers was remarkable for all the morphoagronomic variables. The treatments Ecomic® + Biocep-6 and 150 kg N/ha/year + Ecomic® + Biocep-6 were significantly higher for all the variables. The total and edible dry matter yields obtained with the biological and mineral fertilization were within the range reported by other authors.

The combined use of Ecomic® + Biocep-6 in order to obtain good agricultural yields in *M. alba*, as well as continuing the bromatological composition studies in order to determine the effect these combinations of chemical and biological fertilizers may have, is recommended.

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