

# African Journal of Agricultural Research

Volume 12 Number 48 30 November, 2017

ISSN 1991-637X



*Academic  
Journals*

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## Full Length Research Paper

# Agronomic traits, chemical composition and silage quality of elephant grass fertilized with poultry litter

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Received 4 October, 2017; Accepted 17 November, 2017

The high generation of organic residues from the intensive farming of chickens has induced their use as fertilizer for forage crop production. This aimed to evaluate the effect of poultry litter application on agronomic traits and quality of silage of elephant grass. The experiment was conducted in the experimental field of Boa Vista Farm, in district of Cachoeira de Santa Cruz, Viçosa, State of Minas Gerais, in sandy argisol. The experimental period was from November 2014 to February 2015 and the treatments consisted of four levels of fermented poultry litter (0, 4, 8 and 12 ton.ha<sup>-1</sup>) with five repetitions. Evaluations consisted of measurements of plant height, stem diameter, number of plants.ha<sup>-1</sup> and green mass yield. In relation to silage, measurements of gas losses, effluents and total losses were performed using the four treatments cited and four repetitions. There was a positive linear effect ( $P < 0.01$ ) of poultry litter level on plant height and a quadratic effect ( $P < 0.01$ ) of poultry litter level on the number of plants.ha<sup>-1</sup>, with maximum at 9.1 ton.ha<sup>-1</sup> of poultry litter. There was no treatment effect ( $P > 0.05$ ) on the stem diameter, green mass yield and silage quality.

**Key words:** Organic waste, *Pennisetum purpureum* Schum, poultry litter, productivity, qualitative parameters.

## INTRODUCTION

Elephant grass is widely used in Brazil for cattle production, especially dairy animals, because of its high potential for forage production. However, researches have many times revealed problems such as increase in production costs, mainly for nitrogen fertilizers and rangeland degradation (Olivo et al., 2014).

On the other hand, Brazil as the second largest producer and first largest exporter of broiler meat in the world increased the generation of organic waste from the intensive farming of chickens, which requires proper

environmental disposal, since the disposal at random in the environment causes risks of contamination (Tavares and Ribeiro, 2007; Costa et al., 2009).

Poultry litter has important concentrations of nitrogen, phosphorus, potassium, and micro minerals such as copper and zinc (Oviedo-Rondon, 2008; Lima et al., 2016). According to Costa et al. (2015), there is a necessity for studies on the use of poultry litter as organic fertilizer in the productivity of cultures.

The use of organic fertilizer provides greater

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**Table 1.** Soil chemical properties of the experimental area before the application of treatments with poultry litter for elephant grass planting.

pH	P	K	Ca	Mg	Al	H+Al
(mg/dm <sup>3</sup> )						
5.8	17.3	60	2.6	1.6	0.0	2.97
SB	CTC (t)	CTC (T)	V	M	OM	P-rem
(cmol/dm <sup>3</sup> )						
4.35	4.35	7.32	59	0	4.97	32.0
Zn	Fe	Mn	Cu	B		
(mg/dm <sup>3</sup> )						
5.9	163	78.5	1.9	0.4		

H+Al: Potential acidity; SB: sum of bases; CTC (t): effective cation exchange capacity; CTC (T): potential cation exchange capacity; V: base saturation; m: aluminum saturation, OM: organic matter; P-rem: remaining phosphorus.

environmental sustainability, by preserving natural resources through nutrients recycling, avoiding water contamination and harnessing materials available on the agricultural property, in addition to the reduction of production costs (Ariati et al., 2017).

The benefit of the use of organic waste as fertilizers of forage plants occurs in productivity, directly, through the provision of nutrients readily available in the soil solution, or indirectly, through the modifications of the physical, chemical and biological properties of the soil (Zárate et al., 2010; Mangieri and Tavares Filho, 2015). Therefore, organic fertilizers improve root environment and stimulate the plant development (Kiehl, 1985; Menezes et al., 2004).

Silage making is a strategy to store the excessive production of elephant grass in the rainy season for supplementation during the forage scarcity and weight losses in ruminant livestock at the dry season (Ajayi, 2011). This strategy is especially important in the case of the small farmers with low producing dairy cows, predominant in the tropics (Olivo et al., 2014). However, elephant grass may have high values of moisture, which may affect the quality of the silage by undesirable fermentation (Brant et al., 2017), and indicates the necessity for evaluation of its silage quality.

This study aimed at evaluating the development of elephant grass in response to the application of increasing levels of poultry litter at planting, as well as the quality of the silage produced.

## MATERIALS AND METHODS

### Location and period of the experiment

The experiment was conducted at the Boa Vista Farm belonging to the Federal University of Viçosa, located in the district of Cachoeira de Santa Cruz, municipality of Viçosa, State of Minas Gerais, between October 2014 and January 2015. The geographical

coordinates of the experiment site are 20°45' South latitude, 42°51' West longitude and 703 m altitude.

The climate of the region of Viçosa, according to the classification of Köppen (1948), is Cwa (mesothermal), subtropical, with mild and dry winter, and well-defined dry and rainy seasons. The average annual temperature was 21°C, ranging from 31 to 12°C for the maximum and minimum temperature, respectively. The historical rainfall of the area is 1340 mm. However, in 2014 it was 782 mm, which represented 42% below the historical average of the region. Information on the climatic conditions during the experimental period was recorded at Main Climatological Station of Viçosa located at the Federal University of Viçosa, approximately 15 km from the experimental area.

### Setup of the experiment

Prior to the experiment, *Brachiaria decumbens* and *Brachiaria brizantha* predominated in the area, which were used as reserve pasture for lactating cows. Thus, before planting elephant grass, soil samples were taken at random in several places of the experimental field, with the aid of a probe, at the 0 to 20 cm layer, to analyze the chemical and physical characteristics of the soil, according to the Commission on Soil Chemistry and Fertility (Comissão de Química e Fertilidade do Solo, 2004).

Soil tillage with plowing and harrowing was carried out in the dry season, in September 2014, so that all existing plants in the experimental area entered senescence. Plowing was done at approximately 0.3 m depth and harrowing was done to decompress the initial layer of the soil. In November 2014, the planting was done in rows, spaced 1 m between furrows and 30 cm deep.

The experiment consisted of four treatments (0, 4, 8 and 12 ton.ha<sup>-1</sup> of poultry litter) and five replications, totaling 20 experimental plots. Each experimental unit was five m long and 4 m wide, a total area of 20.0 m<sup>2</sup>, with four rows of plants, the working area was considered 5 m<sup>2</sup>, the two central rows, also discarding 50 cm at each end.

### Soil and fertilizer characteristics

The soil of the experimental area is sandy argisol, suitable for crops with low fertility requirements (Table 1).

The poultry litter used consisted of a flock of broiler chickens and

coffee husks. This organic residue came from an industrial poultry farm located in the municipality of Coimbra, state of Minas Gerais, fermented for 60 days on a wet basis. Five poultry litter subsamples performed a single plot for laboratory analysis.

The results of the analysis of the poultry litter used in this study are the following:

N (%) = 3.48; P (%) = 0.96; K (%) = 2.56; Ca (%) = 1.88; Mg (%) = 0.48; S (%) = 0.45; CO = 21.5; C/N = 6.18; pH (H<sub>2</sub>O) = 7.2; DM (%) = 43.3.

The mineral analyses were determined in the acid extract (nitric acid with perchloric acid), dry matter at 65°C, and oven humidity at 75°C. The levels of poultry litter were determined based on quantities found in the literature (Farias et al., 1986; Arruda et al., 2014).

### Agronomic evaluations

Growth parameters measurements occurred after 110 days of elephant grass planting in a central linear meter of the experimental plot. Among those, include the plant height from the ground level up to the ligule of the highest leaves, the stem diameter close to the ground surface by means of a caliper, the number of plants and weight in a linear meter in order to estimate the total number and yield per hectare. After weighting, the plants were chopped, sampled and frozen for feed analyses, following the methodologies of Detmann et al. (2012).

### Elephant grass silage

Samples of elephant grass harvested at 110 days and chopped were stored in experimental bucket silos with a capacity of 3.8 L, equipped with caps coupled to Bunsen valves to allow the escape of gases from the fermentation. At the bottom of the silos, it was placed in 0.6 kg of dry sand, separated from the forage by a cotton cloth, to quantify the effluent produced.

Elephant grass was compacted with a wooden stick. After compaction, the experimental silos were sealed with adhesive tape, weighed and stored. After 84 days of fermentation, they were again weighed to determine the losses by gases and open. After silage removal, the set (bucket, cap, sand, and cotton cloth) was weighed to quantify the effluent produced.

Silages at the top and bottom of the silos were discarded and the rest homogenized. Sub-samples of 500 g were collected for pre-drying in a forced air ventilation oven at 60°C for 72 h to obtain the dry matter content. These sub-samples were sent to the Plant Tissue Analysis Laboratory for analysis of the total N content (Tedesco et al., 1995), whose value was multiplied by the conversion factor 6.25 to estimate the crude protein content (Association of Official Analytical Chemists, 1995).

The losses of gases and effluent were quantified by weight difference and expressed as percentage of the total ensiled material in natural matter.

### Experimental design

The experimental design of the field experiment was in randomized blocks with four treatments (0, 4, 8 and 12 ton.ha<sup>-1</sup> of poultry litter) and five replications. In the case of silages, the experimental design was completely randomized with four treatments (levels of poultry litter) and four replicates. The experimental data were tested by analysis of variance and regression at 5% of significance, using the Minitab program (Ryan and Joiner, 1994).

## RESULTS

### Soil chemical properties

The use of poultry litter improved the soil chemical properties, with higher values of P, K, Ca, Mg, SB, CTC (t), CTC (T), OM, Zn and Mn (Table 2), even after cultivation and harvest of elephant grass at 110 days of experimental period during the rainy season.

### Production-related variables

There were increasing responses of poultry litter levels on some variables evaluated in elephant grass at 110 days after planting. There was an increasing linear effect ( $P < 0.01$ ) of the poultry litter level on the plant height and quadratic effect ( $P < 0.01$ ) of the poultry litter level on the number of plants.ha<sup>-1</sup>, with maximum value at 9.1 ton.ha<sup>-1</sup> of poultry litter. There was no effect ( $P > 0.05$ ) on stem diameter and green mass yield (Table 3).

The content of protein and K increased and the contents of P, Ca, Mg, Zn, Fe, Mn, Cu and B reduced in the elephant grass plant at 110 days after planting with the use of poultry litter (Table 4).

There was no significant effect ( $P > 0.05$ ) of treatment for gas losses, total loss and pH after 60 days of elephant grass ensiling, but there was a cubic effect on effluent loss (Table 5).

## DISCUSSION

### Soil chemical properties

In general, the soil of the study area had high fertility, meeting the requirements of low-demanding crops, but for elephant grass there is a need for fertility improvements. In the area, there was dominance of *Urochloa decumbens* grass pasture, since the area had not been used for agricultural exploration for more than 10 years. It is noteworthy that the levels of Ca, Mg and K presented high values at the 0 to 20 cm layer (Comissão de Química e Fertilidade do Solo, 2004).

### Fertilizer characteristics

According to Menezes et al. (2004), the chemical composition of the poultry litter can be modified according to the material used to line the poultry houses and the number of times that the bedding material is used. However, according to these authors, the average doses of nitrogen vary from 2 to 5%; P<sub>2</sub>O<sub>5</sub> from 1.5 to 3% and K<sub>2</sub>O from 2 to 4%. In this study, all the three macronutrients are inside the presented ranges.

For soil fertility parameters, organic compounds bring



**Table 2.** Soil chemical properties, after harvesting elephant grass at 110 days, in areas fertilized with increasing levels of poultry litter in the municipality of Viçosa State of Minas Gerais, 2015.

Poultry litter level	pH	P	K	Ca	Mg	Al	H+Al
		(mg/dm <sup>3</sup> )			(cmol <sub>e</sub> /dm <sup>3</sup> )		
0 ton.ha <sup>-1</sup>	6.3	4.1	31	2.6	1.3	0.0	3.46
4	6.4	6.9	42	3.3	1.7	0.0	3.46
8	6.3	7.6	43	3.0	1.6	0.0	3.30
12	6.2	14.9	52	3.2	1.6	0.0	3.63
	SB	CTC (t)	CTC (T)	V	m	OM	P-rem
		(cmol <sub>e</sub> /dm <sup>3</sup> )		(%)		(dag/kg)	(mg/L)
0 ton.ha <sup>-1</sup>	3.48	3.98	7.44	53	0	3.73	18.9
4	5.11	5.11	8.57	60	0	4.54	21.2
8	4.71	4.71	8.01	59	0	3.60	21.9
12	4.73	4.73	8.03	59	0	3.99	21.2
	Zn	Fe	Mn	Cu	B		
	(mg/dm <sup>3</sup> )						
0 ton.ha <sup>-1</sup>	2.5	73.2	54.5	1.5	0.1		
4	4.4	72.0	107	1.7	0.2		
8	4.3	85.0	76.7	1.6	0.2		
12	5.2	74.9	91.8	1.6	0.2		

H+Al: Potential acidity; SB: sum of bases; CTC (t): effective cation exchange capacity; CTC (T): potential cation exchange capacity; V: base saturation; m: aluminum saturation, OM: organic matter; P-rem: remaining phosphorus.

**Table 3.** Growth parameters of elephant grass at 110 days according to organic fertilization with poultry litter at planting in Viçosa, 2015.

Item	Poultry litter (ton.ha <sup>-1</sup> )				SEM	P-value	RE
	0	4	8	12			
Plant height (cm)	61.0	77.8	82.2	96.9	0.04	0.001	1
Stem diameter (mm)	16.0	16.1	16.4	17.0	0.47	0.486	
Plants (x1,000/ha)	90.0	112.0	140.0	126.0	7.86	0.004	2
Yield (ton.ha <sup>-1</sup> GM)	43.4	56.2	48.2	62.5	6.51	0.222	

SEM: Standard error of the mean; RE: regression equation: <sup>1</sup> 62.7 + 2.8X.  $r^2 = 0.62$ ; <sup>2</sup> 87.3 + 10.3X - 0.569X<sup>2</sup>;  $R^2 = 0.52$ ; GM: green mass.

benefits, favoring the increase in pH, SB, CTC (T) and V (%), as well as a decrease in H+Al. In this study, there was a slight increase in pH, Ca, SB, CTC (t), CTC (T) and Mn with poultry litter compared to the soil before the experimental period (Table 2).

The dry matter of the poultry litter with coffee husks has values ranging from 70 to 78%, even derived from the same poultry farm, and may vary depending on the management, the composition of the feed and the waste of the feeders and drinkers (EMBRAPA, 2008). Thus, knowledge of this value is important in the calculation of nutrient replacement required by crops. In the present study, the dry matter content was 43.32%, below the value of 87% obtained by Lima et al. (2016).

The low dry matter content of the poultry litter, which

led to lower mineral content than those reported by EMBRAPA (2008), may have been one of the factors for the lack of response of elephant grass to organic fertilization (Table 3). In this case, higher levels of poultry litter should be used, which could become uneconomical due to the price of this product in the Zona da Mata of Minas Gerais.

### Production-related variables

Forage crop production is affected by, among other factors, the climatic characteristics of the site and the fertilization of the soil. The minimum temperature averages found for the experimental period from October

**Table 4.** Mean content of dry matter (DM), crude protein (CP), macro- and micro-minerals in elephant grass plants fertilized with poultry litter, harvested at 110 days after planting.

Poultry litter level	DM	CP	P	K	Ca	Mg
	%	%DM		( $\text{cmol}/\text{dm}^3$ )		
0 $\text{ton}\cdot\text{ha}^{-1}$	32	7.69	0.16	1.48	0.37	0.53
4	33	7.50	0.13	1.80	0.25	0.36
8	36	6.56	0.14	1.96	0.22	0.41
12	35	8.06	0.13	2.36	0.21	0.34
	S	Zn	Fe	Mn	Cu	B
	( $\text{cmol}/\text{dm}^3$ )			( $\text{mg}/\text{kg}$ )		
0 $\text{ton}\cdot\text{ha}^{-1}$	0.10	37	561	77	8	8.7
4	0.07	22	308	41	5	5.7
8	0.07	24	293	56	4	6.7
12	0.07	17	432	56	4	7.7

**Table 5.** Gas loss, effluent loss and total loss, as percentage of the ensiled material, and pH in elephant grass silage with opening of silos after 84 days.

Item	Poultry litter ( $\text{ton}\cdot\text{ha}^{-1}$ )				SEM	P-value	RE
	0	4	8	12			
Gas (%)	0.02	1.80	-1.02	0.83	1.36	0.15	
Effluent (%)	2.96	2.02	4.76	2.99	0.82	0.04	1
Total loss (%)	2.98	3.81	3.74	3.81	1.55	0.79	
pH	4.28	4.36	4.25	4.18	0.14	0.66	

SEM: Standard error of the mean; RE: regression equation:  $12.96 - 1.38X + 0.372X^2 - 0.0214X^3$ ;  $r^2 = 0.16$ .

2014 to January 2015 were  $18^\circ\text{C}$ , above the  $15^\circ\text{C}$  recommended for the adequate growth of forage plant (Arruda et al., 2014). Nevertheless, the rainfall found for the same period was below the historical average for the region; these data are unfavorable to elephant grass productivity.

The results did not show effect of poultry litter on plant productivity (Table 3). However, the growth of 44% of the elephant grass subjected to organic fertilization is a fact. According to Kiehl (1985), the effect of organic matter on productivity may be direct through the provision of nutrients or changes in soil physical properties, which provides a favorable root environment for the development of plants. In addition to the atypical climatic influence, the greatest benefits may be noticed in the next agricultural years on the productivity of plants.

It is worth mentioning that the use of organic fertilizers promotes a slow and gradual release of nutrients, with the advantage of increasing the organic matter content, gradually solubilizing macro- and micro-nutrients to the soil solution (Menezes et al., 2004). Soil nutrient content, after harvesting elephant grass, at the highest level of fertilization, was the most close to those verified in the soil before planting the grass. Therefore, in the next

years of cultivation, it is expected to maintain productivity with annual maintenance fertilization, unlike control treatment, which should lead to soil depletion and consequent productivity reduction.

According to the earlier observation, the practice of organic fertilization in addition to reducing production costs is important to maintain or increase the stock of organic matter and improve the physical and chemical properties of the soil, which is essential to ensure soil quality and, consequently, the sustainability of agroecosystems (Cardoso et al., 2013; Scotti et al., 2015).

Further, it is worth noting that the lack of fertilization effect on grass productivity can be because the experiment was conducted in a year where the rainfall was 42% below the historical average and the low rainfall strongly affected the growth of plants, causing a reduction in productivity.

The poultry litter contains a high concentration of nutrients, since poultry have low rates of utilization of feed (40 to 60%) and the remainder is eliminated via waste. The producer who decides to use organic fertilization should measure the balance of nutrients annually through soil analysis in order to adjust soil

nutrient contents according to the plant requirement.

Studies indicate that a small fraction found in the soil surface consists of organic matter and that the incorporation of organic residues into the soil provides an increase in nutrient uptake by the plant, by the increase of microbial activity (Maia and Cantaruti, 2004; Rogeri et al., 2015).

### Chemical composition of elephant grass and silage produced

The CP content of elephant grass harvested at 110 days (Table 4) with mean of 7.45% DM obtained in this study is considered adequate and within the standard values recommended by the literature (Queiroz Filho et al., 2000). According to the authors, with the advancing age of the purple elephant grass, from 40 to 100 days there was a quadratic effect on the CP content, with crude protein content estimated at 5.89% at 96 days of age.

According to Van Soest (1994), the minimum CP content in the forage plant is 7% so that there is no reduction in voluntary intake. In this way, the values obtained are within the required standards. The silages presented similar pH values (Table 5). Meantime, only elephant grass silage under fertilization with 12 ton.ha<sup>-1</sup> of poultry litter is within the desirable range of 3.8 to 4.2 as recommended in the literature (Yitbarek and Tamir, 2014).

Higher values of moisture and pH of silages may affect the quality of the silage. Grass silages can be improved by the use of other foods to increase the dry matter content and of compounds that favor fermentation and by the use of microbial inoculants (Santos et al., 2014; Brant et al., 2017).

### Conclusions

Organic fertilization with poultry litter increases the height of the elephant grass plants up to 12 ton.ha<sup>-1</sup> and the number of plants per hectare, with a maximum value of 9.1 ton.ha<sup>-1</sup>. Then, level from 9.1 to 12 ton.ha<sup>-1</sup> of poultry liter seems desirable for elephant grass establishment during the first 110 days of growth. Poultry litter does not influence the silage quality of elephant grass harvested after 110 days of planting.

### CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

### ACKNOWLEDGEMENTS

We thank CNPq, CAPES and FAPEMIG for the financial support of Paula Cristiane Trindade and Rogério de

Paula Lana and by allowing the conduction and publication of this study.

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## Full Length Research Paper

# Evaluation of different sweet potato varieties for growth, quality and yield traits under chemical fertilizer and organic amendments in sandy ferralitic soils

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Received 18 August, 2017; Accepted 10 October, 2017

**Growth, quality and yield components of sweet potato (*Ipomoea batatas* (L.) Lam. Var. Tib1, IRA1112 and Red tuber coat) were investigated under three fertilizer sources (inorganic-NPK (100 kg/ha), green manure (*Eichhornia crassipes*) or poultry manure at 12 t/ha, singly or in combination) with three replications. Application of *E. crassipes* and poultry manure fertilizers in combination significantly ( $P < 0.05$ ) increased the shoot length, tuber yield, chlorophyll content and harvest index in all varieties. The highest plant dry weight (480.60 g/plant), shoot length (263.23 cm), number of leaves (75.33) and tuber yield (18.66 t/ha) harvested were found in Tib1 when plants were supplied with both *E. crassipes* and poultry manure; while the lowest plant dry weight (62.54 g/plant), shoot length (195.45 cm) and tuber yield (2.11 t/ha) were recorded in Red tuber coat when plants were enriched only with inorganic-NPK. Leaf protein content of sweet potato varieties was positively influenced by inorganic-NPK, *E. crassipes* and poultry manure, singly or in combined treatments compared to leaf lipid and carbohydrate contents. A combination of high-yielding sweet potato varieties (Tib1) and adequate use of *E. crassipes* and poultry manure, singly or in combination could enhance sweet potato growth performance in sandy ferralitic soils.**

**Key words:** Field performance, nutritional potential, soil amendments, fertilizer, *Ipomoea batatas*.

## INTRODUCTION

Sweet potato (*Ipomoea batatas*) is a vegetable used as human diets component. It is a source of mineral, vitamins, some hormones precursors, proteins and energy. It is an herbaceous and rambling vegetable with smooth, green-like leaves having a purple pigmentation along their veins. The major economic part of this plant is the starchy tuberous root (Oyenuga and Fetuga, 1975; Antia et al., 2006). Its leaves are used to accompany the

dishes of yam and cocoyam in some parts of Nigeria, namely among the Efik-Ibibio people of South-Eastern Nigeria (Antia et al., 2006). Moreover, it can be used as fodder and browse for cattle, sheep, goats, pigs and other domestic animals (Oyenuga, 1968). Hence, its leaves look like an essential component of diets.

In Sub-Saharan Africa, vegetables are necessary dietary components for soup or sauces that accompany

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carbohydrates. Its tuber crops provide carbohydrates while its leaves are major sources of vitamins, dietary fibers, essential amino acids and antioxidants (Fasuyi, 2006; Nkongho et al., 2014). In Cameroon, sweet potato is traditionally consumed in boiled form with varying accompaniments including cowpea, rice and millet. Plant nutrients are the main source of carbohydrates, proteins, minerals and dietary fibers as well as other bioactive nutrient compounds. The growth and yield of potato and sweet potato is affected by factors such as stem density, plant population and nutrient supply (Masarirambi et al., 2012; Sayanowako et al., 2014).

Despite the importance or the well-known health advantages of sweet potatoes, their disposability and consumption are still insufficient in tropical and sub-tropical Africa. This may be due to low production, seasonality and their vulnerability to various environmental stresses. Hence, organic and inorganic fertilizers are required to improve the yield and the growth rate of sweet potato. However, the use of inorganic fertilizer in the singularized form may have a negative impact on human health and the environment (Arisha and Bardisi, 1999; Basel and Atif, 2008).

Plant metabolism requires minerals such as nitrogen (N), phosphorus (P) and potassium (K). Among these minerals, N amount should be the highest as a deficiency of N will reduce the total dry matter, fruit N intake, protein content and grain yield (Mark et al., 1983). N is needed during leaf formation and contributes to increase the growth and size of tuber; furthermore, it enhances photosynthesis in the leaves (Taffouo, 1994).

Providing N at an early stage of crop development would make up for the overall size of the leaf canopy. When provided at a later stage of growth, N contributes to maintain the greenness and maximize the yield of the canopy. In cowpea plants, the amount of N required for the development of pods comes from root uptake, symbiotic N<sub>2</sub> fixation and N mobilization in vegetative tissues (Douglas and Weaver, 1993). It has been underlined that high dose of P could compensate the loss in grain yield of wheat associated to late sowing, and then it enhances root and seedling development (Blue et al., 1990).

At the vegetative and pod filling stages, cowpea plant supplied with low P fertilization revealed a significantly higher root colonization than the one supplied with medium and high P fertilization (Taffouo et al., 2014). The wheat grain yields increased when supplied with 60 to 120 kg P/ha of crop fertilizer, the maximum being 120 kg P/ha (Hussain et al., 2008).

It has been underlined that when K is in short supply, the enlargement of root or tuber is more depressed than leaf development (Inal, 1997). Length, strength and thinness of fiber in both cotton and ramie may be improved by balancing N and P, with adequate K (Zheng, 1999). Plant height and stem yield were influenced by K fertilization while stem number did not change (Tatar et

al., 2010).

Physical and chemical properties of soil such as water retention, erodibility, cation exchange capacity and nutrients availability are affected by their organic matter amount (Rice, 2002; Deksissa et al., 2008). Moreover, these systems are beneficial for the overall health of agro-environment, development and management of effective fertilization practices like the manipulation of the quantity and type of organic amendments, thus improving soil ecosystems and fertility (Nzguheba et al., 2004; Manqiang et al., 2009). The sustained productivity of agricultural systems is needed for the level of soil organic matter and the optimization of nutrient cycling to be maintained (Odeno et al., 2004; Khan et al., 2013).

Regardless of the provision of nutrients available for plants by organic amendments, nutrients transformation during the decomposition of organic matter strongly interacts with nutrients uptake by plants, leading to competition between soil microorganisms and plants for available nutrients (Kaye and Hart, 1997). Organic manure can be used to replace mineral fertilizer (Wong et al., 1999; Togun and Akanbi, 2003; Naeem et al., 2006) in order to enhance soil structure (Dauda et al., 2008), microbial biomass (Suresh et al., 2004) and plant growth (Radwan et al., 1993). Hence, the use of manures produced by vegetables may improve crop yield and reduce the use of chemical fertilizers.

Nowadays, consumers are more interested in quality and safe food stuff than paying attention to organic products (Bhattacharyya et al., 2008; Basel and Atif, 2008). Farmers' participation in the advanced stages of sweet potato variety selection has been reported to be successful in Ethiopia, Kenya and Uganda (Ndolo et al., 2001; Abidin, 2004; Laurie and Faber, 2008).

The application of 60 kg/ha organic manure with 60 kg/ha inorganic fertilizer can raise the yield of *Brassica oleracea* to the optimum rate (40.05 t/ha) (Ouda and Mahadeen, 2008). The application of foliar fertilizer, urea, *Tithonia* and nitrogen (N), phosphorus (P) and potassium (K) (NPK) recorded the highest growth, and yield must be adopted by farmers to maximize their yields (Kwayep et al., 2017). In a survey on the production and use of sweet potato in 14 areas of South Africa, the main factors hindering sweet potato production were low yields and yield instability resulting from the use of old landraces (Thompson et al., 1999).

The need for worthy changes in the global food system has been underlined: agriculture must overcome both the challenges of feeding a growing population with a rising demand for meat and high calorie diets (Laurie and Faber, 2008; Seufert et al., 2012). Sweet potato contributes highly to the diet of many people in the tropics, more especially in Cameroon. But its production is seriously affected by poor soil fertility. The use of poultry manure as an organic amendment to restore worn-out soils can thus be encouraged (Sanchez-Monedero et al., 2004). The green manure such as *E.*



Figure 1. Map of the study area.

*crassipes* must be also used to replace soil nitrogen and other elements, and to build up soil organic matter content (Hammad et al., 2011; Wamba et al., 2012).

The objective of this study is to evaluate the physiological and agronomic responses of sweet potato (*Ipomoea batatas*) under chemical fertilizer and organic amendments in sandy ferralitic soils for increasing sweet potato production.

## MATERIALS AND METHODS

### Description of the study site

The study was conducted in the coastal region of Cameroon in the experimental field of the University of Douala during 2013 and 2014 cropping seasons. The study site (Figure 1) is located on the geographic coordinates of 4°01' North latitude and 9°44' East longitudes. The altitude is about 13 m. The climate is of equatorial type with Cameroonian features and with two seasons per year: one short dry season (3 months) and a longer rainy season (9 months); it has an average annual rainfall of 3597 mm and an average annual temperature of 26.7°C. The relative humidity is usually high and close to 90% throughout the year. The

experimental site has yellow sandy loam ferralitic soils.

### Experimental design and procedures

Three sweet potatoes (Tib1 and IRA112 a selected variety and Red-tuber coat local variety), inorganic fertilization (100 kg/ha NPK) and two organic fertilizers such as green manure (*E. crassipes*) and poultry manure applied at 12 t/ha were used singly or in combination in a randomized block design experiment with three replications (Wamba et al., 2012). Before the experimentation, the nutrient contents (g/kg) of *E. crassipes* plant samples (127.16 N, 87.62 P and 214.34 K) and poultry manure (21.76 N, 8.74 P and 11.22 K) were determined. N was applied as urea (46% N), whereas P and K were used as simple superphosphate (7.9% P) and potassium chloride (49.8% K), respectively. Each sub-plot had 4 x 4 m area. The potato stem fragments (cuttings) cut to 30 cm with 6 nodes were collected from the apical portions of the plant at maturity (Taffouo, 1994). These selected cuttings were introduced in the ground at the level of the stem's median region with a 30 cm spacing in order to have a population of 90 000 plants/ha. The treatment with the various fertilizers was done three weeks after planting (WAP). Thiodan (endosulfan organochlorate insecticide) was applied to the soil 4 to 8 WAP, as a preventive pre-emergence measure to fight against pests. The parameters assessed at harvest were the plant dry weight, shoot length, number of leaves

**Table 1.** Physico-chemical properties of the soil (0-20 cm).

Parameter	Units	Value
Texture	-	Sandy loam
Clay	(%)	14.20 (1.2) <sup>a</sup>
Sand	(%)	53.50 (1.9)
Silt	(%)	32.30 (1.05)
Nitrogen	(%)	0.32 (0.01)
Organic C	(%)	0.75 (0.05)
Ratio C/N	-	2.34 (0.02)
Phosphorus	(ppm)	4.60 (0.1)
Potassium	(g Kg <sup>-1</sup> )	0.25 (0.02)
Sodium	(g Kg <sup>-1</sup> )	0.07 (0.01)
Calcium	(g Kg <sup>-1</sup> )	0.23(0.01)
Magnesium	(g Kg <sup>-1</sup> )	0.17 (0.01)
Zinc	(g Kg <sup>-1</sup> )	0.29 (0.02)
Cu	(g Kg <sup>-1</sup> )	1.42 (0.01)
Fe	(g Kg <sup>-1</sup> )	3.26 (0.1)
pH- Water	-	6.45 (0.1)

<sup>a</sup>Values in parenthesis represent the standard error of the mean.

per plant, stem diameter, total chlorophyll content, tuber yield and harvest index.

#### Soil, green and poultry manures sampling and determination of soil physical and chemical properties

Soil samples were taken using auger from the experimental site from a depth of 0 to 20 cm. Ten sub-samples were chosen to get a composite sample for the analysis of soil physical and chemical properties (Table 1). *E. crassipes* samples were washed with distilled water, pressed under blotting-paper and their fresh weight recorded. The plant samples were then air-dried, crushed and sieved using a 2 mm sieve. The poultry manure was stocked in an airtight recipient at 22°C before use. The following chemical analyses were done to the soil, the green crop manure and the poultry manure: Organic carbon (C) was determined using humid oxidation procedure (Walkley and Black, 1934) and total nitrogen (N) by Kjeldahl method. Magnesium (Mg) was extracted using Mehlich 3 method and determined by Technicon autoanalysers (Technicon 2). Total available phosphorus (P) was determined by Okalebo et al. (1993) method. The pH of the soil was measured potentiometrically in the supernatant suspension of a 1:2.5 soil to water ratio using a pH meter. Calcium (Ca), potassium (K) and sodium (Na) were determined using a flame photometer (JENWAY) as described by Taffou et al. (2008).

#### Plant sampling and determination of growth and yield characteristics, protein, lipid and carbohydrate contents

The plants were sampled at complete maturity 12 WAP, for their dry weight, shoot length, total chlorophyll content, number of leaves per plant and tuber yield. Ten plants from which measures of shoot length were taken periodically 4, 8 and 12 WAP were identified randomly per plot. Ten plants were also randomly selected in each plot, and their aerial parts were cut at ground level; their fresh

weight was registered. A representative sub-sample of about 1000 g per plot was dried in an oven at 70°C for 72 h in order to determine its dry weight. The fresh tubers were carefully uprooted and weighed. Harvest index was determined as a ratio of yield to total plant biomass (shoots plus roots). Chlorophyll content in leaves was estimated after extracting 20 mg of the ground material, following the procedure described by Arnon (1949). Chlorophyll of samples was extracted with 80% alkaline acetone (v/v). The absorption of the extracts was measured at 663 nm and 645 nm with a spectrophotometer (BECKMAN DU 68). For quantifying tissue lipid content, the Soxhlet was used for extraction with hexane as solvent (Taffou, 1994). The total carbohydrates and protein contents were determined using AOAC (1995).

#### Statistical analysis

Results obtained are expressed as mean ± standard deviation, and analyzed using statistical package for social sciences (SPSS) software. Statistical differences between treatment means were established using the Fisher least significant difference (LSD) test at p values < 0.05. Analysis of variance (ANOVA) was used to estimate whether varieties, fertilization type, singly or in combination had a significant influence on the measured parameters. The multiple comparisons of data in experimental groups compared to those recorded in the control group were done using Dunnett's procedure (Sigma Stat 2.03 software).

## RESULTS AND DISCUSSION

### Plant growth

Means of the growth traits of the sweet potato varieties (12 WAP) are depicted in Table 2 and Figure 2. Application of inorganic-NPK significantly ( $P < 0.05$ ) influenced the dry weight in Tib1, and the shoot length in Tib1 and IRA1112, on the contrary, led to a significant ( $P < 0.05$ ) decrease of shoot length and number of leaves per plant in Red tuber coat compared to untreated plants (Table 2, Figure 2).

Similar results were obtained by Wong et al. (1999), Magnusson (2002), Basel et al. (2008), Ouda and Mahadeen (2008) and Wamba et al. (2012) on several vegetable crops. Numerous studies have reported that inorganic-NPK fertilizer increased growth in some species by enhancing nitrogen, phosphorus and potassium uptake (MMhango et al., 2008; Anyanzwa et al., 2010; Bado et al., 2010; Shehu et al., 2010; Tauro et al., 2010; Bala et al., 2011).

Nitrogen, phosphorus and potassium are among the essential elements required for plant metabolism and the improvement of soil water-holding capacity (Wamba et al., 2012). Nitrogen is largely needed during leaf formation and then for increasing tuber growth and size, when it ensures optimal photosynthate production in the leaves (Taffou, 1994). Nitrogen fed at an early stage of crop development will help build the overall size of the leaf canopy, whereas at later stage of growth, nitrogen helps maintain the greenness of the canopy and maximize yield (Mark et al., 1983).

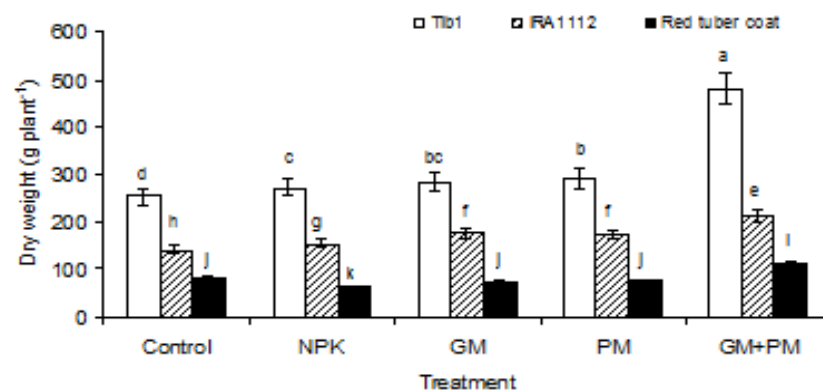
Poultry manure and *E. crassipes* supply singly



**Table 2.** Effect of organic and inorganic fertilization sources on growth characteristics of sweet potato cultivars (12 WAP).

Treatment	Plant growth parameters								
	Shoot length (cm)			Stem diameter (cm)			No. of leaves per plant		
	Tib1	IRA1112	Red tuber coat	Tib1	IRA1112	Red tuber coat	Tib1	IRA1112	Red tuber coat
Control	130.00±7.81 <sup>c</sup>	101.50±5.48 <sup>c</sup>	203.92± 5.68 <sup>c</sup>	8.82±0.35 <sup>a</sup>	6.58±0.15 <sup>a</sup>	5.52±0.26 <sup>a</sup>	68.14±3.60 <sup>b</sup>	33.66±3.05 <sup>b</sup>	38.33±2.51 <sup>a</sup>
NPK	156.58±4.55 <sup>a</sup>	125.86±6.22 <sup>a</sup>	195.45±4.90 <sup>d</sup>	9.21±0.17 <sup>a</sup>	7.37±0.30 <sup>a</sup>	5.32±0.15 <sup>a</sup>	71.45±4.18 <sup>b</sup>	33.40±2.11 <sup>b</sup>	31.65±1.32 <sup>b</sup>
GM	214.20±5.56 <sup>d</sup>	99.50±5.39 <sup>c</sup>	205.11±6.08 <sup>c</sup>	8.65±0.14 <sup>a</sup>	6.60±0.25 <sup>a</sup>	5.24± 0.11 <sup>a</sup>	70.66±2.08 <sup>b</sup>	36.66±2.80 <sup>b</sup>	37.66±1.73 <sup>a</sup>
PM	240.70±3.52 <sup>b</sup>	111.79±5.16 <sup>b</sup>	220.52±5.02 <sup>b</sup>	9.40±0.52 <sup>a</sup>	7.16±0.20 <sup>a</sup>	5.38± 0.18 <sup>a</sup>	72.48±5.01 <sup>b</sup>	37.52±3.31 <sup>b</sup>	37.52±2.67 <sup>a</sup>
GM+PM	263.23±6.24 <sup>a</sup>	121.45±6.35 <sup>a</sup>	235.22±8.08 <sup>a</sup>	10.34±0.22 <sup>a</sup>	7.45±0.22 <sup>a</sup>	5.63±0.26 <sup>a</sup>	75.33±0.21 <sup>a</sup>	40.33±2.51 <sup>a</sup>	38.68±3.71 <sup>a</sup>

Means±SE (n=10) with different letters in the same columns are significantly different ( $P<0.05$ ). NPK: nitrogen, phosphorus and potassium fertilizers, GM: *Eichhornia crassipes*, PM: poultry manure.



**Figure 2.** Effects of organic and inorganic fertilizers on dry weight ( $\text{g plant}^{-1}$ ) of sweet potato cultivars. NPK: nitrogen, phosphorus and potassium fertilizers, GM: *Eichhornia crassipes*, PM: poultry manure. Means followed by the same letter are not significantly different ( $P < 0.05$ ) as determined by Duncan test. Bars indicate standard deviation.

or in combination had significant effects on plant growth compared to untreated plants (Table 2). Application of poultry manure singly or in combination with *E. crassipes* led to a significant ( $P < 0.05$ ) increase in dry weight and shoot length of all varieties compared to untreated plants (Table 2, Figure 2). Poultry manure is a readily

available nutrient source for crop production (Boateng et al., 2006; Schomberg et al., 2011).

Incorporation of poultry manure into soil promoted transformation and mineralization of less-labile inorganic and organic phosphorus into labile- $P_i$  in the rhizosphere, which resulted in higher root phosphorus concentrations and higher

total phosphorus uptake by plants (Waldrip et al., 2011). Regardless of the provision of available nutrients for plants by organic amendments, nutrients transformation during the decomposition of organic matter strongly interacts with nutrients uptake by plants, leading to competition between soil microorganisms and plants for available

**Table 3.** Effect of organic and inorganic fertilization sources on yield components of sweet potato varieties (12 WAP).

Treatment	Yield components								
	Tuber yield (t ha <sup>-1</sup> )			Leaf chlorophyll content (mg g <sup>-1</sup> FW)			Harvest Index (%)		
	Tib1	IRA1112	Red tuber coat	Tib1	IRA1112	Red tuber coat	Tib1	IRA1112	Red tuber coat
Control	10.33 <sup>c</sup>	11.01 <sup>b</sup>	4.10 <sup>b</sup>	30.55 <sup>c</sup>	24.06 <sup>d</sup>	23.13 <sup>c</sup>	0.45 <sup>b</sup>	0.87 <sup>a</sup>	0.57 <sup>a</sup>
NPK	11.77 <sup>c</sup>	9.78 <sup>c</sup>	2.11 <sup>c</sup>	38.25 <sup>b</sup>	30.34 <sup>c</sup>	27.67 <sup>c</sup>	0.48 <sup>b</sup>	0.69 <sup>b</sup>	0.37 <sup>b</sup>
GM	14.00 <sup>b</sup>	10.66 <sup>b</sup>	4.33 <sup>b</sup>	41.10 <sup>ab</sup>	34.47 <sup>bc</sup>	33.59 <sup>b</sup>	0.55 <sup>a</sup>	0.68 <sup>b</sup>	0.66 <sup>a</sup>
PM	14.35 <sup>b</sup>	10.55 <sup>b</sup>	4.19 <sup>b</sup>	43.23 <sup>a</sup>	36.33 <sup>b</sup>	34.67 <sup>b</sup>	0.54 <sup>a</sup>	0.68 <sup>b</sup>	0.63 <sup>a</sup>
GM+PM	18.66 <sup>a</sup>	14.33 <sup>a</sup>	6.00 <sup>a</sup>	47.57 <sup>a</sup>	45.32 <sup>a</sup>	43.66 <sup>a</sup>	0.43 <sup>b</sup>	0.74 <sup>b</sup>	0.61 <sup>a</sup>

Means±SE ( $n=10$ ) with different letters are significantly different ( $P<0.05$ ). NPK: nitrogen, phosphorus and potassium fertilizers, GM: *Eichhornia crassipes*, PM: poultry manure.

**Table 4.** Probabilities of significance for analyses of variance of growth characteristics, yield components and quality in sweet potato varieties grown under organic (*Eichhornia crassipes* and poultry manure, in combination) fertilization.

Trait	Fertilization	Variety	Fertilization x Variety
Dry weight	*	*	*
Shoot length	*	*	NS
Stem diameter	*	*	NS
No. of leaves per plant	*	*	NS
Tuber yield	**	*	*
Leaf chlorophyll content	**	*	*
Harvest Index	**	*	*
Leaf proteins	*	*	*
Leaf lipids	*	NS	NS
Leaf carbohydrates	*	*	NS

\*, \*\* Significant at  $P < 0.05$  and  $P < 0.01$ ,  $P < 0.01$  respectively; ns: not significant.

nutrients (Kaye and Hart, 1997). There were no significant differences in stem diameter and number of leaves per plant in all the varieties except in Tib1 and IRA1112 when poultry manure and *E. crassipes* were supplied in combination (Table 2).

A significant ( $P < 0.05$ ) two-way interaction between the factors variety and organic fertilization sources was observed for dry weight (Table 4). These results could be explained by the fact that the combination of the organic manures activates many microorganisms, which release phytohormones and may stimulate the plant's growth and absorption of nutrients (Arisha et al., 2003). These results could also be due to an increase in organic matter caused by the generation of carbon dioxide during compost decomposition and improvement in the soil structure conditions and root development by improving soil aeration (Arisha et al., 2003).

According to Hossner and Juo (1999), organic matter increases the capacity of the soil to buffer changes in pH, enhances cation exchange capacity (CEC), reduces phosphate fixation and serves as a reservoir for

secondary nutrients and micronutrients.

### Yield traits

The tuber yield, total chlorophyll content and harvest index (12 WAP) were affected by inorganic-NPK, poultry manure and *E. crassipes* fertilizers supply singly or in combination in all varieties (Table 3). Previous studies have shown that high dose of P can compensate for the loss in grain yield of wheat and cowpea, since it helps in rapid development of root and seedling (Blue et al., 1990; Karikari et al., 2015). There was a progressive increase in grain yield of wheat from 60 to 120 kg P/ha from the crop fertilized with 120 kg P/ha (Hussain et al., 2008).

It is commonly observed that root or tube enlargement is depressed relatively more than leaf development, when potassium is in short supply (Inal, 1997). Zheng (1999) stated that balancing nitrogen and phosphorus with adequate potassium improves length, strength and thinness of fiber in both cotton and ramie. Potassium

fertilization impacted plant height and stem yield, whereas stem number did not change (Tatar et al., 2010).

The direct effect of potassium on yield is less marked than nitrogen, which constitutes a part of the organic matter synthesized during growth (Abdel-Motagally and Attia, 2009). In agreement with the results obtained by Christianson and Vlek (1991) and Chukwuka et al. (2015), inorganic-N, P and K in combination is a key element in the production of vegetables as it enhances yield by promoting cell division, expansion in leaves and root development.

According to Maiti and Jana (1985), the combined supply of inorganic-N and K is beneficial for protein metabolism that promotes cell division and enlargement, resulting in higher yield of *sesamum* seeds. Furthermore, inorganic-N, P and K in combination increased root density and proliferation. It aids extensive soil exploration and supply of nutrients and water to the growing plant. This results in increased growth and yield traits, thereby ensuring more dry matter yield (Shehu et al., 2010).

The combination of the poultry manure and *E. crassipes* fertilizers showed significantly ( $P < 0.05$ ) higher tuber yield in Tib1 and IRA1112 than Red tuber coat compared to the plants fed with inorganic-NPK, poultry manure and *E. crassipes* singly (Table 3). Similar results were found by Abdelrazzag (2002) on *Allium cepa* var. Geza. Organic matter is a key component of soils which affects their physical and chemical properties such as water retention, erodibility, cation exchange capacity and nutrient availability (Rice, 2002; Deksissa et al., 2008). The maintenance of soil organic matter levels and the optimization of nutrient cycling are essential for the sustained productivity of agricultural systems (Ayuke et al., 2004; Khan et al., 2013).

Application of inorganic-NPK, poultry manure and *E. crassipes* fertilizers increased significantly ( $P < 0.05$ ) the total chlorophyll in all varieties compared to control (Table 3). These results are similar to those obtained by Wamba et al. (2012) which found that total chlorophyll content increased markedly with the supply of both organic and inorganic fertilizers.

According to Maiti and Jana (1985) and Kilinc et al. (2005), organic and inorganic fertilizers contain macro and trace elements that increase root density and proliferation thereby favoring water and nutrient uptake for the growth of the different plant organs. This favors increase in dry weight and total chlorophyll content. The harvest index decreased significantly ( $P < 0.05$ ) in IRA1112 while no significant differences were found between Tib1 and Red tuber coat except when the poultry manure and green manure fertilizers were supplied singly in Tib1 (Table 3). A significant ( $P < 0.05$ ) two-way interaction between the factors variety and organic fertilization sources was observed for tuber yield, leaf chlorophyll content and harvest index (Table 4). Katwate et al. (2011) reported that grain yield was influenced by varieties and fertilizers in *Sesamum*

*indicum*.

### Proteins, lipids and carbohydrates contents

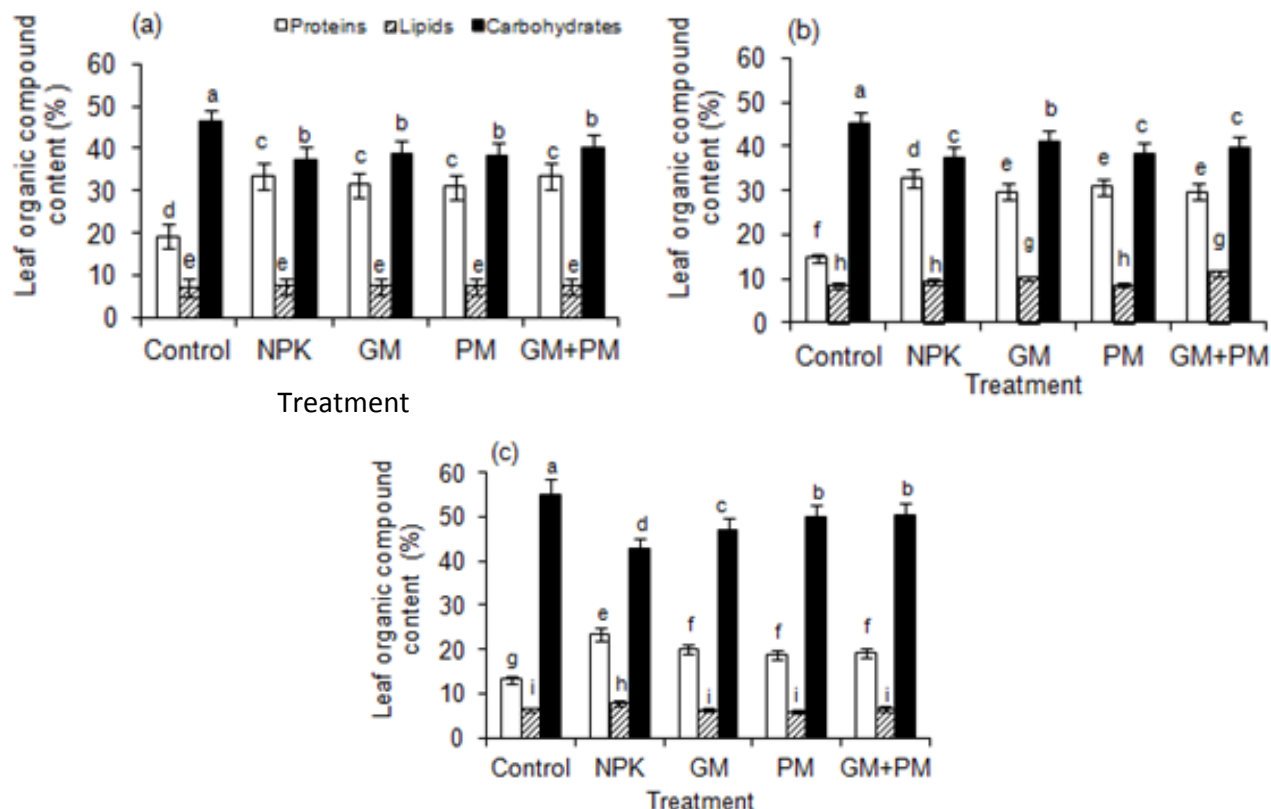
Application of inorganic-NPK, poultry manure and *E. crassipes* fertilizers supply singly or in combination had a positive effect on leaf proteins content, on the contrary, decreased significantly ( $P < 0.05$ ) the carbohydrate contents in all varieties compared to untreated plants (Figure 3). The increase in leaf proteins content with application of inorganic-NPK and organic manure may be due to better nutrient availability and its uptake by plants (Channabasanagowda et al., 2008).

A higher value of leaf proteins content could be attributed to the ability of organic manure to supply nutrients throughout mineralization and improvement of the physical and chemical properties of the soil and the ability of organic fertilizer to release nutrients gradually throughout the growing season (Ouda and Mahadeen, 2008). An adequate supply of nitrogen to plants is beneficial for carbohydrate and protein metabolism resulting in higher yield (Shehu et al., 2010), while nitrogen deficiency may result in the reduction of total dry weight, lower intake of nitrogen into fruits, and less protein content and grain yield (Mark et al., 1983).

Taffouo et al. (2014) reported that nitrogen is directly transferred from the roots towards the leaves of leguminous plants where the nitrogen compounds are used for protein biosynthesis. According to Maiti and Jana (1985), the combined supply of inorganic-N, P and K is beneficial for protein metabolism. No significant differences were found in lipid content of leaves when plants were supplied with inorganic-NPK, poultry manure and *E. crassipes* supply singly except for IRA1112 with poultry manure and green manure combination treatment (Figure 3). This might have led to the increase in lipid and carbohydrates metabolism which helps in increasing the protein content in leaf (Taffouo, 1994; Channabasanagowda et al., 2008).

### Conclusion

The dry weight, shoot length, tuber yield and chlorophyll content were positively influenced by the combination of poultry manure and *E. crassipes* fertilizers in all varieties. Tib1 showed higher dry weight, shoot length, tuber yield and chlorophyll content when plants were supplied with poultry manure and *E. crassipes* fertilizers in combination; while the lowest plant dry weight, shoot length and tuber yield were recorded in red tuber coat when plants were enriched only with inorganic-NPK. Leaf protein contents of sweet potato varieties were positively influenced by inorganic-NPK, poultry manure and *E. crassipes* fertilizers singly or in combination compared to leaf lipid and carbohydrate contents. In the analysis of



**Figure 3.** Effects of organic and inorganic fertilizers on leaf proteins, lipids and carbohydrates ( $\text{g } 100 \text{ g DM}^{-1}$ ) of sweet potato cultivars. (a): Tib1, (b): IRA1112, (c): Red tuber coat, NPK: nitrogen, phosphorus and potassium fertilizers, GM: *Eichhornia crassipes*, PM: poultry manure. Means followed by the same letter are not significantly different ( $p < 0.05$ ) as determined by Duncan test. Bars indicate standard deviation.

combined growth, nutritional potential of leaves and yield components of the parameters measured, Tib1 showed higher field performance than other varieties studied. A combination of high-yielding sweet potato varieties (Tib1) and adequate use of poultry manure and *E. crassipes* fertilizers singly or in combination could enhance sweet potato growth performance in sandy ferralitic soils.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

## Selectivity of herbicides to erva-mate (*Ilex paraguariensis*) plants

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Received 19 July, 2017; Accepted 29 August, 2017

Herbicides can cause negative effects on the morphophysiological characteristics of agricultural crops when proper knowledge about their selectivity is not available. For erva-mate plantations, herbicides which are registered and recommended are scarce. In this sense, this study was carried out to evaluate the selectivity of herbicides applied in different doses to erva-mate. The experiment was installed in a greenhouse at the Federal University of Fronteira Sul (UFFS), Campus Erechim, state of Rio Grande do Sul (RS), Brazil. The experimental design was a randomized block, arranged in a 7 × 4 factorial scheme, with four replications. Factor A comprised the herbicides (tembotrione, chlorimuron-ethyl, oxyfluorfen, sethoxydim + diclosulam, metsulfuron-methyl, Fomesafen + fluzifop-p-butyl and nicosulfuron) and factor B comprised the doses of these herbicides (0, 0.5, 1 and 2-fold the recommended dose for other crops, on the respective herbicide labels). The phytotoxicity, plant height, stem diameter, dry mass of shoots and roots, chlorophyll content, sub-stomatal CO<sub>2</sub> concentration, photosynthetic rate, CO<sub>2</sub> consumed, stomatal conductance of water vapors, transpiration rate, and water use efficiency were assessed. Oxyfluorfen, fomesafen + fluzifop-p-butyl and nicosulfuron affected the morphophysiological characteristics of erva-mate plants at all doses tested. The chlorimuron-ethyl and sethoxydim + diclosulam were presented as being potential to be used in erva-mate plantations until 2-fold the label dose, because they present low plant toxicity and reduced interference in the morphophysiological characteristics of erva-mate.

**Key words:** Chlorimuron-ethyl, alternative crop, chemical management.

### INTRODUCTION

The erva-mate (*Ilex paraguariensis* St. Hil.) is a native tree species in South America. It has 80% of its natural

occurrence in Brazil (Cardozo Jr. et al., 2010) where it is called “erva-mate”, playing an important economic and

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**Table 1.** Herbicides applied post-emergence to erva-mate plants. Federal University of Fronteira Sul, Erechim, Brazil, 2016.

Commercial brand	Active ingredient (a.i.)	Recommended dose ((a.i.) g ha <sup>-1</sup> )
Soberan <sup>®</sup>	Tembotrione	100.8
Classic <sup>®</sup>	Chlorimuron-ethyl	20.0
Goal <sup>®</sup>	Oxyfluorfen	960.0
Poast <sup>®</sup> +Spider <sup>®</sup>	Sethoxydim+diclosulam	276.0 + 25.0
Ally <sup>®</sup>	Metsulfuron-methyl	3.6
Fusiflex <sup>®</sup>	Fomesafen+fluazifop-p-butyl	125.0 + 125.0
Nicosulfuron <sup>®</sup>	Nicosulfuron	60.0

\*All herbicides were added with the adjuvant recommended by the manufacturer.

social role, especially for smallholders. Despite the importance of this plant for the economy, their productivity of 7,650 kg ha<sup>-1</sup> or 510 ha<sup>-1</sup> (Seab, 2014) is far below what could actually be produced, due to the presence of factors limiting the growth and development of the erva-mate plants.

According to Agostinetto et al. (2010), forest crops like any plant community are subjected to a series of ecological factors that directly or indirectly can affect the growth of trees, and in the case of erva-mate, the production of leaves. Among these factors, weed interference which leads to productivity losses due to the competition for environmental resources and allelopathy, may be highlighted. These factors also interfere with the quality of the harvested product (Vargas and Roman, 2005).

Toledo et al. (2000) reported that weed management in reforestation is carried out by mechanical and chemical controls, isolated or combined. The mechanical control is basically done by weeding and brushing, with the advantage of causing little or no injury to the plantation. On the other hand, the widespread use of the chemical weed control is due to the cultivation of large areas, practicality, efficiency, low cost, and mainly the lower demand for human labor compared to other weed control methods.

However, just a few herbicides are registered for the selective post-emergence weed control in erva-mate (MAPA, 2017), and studies regarding the selectivity of herbicides to this crop are scarce. The negative effects of herbicides when applied on cultivated plants are important aspects that must be considered, since they can influence several physiological processes, consequently reflecting on the quantity and the quality of the harvested product.

Intoxication effects of herbicides to plants should not be determined solely by verifying the visual symptoms, as examples of herbicides that may reduce crop productivity without causing visually detectable effects are known. On the other hand, some herbicides can cause severe injuries, which disappear with the development of the crop with little or no impact on productivity (Velini et al., 2000; Negrisoli et al., 2004).

Several factors may influence the growth and development of the crop, which is largely determined by the photosynthetic rate of the plant. This parameter is directly or indirectly influenced by water deficiency, thermal stress (Loreto and Bonghi, 1989), internal and external gas concentration on the leaf environment (Kirschbaum and Pearcey, 1988), composition and light intensity (Sharkey and Raschke, 1981), and mainly stresses caused by the application of herbicides. However, studies related to the effect of herbicides on the physiology of erva-mate are scarce and the effect of these products on the crop has to be deeply reported.

Thus, in order to adopt the chemical weed control method in erva-mate plantations, it is necessary to evaluate the selectivity of commonly available herbicides to these plants. The hypothesis is that the selectivity of herbicides to erva-mate plants is a function of product and dose. Thus, this study was carried out to assess the selectivity of herbicides applied at different doses to erva-mate plants.

## MATERIALS AND METHODS

### Study species

The erva-mate (*I. paraguariensis*) is a tree native to the subtropical regions of South America. Its leaves and thin branches are toasted and consumed as hot or cold tea, which are called "chimarrao" and "terere", respectively. This tree usually reaches 12 m in height, being relatively sensitive to excessive sunlight, demanding some degree of shading in its initial establishment.

### Greenhouse trial

The experiment was carried out in a greenhouse at the Federal University of Fronteira Sul (UFFS), at the campus located in the city of Erechim, state of Rio Grande do Sul (RS), Brazil, in a randomized complete block design arranged in a 7 × 4 factorial scheme, with four replications. Factor A comprised the herbicides (tembotrione, chlorimuron-ethyl, oxyfluorfen, sethoxydim + diclosulam, metsulfuron-methyl, fomesafen + fluazifop-p-butyl and nicosulfuron) (MAPA, 2017), and factor B comprised the doses of these herbicides (0, 0.5, 1 and 2-fold the recommended dose for other crops, on the respective herbicide labels) (Table 1).

Young plants of erva-mate with uniform size of 20 ± 2 cm, native



genotype from the location of Erechim, RS, Brazil, were selected from the same planting lot. The experimental units were composed of polyethylene pots with capacity of 8 dm<sup>3</sup>, filled with red latosol aluminoferric, where one plant was transplanted to the center in the first half of May, 2015. Fertilization was used in accordance with the technical recommendations for cultivation of erva-mate (Rolas, 2004) 15 days after transplanting (DATp), based on soil analysis. Plants were protected with a 50% shading screen in the first 30 DATp.

The application of the herbicides was performed directly to the plants 60 DATp by using a CO<sub>2</sub> pressurized backpack sprayer connected to a single spray tip of the series TT 110.02, operating at 2.0 kgf cm<sup>-2</sup> with volume equivalent to 150 L ha<sup>-1</sup>.

Phytotoxicity was evaluated at 7, 14, 21, 28, 35, 42 and 49 days after application of the treatments (DAT) being assessed visually, assigning scores from zero to 100% by two evaluators, where zero (0%) corresponds to no injury and 100 (100%) to death of plants, according to the methodology proposed by SBCPD (1995).

Fifty DAT plant height (cm) was assessed with a ruler, from soil to the apical meristem; stem diameter (cm) was determined with a digital caliper rule of 5 cm above soil and chlorophyll content (SPAD) was assessed with a digital SPAD meter, evaluating leaves in the lower, middle and upper third of plants.

Physiological parameters were assessed at 51 DAT: sub-stomatic CO<sub>2</sub> concentration (C<sub>i</sub> - μmol mol<sup>-1</sup>), photosynthetic rate (A - μmol m<sup>-2</sup> s<sup>-1</sup>), CO<sub>2</sub> consumed (ΔC - μmol mol<sup>-1</sup>), water use efficiency (WUE - mol CO<sub>2</sub> mol H<sub>2</sub>O<sup>-1</sup>), and transpiration rate (E - mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>). These variables were determined in the middle third of the first fully expanded leaf of the erva-mate plants, by using an infrared gas analyzer (IRGA), ADC-LCA PRO (Analytical Development Co. Ltd, Hoddesdon, UK); each block was assessed per day, between 8 and 10 o'clock in the morning to guarantee homogeneous environmental conditions during the analysis of each block.

Seventy DAT plants were removed from the vases, sectioned with roots and shoots separated, packed in paper bags and put into forced air circulation oven at 65 ± 5°C for four days, to determine the dry mass.

### Statistical analysis

The data set was submitted for analysis of variance by the F-test and for the quantitative factor (doses), linear and non-linear regressions were adjusted. Tukey's test was used for the qualitative factor (herbicides). All tests were performed at 5% probability.

## RESULTS AND DISCUSSION

There was interaction between herbicide and dose for all variables. There was increase in phytotoxicity as herbicide rates were increased, mainly at 7 DAT for doses of fomesafen + fluazifop-p-butyl, with the highest injury occurring with twice the recommended dose (250 + 250 g ha<sup>-1</sup>). The application of sethoxydim + diclosulam had no considerable phytotoxicity for any dose, stabilizing in about 4% (Figure 1A).

For the other herbicides, there were no model adjustments to the data, but the mean phytotoxicity exceeded 6.42%. It should be noted however that, the low levels of phytotoxicity found at 7 DAT is due to the fact that, depending on the mechanism of action to which the herbicide belongs, there is a need for more time for it

to show injury to the plants in which they are applied (Rodrigues and Almeida, 2011).

It should be noted that fomesafen belongs to the mechanism of PROTOX inhibition, with action on broad and narrow leaves, whereas fluazifop-p-butyl inhibits the enzyme acetyl-CoA carboxylase (ACCCase) and therefore is a specific grass killer. Thus, the phytotoxic effects of the fomesafen + fluazifop-p-butyl mixture can be attributed mainly to fomesafen. At 14 DAT, all herbicides showed increased levels of plant damage (Figure 1B) compared to the first evaluation. At 28 DAT (Figure 1C), fomesafen + fluazifop-p-butyl maintained high phytotoxicity levels.

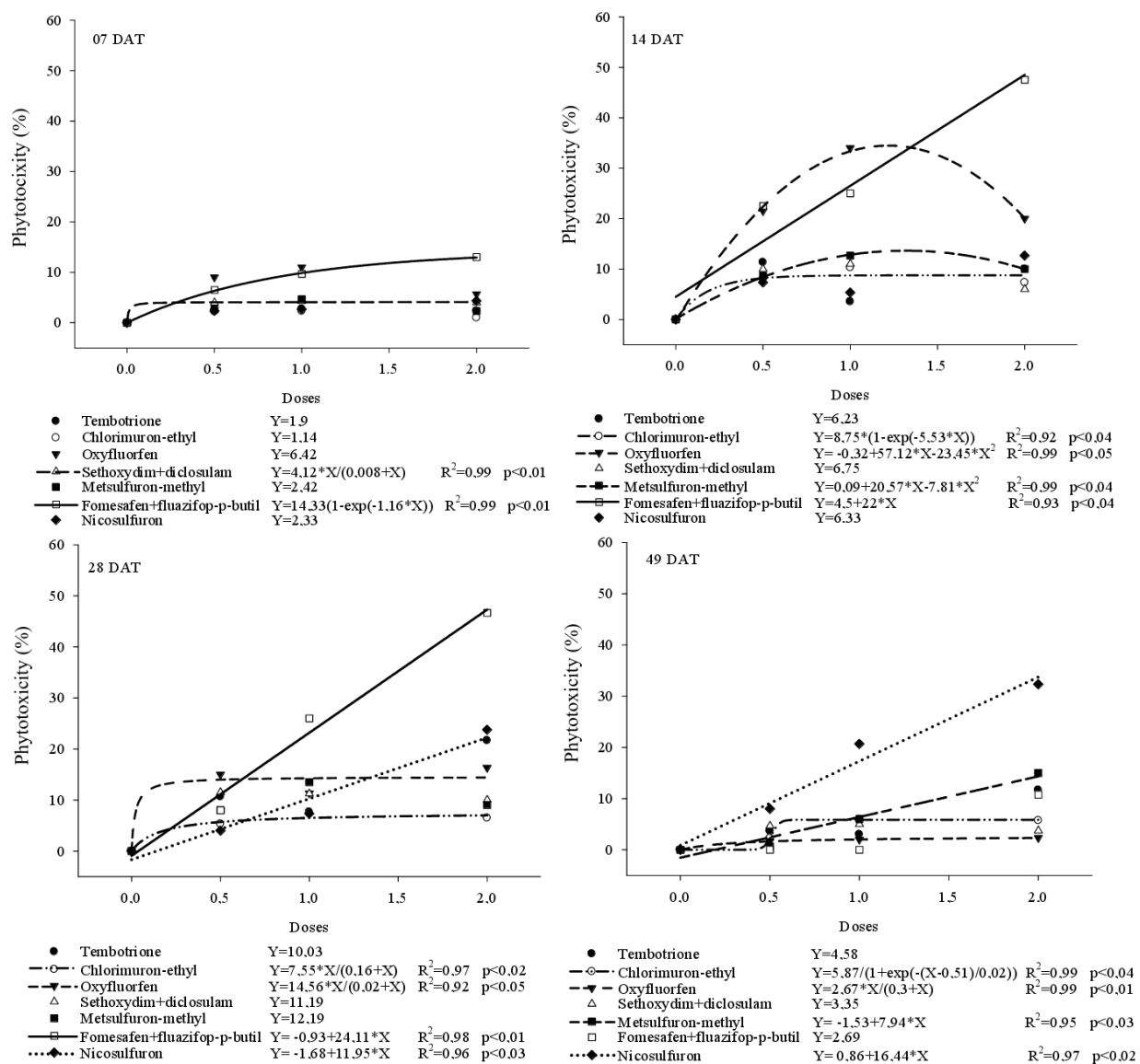
In the last phytotoxicity assessment carried out 49 DAT, the herbicide nicosulfuron presented the highest rate of injury to erva-mate, reaching about 17 and 34% when using the dose and 2-fold the recommended dose, respectively. Similarly to nicosulfuron, metsulfuron-methyl showed a linear increase of about 16% in injury to plants as dose increased, with application of 2-fold the recommended dose (Figure 1D). It was observed that oxyfluorfen and chlorimuron-ethyl caused phytotoxicity between 1 and 5%, even at high doses. There was no regression fit for the increasing doses of tembotrione, sethoxydim + diclosulam and fomesafen + fluazifop-p-butyl (Figure 1D), with averages of 5, 3 and 3% phytotoxicity, respectively.

It was observed that in cases where there was phytotoxicity to the plants, the recovery relied on the emission of new sprouts from the damaged shoots. For the herbicides that present contact effect, new sprouts that appear after the application were not affected (Oliveira Júnior et al., 2011), provided that the injury is not high to the point of causing the death of the growth meristems.

Carotenoid inhibiting herbicides, in general terms, act efficiently in pre- or early post-emergence of plants (Vidal and Merotto, 2001). The fact that the erva-mate is transplanted with a more developed vegetative structure guaranteed plant recovery at 35 DAT with tembotrione.

Although the herbicides chlorimuron-ethyl, metsulfuron-methyl, nicosulfuron and diclosulam are all inhibitors of the enzyme acetolactate synthase (ALS), they showed distinct behavior. Chlorimuron-ethyl, metsulfuron-methyl and sethoxydim + diclosulam showed phytotoxicity below 15% for erva-mate plants (Table 2). This can be explained by the fact that plants were probably able to metabolize the herbicide at such a rate that it prevents it from reaching the site of action (Vidal and Merotto, 2001). Nicosulfuron at 49 DAT showed up to 35% phytotoxicity at 2-fold the label dose (Table 2).

There was fitting for plant height only for doses of chlorimuron-ethyl and metsulfuron-methyl. For both, plant growth stagnation was about 27 and 45% after application of half and the full dose, respectively compared to the control (Figure 2). When comparing herbicides within each dose, nicosulfuron showed lower

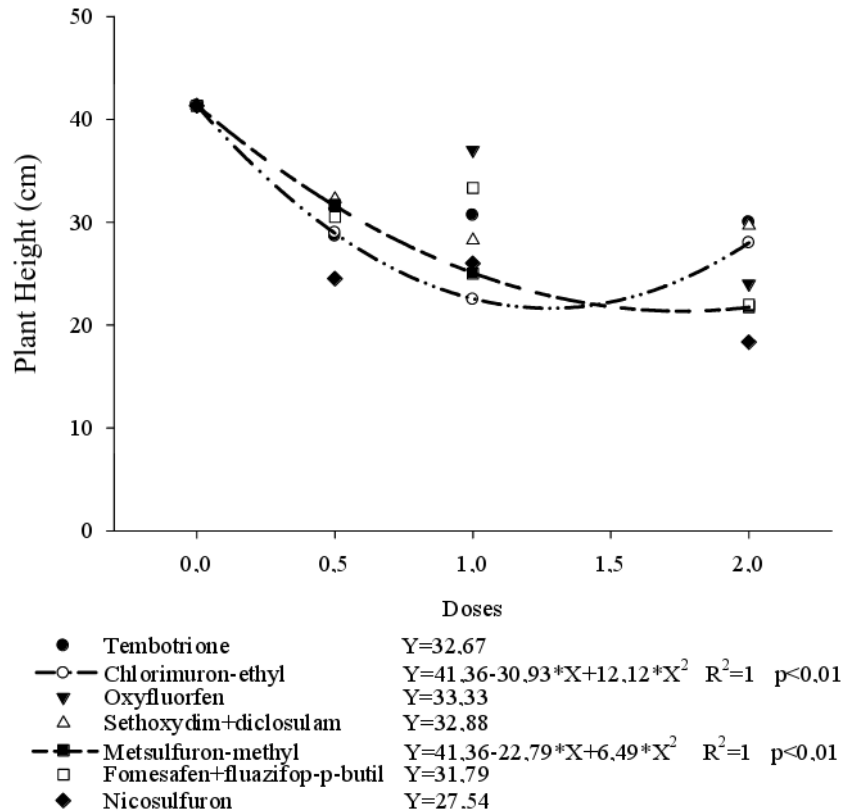


**Figure 1.** Herbicide phytotoxicity (%) to erva-mate, as function of herbicide and dose (Federal University of Fronteira Sul, Erechim, Brazil, 2016).

**Table 2.** Phytotoxicity (%) to erva-mate (native genotype) plants 49 days after treatment (DAT), as function of herbicide and dose (Federal University of Fronteira Sul, Erechim, Brazil, 2016).

Herbicide	Dose			
	0 x	0.5 x	1 x	2 x
Tembotrione	0.0 <sup>a</sup>	3.67 <sup>ab</sup>	3.00 <sup>bcd</sup>	11.67 <sup>bc</sup>
Chlorimuron-ethyl	0.0 <sup>a</sup>	2.25 <sup>b</sup>	6.00 <sup>b</sup>	5.75 <sup>d</sup>
Oxyfluorfen	0.0 <sup>a</sup>	1.67 <sup>b</sup>	2.00 <sup>cd</sup>	2.33 <sup>d</sup>
Sethoxydim + diclosulam	0.0 <sup>a</sup>	4.67 <sup>ab</sup>	5.00 <sup>bc</sup>	3.75 <sup>d</sup>
Metsulfuron-methyl	0.0 <sup>a</sup>	0.67 <sup>b</sup>	6.00 <sup>b</sup>	15.00 <sup>b</sup>
Fomesafen + fluazifop-p-butyl	0.0 <sup>a</sup>	0.00 <sup>b</sup>	0.00 <sup>d</sup>	10.75 <sup>c</sup>
Nicosulfuron	0.0 <sup>a</sup>	8.00 <sup>a</sup>	20.67 <sup>a</sup>	32.33 <sup>a</sup>

<sup>1</sup>Means followed by the same letter, into the same dose, do not differ according to Tukey's test ( $p \leq 0.05$ ).



**Figure 2.** Plant height of erva-mate as function of herbicide and dose (Federal University of Fronteira Sul, Erechim, Brazil, 2016).

plant height at all doses, not differing from tembotrione, chlorimuron, sethoxydim + diclosulam and metsulfuron-methyl. The highest effect was verified with the use of 2-fold the dose, when plant height reduction was approximately 56% compared to control plants (Table 3).

For stem diameter, there was no curve fitting (data not shown). In the comparison between herbicides and doses, nicosulfuron showed the lowest stem diameter (3.52 mm) when under application of 2-fold the dose ( $120 \text{ g ha}^{-1}$ ), which was about 36% less than the control (Table 3). The stagnation in plant height, as well as the remarkable phytotoxicity, also affected the stem diameter of plants treated with nicosulfuron, but not its chlorophyll content (Figure 3).

With regard to the physiological variables, sub-stomatal  $\text{CO}_2$  concentration, photosynthetic rate,  $\text{CO}_2$  consumed, stomatal conductance, transpiration rate and water use efficiency, it was possible to adjust models only for stomatal conductance (Gs), transpiration rate (E), and water use efficiency (Figure 4). For nicosulfuron, an increase in Gs of 24, 42 and 58% was observed with the use of 0.5, 1.0 and 2.0-fold the dose, respectively, compared to the control (Figure 4). It can be verified that the Gs increase is proportional to the phytotoxicity increase caused by nicosulfuron at 49 DAT. When

comparing the effect of the herbicides within each dose, it was observed that oxyfluorfen and nicosulfuron showed higher rates of Gs compared to the other herbicides at all doses. However, twice the dose of oxyfluorfen and fomesafen + fluazifop-p-butyl increased Gs from  $0.43$  to  $0.48 \text{ mol m}^{-2} \text{ s}^{-1}$ , superior to the other herbicides, but not differing from tembotrione and nicosulfuron (Table 4).

For oxyfluorfen and fomesafen+fluazifop-p-butyl, the high rates of Gs are related to the accelerated metabolism of plants for the emission of new sprouts, since the contact effects of these herbicides do not act on new vegetative formations. The results corroborate with those reported by Concenço et al. (2014), who reported that 25% of the dose of fluazifop-p-butyl, clethodim and the commercial mixture of bentazon + imazamox, promoted increases in stomatal conductance of *Crambe abyssinica*.

For the transpiration rate (E), linear and quadratic models were adjusted for oxyfluorfen and metsulfuron-methyl, respectively (Figure 4). For oxyfluorfen, the increase in doses resulted in a linear increase in the E of erva-mate; with double the dose there was increase of about 66% in the evaluated variable (Figure 4). Metsulfuron-methyl showed a distinct behavior, with an increase of approximately 52% in E when the

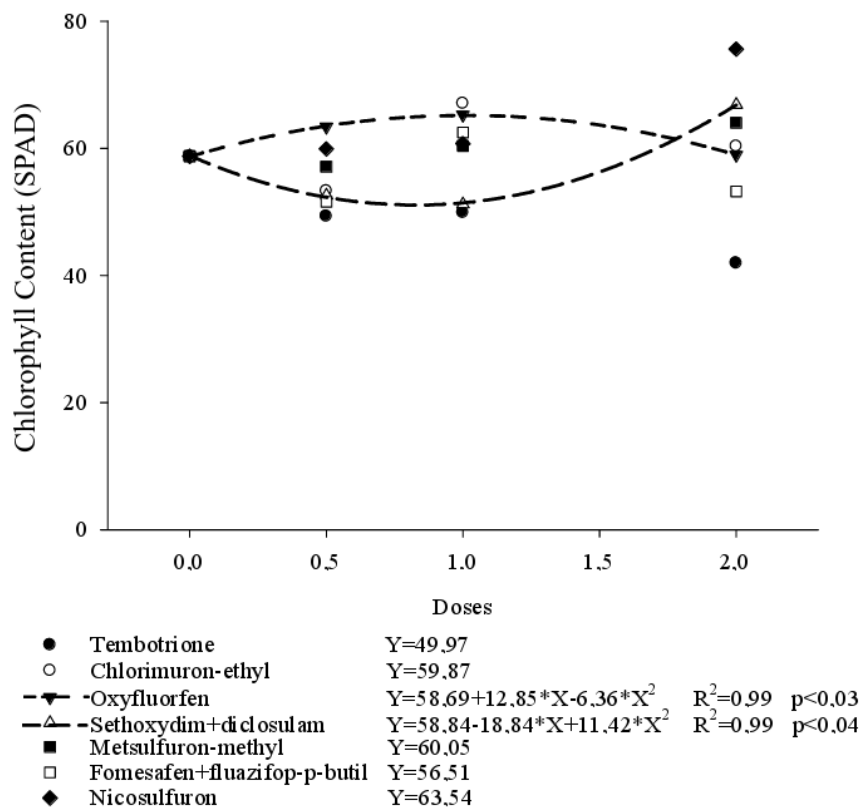
**Table 3.** Plant height, stem diameter and chlorophyll content of erva-mate (native genotype) plants 49 days after treatment (DAT), as function of herbicide and dose (Federal University of Fronteira Sul, Erechim, Brazil, 2016).

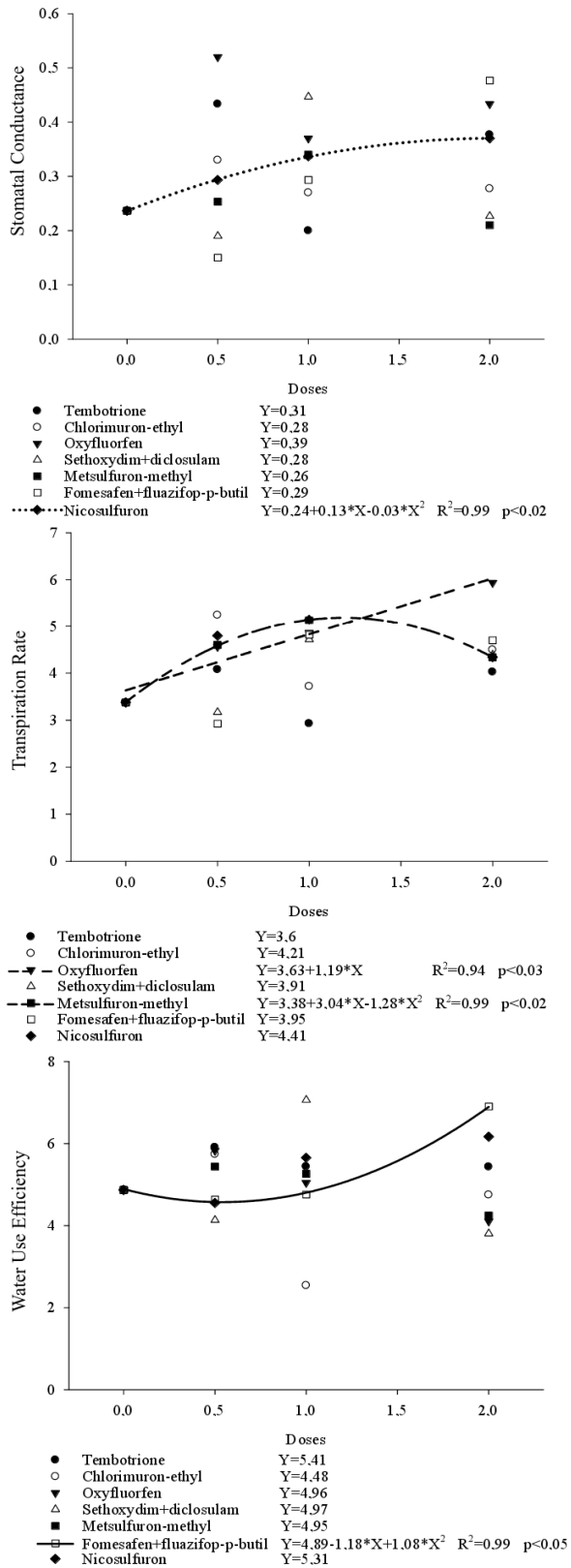
Herbicides	Plant height (m)	Stem diameter (mm)	Chlorophyll content (SPAD)	Plant height (m)	Stem diameter (mm)	Chlorophyll content (SPAD)
Tembotrione	41.33 <sup>a1</sup>	5.51 <sup>a</sup>	58.73 <sup>a</sup>	28.67 <sup>c</sup>	4.99 <sup>ab</sup>	49.30 <sup>d</sup>
Chlorimuron-ethyl	41.33 <sup>a</sup>	5.51 <sup>a</sup>	58.73 <sup>a</sup>	29.00 <sup>bc</sup>	3.67 <sup>c</sup>	53.30 <sup>cd</sup>
Oxyfluorfen	41.33 <sup>a</sup>	5.51 <sup>a</sup>	58.73 <sup>a</sup>	31.00 <sup>abc</sup>	4.18 <sup>bc</sup>	63.40 <sup>a</sup>
Sethoxydim + diclosulam	41.33 <sup>a</sup>	5.51 <sup>a</sup>	58.73 <sup>a</sup>	32.25 <sup>a</sup>	5.26 <sup>a</sup>	52.57 <sup>cd</sup>
Metsulfuron-methyl	41.33 <sup>a</sup>	5.51 <sup>a</sup>	58.73 <sup>a</sup>	31.67 <sup>ab</sup>	5.27 <sup>a</sup>	57.10 <sup>bc</sup>
Fomesafen + fluazifop-p-butyl	41.33 <sup>a</sup>	5.51 <sup>a</sup>	58.73 <sup>a</sup>	30.50 <sup>bc</sup>	4.66 <sup>abc</sup>	51.59 <sup>cd</sup>
Nicosulfuron	41.33 <sup>a</sup>	5.51 <sup>a</sup>	58.73 <sup>a</sup>	24.50 <sup>d</sup>	4.52 <sup>abc</sup>	59.90 <sup>ab</sup>

Herbicides	Dose 1 x			Dose 2 x		
	Tembotrione	30.67 <sup>abc</sup>	5.49 <sup>a</sup>	49.90 <sup>b</sup>	30.00 <sup>a</sup>	4.34 <sup>bc</sup>
Chlorimuron-ethyl	22.50 <sup>c</sup>	4.33 <sup>bc</sup>	67.10 <sup>a</sup>	28.00 <sup>a</sup>	4.43 <sup>abc</sup>	60.35 <sup>bc</sup>
Oxyfluorfen	37.00 <sup>a</sup>	4.19 <sup>c</sup>	65.27 <sup>a</sup>	24.00 <sup>b</sup>	3.77 <sup>cd</sup>	58.93 <sup>cd</sup>
Sethoxydim + diclosulam	28.25 <sup>bc</sup>	4.89 <sup>ab</sup>	51.20 <sup>b</sup>	29.67 <sup>a</sup>	5.06 <sup>a</sup>	66.87 <sup>b</sup>
Metsulfuron-methyl	25.00 <sup>c</sup>	4.16 <sup>c</sup>	60.37 <sup>a</sup>	21.75 <sup>b</sup>	4.66 <sup>ab</sup>	64.00 <sup>bc</sup>
Fomesafen + fluazifop-p-butyl	33.33 <sup>ab</sup>	4.63 <sup>bc</sup>	62.50 <sup>a</sup>	22.00 <sup>b</sup>	4.25 <sup>bc</sup>	53.20 <sup>d</sup>
Nicosulfuron	26.00 <sup>bc</sup>	4.98 <sup>ab</sup>	60.73 <sup>a</sup>	18.33 <sup>c</sup>	3.52 <sup>d</sup>	75.60 <sup>a</sup>

<sup>1</sup>Means followed by the same letter, into the same dose and variable, do not differ according to Tukey's test ( $p \leq 0.05$ ).

**Figure 3.** Chlorophyll content (SPAD) of erva-mate plants as function of herbicide and dose (Federal University of Fronteira Sul, Erechim, Brazil, 2016).



**Figure 4.** Stomatal conductance ( $\text{mol m}^{-1} \text{s}^{-1}$ ), transpiration rate ( $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ ) and water use efficiency of erva-mate plants as function of herbicide and dose (Federal University of Fronteira Sul, Erechim, Brazil, 2016).

recommended dose ( $3.6 \text{ g ha}^{-1}$ ) was applied, compared to control (Figure 4). However, with 2-fold dose, an increase of about 28% was observed. The differentiated behavior of oxyfluorfen and metsulfuron-methyl with respect to E of erva-mate can be associated with the different mechanisms of action of these herbicides.

As the E is mainly determined by Gs, it can be hypothesized that there was an increase in the metabolism and in E for the emission of new sprouts under application of oxyfluorfen (contact herbicide) and increase in metabolism for detoxification of metsulfuron-methyl. Similar results were found by Concenço et al. (2014), who reported increase in E of *C. abyssinica* with 75% of the dose of bentazon + imazamox compared to the lowest dose tested. Similarly, Galon et al. (2014) found that ryegrass plants showed an increase in Gs and E rates when they grew under application of the recommended dose of imazethapyr + imazapic.

Regarding water use efficiency, fomesafen + fluazifop-p-butyl presented quadratic behavior as a function of dose increase. It was observed that the WUE practically did not change until the recommended dose, however with the application of 2-fold the dose, an increase of approximately 40% was verified (Figure 4).

Fluazifop-p-butyl is an inhibitor of the enzyme ACCase and has no effect on dicotyledons (Vidal and Merotto, 2001), while fomesafen, as a contact-effect herbicide (Oliveira Júnior et al., 2011) destroys mainly meristematic tissues. The commercial mixture of fomesafen + fluazifop-p-butyl caused high phytotoxicity 07 to 42 DAT; the largest WUE for this treatment occurred as the plants showed intense development of new apical shoots after 42 DAT.

In general, oxyfluorfen and fomesafen + fluazifop-p-butyl reached the highest rates of sub-stomatal  $\text{CO}_2$  ( $C_i$ ), photosynthetic rate (A) and  $\text{CO}_2$  consumed ( $\Delta C$ ) at all doses evaluated (Table 4). However, with application of the label dose, sethoxydim + diclosulam presented higher  $\Delta C$  and A, not statistically differing from metsulfuron-methyl and nicosulfuron. Chlorimuron-ethyl and nicosulfuron, at the label dose, had respectively the highest and lowest  $C_i$ , with the other herbicides being intermediary.

The selectivity of herbicides to plants of the same genotype may be related to several factors like the physiological detoxification capacity of some plants, the environmental influences and also the physical, chemical and biological characteristics of the herbicides. Brandão et al. (2014) reported that the greater the genetic variability within the same species, the greater the diversity of responses that may occur.

A reduction was observed in the accumulation of shoot dry mass of erva-mate with the increase of the doses of oxyfluorfen and fomesafen + fluazifop-p-butyl; for the other herbicides, it was not possible to adjust a regression to the data. Oxyfluorfen caused a fall in the dry mass accumulation of about 26, 42 and 45% when

**Table 4.** Consumed CO<sub>2</sub> ( $\Delta C$  -  $\mu\text{mol mol}^{-1}$ ), sub-stomatal CO<sub>2</sub> concentration ( $C_i$  -  $\mu\text{mol mol}^{-1}$ ) and photosynthesis rate ( $A$  -  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) of erva-mate plants, native genotype, as function of herbicide and dose (Federal University of Fronteira Sul, Erechim, Brazil, 2016).

Herbicides	$\Delta C$	$C_i$	$A$	$\Delta C$	$C_i$	$A$
	0 x			0.5 x		
Tembotrione	51 <sup>a1</sup>	201.33 <sup>a</sup>	16.45 <sup>a</sup>	74.50 <sup>ab</sup>	230.00 <sup>ab</sup>	23.98 <sup>ab</sup>
Chlorimuron-ethyl	51 <sup>a</sup>	201.33 <sup>a</sup>	16.45 <sup>a</sup>	94.00 <sup>a</sup>	190.00 <sup>bc</sup>	30.16 <sup>a</sup>
Oxyfluorfen	51 <sup>a</sup>	201.33 <sup>a</sup>	16.45 <sup>a</sup>	83.00 <sup>ab</sup>	184.66 <sup>bc</sup>	26.55 <sup>ab</sup>
Sethoxydim + diclosulam	51 <sup>a</sup>	201.33 <sup>a</sup>	16.45 <sup>a</sup>	41.00 <sup>c</sup>	239.33 <sup>a</sup>	13.13 <sup>c</sup>
Metsulfuron-methyl	51 <sup>a</sup>	201.33 <sup>a</sup>	16.45 <sup>a</sup>	78.00 <sup>ab</sup>	180.50 <sup>c</sup>	25.05 <sup>ab</sup>
Fomesafen + fluazifop-p-butyl	51 <sup>a</sup>	201.33 <sup>a</sup>	16.45 <sup>a</sup>	41.66 <sup>c</sup>	224.33 <sup>abc</sup>	13.41 <sup>c</sup>
Nicosulfuron	51 <sup>a</sup>	201.33 <sup>a</sup>	16.45 <sup>a</sup>	67.66 <sup>b</sup>	226.00 <sup>ab</sup>	21.74 <sup>b</sup>
	1 x			2 x		
Tembotrione	49.50 <sup>cd</sup>	235.00 <sup>bc</sup>	15.86 <sup>c</sup>	68.50 <sup>bcd</sup>	233.50 <sup>ab</sup>	21.91 <sup>bc</sup>
Chlorimuron-ethyl	29.33 <sup>d</sup>	307.33 <sup>a</sup>	9.36 <sup>c</sup>	66.50 <sup>bcd</sup>	208.67 <sup>bc</sup>	21.36 <sup>bc</sup>
Oxyfluorfen	83.75 <sup>ab</sup>	246.33 <sup>b</sup>	24.39 <sup>b</sup>	77.67 <sup>abc</sup>	252.75 <sup>a</sup>	24.33 <sup>bc</sup>
Sethoxydim + diclosulam	102.67 <sup>a</sup>	206.00 <sup>cd</sup>	32.95 <sup>a</sup>	53.00 <sup>d</sup>	235.00 <sup>ab</sup>	16.98 <sup>c</sup>
Metsulfuron-methyl	83.50 <sup>ab</sup>	223.00 <sup>bc</sup>	26.85 <sup>ab</sup>	57.67 <sup>cd</sup>	187.00 <sup>c</sup>	18.54 <sup>c</sup>
Fomesafen + fluazifop-p-butyl	71.50 <sup>bc</sup>	229.00 <sup>bc</sup>	22.89 <sup>b</sup>	100.00 <sup>a</sup>	201.33 <sup>c</sup>	32.10 <sup>a</sup>
Nicosulfuron	90.50 <sup>ab</sup>	186.67 <sup>d</sup>	29.08 <sup>ab</sup>	83.67 <sup>ab</sup>	232.00 <sup>ab</sup>	26.75 <sup>ab</sup>

<sup>1</sup>Means followed by the same letter, into the same dose and variable, do not differ according to Tukey's test ( $p \leq 0.05$ ).

applied, respectively at 0.5-, 1- and 2-fold the recommended doses (Figure 5A). Fomesafen + fluazifop-p-butyl was shown to be more phytotoxic to the crop, especially when applying 2-fold the dose (250 + 250 g ha<sup>-1</sup>).

Compared with the control that produced around 16 g of dry mass per plant, the application of 2-fold the dose of fomesafen + fluazifop-p-butyl accumulated 5.3 g plant<sup>-1</sup>, that is 67% drop in the variable (Figure 5A). When comparing herbicides used at twice the dose, it is noted that the lowest accumulation of shoot dry mass occurred when oxyfluorfen was used, but did not differ statistically from fomesafen + fluazifop-p-butyl and nicosulfuron. At the same dose, the herbicide that presented the highest shoot dry mass was chlorimuron-ethyl, not differing from tembotrione (Table 5). The lower accumulation of dry mass is directly related to dose increase of herbicides, making an inversely proportional relation. Oxyfluorfen, fomesafen + fluazifop-p-butyl, metsulfuron-methyl and nicosulfuron caused greater phytotoxicity with necrosis, death of meristems and leaf abscission. In general, the application of herbicides reflected in lower dry mass of shoots in erva-mate plants.

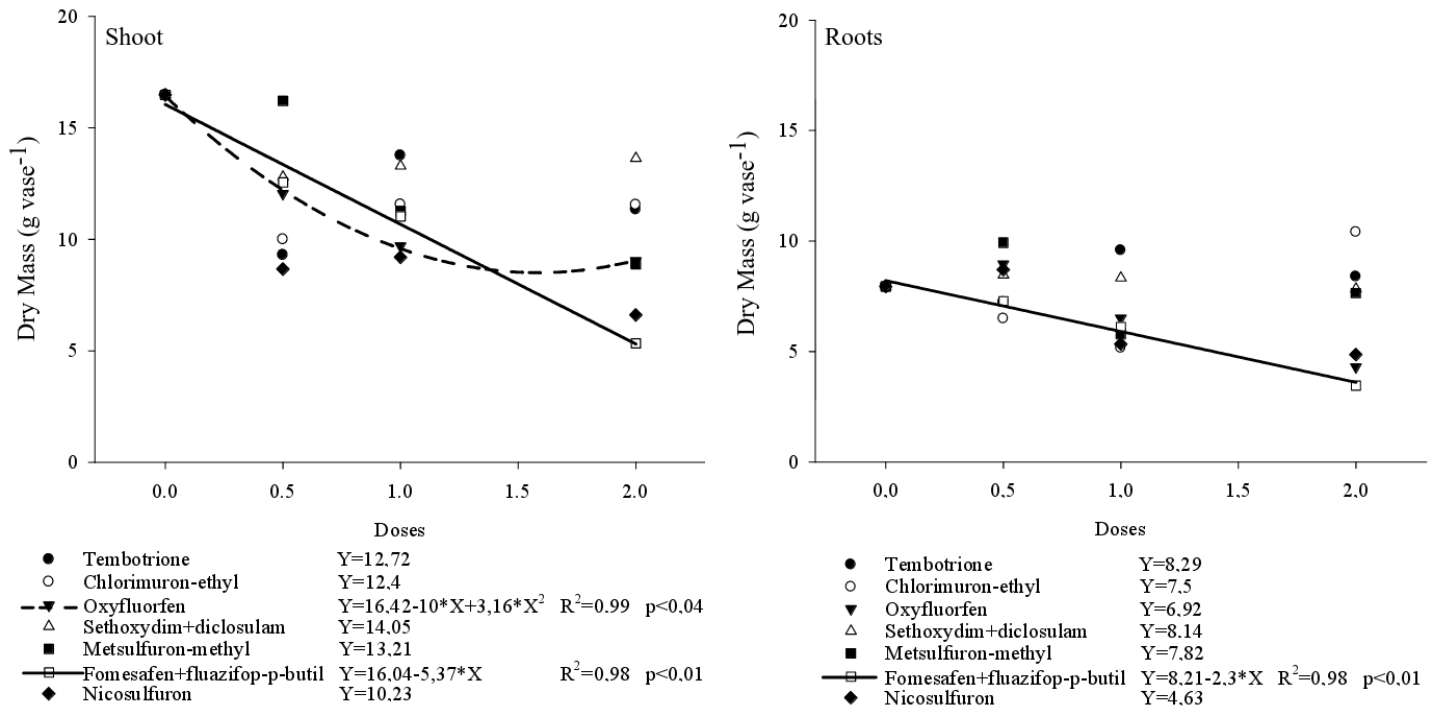
Root dry mass presented similar results to the shoot dry mass (Figure 5B). Reduction of root dry mass was observed with increasing dose of fomesafen + fluazifop-p-butyl; the application of 0.5-f, 1- and 2-fold the label dose resulted in a reduction of approximately 14, 28 and 56% accumulation of root dry mass, respectively for the doses (Figure 5B).

The herbicides that caused the most damage to

the plant root system were fomesafen + fluazifop-p-butyl, nicosulfuron and oxyfluorfen, which did not differ statistically in the highest dose tested (Table 5). It can be inferred that the contact effect of the herbicide reduced the photosynthetically active area and consequently the production of photoassimilates, directly influencing root development.

Brighenti and Muller (2014) observed reductions of up to 86% of Australian cedar root dry mass under the effect of different herbicide doses. Similar results were found by Brandão et al. (2014), who observed reduction of up to 50% in root dry mass of açai plants that received application of herbicides in 3-leaf stage. Tuffi-Santos et al. (2006) found a decrease of approximately 60% in the root dry mass of *Eucalyptus* under application of 172 g ha<sup>-1</sup> of glyphosate.

The herbicides interfere differently with erva-mate development. The increase in doses caused an increase in phytotoxicity, mainly for oxyfluorfen and fomesafen + fluazifop-p-butyl until 28 DAT. After this period, metsulfuron-methyl and nicosulfuron were more phytotoxic, with long-lasting effects so that at the end of the evaluated period (49 DAT) the lowest phytotoxicity was observed in plants applied with chlorimuron-ethyl and sethoxydim + diclosulam, which even at the highest dose did not cause more than 12% phytotoxicity. On the other hand, the highest level of injury was found mainly with the use of oxyfluorfen, fomesafen + fluazifop-p-butyl and nicosulfuron. In an attempt to detoxify the herbicides and emit new sprouts, an increase in plant metabolism and a consequent increase in the physiological



**Figure 5.** Dry mass of shoot (A) and roots (B) of erva-mate plants as function of herbicide and dose (Federal University of Fronteira Sul, Erechim, Brazil, 2016).

**Table 5.** Dry mass of shoot and roots of erva-mate plants, native genotype, as function of herbicide and dose (Federal University of Fronteira Sul, Erechim, Brazil, 2016).

Herbicides	Shoot dry mass (g)		Root dry mass (g)	
	0 x	0.5 x	0 x	0.5 x
Tembotrione	16.47 <sup>a1</sup>	9.29 <sup>d</sup>	7.95 <sup>a</sup>	7.24 <sup>bc</sup>
Chlorimuron-ethyl	16.47 <sup>a</sup>	10.00 <sup>cd</sup>	7.95 <sup>a</sup>	6.49 <sup>c</sup>
Oxyfluorfen	16.47 <sup>a</sup>	12.05 <sup>bc</sup>	7.95 <sup>a</sup>	8.95 <sup>ab</sup>
Sethoxydim + Diclosulam	16.47 <sup>a</sup>	12.80 <sup>b</sup>	7.95 <sup>a</sup>	8.46 <sup>abc</sup>
Metsulfuron-methyl	16.47 <sup>a</sup>	16.21 <sup>a</sup>	7.95 <sup>a</sup>	9.93 <sup>a</sup>
Fomesafen + Fluazifop-p-butyl	16.47 <sup>a</sup>	12.55 <sup>b</sup>	7.95 <sup>a</sup>	7.28 <sup>bc</sup>
Nicosulfuron	16.47 <sup>a</sup>	8.66 <sup>d</sup>	7.95 <sup>a</sup>	8.70 <sup>ab</sup>
1 x				
Tembotrione	13.77 <sup>a</sup>	11.35 <sup>b</sup>	9.58 <sup>a</sup>	8.39 <sup>ab</sup>
Chlorimuron-ethyl	11.57 <sup>ab</sup>	11.55 <sup>b</sup>	5.16 <sup>c</sup>	10.41 <sup>a</sup>
Oxyfluorfen	9.69 <sup>ab</sup>	9.04 <sup>c</sup>	6.50 <sup>bc</sup>	4.3 <sup>c</sup>
Sethoxydim + Diclosulam	13.29 <sup>ab</sup>	13.64 <sup>a</sup>	8.33 <sup>ab</sup>	7.83 <sup>b</sup>
Metsulfuron-methyl	11.27 <sup>ab</sup>	8.88 <sup>c</sup>	5.78 <sup>c</sup>	7.64 <sup>b</sup>
Fomesafen + Fluazifop-p-butyl	11.03 <sup>ab</sup>	5.33 <sup>e</sup>	6.10 <sup>bc</sup>	3.45 <sup>c</sup>
Nicosulfuron	9.19 <sup>b</sup>	6.61 <sup>d</sup>	5.33 <sup>c</sup>	4.86 <sup>c</sup>

<sup>1</sup>Means followed by the same letter, into the same dose and variable, do not differ according to Tukey's test ( $p \leq 0.05$ ).

characteristics were observed.

As the erva-mate is a plant that has not yet undergone

genetic improvement processes thus presenting high heterogeneity, there is a need to carry out additional

studies, especially concerning herbicide selectivity.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

The authors appreciate CNPq and FAPERGS for the scholarships and financial support to this research.

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