

Quality of yerba-mate leaves originating from male and female plants

Miroslava Rakocevic⁽¹⁾, Moacir José Sales Medrado⁽²⁾, Osmir José Lavoranti⁽²⁾, Alice Teresa Valduga⁽³⁾

⁽¹⁾Embrapa Informática Agropecuária, Av. André Tosello, n°. 209, Barão Geraldo, Caixa Postal 6041, CEP 13083-886, Campinas-SP. E-mail: mima@cnpia.embrapa.br ⁽²⁾Embrapa Florestas, Estrada da Ribeira, Km 111, Caixa Postal 319, CEP 83411-000, Colombo-PR. E-mail: medrado@cnpf.embrapa.br, osmir@cnpf.embrapa.br ⁽³⁾Universidade Regional Integrada de Alto Uruguai e Missões - URI, Av. Sete de Setembro, 1558 - 2º e 3º Andares, Caixa Postal 290, CEP 99.700-000, Erechim-RS. E-mail: valice@uri.com.br.

Abstract - The aim of this study was to assess the taste of beverage prepared from yerba-mate (*Ilex paraguariensis*) leaves, originated from male and female plants, by taking into account leaf gas exchange and the content of macro-and-trace-elements. Ecophysiological, chemical and sensorial properties were evaluated in one of the sprouting phases and in two fruit ripening phases. Male and female plants did not differ in fresh and dry matter production per plant, nor in the content of micro- and trace elements. However, female plants showed higher leaf net photosynthesis (A) and stomatal conductance (g_s) than male plants. The beverage prepared with male plant leaves was less bitter than that prepared with female plant leaves, especially when leaves from the branch tips were used. The intensity of bitterness was positively correlated with Zn and Mg contents in female plants and with Mn content and g_s in male plants. The bitterness of the beverage prepared with male plant leaves was negatively correlated with K content. The idea about “masculinization” of yerba-mate stands appeared as a possible solution for the production of yerba-mate of normal to soft taste.

Index terms: Macronutrients, photosynthesis, productivity, stomatal conductance, bitterness, trace elements.

Qualidade das folhas de erva-mate oriundas de plantas masculinas e femininas

Resumo - Este estudo teve por objetivo avaliar o sabor da bebida preparada a partir de folhas de erva-mate, de plantas masculinas e femininas. As propriedades ecofisiológicas, químicas e sensoriais foram avaliadas em uma das fases de brotação e em duas fases de frutificação. As plantas masculinas e femininas não diferiram em produção de matéria fresca e seca, nem nos teores de macro-e-micro-elementos. Mas, as plantas femininas apresentaram maior fotossíntese líquida (A) e condutância estomática (g_s). A bebida preparada com folhas oriundas de plantas masculinas foi menos amarga do que das femininas, particularmente quando coletadas das ponteiros dos ramos. A intensidade de amargor apresentou correlação positiva com o teor de Zn e Mg nas plantas femininas e com g_s e teor de Mn nas plantas masculinas. O amargor da bebida preparada de plantas masculinas mostrou-se negativamente correlacionada com teor de K. A “masculinização” de erval pode ser uma solução para se produzir erva-mate de sabor normal a suave.

Termos para indexação: Amargo, condutância estomática, fotossíntese, macro-elementos, micro-elementos, produtividade.

Introduction

Yerba-mate (*Ilex paraguariensis* St. Hil., Aquifoliaceae) is an evergreen subtropical tree that can reach 12-15 m in height. It occurs in nature at the subcanopy level in the subtropical rainforest with *Araucaria angustifolia* (PETERSEN et al., 2000). Industrially processed leaves of yerba-mate are used to prepare a South-American tea, which is called “chimarrão” in Brazil. For production, yerba-mate plants are trimmed annually, or every second or third year, and the plants are managed as shrubs of up to 3 m in height. In

modern production systems, yerba-mate shrubs are managed as pure monocultures or associated with cover crops (PICCOLO et al., 2004), in agroforestry systems (EIBL et al., 2000) or under shade in the forest understory.

Yerba-mate is a cryptic dioecious species, diploid, with $2n=40$ chromosomes (SCHERER, 1997). Although there are thousands of dioecious species, only a few of them have unambiguously demonstrated heteromorphic sex chromosomes (VYSKOT; HOBZA, 2004). There is yet neither an evidence of sex chromosomes in yerba-mate, nor any successful attempt to isolate possible genes

involved in sex determination (WENDT, 2005). It has been difficult or rather impossible to determine sex in yerba-mate plants before they reach flowering age (SCHERER, 1997). The analyses of genetic basis of sex in plants have shown, in some species, that it depends on the hormone biosynthesis pathways. In some species, sex expression is sensitive to the exogenously applied hormones: in *Mercurialis*, for example, auxins produced a “masculinizing” effect whereas cytokinins a “feminizing” effect (AINSWORTH, 2000). In contrast, in *Cannabis*, auxins induced female flowers on male plants (MOHAN RAM; JAISWAL, 1970), whereas gibberellins induced male flowers on females plants (MOHAN RAM; JAISWAL, 1972). Some cases of feminizing effects were observed in yerba-mate but without any experimental proof.

The need to allocate chemical elements during fruit ripening and seed maturation in plants explains the functional sex dimorphism in dioecious species and morphological differences between male and female plants. In yam (*Dioscorea* sp.), female plants produce more tubers (AKORODO et al., 1984) than do males. Male plants are reported to be more productive than the females in *Populus* sp. (TSCHAPLINSKI; TUSKAN, 1994) and asparagus (BENSON, 1982). In yerba-mate, female plants produced 15.8 % more leaves than did the males (STURION et al., 1995) in the winter harvesting.

Yerba-mate has been studied intensively in the aspects of management, production and genetic selection. However, the aspects of growth pattern, carbon acquisition and physiological dimorphism are still very poorly known. Two cycles of growth flushes and pauses during the year have been observed in yerba-mate plants (BAZZO; RAKOCEVIC, 2005). Growth ceased completely in the winter (June to September). Two regrowth flushes occurred from late September to early December and from March to June. The second growth pause, total or partial, occurred during the summer (from December to February). Seasonality in gas exchange has also been observed, following the periodical pattern in growth flushes and pauses in young yerba-mate plants in the field (RAKOCEVIC et al., 2005).

The Brazilian market for yerba-mate value green beverage color and normal (mild) to soft taste. However, no chemical or sensorial pattern has been defined yet in Brazil nor in any other South American country, in a manner similar to the established for black tea (*Camellia*

sinensis). Flavor and chemical qualities in black tea has been related to terpenoid biogenesis and to climatic factors at the harvesting time (WICKREMASINGHE, 1974). In yerba-mate, there is no evidence of correlation between production traits or chemical properties with environmental changes, physiological aspects, sex dimorphism, development phases, or harvest season.

It was hypothesized that yerba-mate plants would invest more energy than male plants in reproductive functions during the fruit ripening phase. This would lead to changes in leaf chemical composition and, thereby, in altered beverage sensorial properties. In addition, it was supposed that female plants would produce less leaf mass than do male plants. Therefore, this study was carried out to investigate the relation between the growing conditions, as well as the chemical and physiological properties of yerba-mate leaves produced by male and female plants with taste qualities of the beverage.

Material and methods

The field trial was established in Erechim, State of Rio Grande do Sul, Southern Brazil (27° 29' 6" S, 52° 21' 3" W, average altitude of 820 m). The climate is humid subtropical (Cfa), according to Köppen's climate classification system, with rainfalls regularly distributed along the year and average temperature greater than 22° C in the hottest month. The soil was classified as Rhodic Haplodux. Soil analysis from samples taken from around the yerba-mate plants showed pH (in CaCl) ranging from 3.85 to 4.22, and high levels of Mn (175-420 mg.dm⁻³) and exchangeable Al³⁺ (3.62-5.04 cmolc.dm⁻³).

Yerba-mate was grown in isolated rows. Four seven-year-old plants of each sex were marked in February 2003. Pruning had been done in the winter of 2000 and their crowns had grown to approximately 2.5 m in height. After measurements in February 2003, the trees were pruned to 0.8 m to 1.2 m in height. Only a few branches with bearing leaves were left for the maintenance of photosynthesis. The second production assessment was done in January 2005. Branches, leaves and fruits in each plant were harvested separately by position in the crown and the fresh and dry matters were determined.

Leaf production, leaf gas exchange, nutrient content, and sensorial tests were assessed in samples taken from two positions of foliated tree crown: the interior of the

middle third part of the tree (IN) and the branch tips (PO).

Ten leaves from each position in the crown, from four cardinal points of the plant were collected to form a composite sample with 40 leaves per position. Fruits were collected by following the same sampling procedure. The plant material was then oven-dried at 70° C until a constant weight was reached.

Calcium, Mg, and trace element contents were determined by using the procedure of flame atomic absorption spectrometry; P was determined with UV spectral photometry; K with flame photometry; and N with the classic Kjeldahl method.

Net photosynthesis ($A - \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and stomatal conductance ($g_s - \text{mol m}^{-2} \text{ s}^{-1}$), were measured in a LI-6200 analyzer (LICOR, Nebraska, USA). Field measurements were taken in natural stands (“*in situ*”), on leaves located inside (IN) of the middle third part of the trees, and at the tips (PO) of branches. The leaves at the tips of yerba-mate branches are considered to be “sun” leaves, which are not subjected to reduction in light by self-shading. Previous findings about patterns of leaf gas exchanges during the day indicated the need to have at least four replicates of observations in each leaf position. Measurements were taken hourly between 10:00 h and 15:00 h on the same leaves. The first assessment was made in February 2003, at fruit ripening phase when summer growth pause occurred; the second assessment was in March 2004, at the beginning of autumn regrowth; the third assessment was during the summer growth pause in January 2005 (fruit ripening phase and summer growth pause).

The sampling procedure used for the assessment of bitterness was similar to the case of nutritional analysis. The leaf position was not considered in 2003 and 2004 assessments. Only in 2005 were positions of the sample tissues within the crown considered.

Soluble extracts were prepared by percolating water through ground leaves of processed yerba-mate (VALDUGA et al., 2003). The sensorial hedonic tests of the extract was performed in cabins lighted with blue, green and red light.

The bitterness was assessed according to a 1 (soft) to 10 (bitter) scale (AMARINE et al., 1965; LAMOND, 1977) filled out by a group of 21 semi-trained persons who tasted the beverage during the period from 15:00 h to 17:00 h. Yerba-mate infusions at temperature of 50°

C to 60° C were served in volumes of 100 ml.

The productions of green and dry matter of male and female plants collected from two crown positions (inside and tips) were compared and tested by Levene’s test for homogeneity prior to the analyses of variance (MONTGOMERY, 1991). A completely random design was used, with four replications in a 2 x 2 factorial distribution (two sexes and two positions in the crown). The assumed model was verified by performing the analysis of residue, the test for the homogeneity of variances, and for the normality of errors.

The experiment for the analysis of macronutrients and trace elements followed a completely random design with four replications in a 2 x 2 x 3 factorial distribution (two sexes, two positions in the crown, and three harvesting periods). Orthogonal contrasts (STELL; TORRIE, 1980) were planned to test the effects of harvests performed in different phenophases (fruit ripening periods vs. period of regrowth or Feb2003 and Jan2005 vs. Mar2004) and to test the environmental effects of harvests performed in the same phenophase (Feb2003 vs. Jan2005) for each crown position.

The scores attributed for beverage bitterness of yerba-mate from male and female plants were analyzed by the generalized linear model with gamma distribution and correlated with gas exchange (A, g_s), macronutrient and trace element data.

Results

The yerba-mate trees in production did not show sexual phenotypic dimorphism, until at the time of flowering and fruit ripening phases, when it is easy to distinguish male plants from the females. No difference in total green and dry matter production was observed between sexes (Table 1). The analysis of variance did not reveal significant variation in production variables (p -values > 0.1) between sexes. Branches in male plants produced more leaves in the interior (older leaves) than at the tips; in female plants, the branches carried more fruits in the interior than at the tips (Table 1, marked in bold).

There was no apparent sexual dimorphism nor variation among plants of the same sex, and among positions in the crown with respect to concentrations of macro- (N, P, K, Ca, Mg) and micro-elements (Cu, Fe, Mn, Zn) (Tables 2 and 3). Although leaves from the tip of the branches are expected to contain less N due to their younger age than the leaves at the interior, no significant variation in N concentration among leaf positions was detected.

Table 1. Fresh and dry matter productions in female and male maté plants

Sex	Position on tree crown	Production parameters (kg/plant)																
		Green leaves		Dry leaves		Green branches		Dry branches		Fresh fruits		Dry fruits		Total / FM ⁴		Total / DM ⁴		
		Mean	Error ¹	Mean	Error ¹	Mean	Error ¹	Mean	Error ¹	Mean	Error ¹	Mean	Error ¹	Mean	Error ¹	Mean	Error ¹	
Females																		
	Inside	1.1872	0.1431	0.5152	0.0618	1.5400	0.1999	0.7069	0.1214	0.2235	0.0473	0.6731	0.0289	2.9507	0.3730	1.2894	0.1807	
	Tips	1.3417	0.1107	0.5642	0.0540	2.0937	0.3521	0.8288	0.1170	0.1399	0.0475	0.4761	0.0118	3.5753	0.4046	1.4407	0.1280	
	Shapiro-Wilk ²	W	p-value	W	p-value	W	p-value	W	p-value	W	p-value	W	p-value	W	p-value	W	p-value	
	Inside	0.9684	0.8320	0.9502	0.7179	0.8933	0.3985	0.8730	0.3098	0.9083	0.4736	0.8399	0.1951	0.9652	0.8120	0.8965	0.4141	
	Tips	0.9563	0.7561	0.9269	0.5763	0.8947	0.4053	0.9778	0.8893	0.8427	0.2036	0.8837	0.3548	0.9664	0.8193	0.9227	0.5524	
	F value	p-value	F value	p-value	F value	p-value	F value	p-value	F value	p-value	F value	p-value	F value	p-value	F value	p-value	F value	p-value
	Levene's Test ³	0.44	0.5323	0.13	0.7321	2.62	0.1566	0.01	0.9172	0.00	0.9924	8.97	0.0241	0.06	0.8191	0.76	0.4163	
Males																		
	Inside	1.4279	0.0522	0.5891	0.0428	1.8790	0.3791	0.8781	0.1734					3.3070	0.4304	1.4673	0.2136	
	Tips	1.2735	0.1186	0.4837	0.0429	2.0637	0.3049	0.8219	0.1185					3.3372	0.4189	1.3056	0.1575	
	Shapiro-Wilk ²	W	p-value	W	p-value	W	p-value	W	p-value					W	p-value	W	p-value	
	Inside	0.7716	0.0485	0.9308	0.4917	0.9193	0.4501	0.8540	0.2513					0.9050	0.4017	0.7964	0.1060	
	Tips	0.9912	0.8201	0.8198	0.1628	0.9458	0.5513	0.9966	0.8883					0.9796	0.7263	0.9949	0.8632	
	F value	p-value	F value	p-value	F value	p-value	F value	p-value	F value	p-value				F value	p-value	F value	p-value	
	Levene's Test ³	2.50	0.1817	0.00	0.9957	0.35	0.5848	0.93	0.3889					0.01	0.9427	0.64	0.4678	
	Mean	Error ¹	Mean	Error ¹	Mean	Error ¹	Mean	Error ¹	Mean	Error ¹	Mean	Error ¹	Mean	Error ¹	Mean	Error ¹	Mean	Error ¹
	Female	1.2644	0.0887	0.5397	0.0391	1.8169	0.2146	0.7678	0.0813	0.1817	0.0348	0.5746	0.0067	3.2630	0.2807	1.3651	0.1064	
	Male	1.3507	0.0674	0.5364	0.0359	1.9714	0.2214	0.8500	0.0947					3.3220	0.2686	1.3864	0.1241	
	F value	p-value	F value	p-value	F value	p-value	F value	p-value	F value	p-value				F value	p-value	F value	p-value	
	Levene's Test ³	0.96	0.3472	0.41	0.5322	0.25	0.6230	0.00	0.9593					0.48	0.5018	0.00	0.9675	

¹ Stand error mean; ² Tests for normality, ³ Levene's test for homogeneity of variance - ANOVA of squared deviations from group means; ⁴ Total fresh (FM) and dry matter (DM) were calculated with fruits in females

Table 2. Analysis of variance for macronutrients in maté leaves collected from male and female plants in different tree crown positions and harvesting times. split-split-plot design, four-plant plot, with whole-plot in a completely randomized design. Factor sex on the whole-plot was split by position on the sub-plot, which was split by factor harvesting time on the sub-sub plot.

Source of variance	N	Pr > F	P	Pr > F	K	Pr > F	Ca	Pr > F	Mg	Pr > F		
											DF	Sum of Squares
Model	(21)	(358.0067)	(37.0704)	(521.4110)	(97.5644)	(122.8474)						
Plants (Sex)	Sex	1	6.2489	0.4812	0.0566	0.8883	2.6680	0.8366	4.1311	0.3837	4.9227	0.5495
		5	54.0063		12.9633		282.7882		22.6805		59.8322	
	Position	1	14.0709	0.4171	0.2438	0.3969	1.5200	0.4916	1.7856	0.5836	2.4869	0.1141
	Sex x Position	1	5.2501	0.5832	0.0213	0.8004	15.3756	0.1450	0.0113	0.9640	0.1198	0.6975
Plants (Sex x Position)		5	76.3949		1.5017		25.8153		25.1925		3.5312	
	Harvesting time	(2)	(107.2396)	0.0586	(21.4056)	<0.0001	(80.2208)	0.1976	(11.5275)	0.1392	(39.3198)	0.0016
Feb-2003 e Jan-2005 vs Mar-2004		1	18.7721	0.2909	19.6199	<0.0001	71.2116	0.1075	4.5101	0.2531	0.3748	0.6961
Feb-2003 vs Jan-2005		1	88.4675	0.0289	1.7857	0.0912	9.0092	0.5556	7.0173	0.1577	38.9450	0.0006
	Sex x Harvesting time	2	4.1711	0.8809	0.0914	0.9213	83.3812	0.1863	5.3501	0.3815	2.7826	0.5360
	Position x Harvesting time	2	59.3267	0.1887	0.4566	0.6682	28.0513	0.5502	10.6289	0.1602	6.0618	0.2693
	Sex x Position x Harvesting time	2	31.2981	0.4008	0.3301	0.7460	1.5907	0.9658	16.2569	0.0685	3.7905	0.4316
Error		20	326.9465		11.1010		455.6541		52.8884		43.2410	
Corrected Total		41	684.9532		48.1714		977.0652		150.4528		166.0884	

The contents of nutrient elements in the leaves, except K, Ca, and Zn, varied throughout the harvesting times (Tables 2 and 3) but they followed no definite pattern (Figures 1 and 2). The concentration of phosphorus in the leaves was higher in the regrowth phase (March 2004) than in the fruit ripening (Figure 1B). The lower concentrations of N and P were shown in February 2003 rather than in January 2005 (two fruit ripening phases), in opposite to Mg, Cu, Fe and Mn concentrations which were more important in February 2003 than in January 2005 (Figures 1 and 2, Table 2).

The differences in the macronutrient contents between fruits and leaves (Table 4) were more evident at the second fruit ripening phase (January 2005, Figure 1). As observed in leaves, the macro-element concentration also varied in fruits but without a defined pattern (Figure 1). Ca and Mg were exceptions, and showed the same pattern of higher concentration in leaves than in fruits (Figure 1B), in two fruit ripening phases (Table 4). The N content was also lower in fruits than in leaves, whereas K and P contents in fruits were higher than in leaves (Figures 1A and 1B).

Table 3. Analysis of variance (p-value) for trace elements in maté leaves collected from male and female plants in different tree crown positions (inside – IN, and tips – PO) and harvesting times (details in text).

Source of variance	DF	Cu		Fe		Mn		Zn	
		Sum of Squares	Pr > F	Sum of Squares	Pr > F	Sum of Squares	Pr > F	Sum of Squares	Pr > F
Model	(17)	(120.1900)		(2.0509E6)		(4.0882E7)		(6.7595E4)	
Plants (Sex)	1	5.8936	0.511	3.5629E2	0.851	1.4171E6	0.415	3.3870E3	0.403
	5	58.9285		4.5945E4		8.9779E6		2.0339E4	
	1	5.6700	0.141	2.2857E2	0.703	1.2917E6	0.500	9.4723E1	0.877
Plants (Sex x Position)	1	0.5265	0.602	2.4537E3	0.384	9.2037E4	0.848	8.6528E2	0.732
	5	8.5085		1.3494E4		1.1327E7		3.3175E4	
	1	33.8800	0.028	1.9641E6	<0.000	1.3778E7	0.024	4.5441E3	0.370
Sex x Harvesting time	1	6.1615	0.301	2.0868E4	0.044	1.0064E6	0.489	4.5261E3	0.371
Position x Harvesting time	1	0.2414	0.833	3.0228E2	0.787	1.1546E6	0.459	3.5437E1	0.935
Sex x Position x Harvesting time	1	0.3800	0.792	3.0842E3	0.397	1.8356E6	0.355	6.2757E2	0.734
Error	10	5.18870		3.9497E4		1.9542E7		5.1760E4	
Corrected Total	27	172.0771		2.0904E6		6.0425E7		1.1935E5	

Table 4. Analysis of variance (p-value) of A/ macronutrients and B/ trace elements in maté fruits and leaves collected from female plants in different tree crown positions (inside and tips) and two phases of fruit ripening (February 2003 and January 2005).

A/ Source of variance	DF	N		P		K		Ca		Mg	
		Sum of Squares	Pr > F	Sum of Squares	Pr > F	Sum of Squares	Pr > F	Sum of Squares	Pr > F	Sum of Squares	Pr > F
Model	(13)	(190.1104)		(3.8792)		(302.8556)		(83.2872)		(59.1774)	
Organ	1	92.9244	0.006	1.5311	0.021	95.3325	0.038	63.2333	<0.000	34.4313	0.002
Position	1	3.9200	0.510	0.0122	0.815	18.7027	0.319	1.1340	0.434	0.0101	0.947
Plants (Organ x Position)	11	93.2660		2.3359		188.8204		18.9199		24.7360	
Error	3	71.9989		1.1459		5.4545		1.2869		3.6373	
Corrected Total	16	262.1093		5.0251		308.3101		84.5740		62.8147	

B/ Source of variance	DF	Cu		Fe		Mn		Zn	
		Sum of Squares	Pr > F	Sum of Squares	Pr > F	Sum of Squares	Pr > F	Sum of Squares	Pr > F
Model	(13)	(33.5304)		(8.7062E5)		(1.5470E7)		(1.2307E4)	
Organ	1	21.1407	0.001	4.7193E4	0.427	8.0203E6	0.005	6.8725E3	0.002
Position	1	0.0032	0.958	5.8345E4	0.379	6.0621E4	0.769	6.0273E2	0.266
Plants (Organ x Position)	11	12.3865		7.6508E5		7.3891E6		4.8326E3	
Error	3	0.2800		4.0636E5		1.0583E6		1.9189E3	
Corrected Total	16	33.8104		1.2769E6		1.6528E7		1.4226E4	

The microelement concentrations showed a defined pattern (Table 4B). The concentrations of Zn and Mn in the fruits were lower than in leaves (Figures 2A and 2B), whereas Cu was in higher concentration in fruits than in leaves (Figure 2A). Manganese concentrations in fruits were in the order of 695 mg.kg⁻¹ to 800 mg.kg⁻¹, but always in concentrations significantly lower than in leaves. These values were extremely high (one sample showed 5,500 mg.kg⁻¹), especially in leaves collected in February 2003 (Figure 2B).

The general pattern of gas exchange response in yerba-mate showed a higher net photosynthesis (*A*) in female than in male plants, whereas stomatal conductance (*g_s*) was high only at the branch tips (Figure 3). Gas exchange response was highly dependent on the phenophases. The highest rate of gas exchange were observed during the branch regrowth. The lowest values were observed during fruit ripening (Figure 3). The *A* and *g_s* varied according to the phases of fruit ripening.

The beverages obtained from leaves of either male or female plants were classified as normal (mild) in the bitterness scale. The assessments by individual testers showed significant variation in the evaluation process (Table 5). Generally, plant sex did not affect the bitterness of “chimarrão”. There was an interaction between sex and position in the crown in the aspect of beverage bitterness (Table 5). Leaves from male plants at branch tips produced softer beverages than leaves from the inside the crowns (Figure 4A). Leaves from inside the crown of male trees produced beverages with bitterness similar to those from the whole crown of female trees.

Table 5. The gamma scale parameter estimated by DOF/Pearson's chi-square for taste of maté beverage obtained from leaves collected from female plants in different tree crown positions (inside and tips) in three harvesting times (February 2003, March 2004 and January 2005).

Source of variance	Deviance	DF	Chi-Square	Pr > ChiSq
Intercept	25.7716			
Tester	24.5535	20	44.91	0.0011
Sex	24.5435	1	0.37	0.5433
Plants (Sex)	22.3851	6		
Position	23.3345	1	8.76	0.0031
Sex x Position	22.3035	(1)	4.14	0.0418
Females - Position		1	1.14	0.2856
Males - Position		1	9.52	0.0020
Plants (Sex x Position)	21.8806	6		
Harvesting time	21.2697	(2)	22.52	<0.0001
Feb-2003 e Jan-2005 vs Mar-2004		1	8.49	0.0036
Feb-2003 vs Jan-2005		1	17.43	<0.0001
Sex x Harvesting time	20.1755	(2)	40.34	<0.0001
(Females)				
Feb-2003 e Jan-2005 vs Mar-2004		1	0.18	0.6718
Feb-2003 vs Jan-2005		1	0.43	0.5105
(Males)				
Feb-2003 e Jan-2005 vs Mar-2004		1	16.62	<0.0001
Feb-2003 vs Jan-2005		1	58.66	<0.0001
Position x Harvesting time	20.1129	2	2.31	0.3152
Sex x Position x Harvesting time	20.0871	2	0.95	0.6222
Scaled Deviance	740.5219666			
Pearson Chi-Square	18.0657			

The bitterness of “chimarrão” prepared with leaves from male trees was affected also by the harvesting time (Table 5). The most intense bitterness was observed in male plants harvested in February 2003 (phase of fruit ripening and growth pause - the time recommended for harvesting) in comparison to other harvesting periods (Figure 4B). While male trees tended to produce softer leaves at the branch tips than the females, opposite patterns were observed at some harvesting periods indicated evidence of “sex and harvesting time” interactions (Table 5).

The bitterness of yerba-mate “chimarrão” was positively correlated *g_s* and Mn content in the leaves. The correlation of bitterness with K content in the leaves of male trees was negative (Table 6). In female trees, bitterness was positively correlated with Mg and Zn contents in the leaves.

Discussion

Green and dry matter production per plant did not vary between sexes in yerba-mate trees (Table 1). Similar results were reported in Argentina (BELINGHERI; PRAT KRICUN, 1992). Evaluation of 31 families in three locations in the State of Paraná, Brazil (STURION et al., 1995) showed that female plants produce 15.1 % more leaf mass than male plants. The pattern of leaf distribution on the branches of male plants (more leaves at the branch tips - Table 1) differs from female plants, which suggests more intensive leaf shed in females than in males (RAKOCEVIC et al., 2006), which, in some cases, can contribute to the sexual differentiation of the final leaf production. All previous experiments considered individual plants without regard to progeny or provenance. The distinction in production between sexes (dimorphism) can be ascertained by comparing the performance of both sexes from the same family.

The requirement levels of micro- and macronutrients by yerba-mate plants were classified in a decreasing order as N, Ca, P, K, Mg, Zn, Cu, and Fe (BELLOTE; STURION, 1985). Observed variations in P content among seasons (Table 2) are due to a higher mobilization during the period of regrowth (March) than during fruit ripening (January-February). Yerba-mate shows seasonal variation in N, P and K contents. There is a higher export in October (regrowth) and stability from May to August (REISSMANN et al., 1985).

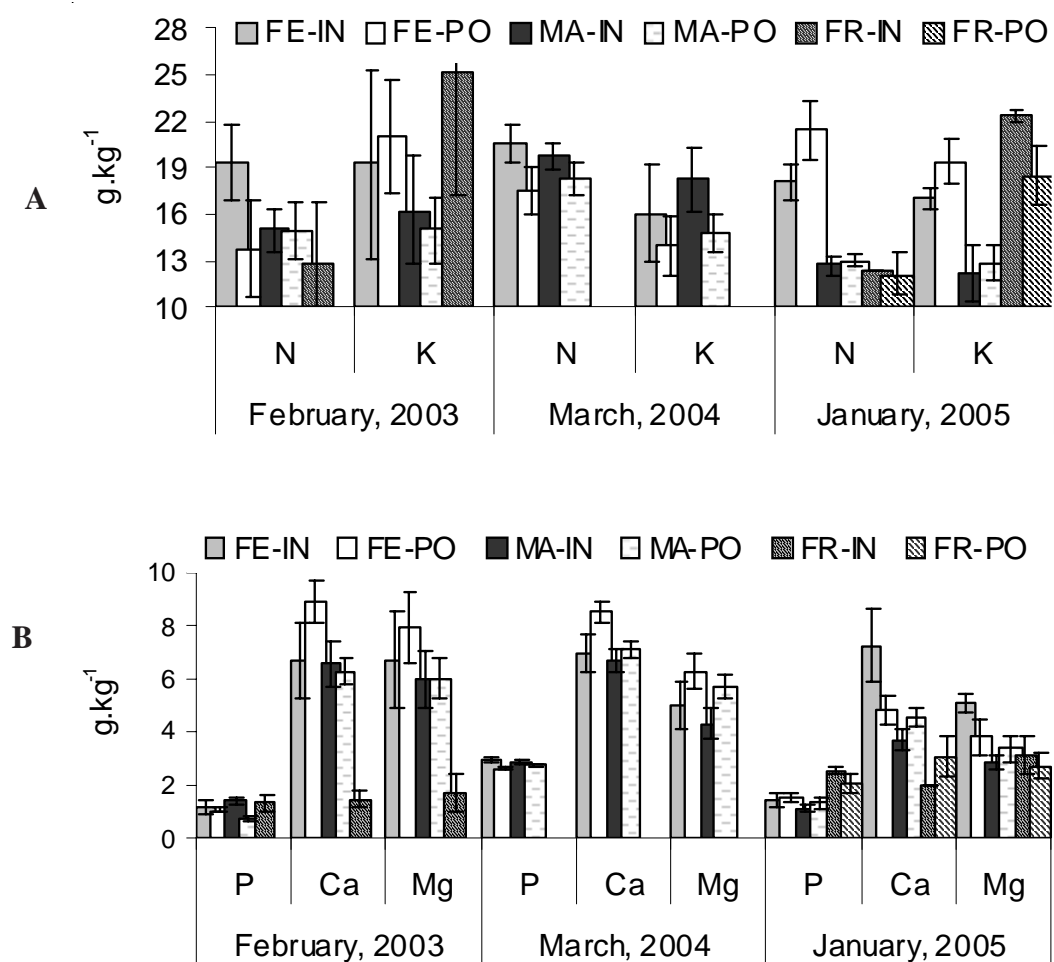


Figure 1. Standard error and average content of macronutrients **A/** N and K; **B/** P, Ca and Mg in maté leaves from males (MA), females (FE), and (fruits FR) collected inside (IN) and in tips (PO) of tree crowns in two phases of fruit ripening (February 2003 and January 2005) and one phase of regrowth (March 2004).

Yerba-mate occurs on soils of medium to clay texture, without water deficiency, in places not prone to flooding, generally on sites with low content of nutrients and high contents of Al (OLIVEIRA; ROTTA, 1985). It does not grow well on shallow soils (DEDECEK, 1997). The application of N-NH₄ fertilizer tends to acidify the rhizosphere. This enhances the absorption of K, Mg, Fe, and Mn (GAIAD et al., 2006) and, consequently, stimulates growth in leaf area, leaf number and net photosynthesis. Several species, when grown on soils with high amounts of available metal ions, accumulate the excess of ions in vacuoles, in concentrations higher than the required for their physiological processes. Because yerba-mate is a Al-Mn tolerant species and Mn mobilization is enhanced in acid subtropical soils, Mn

tends to accumulate in high concentration in yerba-mate leaves (Figure 2B), far above the average in other subtropical species (BOEGER et al., 2005). Mn concentration in yerba-mate leaves reaches over 100-fold the concentration found in peach leaves (BASAR, 2006). By considering that storage organs in plants can be used for human nutrition and phytotherapy, high metal ion concentrations can become a threat to human health. The World Health Organization has defined maximum allowable levels only of arsenic, cadmium and lead in the amounts of 1.0, 0.3, and 10.0 mg/kg, respectively, in foodstuff (EOZAK et al., 2002). The high Mn accumulation in yerba-mate may require more research to determine the consequences of ingesting such amounts of the element.

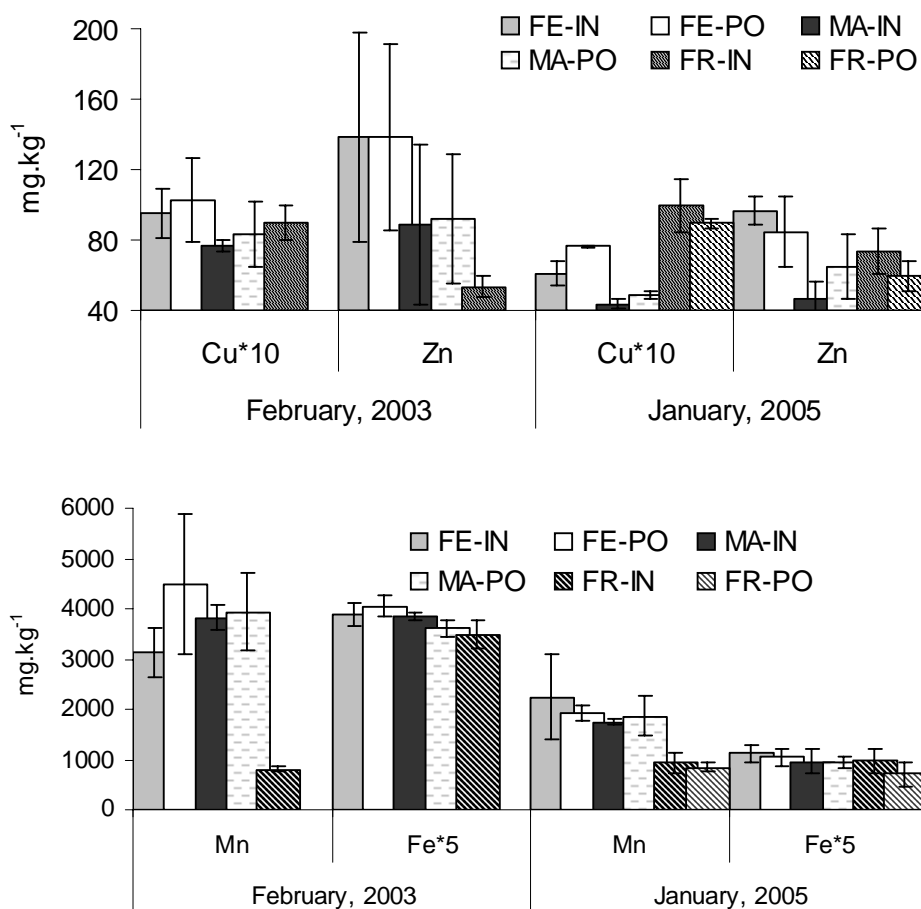


Figure 2. Standard error and average content of trace elements A/ Cu and Zn; B/ Mn e Fe in maté leaves from males (MA), females (FE), and (fruits FR) collected inside (IN) and in tips (PO) of tree crowns in two phases of fruit ripening (February 2003 and January 2005).

Phosphorous, K and Cu contents were higher in fruits than in leaves of female plants (Table 4, Figures 1A, 1B and 2A). A higher accumulation of these ions were observed in regions with higher physiological activities (HO, 1988). The lower N concentration in fruits than in the leaves (Table 4) was expected (THORNE, 1985) because a large portion (up to 75 %) of N contained in leaves is used in photosynthesis and fruits are major importers of N.

In populations of yerba-mate constituted by advanced-age plants the predomination of heterozygotes in females was observed, whereas in males an important level of endogamy was noted (WENDT, 2005). The excess of heterozygotes in females could be justified by the premature death of endogamic individuals through the natural selection and survival of the most competitive heterozygote females and elimination of female

homozygotes. Obeso et al. (1998) observed that the efficiency of the photosynthesis of leaves on nonfruiting branches of females of *Ilex aquifolium* (another species from the same genus as yerba-mate) was higher than the ones for leaves on branches of male plants under low-light conditions. This observation is in agreement with higher stomatal conductance and net photosynthetic rate of females than males in yerba-mate (Figure 3), and the variation observed on leaves under self-shading effect. This expression of sexual dimorphism can be attributed to the higher reproduction cost in females as concluded by Obeso et al. (1998).

Up to now, the hedonic test has been the unique method applied in yerba-mate beverage sensorial properties evaluation. This method did not ensure precision because of subjectivity, even when a large number of testers is involved (Table 5). The experience

showed that hedonic evaluations were affected by personal sensibility, motivation, physical and mental conditions of the testers. Some electronic instruments such as artificial neural networks outfitted with a set of sensors have been developed for complex aroma and taste analyses (DUTTA et al., 2003; BURATTI et al., 2007). These have been extensively used for the classification of foodstuffs such as eggs, beverages (coffee, tea), fish and meat. The great subjectivity of human tester panels indicates the need to establish an objective method for yerba-mate taste evaluation, as is the establishment of standards for recognition with the use of an electronic tongue.

A positive correlation was found between bitterness and Zn concentration in female plants (Table 6). Zinc is a highly fixed metal ion in plant cells. It has a restricted mobility,

especially in older yerba-mate leaves (REISSMANN et al., 1994). In tea plants (*Camellia sinensis* L.), Zn concentration increases rapidly in new spring sprouts and, in a few weeks, its absorption diminishes gradually, whereas Cu is absorbed in low intensity but constantly (TSUSHIDA; TAKEO, 1977). These authors concluded that green tea processed with young leaves (rich in Zn) was of a higher quality than the tea processed with old leaves. The fact that leaves on branch tips of male yerba-mate plants are less bitter than the leaves of female plants (Figure 4A) is consistent with the pattern found in tea (*Camellia sinensis* L.) plants, and suggests the need to include leaf age as a factor in future research on the influence of sex on yerba-mate taste.

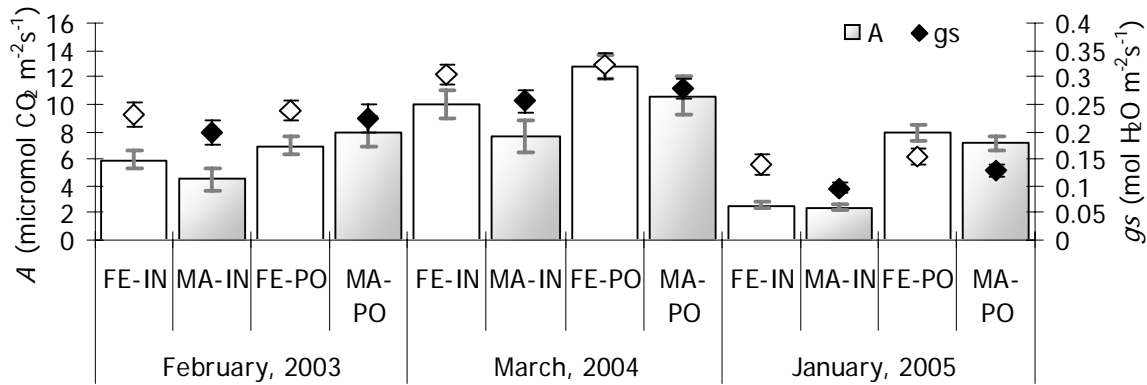


Figure 3. Standard error and average net photosynthesis (A) and stomatal conductance (g_s) measured in maté leaves on females (FE) and males (MA) in interior (IN) and tips (PO) of tree crowns in two phases of fruit ripening (February 2003 and January 2005) and one phase of regrowth (March 2004).

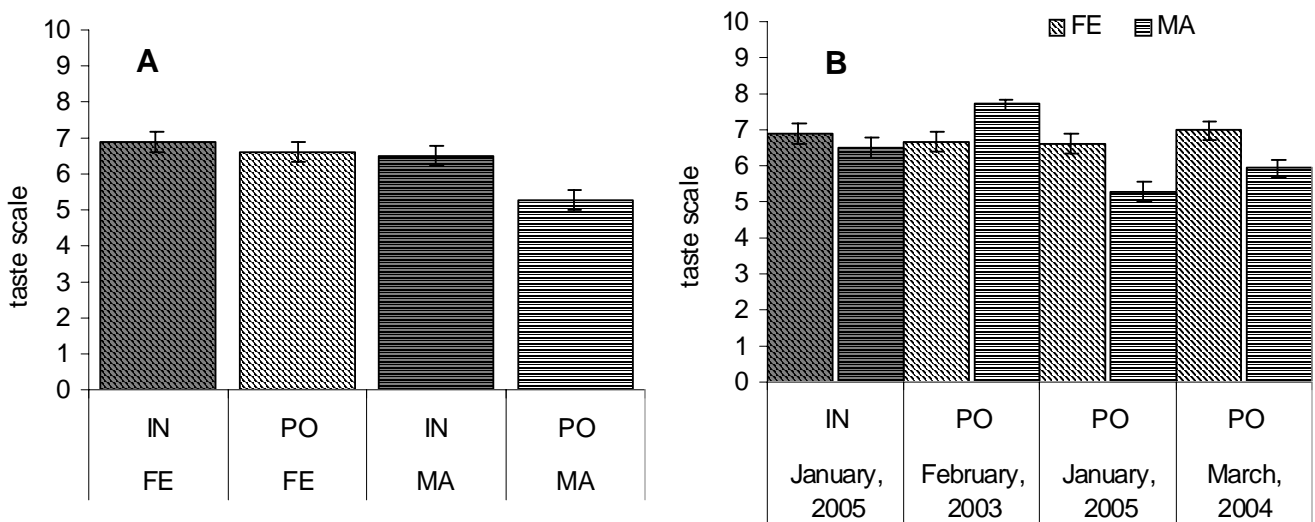


Figure 4. Standard error and average note for bitterness of maté leaves **A/** General analyses; **B/** Leaves collected in different harvesting times from males (MA), females (FE), from inside (IN) and tips (PO) of tree crowns in the phases of fruit ripening (February 2003 and January 2005) and regrowth (March 2004).

The positive correlation found between bitterness and stomatal conductance (g_s) (Table 6), and the low gas exchange during the phases of growth pause and fruit ripening (Figure 3) can be compared with correlations found in tea (*Camellia sinensis* L.). Wirckremasinghe (1974) associated tea quality with climatic conditions and flavour development. The best Ceylon tea quality was obtained when leaves were harvested during dry and cool seasons, in windy, bright, and cloudless days, followed by cold (6-10° C) nights. The flavour was associated with terpenoids normally derived from acetate pathways inside and outside the chloroplasts. The impermeability of the chloroplast membrane to mevalonic acid was associated with a better tea quality. Tea flavour

was also correlated with photosynthesis rate, closed stomata and chlorophyll content. The positive correlation found between Mg content and bitterness in yerba-mate (Table 6) can be due to a higher chlorophyll content in bitter yerba-mate (REISSMANN et al., 1994). This aspect must be confirmed in future works. The bitterness of yerba-mate was negatively correlated with K content in the leaf (Table 6). Potassium plays a relevant role in biochemical functions in plants (BEDNARZ; OOSTERHUIS, 1999), and in the stomatal opening (HUMBLE; RASCHKE, 1971). It has been associated with stomatal conductance and photosynthetic rate (EGILLA; DAVIES, 1995; BASILE et al., 2003).

Table 6 (A). Biserial correlation between soft, normal and bitter taste of maté beverage with gas exchange (A, g_s), macronutrients and trace elements in females and Pearson correlation coefficient between sensorial analysis of maté beverage with gas exchange (A, g_s), macronutrients and trace elements in female plants.

Taste	Statistics	Gas exchange				Macronutrients				Trace elements		
		A	g_s	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
Soft	R	0.324	0.355	-0.218	-0.500	-0.319	0.425	0.628	0.224	0.066	0.303	0.790
	p-value	0.303	0.256	0.495	0.097	0.311	0.167	0.028	0.483	0.837	0.337	0.002
Normal	R	0.420	0.317	-0.261	-0.174	-0.318	0.413	0.755	0.160	0.055	0.358	0.821
	p-value	0.173	0.314	0.412	0.588	0.313	0.182	0.004	0.617	0.864	0.253	0.001
Bitter	R	0.406	0.264	-0.262	-0.093	-0.214	0.505	0.874	0.143	0.161	0.365	0.754
	p-value	0.189	0.405	0.409	0.772	0.502	0.093	0.000	0.656	0.615	0.242	0.004
Sensorial Analysis	R	0.370	0.306	-0.328	-0.402	-0.405	0.411	0.857	0.093	0.027	0.380	0.775
	p-value	0.235	0.332	0.297	0.195	0.190	0.184	0.000	0.773	0.933	0.222	0.002

Table 6 (B). Biserial correlation between sweet, normal and bitter taste of maté beverage with gas exchange (A, g_s), macronutrients and trace elements in males and Pearson correlation coefficient between sensorial analysis of maté beverage with gas exchange (A, g_s), macronutrients and trace elements in male plants.

Taste	Statistics	Gas exchange				Macronutrients				Trace elements		
		A	g_s	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
Soft	R	0.121	0.967	-0.229	-0.045	-0.974	-0.207	0.362	0.250	0.260	0.903	0.151
	p-value	0.706	<0.000	0.474	0.887	<0.000	0.518	0.246	0.433	0.414	0.000	0.637
Normal	R	0.153	0.802	-0.504	-0.388	-0.850	-0.261	0.287	0.158	0.557	0.933	0.157
	p-value	0.633	0.001	0.094	0.212	0.000	0.412	0.365	0.622	0.059	<0.000	0.624
Bitter	R	-0.258	0.886	-0.332	-0.221	-0.943	-0.261	0.212	0.330	0.260	0.929	0.131
	p-value	0.416	0.000	0.291	0.489	0.000	0.411	0.507	0.293	0.413	<0.000	0.682
Sensorial Analysis	R	-0.020	0.980	-0.496	-0.115	-0.931	-0.243	0.352	0.182	0.463	0.972	0.085
	p-value	0.957	<0.000	0.174	0.767	<0.000	0.528	0.352	0.638	0.209	<0.000	0.826

Conclusions

Our analyses indicated that the taste of yerba-mate was influenced by the plant sex, mineral composition, harvesting time and gas exchange properties. In spite of the small number of individuals involved in this experiment it can be concluded that:

- Male plant leaves appeared to be less bitter than the female plant leaves, especially those produced at the tips of the branches. Therefore, the masculinization of yerba-mate plantation could be an alternative to improve the taste of yerba-mate beverage;

- The positive correlation between bitterness of yerba-mate beverage with stomatal conductance in males, and with Zn and Mg contents in females, besides the negative correlation with K content in the leaves of male plants, indicates the association of mineral content with some physiological functions related to gas exchange in a sexual dimorphism expression;

- More objective methods for yerba-mate sensorial testing is needed, such as the standard recognition by using an electronic tongue;

- More individuals, plants or clones of both sexes must be observed in future studies, in attempt to obtain more accurate data;

- More research on the relation of beverage taste with plant sex, climate, biochemistry, and physiology of yerba-mate are required in order to develop new practices in yerba-mate cultivation.

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