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# Growth and nutrient uptake patterns in plants of *Duboisia* sp

## Crescimento e marcha de absorção de nutrientes em plantas de *Duboisia* sp

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### Abstract

Characterizing growth and nutrient uptake is important for the establishment of plant cultivation techniques that aim at high levels of production. The culturing of *Duboisia* sp., although very important for world medicine, has been poorly studied in the field, since the cultivation of this plant is restricted to a few regions. The objective of this paper is to characterize growth and nutrient absorption during development in *Duboisia* sp. under a commercial cultivation system, and in particular to assess the distribution of dry matter and nutrients in the leaves and branches. Our work was performed on a commercial production farm located in Arapongas, Paraná, Brazil, from March 2009 to February 2010. A total of 10 evaluations took place at approximately 10-day intervals, starting 48 days after planting and ending at harvesting, 324 days after planting. The growth parameters analyzed were the height of the plants and the dry matter of the leaves and of branches, while the chemical composition of the leaves and branches was used to study nutrient absorption. Data were submitted to regression analysis. Growth in height followed Richards' model, mirroring the rise in air temperature and water availability. Phytomass accumulation in the aerial parts of the plants was slow during the first 150 days, but grew 25 times from that point to harvesting. Starting from 260 days, phytomass accumulation in the leaves began to be notable, while in the branches, a growth of 169% occurred, a pattern of biomass partitioning that is unfavorable for the producer. Accumulation of nutrients mirrored the accumulation of phytomass, showing the same unfavorable partition. The nutritional demand for macronutrients and micronutrients was, respectively,  $N < K < Ca > P > Mg > S$  and  $Fe > Mn > Zn > B > Cu$ .

**Key words:** Dry matter. Medicinal plants. Nutrition. Partition. Physiology.

### Resumo

A caracterização do crescimento e da absorção de nutrientes de uma cultura é de grande importância para o estudo e o estabelecimento de técnicas de cultivo que visam à obtenção de elevado desempenho produtivo. A cultura da *Duboisia* sp., apesar de muito importante para a medicina mundial, tem seus aspectos da produção em campo pouco estudados, pois se trata de um cultivo restrito a poucas regiões. O objetivo deste trabalho foi caracterizar o crescimento e a marcha de absorção de nutrientes em plantas de *Duboisia* sp. sob condição comercial de cultivo, bem como a partição de matéria seca e nutrientes em folhas e ramos. O trabalho foi realizado em uma fazenda de produção comercial, localizada no município de Arapongas, PR, no período de março de 2009 a fevereiro de 2010. As avaliações em intervalos de aproximadamente 30 dias, iniciando-se aos 48 dias após o plantio e finalizando com a colheita, 324 dias após o plantio, totalizando 10 avaliações. Os parâmetros de crescimento analisados foram altura de planta, massa seca de folhas e massa seca de ramos, enquanto a composição química de folhas e ramos foi utilizada para estudar a absorção de nutrientes. Os dados foram submetidos à análise de regressão. O crescimento em altura ocorreu segundo o modelo de Richards, acompanhando a elevação da temperatura do ar e a disponibilidade hídrica. O acúmulo de fitomassa da parte aérea foi

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lento nos primeiros 150 dias e apresentou incremento de 25 vezes a partir desse ponto até a colheita. A partir dos 260 dias, o acúmulo de fitomassa de folhas passou a ser pouco significativo, enquanto o de ramos teve um incremento de 169%, evidenciando uma partição de biomassa desfavorável ao produtor. O acúmulo de nutrientes acompanhou o acúmulo de fitomassa e, portanto, também mostrou a mesma partição desfavorável. A ordem de demanda nutricional para macronutrientes e micronutrientes foi, respectivamente,  $N > K > Ca > P > Mg > S$  e  $Fe > Mn > Zn > B > Cu$ .

**Palavras-chave:** Fisiologia. Matéria seca. Nutrição. Partição. Plantas medicinais.

## Introduction

The genus *Duboisia* belongs to the Solanaceae family, which includes important species for world medicine, such as *D. myoporoides* and *D. leichhardtii*, two species that naturally occur only in Australia and New Caledonia. *Duboisia* plants are perennial woody shrubs and can reach up to 14 meters in height. Their importance comes from the tropane alkaloids in the leaves, which have been used primarily by the native peoples living in the plants' original range, and also in war medicines, and nowadays in anti-spasmodic and mydriatic drugs by the pharmaceutical industry (FOLEY, 2006; OHLENDORF, 1996).

Because laboratory production of the plants' main active compounds is difficult, they are usually collected exclusively from field cultivation of the plants (FOLEY, 2006).

Because *Duboisia* is restricted to only a few regions in the world, unlike cereals and oleaginous plants, few studies have been made of its field management. Furthermore, because the supply of its active compounds to industry depends upon the success of agricultural production, it is fundamental that an adequate production system be established. Understanding and characterizing the ecophysiology of these plants is therefore critical for the production process.

The study of phytomass accumulation, growth patterns, and nutrient absorption permits the identification of periods of greater and lesser nutritional demand, as well as the distribution of photosynthates among the organs of the plant according to its age and the time of the year, while also making possible comparisons among genotypes,

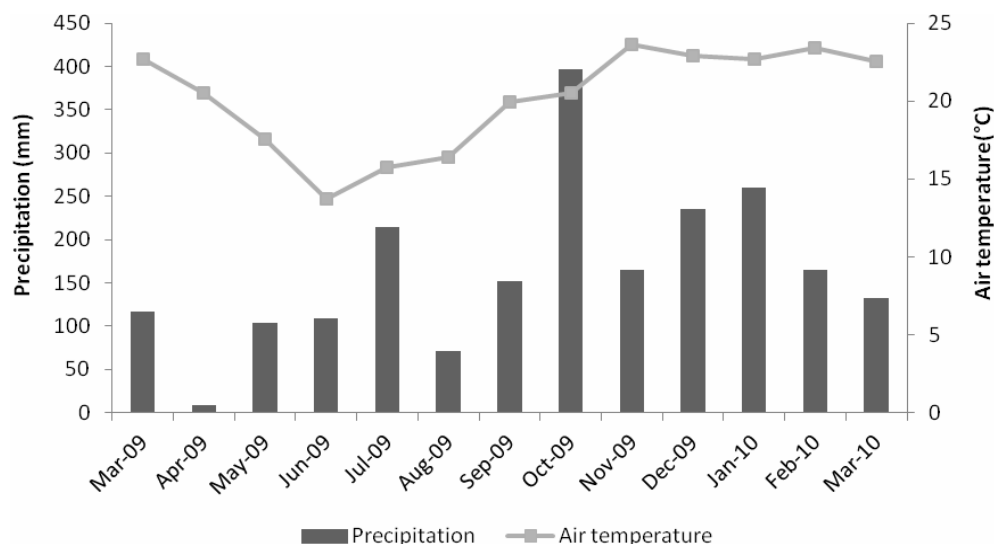
and times for planting and fertilizing. As a result, it will be possible to establish effective agronomical practices, determine critical management times, predict the nutrient consumption and destination of photosynthates, create strategies for the positioning of genotypes, estimate harvest time, and evaluate new production systems (DUARTE et al., 2003; GONDIM et al., 2011; MAUAD et al., 2013; OLIVEIRA et al., 2000; ZABOT et al., 2004).

Moreover, as the normal patterns of growth and development for a plant are documented, easily measured parameters can be established that can be practically evaluated during production, so as to follow the development of the plants and identify ongoing irregularities, a procedure that will create a routine for continuous improvement.

The objective of this study was to characterize the accumulation of phytomass, the growth in plant height, the nutrient absorption rate, and the biomass and nutrient partitioning among the leaves and branches in *Duboisia* sp. under commercial cultivation.

## Materials and Methods

Our work was performed in a *Duboisia* commercial production area in Arapongas, Paraná, Brazil (23°24'27" S; 51°21'55" E), between March 2009 and February 2010. According to the Köppen climatic classification (KÖPPEN, 1936), the region's climate is Cfa (humid temperate with hot summer), with an annual average temperature of 19.9°C and average annual precipitation of 1497 mm. Figure 1 shows the meteorological data on precipitation and air temperature collected between March 2009 and March 2010 by sensors located on the farm.

**Figure 1.** Precipitation (mm) and air temperature (°C) during the experimental period. Arapongas, PR.

The soil was classified as dystrophic oxisoil (EMBRAPA, 2006) of a very clayey texture, and was chemically analyzed according to EMBRAPA (2009). Results of chemical analysis are shown in Table 1.

**Table 1.** Soil test results of the experimental area with *Duboisia* sp. plants. Arapongas, PR.

Depth	pH	OM*	H+Al	Ca <sup>2+</sup>	Mg <sup>2+</sup>	K <sup>2+</sup>
---cm---		---g dm <sup>-3</sup> ---		-----cmol <sub>c</sub> dm <sup>-3</sup> -----		
00 a 10	4,26	38,21	6,99	0,89	0,49	0,78
10 a 20	4,25	33,49	6,84	1,10	0,40	0,45
20 a 40	4,35	20,75	5,89	1,06	0,40	0,21

Depth	Extractable-P	Rem-P**	Base Saturation	Al <sup>3+</sup>	CEC
---cm---	---mg dm <sup>-3</sup> ---	---g dm <sup>-3</sup> ---	-----%-----		--cmol <sub>c</sub> dm <sup>-3</sup> --
00 a 10	5,98	17,68	23,70	25,93	9,16
10 a 20	4,72	15,44	22,25	30,20	8,79
20 a 40	1,45	12,49	22,11	26,60	7,56

\*Organic matter. \*\*Remaining phosphorus. Extractors: pH, CaCl<sub>2</sub>; OM, Walkley-Black; Ca-Mg-K, KClIN; Extractable-P, Mehlich-1; Rem-P, Mehlich-1.

The experimental area was 17600 m<sup>2</sup>, with 5 m borders. Seedlings were planted on March 21<sup>st</sup>, 2009, at 3.50 × 1.30 m spacing, for a total of 3968 plants in the entire area and 3393 plants in the useful area.

Evaluations were made of both dry phytomass of leaves and branches (DPL and DPB), chemical composition of leaves and branches, and also the

height of the plants, measured 10 different times at intervals of approximately 30 days, starting 48 days after planting (48, 79, 109, 140, 201, 232, 262, 293, and 324 days after planting). Dry phytomass of the aerial parts (DPAP) was calculated as the sum of DPL and DPB, and the relative height of the plants was calculated by treating the final height measurement as 100%.

The experimental design used was totally randomized, with 12 replications for plant height and dry phytomass of leaves and branches, and three replications for chemical composition. Phytomass and chemical composition data were used to calibrate the growth curves and the rates of nutrient absorption, respectively.

The soil was amended before planting, with the objective of achieving saturations of 65%  $\text{Ca}^{2+}$  and 15%  $\text{Mg}^{2+}$  (KINSEY; WALTERS, 2009), and 150% of relative phosphate (ALVAREZ et al., 2000), as well as neutralizing the toxic aluminum on the surface, so as to allow for the deepening of the root system, and increasing the bioavailability of micronutrients and sulfur. For that purpose, 3000  $\text{kg ha}^{-1}$  of calcite limestone (44%  $\text{CaO}$ , 4%  $\text{MgO}$ , 88% PRNT), 1100  $\text{kg ha}^{-1}$  of dolomitic limestone (29%  $\text{CaO}$ , 18%  $\text{MgO}$ , 86% PRNT), 1500  $\text{kg ha}^{-1}$  of agricultural gypsum (17%  $\text{Ca}$ , 14%  $\text{S}$ ), 220  $\text{kg ha}^{-1}$  of thermal phosphate (10%  $\text{P}_2\text{O}_5$  sol. citric acid 2%), and 130  $\text{kg ha}^{-1}$  of simple superphosphate (18%  $\text{P}_2\text{O}_5$  sol.  $\text{CAN}+\text{H}_2\text{O}$ ) were distributed by hauling, followed by moldboard plowing to a depth of 20 cm, and then application of 2600  $\text{kg ha}^{-1}$  of calcite limestone, 1200  $\text{kg ha}^{-1}$  of dolomitic limestone, 240  $\text{kg ha}^{-1}$  of thermal phosphate, and 140  $\text{kg ha}^{-1}$  of simple superphosphate (18%  $\text{P}_2\text{O}_5$  sol.  $\text{CAN}+\text{H}_2\text{O}$ ), and finally leveling harrowing. On October 10<sup>th</sup>, 2009, 100  $\text{kg ha}^{-1}$  of urea (45%  $\text{N}$ ) was made upon the tree top projection.

The height of the plants was measured with a 2.50 m wooden ruler, which was positioned next to the central axis of the plant so as to measure the distance between the soil surface and the tree top.

For the biomass evaluation, the aerial part was collected by cutting the neck of the plant close to the soil with pruning scissors. The material was placed on a shading screen and desiccated at 65°C for 24 h in an industrial desiccator. After drying, branches and leaves were manually separated for weighing and then milled for chemical analysis.

For the evaluation of the nutrient absorption rate, samples of leaves and branches were taken to the laboratory for chemical analysis of  $\text{N}$ ,  $\text{P}$ ,  $\text{K}$ ,  $\text{Ca}$ ,  $\text{Mg}$ ,  $\text{S}$ ,  $\text{B}$ ,  $\text{Cu}$ ,  $\text{Fe}$ ,  $\text{Mn}$ , and  $\text{Zn}$ , following the methods specified by EMBRAPA (2009).

Data were submitted to a regression analysis by Curve Expert 1.4 for Windows (HYAMS, 1997), so as to find the best mathematical model for each variable. The eventual choice of the model was made based on the greatest coefficient of determination ( $r^2$ ) and minimal pattern error of estimation ( $S$ ), together with a satisfactory explanation for the biological behavior.

The data of DPL and DPB were split into periods (up to 201 days and after 201 days), to which different models were fitted. For the height of the plants (cm), relative height of the plants (%), the DPAP ( $\text{g plant}^{-1}$ ), and the DPL after 201 days ( $\text{g plant}^{-1}$ ), the chosen model was Richards', expressed by Equation 1, in which  $y$  is the analyzed variable,  $x$  is the age (days) and  $a$ ,  $b$ ,  $c$ , and  $d$  are coefficients to be estimated.

$$y = \frac{a}{(1 + e^{b-cx})^{1/d}} \quad (1)$$

The best fits for DPL up to 201 days ( $\text{g plant}^{-1}$ ) and DPB up to 201 days ( $\text{g plant}^{-1}$ ) were obtained by means of the Geometric Model (Equation 2), while for DPB after 201 days ( $\text{g plant}^{-1}$ ), the Gaussian Model presented the best fit. In these equations, as in Equation 1,  $y$  is the analyzed variable,  $x$  is the age (days), and the other symbols are coefficients to be estimated.

$$y = ax^{bx} \quad (2)$$

$$y = ae^{\frac{-(b-x)^2}{2c^2}} \quad (3)$$

The model chosen for the absorption rates of  $\text{N}$ ,  $\text{K}$ ,  $\text{B}$ ,  $\text{Cu}$ ,  $\text{Fe}$ ,  $\text{Mn}$ , and  $\text{Zn}$  was the Gaussian

(Equation 3). For P, Ca, and S, the best model was the Modified Exponential (Equation 4), while the model identified as Vapor Pressure (Equation 5) was the one chosen for Mg. As in the other equations,  $y$  is the analyzed variable,  $x$  is the age (days), and the remaining symbols are coefficients to be estimated.

$$y = ae^{b/x} \quad (4)$$

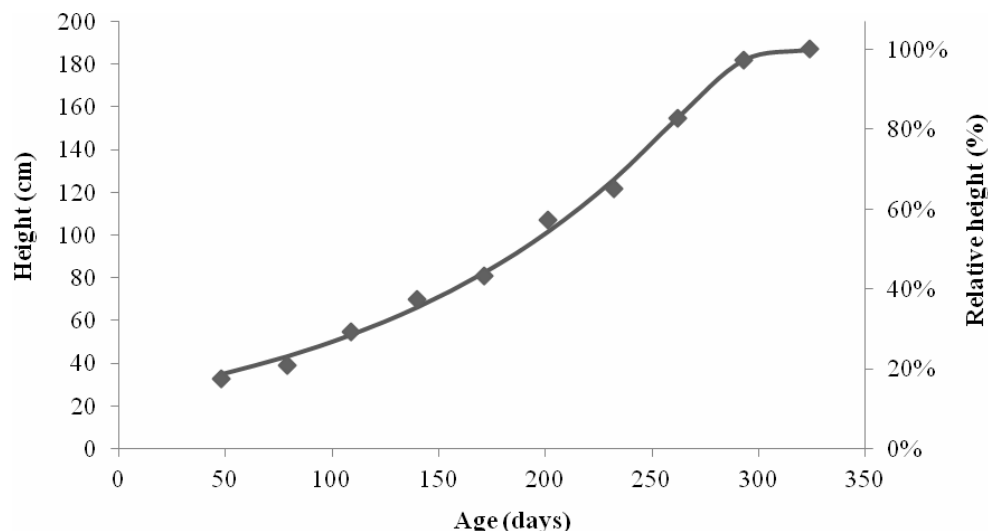
$$y = e^{a + \frac{b}{x} + c \ln x} \quad (5)$$

Because the analyzed period included the complete cycle from planting to first harvest, the adjusted models could not be extrapolated to other periods.

## Results and Discussion

The growth in height of the *Duboisia* plants, based on age, was satisfactorily explained by Richards' Model (Figure 2) at a coefficient of determination ( $r^2$ ) greater than 0.99, apparently mirroring the rise in temperature and water availability, with which variables this growth exhibited correlation coefficients of 0.88 and 0.43, respectively. The three first measurements were an exception: in them, growth did not appear to be considerably affected by the lower temperatures and lower rainfall volumes. The mathematical models that describe the growth in height, with their respective estimated coefficients, the determination coefficient, and the pattern error of estimate, are presented in Table 2.

**Figure 2.** Plant height of *Duboisia* sp. as a function of age (days). Arapongas, PR.



**Table 2.** Coefficient of determination ( $r^2$ ), standard error of the estimate (S) and estimated coefficients of the mathematical models adjusted for height (cm) and relative height (%) of *Duboisia* sp. plants as a function of age (days). Arapongas, PR.

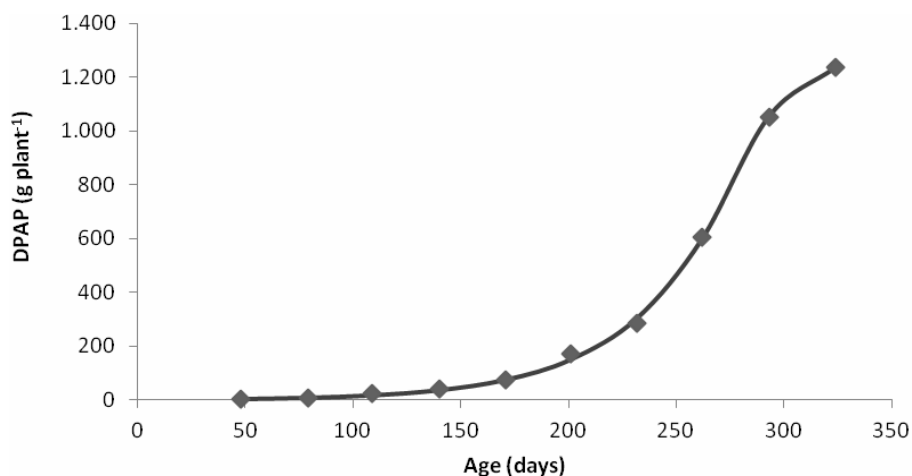
Parameter	Model <sup>1</sup>	a	b	c	d	$r^2$	S
Height (cm)	Richards	187,20	33,76	0,12	0,06	0,9970	3,7952
Relative Height (%)	Richards	100,12	31,70	0,11	0,06	0,9974	1,9039

<sup>1</sup> Nomenclature of *software* Curve Expert 1.4 for Windows (HYAMS,1997).

Accumulation of DPAP was quite slow up to approximately 150 days after planting (Figure 3), probably due to low temperatures and low rainfall (Figure 1). Nevertheless, from 150 days on, one could notice a significant accumulation, matching the temperature and rainfall increase with the

approach of spring. In just 60 days (between day 150 and day 210), the plants almost tripled their phytomass. From 150 days up to the last harvest, there was a gain of 25 times, with 88% of the total DPAP accumulation occurring between 200 days and the harvest. That increase led to a concomitant increase in nutrient absorption.

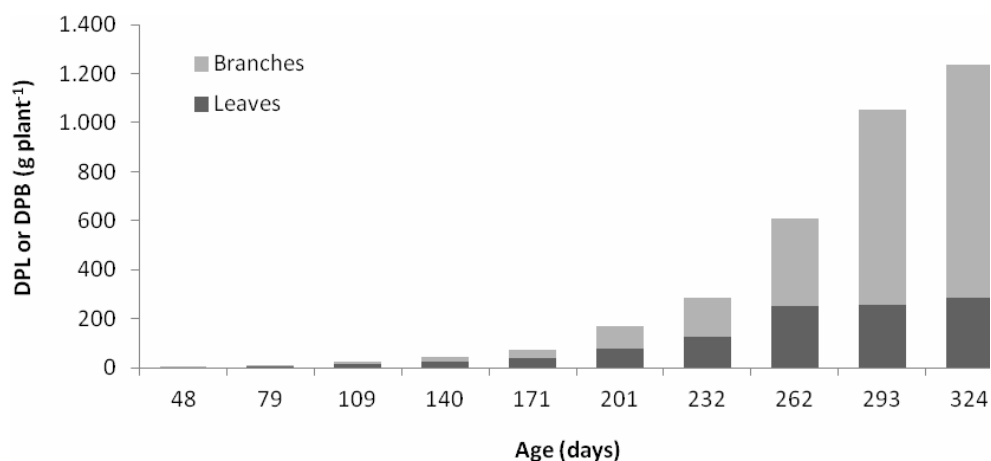
**Figure 3.** Dry phytomass of aerial part (DPAP) of *Duboisia* sp. as a function of age (days).



In Figure 4, the DPAP accumulation curve was decomposed into DPL and DPB to highlight these partitions within the plant. From the 200<sup>th</sup> day after planting onward, accumulation occurred predominantly in branches rather than leaves, so that in the last three evaluations, comprising a period of approximately three months, biomass gain in the

leaves was nearly null. At 324 days, the branches accounted for 77% of the DPAP, while the leaves accounted for 23%. That pattern of divergence was also observed by Bragança et al. (2010) when studying the accumulation and the partitioning of biomass in *Conilon* coffee.

**Figure 4.** Dry phytomass of leaves (DPL) and branches (DPB) of *Duboisia* sp. as a function of age (days). Arapongas, PR.





In contrast to coffee, in which the branches are expected to bear fruit, the target organ in *Duboisia* is the leaf, which is the source of the alkaloids of interest in medicine. The final three months of cultivation therefore represent unnecessary costs as well as a waste of resources, since the harvest could have been done at the 260<sup>th</sup> day after planting when DPL accumulation was stabilized. If that pattern

repeats in subsequent years, a significant time reduction in the production cycle can be foreseen, greatly increasing the efficiency of soil, water, light, and nutrient use. Mathematical models adjusted for DPAP, DPL, and DPB and their respective estimated coefficients, coefficients of determination, and standard errors of the estimate are presented in Table 3.

**Table 3.** Coefficient of determination ( $r^2$ ), standard error of the estimate (S) and estimated coefficients of the mathematical models adjusted for dry phytomass of aerial part (DPAP), dry phytomass of leaves (DPL) and dry phytomass of branches (DPB) of *Duboisia* sp. as a function of age (days). Arapongas, PR.

Parameter	Model <sup>1</sup>	a	b	C	d	$r^2$	S
DPAP	Richards	1.251,41	28,87	0,10	4,32	0,9995	0,3703
DPL (up to 201 days)	Geometric	2,04	0,003	-	-	0,9938	2,2925
DPL (after 201 days)	Richards	269,50	1.322,40	4,98	236,44	0,9870	20,9057
DPB (up to 201 days)	Geometric	0,60	0,005	-	-	0,9940	3,1018
DPB (after 201 days)	Gaussian	963,50	324,99	47,54	-	0,9878	60,2659

<sup>1</sup> Nomenclature of *software* Curve Expert 1.4 for Windows (HYAMS, 1997).

Figure 5 presents the rate of absorption of macronutrients (N, P, K, Ca, Mg, and S) and essential micronutrients (B, Cu, Fe, Mn, and Zn). The mathematical models adjusted for each nutrient, along with estimated coefficient, determination coefficient, and pattern error of the estimate, are presented in Table 4. The nutrient accumulation pattern was similar to the pattern for phytomass, showing that nutritional demand followed plant growth. Similar findings have been made by a number of authors such as Prado et al. (2011) in tomato plants, Duarte et al. (2003) in corn, Mauad

et al. (2013) in crambe, and Pedrinho Júnior et al. (2004) in soy. Macronutrients and micronutrients both came to be in high demand from the 200<sup>th</sup> day after planting onward. The mathematical models that describe the accumulation of each nutrient, their determination coefficients, coefficients of determination and standard errors of the estimate are presented in Table 4. Overall, the models possess greater accuracy from the 170<sup>th</sup> day after planting, and underestimate the phytomass during the earlier period. The relative order of demand for macronutrients and micronutrients, respectively, was  $N < K < Ca < P < Mg < S$  and  $Fe < Mn < Zn < B < Cu$ .



**Table 4.** Coefficient of determination ( $r^2$ ), standard error of the estimate (S) and estimated coefficients of the mathematical models adjusted for the absorption rate of macronutrients (N, P, K, Ca, Mg and S) and micronutrients (B, Cu, Fe, Mn and Zn) in *Duboisia* sp. as a function age (days). Arapongas, PR.

Parameter	Mathematical Model <sup>1</sup>	$r^2$
N	$y=25876,7482*\exp(-((x-337,1493)^2/10824,4718))$	0,9980
P	$y=48836,2616*\exp(-1057,3055/x)$	0,9782
K	$y=17211,1510*\exp(-((x-364,0114)^2/14615,0627))$	0,9979
Ca	$y=146650,5258*\exp(-794,5195/x)$	0,9619
Mg	$y=\exp(-7,4651-188,7311/x+2,6615*\ln(x))$	0,9958
S	$y=10157,5844*\exp(-701,9546/x)$	0,9598
B	$y=\exp(83,7092-3499,9307/x-10,8705*\ln(x))$	0,9662
Cu	$y=21282,9847*\exp(-((x-325,1322)^2/7669,4113))$	0,9986
Fe	$y=602644,1867*\exp(-((x-310,2383)^2/4983,3339))$	0,9989
Mn	$y=89609,2216*\exp(-((x-345,4158)^2/15587,5996))$	0,9925
Zn	$y=26244,1245*\exp(-((x-354,1529)^2/14447,7221))$	0,9965

<sup>1</sup> Nomenclature of *software* Curve Expert 1.4 for Windows (HYAMS, 1997).

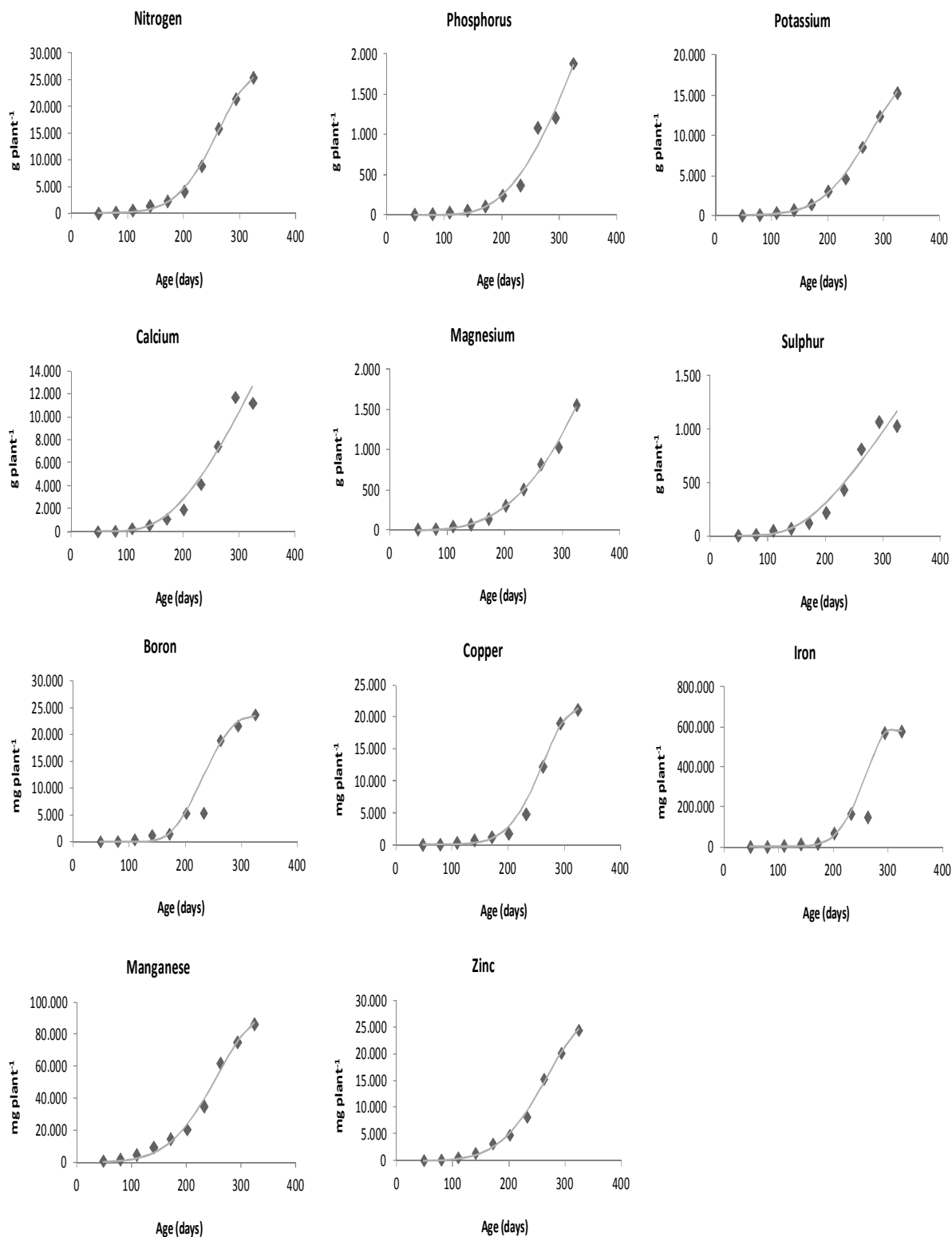
In parallel with the accumulation pattern of phytomass in leaves and branches, nutrient accumulation was greater in the branches than in the leaves from around the 250<sup>th</sup> day after planting, sometimes in a remarkable way (Figure 6). This was the case with phosphate, potassium, calcium, copper, and iron, which latter nutrient had only 4% remaining in the leaves versus 96% in the branches. This pattern enhances the waste of resources, and in particular of fertilizers in the last three months of cultivation, when nutrients that could have been used in a second cycle for leaf production were extracted from the soil and allocated to the branches of the plants, an organ of no commercial interest.

Nutrients displayed some differences among themselves with respect to their levels in the leaves and branches due to age (Figure 7). Most of them had a decreasing trend, while some rose and others oscillated around an average throughout the cycle, not presenting a clear trend toward concentration

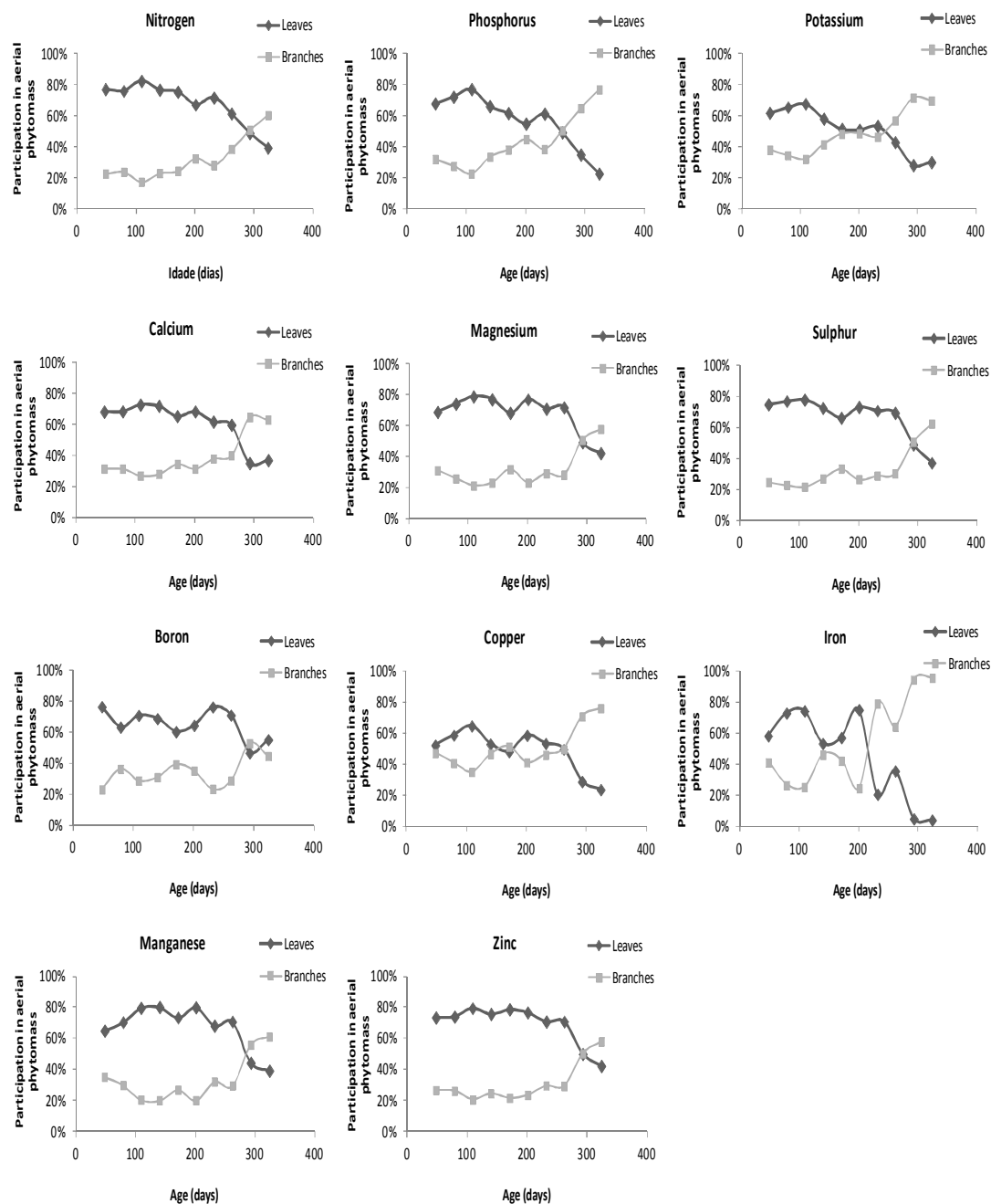
or dilution. Content levels of P, B, and Cu had a trend toward concentration, while K, Mg, Fe, Mn, and Zn underwent a decrease along the cycle, showing dilution. In branches, the content variation of most nutrients exhibited the same trends as in the leaves, except for Ca, B, and Fe, with the two first presenting a decreasing trend, and the last one an increasing trend.

Decreasing nutrient contents indicate that the rate of accumulation of phytomass is greater than the nutrient absorption rate, while increasing nutrient content levels indicate that absorption occurs faster than phytomass accumulation. Such variation can occur either due to soil supply or for natural physiological reasons, as happens with potassium in corn, which has completed, by about 70 days after planting, 90% of its potassium absorption, followed almost exclusively by redistribution (EMBRAPA, 2010).

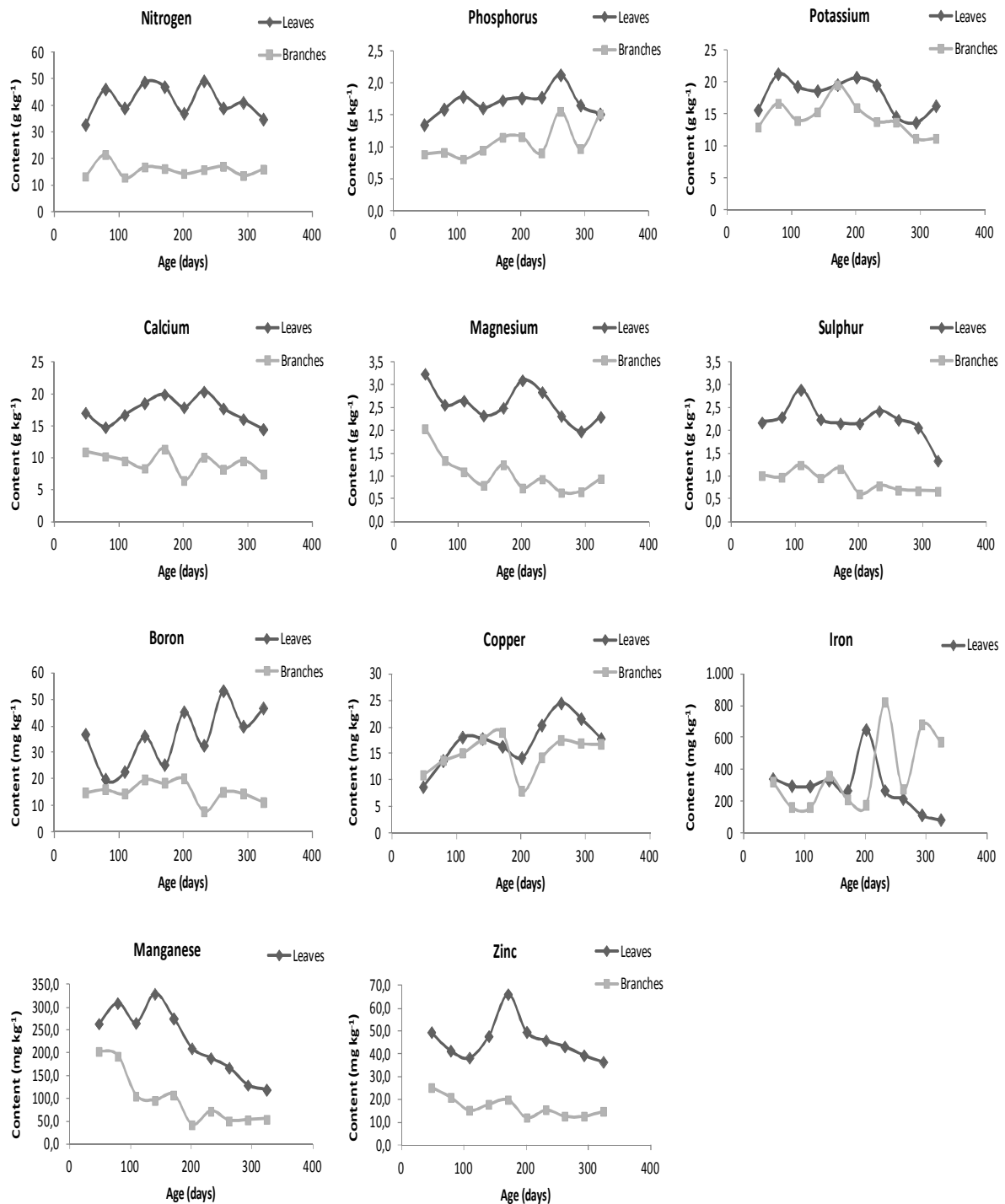
**Figure 5.** Accumulation curves of macronutrient (N, P, K, Ca, Mg and S) and micronutrients (B, Cu, Fe, Mn e Zn) in *Duboisia* sp. as a function of age (days). Arapongas, PR.



**Figure 6.** Dry phytomass partition (percentage of phytomass in the aerial part) of leaves and branches in *Duboisia* sp. as a function of age (days). Arapongas, PR.



**Figure 7.** Macronutrient (N, P, K, Ca, Mg, and S) and micronutrient (B, Cu, Fe, Mn and Zn) contents in leaves and branches of *Duboisia* sp. as a function of age (days). Arapongas, PR.



When nutrient relocation occurs, and one nutrient is extracted from an organ and supplied to another, a decrease occurs in the source organ's nutrient content, allowing for an increase in the receptor organ, with no alteration in the overall nutrient content in the plant, but this was not the case in the studied *Duboisia* plants, where the content levels of all the nutrients was always increasing.

## Conclusions

Dry phytomass accumulation in the aerial part of *Duboisia* plants, and the absorption rate of nutrients, occurred in a manner similar to that found in other species of economic importance, and was similarly subject to climate dynamics.

The partitioning of the phytomass made the harvesting of leaves favorable over the harvesting of branches only up to 260 days after planting.

Nutrient demand in *Duboisia* followed the same pattern seen in phytomass accumulation.

The relative demand levels for macronutrients and micronutrients in *Duboisia* was, respectively,  $N < K < Ca > P < Mg > S$  and  $Fe > Mn > Zn > Cu$ .

## Acknowledgments

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