

Leaf gas exchange differences between males and females of yerba-mate

Miroslava Rakocevic⁽¹⁾, Moacir José Sales Medrado⁽²⁾, Osmir José Lavoranti⁽²⁾

⁽¹⁾Embrapa Informática Agropecuária, Av. André Tosello, n°. 209, Barão Geraldo, Caixa Postal 6041, CEP 13083-886, Campinas,-SP, Brazil. E-mail: mima@cnpia.embrapa.br ⁽²⁾Embrapa Florestas, Estrada da Ribeira Km 111, Caixa Postal 319, CEP 83411-000, Colombo-PR, Brazil. E-mail: medrado@cnpf.embrapa.br, osmir@cnpf.embrapa.br

Abstract - A preliminary study was developed on leaf gas exchange properties of males and females in yerba-mate (*Ilex paraguariensis* St. Hil.), planted in open growth conditions. The photosynthetic photon flux density (PPFD) on leaf level, stomatal conductance (g_s), net photosynthesis (A), transpiration (E), and leaf temperature (T_l) was measured during the biennial production period: in the summer during the fruit ripening when there is a pause in its growth (January, February), spring sprouting (November) and autumn sprouting (March, Jun). The measurements have been taken in different tree crown positions (inside, exterior and tips). First indications of sex physiological dimorphism in yerba-mate have been shown. Conductance was superior on female plants, with exception on tips, whereas the A and E were superior on females, on the exterior of the third medium of the tree crown. Positive correlations among gas exchange properties and PPFD were established. The seasonality in gas exchange was observed. The maximum gas exchange values were registered in active sprouting whereas the minimum values were registered during fruit ripening, in full summer. A and E were positively correlated with g_s for both males and females. The functional strategy of females, aiming to finish the reproductive process, is to proportionate the relative increase of A and E on self-shaded leaves, through the superior g_s compared to males.

Index terms: Light, photosynthesis, stomatal conductance, temperature, transpiration.

Diferenças da troca gasosa foliar entre plantas masculinas e femininas de erva-mate

Resumo - Neste estudo preliminar foram determinadas as propriedades ecofisiológicas de plantas femininas e masculinas da erva-mate (*Ilex paraguariensis* St.Hil.) cultivadas no campo. Fluxo de fótons ativo na fotossíntese (PPFD), condutância estomática (g_s), fotossíntese líquida (A), transpiração (E) e temperatura foliar (T_l) foram avaliados em um ciclo bienal de produção: no verão (janeiro e fevereiro) durante o período de maturação dos frutos quando ocorre pausa no crescimento; na primavera (novembro) e no outono (março a junho) durante emissão de novas brotações. As medições foram efetuadas em diferentes posições na copa (interior, exterior e ponteiros). Resultados indicam existência de dimorfismo sexual fisiológico na espécie. A condutância foi superior nas plantas femininas, exceto nas ponteiros. A e E foram superiores na parte exterior do terço médio das copas das plantas femininas. Foi detectada sazonalidade na troca gasosa, com os maiores valores registrados na época de emissão de novas brotações e menores durante a fase de maturação dos frutos. Observaram-se correlações positivas entre trocas gasosas e PPFD. A e E correlacionaram-se positivamente com a g_s , tanto para plantas masculinas quanto femininas. A estratégia funcional das plantas fêmeas, com o propósito de finalização do processo de reprodução, é de proporcionar o aumento relativo da fotossíntese e da transpiração das folhas sombreadas por intermédio de um maior valor de g_s , quando comparado com indivíduos masculinos.

Termos para indexação: Condutância estomática, fotossíntese, luz, temperatura, transpiração.

Introduction

Yerba-mate (*Ilex paraguariensis* St. Hil., Aquifoliaceae) is a subtropical, evergreen tree. It grows in the sub-canopy (CARPANEZZI, 1995) of subtropical rainforest with *Araucaria angustifolia* (PETERSEN et al., 2000). The dioecy of yerba-mate was observed only at the end of the nineteenth century (FERREIRA et al., 1983). It is an entomophilous species, with no

specialized pollinators, visited by unnumbered Hymenoptera, Coleoptera, Hemiptera and Diptera, and presents a high level of parthenocarpy (FERREIRA et al., 1983). Thompson e Brunet (1990) suggested that dioecy, greenish flowers and fleshy fruits, all unite in an understory syndrome, associated with pollination conferred to small, unspecialized pollinators that typify such habitats, favoring dioecy through an outcrossing advantage.

In natural yerba-mate habitats, the sex ratio was found to be 1:1 (FLOSS, 1994), whereas in populations with older yerba-mate plants this ratio was 7-8 : 5, where males prevailed (FERREIRA et al., 1983; STURION et al., 1995). In dioecious species, the morphophysiology of the two sexes can differ in leaf production as a function of investment and allocation of nutrients for fructification, fruit and seed ripening (HO, 1988). In a whole plant level, the reproductive organs serve as the strongest sinks (THORNE, 1985; KORPELAINEN, 1994; PATRICK, 1997), which can induce the physiological modification in leaves.

Differences in physiological specialization may help each sex in meeting different resource demands associated with reproduction, but the results depend on species without any general pattern. Dimorphism in physiological traits is also variable and different sexes and ecotypes might employ different survival strategies under environmental stress, as has been shown in *Hippophae rhamnoides*, where males were more responsible in the development of freezing tolerance than females (LI et al., 2005). The environment affected plant morphology, and it can influence allocation patterns of dioecious *Rubus chamaemorus* (KORPELAINEN, 1994) and *Carica papaya* (ALLAN et al., 1987). In *Enhalus acoroides*, one tropical seagrass, females showed higher requirements for production of reproductive structures than males (ROLLÓN et al., 2003).

The leaves of yerba-mate are economically important, representing the primary material for harvesting. After their industrial processing, they are used for the beverage preparation, one kind of Latin-American tea, named “chimarrão” in Brazil. Until today, yerba-mate was studied for management, production and selection, but growing pattern, carbon acquisition and physiological dimorphism of yerba-mate are very poorly known. Recently, it was shown that yerba-mate represented annual growing periodicity, consisted of two growth pauses, and two flushes of regrowth (BAZZO; RAKOCEVIC, 2005). The total growth pause occurred in winter (from the end of June until the beginning of September), whereas the second, total or partial, occurred in a summer period (from the end of December to February). Two flushes of regrowth occur from the end of September until the beginning of December and from March until the beginning of June. Moreover, the seasonality in gas exchange of young yerba-mate plants

grown in the field, which accompanied the growth flushes and pauses was observed (RAKOCEVIC et al., 2005).

It could be expected that yerba-mate females, in a function of investment to fructify and fruit ripening, represent some functional adaptations different than the ones for males. There are some studies where females have suffered more in stress conditions, as happened with *Hebe subalpina* (DELPH, 1990). In other cases, it was observed that greater reproductive effort in females did not constrain growth and reproduction relative to males of *Silene latifolia*, under low resource condition (GEHRING; LINHART, 1993).

Considering the lack of functional information in yerba-mate, the aim of this study was to determine if there is a physiological sex dimorphism in gas exchange properties between males and females in different phases of growth and development during the biennale productive interval.

Material and methods

The trial was conducted on an experimental field of “Universidade Regional do Alto Uruguai e Missões” (URICER) in Erechim (S 27° 29' 6”, W 52 ° 21' 3”, 820 m above sea level), in the Rio Grande do Sul State, Brazil. The local climate is defined as subtropical humid (Cfa) by Köppen's classification, with a regularly distributed rainfall during the year, and an average temperature above 22 °C in the hottest month.

Yerba-mate has been cultivated on isolated plant lines (bounded lines in open field, 1.2-1.5 m plant to plant). Four adult seven-year-old plants of each sex were marked in February 2003. The last pruning was done in winter 2000, remaining 10-15 % of foliar branches on a 0.8-1.2 m height. In the triennial growing period yerba-mate plants reformed tree crowns of approximately 2.5 m height.

Photosynthetic photon flux density (PPFD - $\mu\text{mol photons m}^{-2}\text{s}^{-1}$), leaf temperature (T_l - °C), net photosynthesis (A - $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$), stomatal conductance (g_s - $\text{mol m}^{-2}\text{s}^{-1}$), and transpiration (E - $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$) were measured with a LI-6200 analyzer (LICOR, Nebraska, USA). Field measurements were carried out in natural conditions, “*in situ*”, on leaves situated on the inside (IN) and exterior (EX) of the middle third part of the tree crown, and on the top (PO) of superior third part of the tree crown. Leaves on the top of yerba-mate trees are considered “sun” leaves, not submitted to reduction of light induced by self-shading. Considering that yerba-mate showed little variation in

leaf gas exchange during the diurnal higher assimilation (RAKOCEVIC, et al., 2007), four repetitions for each leaf position were effectuated from 10:00-15:00 in each measuring data, for each plant and leaf position, returning each hour to the same leaf.

The first measurement was conducted in February 2003, during the summer growth pause of yerba-mate, when females passed the phase of fruit ripening. The second measurement was taken in June 2003, in the beginning of winter growth pause. The third measurement was conducted in March 2004, in the beginning of the autumn regrowth; the fourth in the full spring regrowth, in November 2004; the fifth in the summer growth pause when fruits were ripening (January 2005).

To check differences between sexes, plants, leaf position, and measuring time, factorial analysis of variance was applied in relation to five ecophysiological parameters (A , g_s , E , PPF and T_l). The significant effects of interaction were decomposed orthogonally, and analyzed by the F test. The average effects of measuring time during biennial interval (from February 2003 to January 2005) were analyzed by the orthogonal contrast of the F test, grouping the periods of regrowth *versus* growth pauses.

The grouping of gas exchange for leaves in different positions (inside, exterior and tips) for males and females, during the biennial interval of observations, was analyzed by Ward's method (WARD, 1963), based on principal components obtained by Pearson's correlation analysis (JOHNSON; WICHERN, 1998).

Results

Measurements taken in cloudy days In February 2003, justified an average PPF of the order of $800 \mu\text{mol m}^{-2} \text{s}^{-1}$, that reached the leaves on crown tips (Figure 1A). In subtropical climate, in the middle of a sunny summer day, the photon flux density attains $2400 \mu\text{mol m}^{-2} \text{s}^{-1}$. The average PPF reaching leaves exposed to direct sun radiation without self-shading (exterior and tips) was about $1400\text{-}1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ in other periods of evaluation.

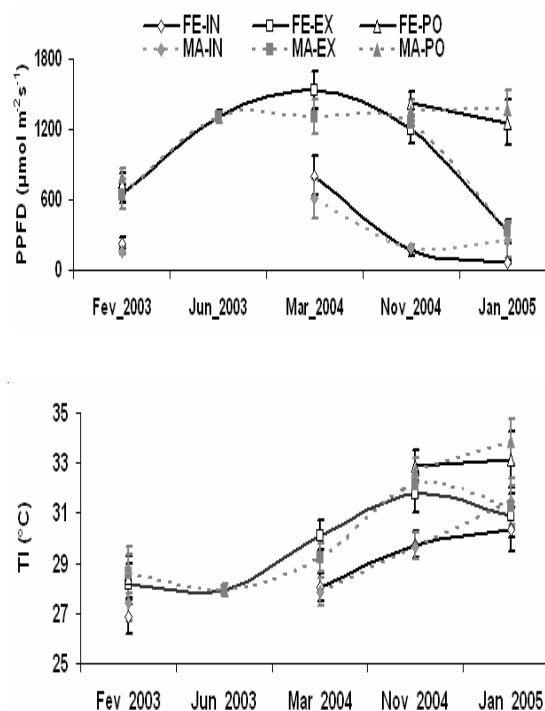


Figure 1. PPF and temperature of mate leaves (T_l) according to different tree crown positions – inside (IN), exterior (EX) and tips (PO) of males (MA) and females (FE), in a period between two sub-sequential prunings.

The PPF difference between leaf positions on tree crown (inside, exterior and tips) was registered (Table 1A). It was remarkable for January 2005 in relation to other periods (Figure 1A), when the light availability was reduced up to sixteen folds on the inside (IN) of the tree crown compared to the tree tips (Figure 1A). The light resource limitation on the plant inside reduced significantly the gas exchange (Table 1A), as compared to other leaf positions.

Leaf temperature has changed with the measurements time according to seasons (Figure 1B), and significant differences were also shown between leaf positions (Table 1A). The higher values were registered in the 2005 summer, and the lower in the 2003 winter. Paradoxally, low temperatures in June 2003 did not decrease the gas exchange values, and they have shown to be superior to those in the summer (Figure 3). However, in the summer (February 2003 and January 2005), when females passed the phase of fruit ripening, leaf gas exchange was the lowest (Figure 2, Table 2) compared to the other measurement periods.

Gas exchange parameters have been influenced by the plant sex, showing higher values in females than in males (Figure 2). The stomatal conductance (g_s) has been

influenced by sex with a 1% probability, whereas the net photosynthesis (A) and transpiration (E) showed significant differences between sexes in the order of 10% (Table 1A).

When the gas exchange was analyzed considering the position of leaves in the plant crown, the A and E were superior in females than in males on the exterior of the middle third of the tree (Table 1C, Figures 2A and 2C). Generally, leaves on the exterior (EX) are younger than the ones inside (IN). The stomatal conductance in the middle third of the tree crown (exterior and inside) has also been superior in females (Table 1B and 1C). The gas exchange varied in plant individuals of the same sex (Table 1), especially on plant tips (Table 1D), which are constituted by the youngest leaves in the tree crown.

Table 1. Variance analyses (p-value) of gas exchange and microclimate conditions of yerba-mate leaves according to different leaf positions on the tree crown and times of measurement.

Source of variation	D. F.	Pr > F for gas exchange and microclimate				
		A	g_s	E	PPFD	TI
A/ General analysis						
Sex	1	0.0998	0.0072	0.0698	0.2448	0.9981
Plant	3	0.0061	<.0001	0.0192	0.0677	0.0454
Position	2	<.0001	0.7264	<.0001	<.0001	<.0001
Measurement time	4	<.0001	<.0001	<.0001	<.0001	<.0001
Sex*Position	2	0.0879	0.1163	0.1081	0.8308	0.7673
Sex*M. time	4	0.0528	0.0518	0.0405	0.2178	0.8712
Position*M. time	5	<.0001	0.0035	0.1329	<.0001	0.1317
B/ Inside (IN)						
Sex	1	0.1699	0.0038	0.3922	0.5455	0.6758
Plant	3	0.0932	0.1184	0.3313	0.1633	0.3619
Measurement time	3	<.0001	<.0001	<.0001	<.0001	<.0001
Sex* M. time	3	0.4048	0.1788	0.2307	0.2752	0.8916
Plant* M. time	9	0.4168	0.4918	0.4711	0.9394	0.9950
C/ Exterior (EX)						
Sex	1	0.0035	0.0003	0.0003	0.5383	0.6931
Plant	3	0.3272	0.0963	0.5717	0.2464	0.1902
Measurement time	4	<.0001	<.0001	<.0001	<.0001	<.0001
Sex* M. time	4	0.7452	0.9543	0.8877	0.7199	0.9814
Plant* M. time	12	0.0507	<.0001	0.0022	0.8516	0.9155
D/ Tips (PO)						
Sex	1	0.5154	0.8607	0.3474	0.6625	0.9565
Plant	3	0.2050	0.0008	0.1589	0.6168	0.6050
Measurement time	2	<.0001	<.0001	<.0001	<.0001	<.0001
Sex* M. time	2	0.3056	0.0765	0.4569	0.9726	0.9504
Plant* M. time	6	0.0063	0.0009	0.3930	0.7042	0.9048

In bold are emphasized the significant values ($p < 0.1$)

Gas exchange was strongly dependent on leaf position

and evaluation time (Table 1A). In all leaf positions, A , g_s and E were lower during summer growth pauses, compared to an active vegetative growth (Table 2). The leaves on the tips (Table 2C) showed differences in temperature and PPFD comparing the regrowth and growth pauses phases (Table 2C).

The rural producers practice is to cut yerba-mate when no new sprouts appear, during the summer and the winter growth pauses. Significant variations in gas exchange were observed between the summer growth pauses observed in this experiment. Lower PPFD and higher temperatures in January 2005 than in February 2003, for all leaf positions (Table 2, Figure 1), influenced those variations. However, when the phases of active vegetative regrowth were compared, (first in spring – November, and second in autumn – March), A and g_s records were higher in March compared than in November (Table 2A and Table 2B, Figure 2).

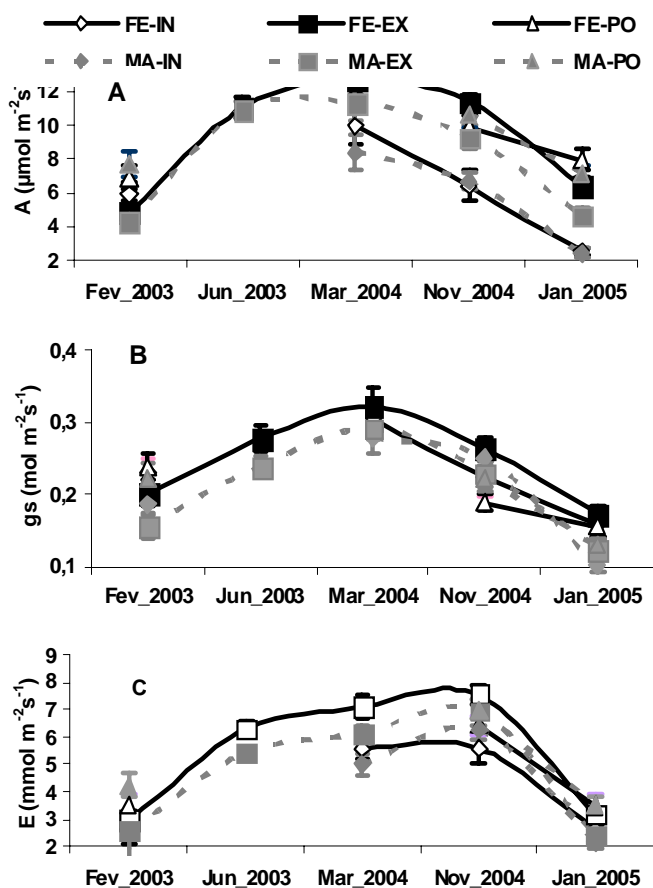


Figure 2. Net photosynthesis (A), stomatal conductance (g_s), and transpiration (E) on yerba-mate leaves according to different tree crown positions – inside (IN), exterior (EX) and tips (PO) of males (MA) and females (FE), in a period between two sub-sequential prunings.

Table 2. Orthogonal contrasts for gas exchange and microclimate of leaves in males and females of yerba-mate.

Contrasts between measurement times	Pr > F for gas exchange and microclimate				
	A	g_s	E	PPFD	TI
A' Inside (IN)					
Feb2003 e Jan2005 vs Mar2004 e Nov2004	<.0001	<.0001	<.0001	0.0010	0.2448
Feb2003 vs Jan2005	0.0002	<.0001	0.8765	0.5748	<.0001
Mar2004 vs Nov2004	0.0032	0.0290	0.0469	<.0001	0.0183
B' Exterior (EX)					
Feb2003, Jun2003 e Jan2005 v s Mar2004 e Nov2004	<.0001	<.0001	<.0001	<.0001	0.1773
Feb2003 e Jan2005 vs Jun2003	0.0030	<.0001	<.0001	<.0001	0.0003
Feb2003 vs Jan2005	0.1442	0.0444	0.3541	0.0171	<.0001
Mar2004 vs Nov2004	0.0740	0.0018	0.0167	0.1771	0.8136
C' Tips (PO)					
Feb2003 e Jan2005 vs Nov2004	0.0160	<.0001	<.0001	0.0074	<.0001
Feb2003 vs Jan2005	0.0002	0.0331	<.0001	<.0001	<.0001

In bold are emphasized the significant values ($p < 0.1$)

High significant tendencies and strong positive correlations were observed among A, g_s and E (Table 3).

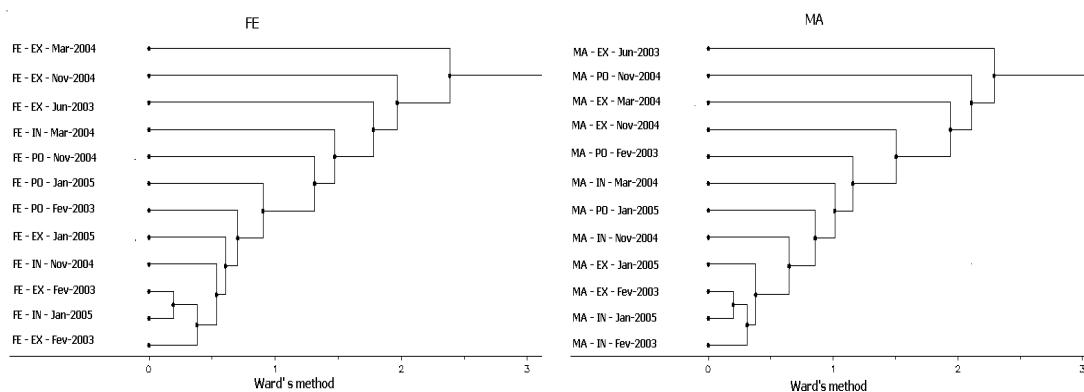
The higher values of A and E on females can be explained by superior g_s on the same plants and conditions. Net photosynthesis and transpiration were

positively correlated with PPFD, showing high probability ($p < 0.0001$), and low to medium values for correlation coefficients (in females 0.5866 and 0.5297, in males 0.5018 and 0.4445 respectively). Correlation between PPFD and g_s in yerba-mate showed high probability, but very low correlation coefficients (0.2451 in females and 0.1463 in males). E and g_s were correlated with leaf temperature, whereas tendencies were not clear (correlation coefficients from 0.3574 to 0.2389). This situation could be related to colored palette in a different situation. Meanwhile, in decomposition analysis (not shown), separating measuring time and position effects, very similar results have been obtained for correlation between gas exchange and microclimate factors.

The gas exchange in a two-year period, analyzed by the multivariate cluster method, showed a very similar pattern for males and females (Figure 3). The agglomeration for the gas exchange responses occurred by leaf positions (EX and PO on superior, and EX and IN on the inferior portion of Ward's diagram, Figure 3), and by periods of regrowth (superior portion of Ward's diagram) and growth pauses (inferior part of diagram). The procedure allowed the separation of the self-shading effect (PO and IN), and the measuring time associated to growth and development of pruned yerba-mate plants effect.

Table 3. Pearson's correlation coefficients (diagonal below) and p-value (diagonal above), between the microclimate (PPFD and TI) and gas exchange properties (A, g_s and E), for females (FE) and males (MA) in yerba-mate plants

	A		g_s		E		PPFD		TI	
	FE	MA	FE	MA	FE	MA	FE	MA	FE	MA
A			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.8394	0.2239
g_s	0.69659	0.72313			<0.0001	<0.0001	0.0005	0.0562	<0.0001	<0.0001
E	0.73820	0.69717	0.63705	0.70270			<0.0001	<0.0001	0.0007	0.0869
PPFD	0.58660	0.50183	0.24512	0.14632	0.52971	0.44454			<0.0001	<0.0001
TI	-0.01450	-0.09350	-0.31600	-0.35740	0.23895	0.13133	0.37815	0.36548		

**Figure 3.** Dendrogram of gas exchange (A, g_s e E) of mate leaves in different positions (inside - IN, exterior - EX and tips - PO), in males and females, for biennial period (February 2003 to January 2005).

Discussion

In spite of the low number of plants observed, yerba-mate showed a sex physiological dimorphism in gas exchange properties (Figure 2). In heterogeneous environments it would be adaptive for plants to possess the ability to vary the proportions of resources allocated to different functions (KORPELAINEN, 1994). Leaves of yerba-mate females were more active in photosynthesis (Figure 2A), but used more water in gas exchange process (Figure 2C). The strong positive correlations between A , E and g_s (Table 3) enabled the conclusion that female individuals, with reproductive function, achieved higher A through more potent g_s than males. The superior female effort in yerba-mate during a whole biennial period observed until the plants attained their “productive maturity”, has its genetic justification. In dioecious woody species there is one big effort in females, employing a great part of resources in reproduction. Consequently, the mortality rate is superior in yerba-mate females. Natural selection acts eliminating homozygotes, the less productive ones in population. The excess of heterozygotes in yerba-mate females and significant endogamy in males were noted (WENDT, 2005). Also, in self-incompatible hermaphroditic species it is possible that large plants become complete female, when the size variation is large enough (SATO, 2004).

Higher g_s on the third middle tree part could justify higher A (Tables 1B and 1C) in yerba-mate females. Justification of this strategy could be the aim of females related to investment in fruits. Meanwhile, plants of two sexes showed reduced gas exchange values during the phase of fruit ripening even when the resource investment of males is limited to flowering stage. The seasonality of gas exchange in yerba-mate was detected recently (RAKOCEVIC et al., 2005) when the ecophysiological properties of progenies in breeding process were compared. Summer growth pauses (BAZZO; RAKOCEVIC, 2005) coincided with fruit ripening phase when a remarkable decrease in gas exchange occurred (Figure 2). PPFD was not a limiting factor (Figure 1A) during this stage and it was supposed that high summer temperatures and short night length could regulate the growth pauses in yerba-mate both in males and females. Leaf gas exchange was low in two periods of fruit ripening (January and February) meanwhile only during January, 2005 excessively high temperatures were

registered. Our recent work showed that other mechanism than temperature is regulating the expression of growth summer pauses in yerba-mate (BAZZO; RAKOCEVIC, 2005), as night length, which “recognizes” the occurrence of physiological phases. In the mean time, the proper physiological expression is influenced by microclimatic conditions and by definition of gas exchange parameters (CAEMMERER; FARQUHAR, 1981).

More recent tests of sex-allocation theory separate allocation at the time of flowering from seed production, to allow for differences in the timing of investment (CAMPBELL, 2000). In this study, the time of flowering (November) was separated from fruit ripening time (January and February). Gas exchange was clearly superior in females at the time of flowering than in fruit ripening (Figure 2, Table 3). The analog response was surprisingly obtained for males comparing flowering phase and summer growth pause.

The seasonality of photosynthesis in subtropical climate was observed and well analyzed in coffee trees (SILVA et al., 2004). Throughout the active growth period A was superior than during the period of reduced growth. The correlation between photosynthesis and air temperature in coffee was positive in the active period (October-March); growth, unlike A , was strongly negatively correlated with air temperature. In contrast, growth and A were both correlated positively with air temperature during the reduced growth period in coffee (April-September).

In different clones of tea culture (*Camelia sinensis* (L.) O. Kuntze), similar to yerba-mate in production proposal, the optimum light intensity for photosynthesis and transpiration was about $800-900 \mu\text{mol m}^{-2} \text{s}^{-1}$, and higher water use efficiency was referred to clones with higher water stress tolerance (JOSHI; PALNI, 1998). Analogically, the females of *Ilex paraguariensis* demonstrated higher g_s and E (Figures 2B and 2C), and could represent lower water stress tolerance, especially during the fruit ripening. Differences in gas exchange between plants, pointed in leaves assimilating in crown tips (Table 1D), showed the importance of yerba-mate breeding in direction of most productive plants, with high photosynthesis rate and low water demand (transpiration rate).

The physiological knowledge could contribute on the breeding program selecting the best yerba-mate individuals for culture plantations, and on environmental

monitoring, as is followed in tea culture (ANANDACOOMARASWAMY et al., 2000). The evaluation of ecophysiological components was shown as good tool on selective process and breeding of yerba-mate (RAKOCEVIC et al., 2005).

Conclusions

1) Yerba-mate has shown physiological dimorphism in gas exchange properties between sexes. Net photosynthesis, stomatal conductance and transpiration were superior on females than on males. A g_s was superior on female plants, except on tips, whereas the A and E were superior on females, on exterior of the third medium of the tree crown. Reduced functioning of leaves in inside of a tree crown was a consequence of PPFD reduction in conditions of self-shading.

2) The yerba-mate breeding process, towards the most productive plants demonstrating the best adaptive ecophysiological properties (high photosynthetic and low transpiration rate) might be recommended to lead the gas exchange measurements on plant tips.

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