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RESEARCH PAPER

Vegetative growth of two mulberry species (*Morus multicaulis* and *M. alba*) under greenhouse and field conditions

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Abstract

M. Johnston, A. Olivares, and Y. Bustos. 2011. Vegetative growth of two mulberry species (*Morus multicaulis* and *M. alba*) under greenhouse and field conditions. Cien. Inv. Agr. 38(1): 41- 51. *Morus alba* and *Morus multicaulis*, common ornamental species in Chile, were studied regarding their growth and dry matter production. In Santiago (Metropolitan Region), plants from vegetative propagation, one part growing under field conditions and the other in a plastic greenhouse were used; both with similar soil, water availability and cutting system (0.20 and 0.40 m). Periodically, four shoots per selected plant were measured for length, number of leaves and dry weight. Previously, a correlation between dry matter and foliar size (length, width, area) of each species was established to estimate the latter. Correlation coefficients obtained had a R^2 higher than 0.60 whereas the length and width used to estimate foliar area had an R^2 of 0.96 to *M. multicaulis* and 0.95 to *M. alba* when correlated to the real area measured. The growth curve for total weight was superior for *M. multicaulis* at the beginning, but at the end was greatest for *M. alba*. In the greenhouse there were more leaves per shoot in *M. alba,* and there were no differences between species in the field. The shoot length changed with environmental conditions, as it was better with *M. alba* in the greenhouse and with *M. multicaulis* in the field. It was concluded that *M. alba* is more sensitive to the temperature regimes used in this study.

Key words: *Morus multicaulis*, *M. alba*, mulberries, temperature regimes, greenhouse, vegetative growth.

Introduction

Development of animal production systems currently demands to be more economical and profitable. One of the alternatives for achieving this is forage species of high nutritional quality and yield. Studies carried out to evaluate new options for improvement of profitable animal production in tropical areas indicate that mulberry has a good potential for milk and meat production in ruminants (Sangines *et al*., 2000).

Mulberry, an Urticales order tree in the Moraceae family and *Morus* genus, was selected because of its ancient use as food for silk worms, along with an improved nutritive value and foliar yield. It has been introduced and cultivated in a wide range of environments: in regions of Asia, Europe, Africa, and America, from sea level to 4,000 meters and from the humid tropics to semiarid and template zones, where specimens from different species may be found (Sánchez, 2000).

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The interest on intensive cultivation and livestock feed began in the eighties (Benavides, 2003). It develops adequately in tropical zones with temperatures between 18º and 38 ºC, with optimal temperature between 24º and 28 ºC; it tolerates abrupt temperature changes and conditions under 0 ºC in Mediterranean climates; with precipitations between 600 and 2,500 mm, with a photoperiod of 9 to 13 hours a day and a relative humidity of 65 to 80% (Datta, 2002).

In Chile, there are specimens established between the IV Region to the X Region which have been used for ornamental purposes for decades. The dominant species are *Morus alba* and *Morus multicaulis,* which present quite similar quality and acceptability features, but differ in growth habit and speed (Hernández, 2003). *M. multicaulis* grows fast and it has large, elliptical, soft and bright green leaves; *M. alba* is characterized by fine small leaves, with a light green plain surface, with branches that are flexible, very numerous, rough and slightly prickly (Machii *et al*., 2002). One of the differences observed in Chile, is that *M. alba* presents a period of foliar abscission with winter dormancy, while *M. multicaulis*, only stops growing in presence of frost, causing necrosis and a subsequent fall of leaves.

Due to the aforementioned characteristics, mulberry seems to represent an interesting alternative as a supplement for animal production, as a substitute for other sources of supplementation, like concentrates, which might not always be used by small or medium farmers due to the cost (Sangines *et al*., 2000). Therefore, studies on adaptation and propagation of mulberries as a possible forage species have been initiated. Thus, it has been determined that leaves reach values of 25% gross protein, 85% apparent digestibility and 28% acid detergent fiber and, that these values vary with the frequency of use (Hernández, 2003).

It has also been observed that edaphoclimatic conditions (soil, humidity, temperature, brightness) determine the behavior of this species and, that different mulberry species present different potential production. Therefore, the need arises to know and evaluate the growth of the two dominant species in Chile, *M. multicaulis* and *M. alba*, in different conditions of temperature and management in order to determine a possible adaptation to different agroecological zones and exploit the production potential of both species properly.

The hypothesis of this work states that the behavior, in regard to the growth dynamics of *Morus multicaulis* and *Morus alba*, differs according to the environmental conditions, particularly the thermal regime will determine where they develop. Therefore, the objective of the study is to evaluate the growth in both species, in different environmental conditions (natural and greenhouse) and with different cutting intensities (0.40 and 0.20 m-residuals).

Materials and methods

The work was carried out in the Faculty of Agronomic Sciences of the Universidad de Chile, located in the Metropolitan Region (33º 40’ LS and 70º 38’ LO), in a alluvial soil belonging to the Santiago soils series (Chile), with a mean depth of 60 cm, plain topography, clay loam texture and good drainage (Comisión Nacional de Riego, 1981). The climate is temperate mesothermal stenothermal semiarid Mediterranean, with a thermal regime characterized by temperatures varying between a maximum mean of 28.7 ºC in January, and a mean minimum of 3.4 ºC in July (Santibáñez and Uribe, 1990). The water regime presents precipitations concentrated in winter months, with an average of 330 mm, a dry period of eight months (September to April) and a water deficit of 1,030 mm, with hot and dry summers and cold winters.

Two-year old *Morus multicaulis* and *Morus alba* plants were used from cuttings; one portion was placed inside a polyethylene greenhouse, the other in a natural environment, with equal soil, and weekly irrigation by furrows. The thermal conditions in the field varied between 4.1º and 31.9 ºC as weekly average and between 5.3º and 48.9 ºC in the greenhouse (Table 1).

Ten plants for each condition were randomly selected from a total of 119 individuals of *Morus*

Table 1. Maximum and minimum mean temperatures (°C) per week in greenhouse and in field of Antumapu Experimental Station.

Weeks		Greenhouse		Field	
		Minimum	Maximum	Minimum	Maximum
Jul.	4	5.3	34.4	4.1	14.8
Aug.	1	5.7	36.0	5.3	13.2
2006	2	5.3	36.4	4.8	15.7
	3	6.9	37.2	7.1	21.7
	4	6.0	39.1	6.0	16.3
Sept.	1	6.0	42.4	5.4	19.3
2006	2	7.4	40.6	7.1	17.0
	3	5.6	46.7	7.2	18.8
	4	6.9	46.1	8.3	24.2
Oct.	1	7.0	35.8	7.7	19.4
2006	2	8.9	38.7	8.7	22.8
	3	9.0	40.1	7.7	20.4
	4	11.6	47.9	9.9	24.2
Nov.	1	7.3	41.4	7.7	21.5
2006	2	6.9	41.4	9.1	25.8
	3	9.6	44.9	9.0	23.1
	4	11.0	47.6	10.8	28.9
Dec.	1	10.0	44.3	10.7	27.0
2006	2	11.9	44.6	10.3	29.1
	3	11.4	45.0	10.9	27.8
	4	10.4	44.6	10.5	27.5
Jan.	1	12.6	46.6	14.4	31.9
2007	2	15.3	47.6	13.7	30.7
	3	16.1	48.9	14.3	29.2
	4	15.6	48.6	12.4	28.4
Feb.	1	17.0	47.4	12.7	30.8
2007	2	16.1	44.7	13.4	28.7
	3	13.6	43.4	11.5	26.2
	4	13.6	43.1	10.6	26.5

multicaulis in the field plantation, and were permanently managed with different cutting heights (0.20 and 0.40 m). Simultaneously, 10 plants were selected for each condition in a population of 46 individuals planted inside a polyethylene greenhouse and were managed with the same cutting intensities. In the case of *M. alba,* the same procedure was applied to a population of 81 plants in the field and 46 individuals in the greenhouse.

Eight treatments with 10 repetitions each were established with this material:

- T_1: *Morus alba*, in field with 0.20 m-residual.
- T_2: *Morus alba*, in field with 0.40 m-residual.
- T_3: *Morus alba*, in the greenhouse with 0.20 m-residual.
- T_4: *Morus alba*, inside the greenhouse with 0.40 m-residual.
- T_5: *Morus multicaulis*, in field with 0.20 m -residual.
- T_6: *Morus multicaulis*, in field with 0.40 m -residual.
- T_7: *Morus multicaulis*, in the greenhouse with 0.20 m -residual.
- T_8: *Morus multicaulis*, in the greenhouse with 0.40 m -residual.

Correlations between dry weight and foliar size were determined by specifying the determination coefficients (R^2) and the respective equation before the beginning of the test because there were a limited number of individuals to measure dry matter by harvest of the vegetative material (length - width – foliar area) for each species.

In order to obtain the growth curve, condition and management in each species, four representative shoots from each selected plant were marked, one for each orientation (North-South-East-West) at the beginning of the season and the number of new shoots or ramifications, their length, the leaves number formed in each, the length and maximum width of each leaf and their dry weight were measured every 20 days. The mean values of the four orientations were multiplied by the total number of plant shoots to obtain the plant dry matter.

Additionally, the temperatures presented in each environment were recorded daily with a maximum – minimum thermometer.

A completely randomized statistical analysis was used for each species, with a factorial structure of 2x2x2 (species, environmental condition, cutting height), with eight treatments and ten repetitions per treatment. The experimental unit was the mulberry plant. The experimental model used was:

$$Y_{jklmn} = \mu + S_i + E_j + A_k + O_l + e_{ijklmn}$$

where:
Y= response.
S_i= effect of the species (i = *M. multicaulis, M. alba*).
E_j= effect of the environment (j=greenhouse, in field).
A_k= effect of the height -residual (k= 20, 40 cm).
O_l= effect of the orientation (l= North, South, East, West).
e_{ijklmn}= sampling error.
μ= general average.

The analyzed variables-response were: number of shoots per plant, length of new shoots (in each orientation), average leaf length, maximum average of leaf width, number of leaves per shoot and dry weight of the shoot. The number of shoots per plant does not have any sampling error as it corresponds to only one datum. The statistical analysis of these variables was made through the program Keditw of the Statistical Analysis System (SAS). The double interactions, showing significant differences in a preliminary analysis, were included.

Results and discussion

From the values obtained for the estimation of the determination coefficient (R^2) and the respective equations relating leaf width and length with dry weight (Table 2), it was deduced that all the parameters had a determination coefficient higher than 60; therefore, the width and length were used to calculate the leaf area, considering it as an ellipsis, due to the shape, according to the following equation:

$$\text{Leaf area} = \frac{\text{maximum leaf width}}{2} \times \frac{\text{leaf length}}{2} \times \pi$$

The determination coefficient was high for both species (Table 2); then, the leaf area was estimated with these values and therefore, the dry weight foliar was estimated. When the foliar area was measured with a leaf area meter ADC BioScientific model AM200, and it was calculated with the correlations, a high relation of the area was ratified with the dry weight in both species, with a R^2 of 0.96 for *M. multicaulis* and 0.95 for *M. alba*.

Table 2. Determination coefficients for dry weight to maximum leaf width, length or leaf area in *Morus multicaulis* and *Morus alba*.

Species	Relation	R^2	Type equations
M. multicaulis	Maximum width.- Dry weight	0.90	$y = 0.133x_1 – 0.4453$
	Maximum length - Dry weight	0.70	$y = 0.0099x_2 – 0.4284$
	Area – Dry weight	0.94	$y = 0.0062x_3 + 0.0884$
	Maximum width – Dry weight	0.85	$y = 0.142x_4 – 0.5661$
M. alba	Maximum length– Dry weight	0.83	$y = 0.0088x_5 – 0.4069$
	Area – Dry weight	0.87	$y = 0.0076x_6 + 0.002$

y = leaf dry weight; x_1 and x_4 = maximum width; x_2 and x_5 = maximum length; x_3 and x_6 = leaf area calculated for *M. multicaulis* and *M. alba*.

Shoot growth

In regard to the growth dynamics, it is equal for both species, as the gradients are equal. However, when the effect of the species on the production of foliar dry matter (FDM) per shoot was analyzed, a higher production in *Morus multicaulis* was observed from the beginning of the process, reaching 22.5% more at the end of the period (Figure 1A). This could be due to the fact that *M. alba* presents in Chile total foliar abscission with winter dormancy between June and September. On the other hand, *M. multicaulis* reinitiates growing immediately after the cutting that took place in May (Hernández, 2003). Additionally, *M. multicaulis* has larger leaves, which affect the amount of dry matter directly.

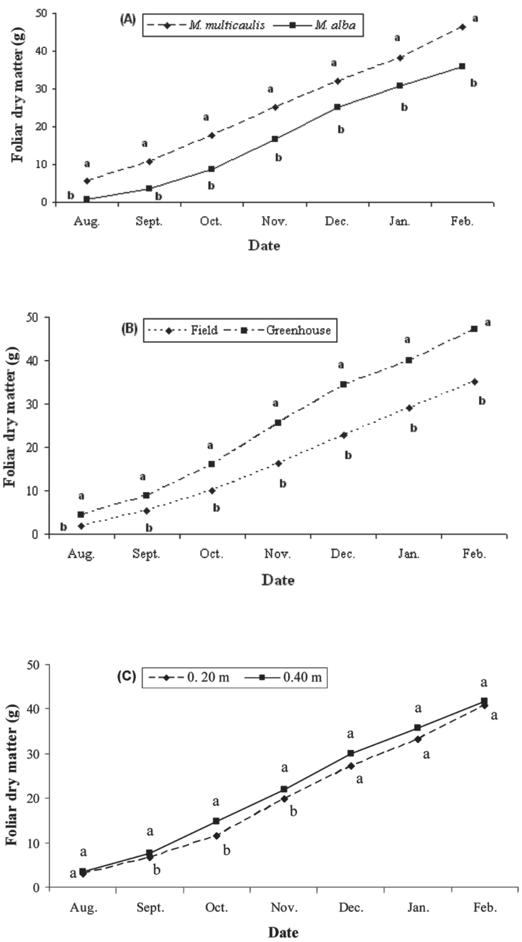

Figure 1. Mean production of leaves dry matter according to species (A), environment (B) and initial height of remains (C).

The controlled environment (greenhouse) was significantly positive for both species, resulting in a growth superior to that in the field by 25.3% (Figure 1B). This could be due to the temperatures recorded in the field, with an average of 8.4 ºC in winter and 21.5 ºC in the summer, as compared to the average temperature in the greenhouse of 10º and 42 ºC, respectively. Similarly, according to Sánchez (2000), these species express a higher growth with temperatures between 18º and 38º C, with an optimal temperature between 24º and 28º C.

The highest residual height (Figure 1C) provoked a higher increase in the production of foliar dry matter only at the beginning of the growth period (August – October). This was possibly related to wood-like plants storing food reserves in the trunk, and, therefore, 0.40 m-residual plants, would have higher reserves than 0.20 m-residual plants, starting their recovery earlier. However, this initial advantage would later be lost due to the magnitude reached by the final foliar area.

The shoot lengths were also superior in *M. multicaulis,* reaching 78% more in the first measurement, but this difference dissipated with the course of the season as it reduced to only 2.1% (Figure 2A). The effect of the environment was significant for both species, which were longer in the greenhouse (Figure 2B). The initial cutting height presents differences every two months in the first growth stage of the shoots (Figure 2C), suggesting an important role of the reserves availability.

The average number of leaves per shoot was superior in *M. alba* since September, except by the initial measurement (Figure 3A), probably due to the early growth in *M. multicaulis*; this difference is equivalent to 24.4% and it is related to the botanical features of each species (Alonso *et al*., 2000). As most of the species of the genus *Morus* are considered tropical plants (Pizarro, 1997) and, regardless the genus presents high plasticity by their capacity to adapt to different climates and altitudes (Benavides, 1995), the average leaf number in all cutting heights and species was superior inside the greenhouse, with a difference of 32.5% (Figure 3B). In the factor of initial cutting height, there was no effect on the number of leaves developed (Figure 3C), which is coherent with the results obtained

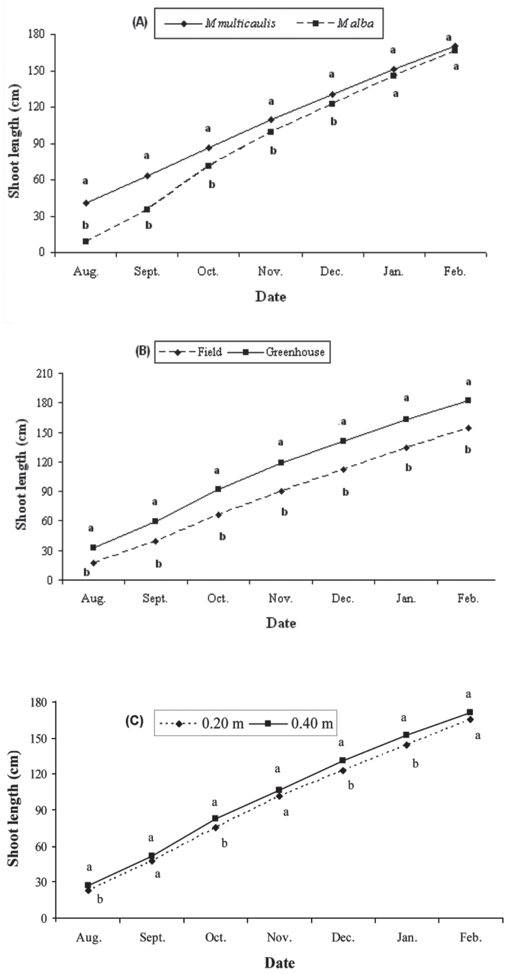

Figure 2. Mean length of shoots according to species (A), environment (B) and initial height of remains (C).

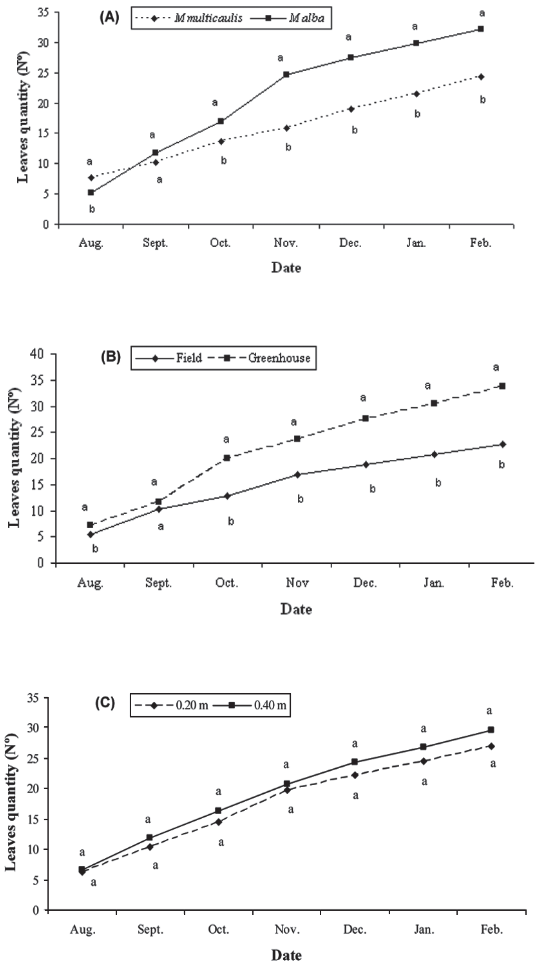

Figure 3. Mean number of leaves per shoot according to species (A), environment (B) and initial height of remains (C).

by Noda *et al*. (2007), who evaluated the effect of a cut at 50 and 100 cm.

The statistical analysis showed different interactions. The first was between species and environment, for the production of foliar dry matter per shoot (Figure 4A) and for the shoot lengths (Figure 4B). Therefore, it was observed that the *M. multicaulis* plants present scarce differences for both parameters as the environment changes; on the other hand, *M. alba* increases its FDM production and shoots length when it grows in the greenhouse. It is noteworthy that a lower growth rate was produced inside the greenhouse in mid-December, possibly due to the maximum temperatures over 45 ºC (Table 1); this condition affected greatly *M. multicaulis*. It is well known that most of the mesophytic plants slow down their growth over 40 ºC and only xerophytic plants are able to survive under those conditions. In this regard, it was observed that leaves from *M. alba* showed a more coriaceous condition. On the other hand, it has been determined that the growth rate and the phenology of three wood-like species from temperate zones (fraxinus, beech, oak) were equally related to the annual temperature from their places of origin (Vitasse *et al*., 2009).

There was also an interaction between species and initial cutting height with the FDM parameter, as the shoots of *M. multicaulis* with 40 cm-residual, presented productions significantly higher (Figure 5) than the productions for *M.*

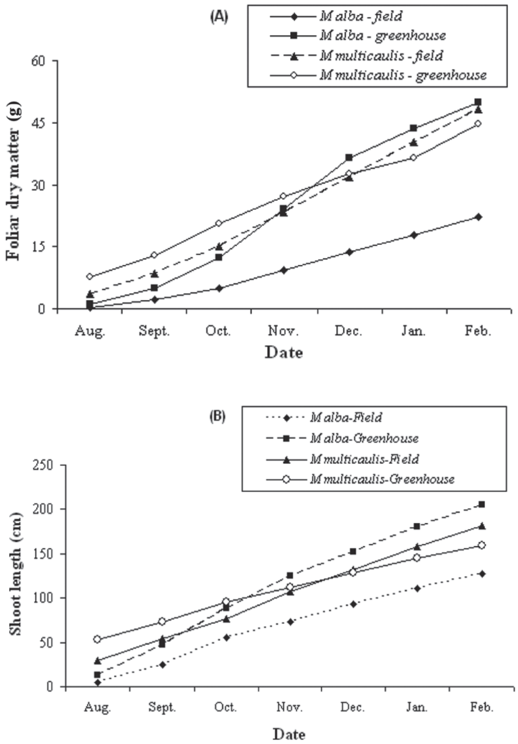

Figure 4. Interaction species x environment in leaves dry matter production (A) and in shoots length (B).

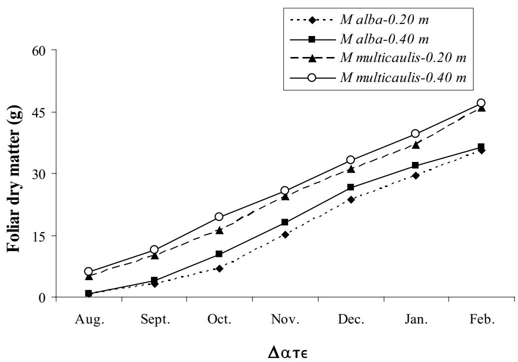

Figure 5. Interaction species x initial height remains in foliar dry matter production.

alba, at both cutting heights. This might suggest that both species differ in magnitude and/ or localization of their reserves.

Additionally, there was interaction between the environment and the initial cutting height as the foliar dry matter; the shoot length and the number of leaves per shoot were evaluated (Figures 6A, B and C). The plants in the greenhouse presented the highest productions, at both cutting heights, where the 0.40 m-residual plants were superior; in the field, the cutting treatment did not produce differences. Similarly, the shoot length and the leaf number formed in them showed the same, where the 0.40 m-residual treatment was always superior in the field. These results suggest that *M. multicaulis* and *M. alba* would have a higher yield, in conditions of high temperature and humidity with a 0.40 m-residual after cutting.

Plant growth

The total of shoots present in the plants through time was considered for these evaluations, according to the growth obtained for each parameter, with averages for the four shoots analyzed (one for each orientation).

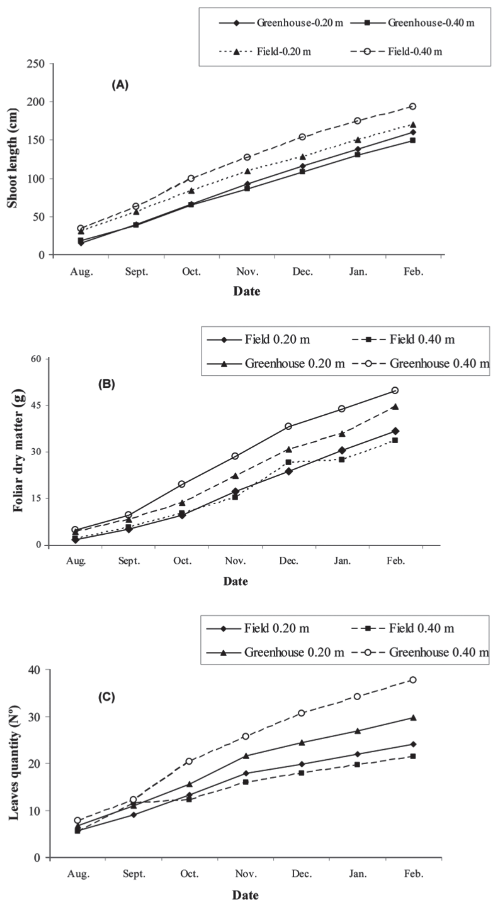

Figure 6. Interactions environment x initial height remains in shoots length (A), x foliar dry matter production (A) and x leaves number (C).

The shoot number was superior in *M. multicaulis* only at the beginning of the growth season (August) and then it stabilized. On the other hand, *M. alba* increased significantly until November. Subsequently, it stabilized in a superior level (75.8%) to the total shoots from *M. multicaulis* (Figure 7A), supporting the different growth habit of these two species. This response is coherent with data from Yongkang (2000), who indicated a big difference in ramification for both species. The effect of the environment on branch formation, regardless of the species, showed superior values in the greenhouse with a strong increase during the three first months of growth, which soon stabilized, a tendency that was repeated in the field (Figure 7B). When the initial residual was included without species discrimination, the average number of shoots was always superior when it was cut at 0.40 m (Figure 7C). This result could be attributed to the existence of a higher level of reserves in the trunk, along the results obtained for other parameters.

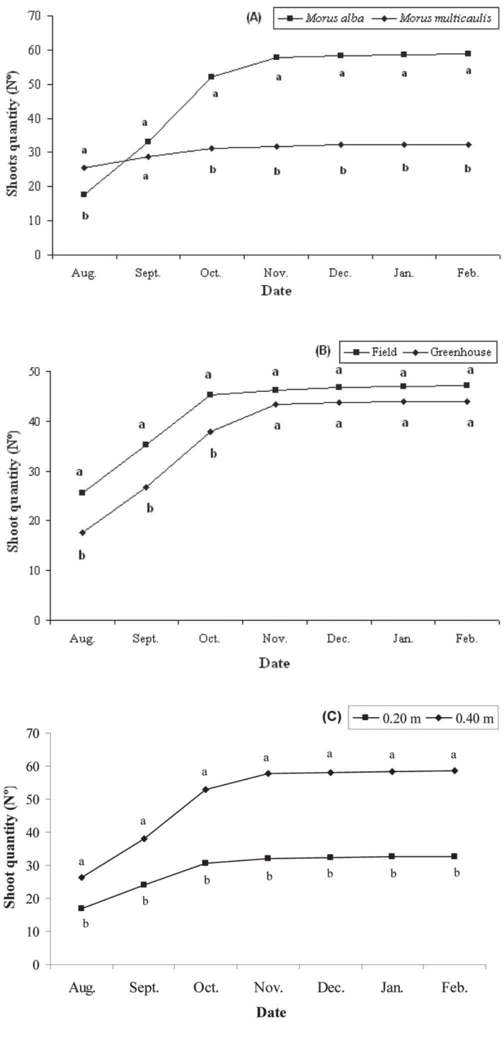

Figure 7. Mean shoots quantity per plant according to species (A), environment (B) and initial height remains (C).

The production of foliar dry matter per plant was affected differently, according to the considered factor (Figures 8A, B and C). Therefore, in regard to the species, it was observed that *M. multicaulis* had a higher initial growth, but it was overcome by *M. alba* in November by 28.5%, due to a higher number of shoots and amount of leaves forming with that species. Therefore, the larger size of *M. multicaulis* leaves is compensated. These values also allow a better understanding of the results by Noda *et al*. (2007) who evaluated two frequencies and two cutting heights in field plantations of *M. alba* in Cuba, obtaining higher productions with cuts every 90 days and with 50 cm-residual heights. The greenhouse environment improved the production of foliar dry matter (Figure 8B), regardless of the other factors studied, which is understandable due to the higher temperatures and humidity presented. However, it is noteworthy that production reached in the field (1,537.3 g), should be considered as good, when compared with other studies involving *M. alba* (García *et al*., 2000; Boschini, 2001), which supports mulberry as a viable alternative for the central zone of Chile.

When the production of foliar dry matter per plant was included, interactions between species-environment and between initial cutting environment-height were observed. The environment did not affect the FDM *M. multicaulis* production, but significantly influenced *M. alba*, which developed exceptionally well inside the greenhouse (Figures 9A); this might show that *Morus alba* is more sensitive to the environment where it grows. In *M. alba* (Figure 9B) there was an effect of the initial height on the FDM production, as the plants with 0.40 m-residual grew better. On the other hand, *M.* multicaulis did not present higher differences with both cutting heights, although higher values with 0.40 m were observed. It is deduced from these results that *Morus alba* would grow higher in warmer conditions, only with sufficient water level, and it would have a better tolerance to colder conditions.

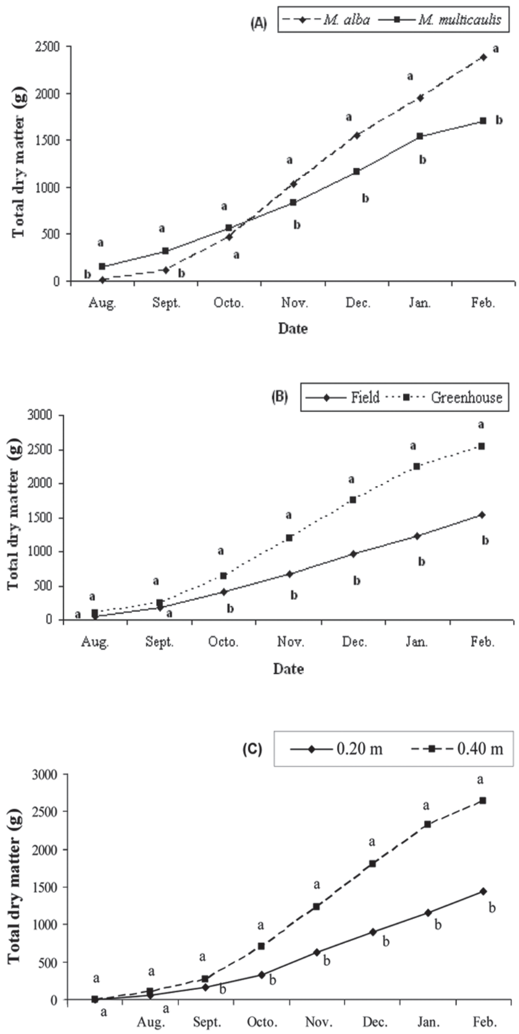

Figure 8. Mean total dry matter per plant during growth period according to species (A), environment (B) and initial height of remains (C).

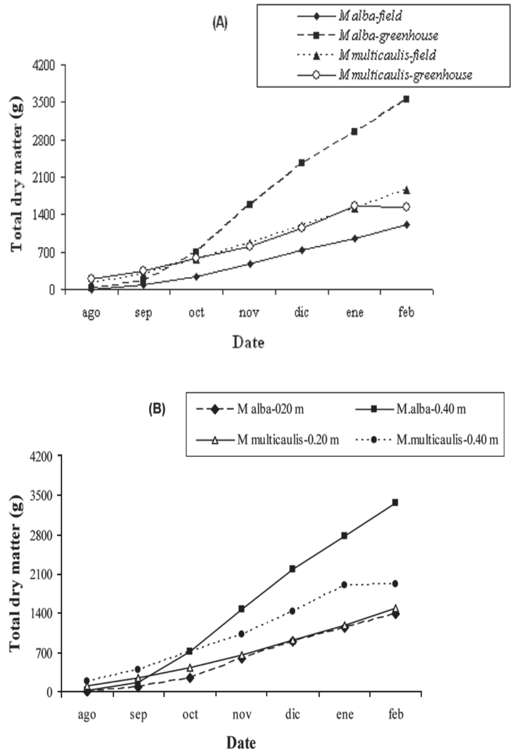

Figure 9. Foliar dry matter per plant in the interaction species x environment (A) and in species x initial height remains (B).

Thermal regimes of the environment are essential for the growth dynamics and magnitude for both species; where *M. alba* presented higher sensitivity.

In *M. alba,* a higher residual (0.40 m cutting height) also increases the production of foliar dry matter.

Resumen

M. Johnston, A. Olivares e Y. Bustos. 2011. Crecimiento de dos especies de morera (*Morus multicaulis* y *M. alba*), en condición controlada de invernadero y en condición de campo. Cien. Inv. Agr. 38(1): 41- 51. En *Morus alba y M. multicaulis,* especies ornamentales comunes en Chile, se estudió su crecimiento y productividad en dos ambientes. Se trabajó en la Región Metropolitana, con plantas propagadas vegetativamente, la mitad plantada dentro de un invernadero de polietileno y la otra en ambiente natural, ambas con condiciones de suelo, disponibilidad de agua y sistema de corte (0,20 y 0,40 m) similares. Se midió periódicamente en cuatro brotes marcados por planta: el largo de éstos, el número de hojas formadas y su peso seco. Previamente se estableció una correlación entre el peso seco y el tamaño foliar (largo, ancho, área), en cada especie, con el fin de usar alguno de estos parámetros para estimar la materia seca. Los coeficientes de correlación obtenidos mostraron un R^2 superior a 0,60, por lo que se usó el largo y ancho, para estimar el área de hoja, y al correlacionar el área calculada con el área real medida se obtuvo 0,96 y 0,95, para *M. multicaulis y M. alba*, respectivamente. La curva de crecimiento en función del peso total por planta así estimado fue inicialmente superior en *M. multicaulis*, pero avanzada la estación, fue mayor en *M. alba*. En condiciones de invernadero se obtuvo una mayor cantidad de hojas formadas por brote para *M. alba*, y no se obtuvo diferencias entre ambas especies en el exterior. El largo de los brotes difiere en cuanto a la mejor condición ambiental para su crecimiento, que es el invernadero para *M. alba* y el exterior para *M. multicaulis*. Se concluye que *M. alba* es más sensible a los regímenes térmicos con que se trabajó.

Palabras clave: *Morus multicaulis*, *M. alba*, moreras, regímenes térmicos, invernadero, crecimiento vegetativo.

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