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Effect of cover on the development and production of secondary compounds of *Maytenus ilicifolia* and *Ilex paraguariensis* in agroforestry systems

Efeito da cobertura do dossel no desenvolvimento e produção de compostos secundários de *Maytenus ilicifolia* e *Ilex paraguariensis* em sistemas agroflorestais

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Abstract

The Midwest regions of Santa Catarina state, in Brazil, include a great diversity of forest essences with medicinal application; however, many of these resources are poorly exploited or managed in an extractive manner. This research aimed to assess the effects of canopy cover in agroforestry systems on development and production of secondary composites of espinheira-santa *(Maytenus ilicifolia)* and yerba mate *(Ilex paraguariensis*). Two experimental areas were planted following the same spacing and arrangement for the plants. Experimental blocks were set up by pruning the pioneer plants, with three variable levels of canopy cover (0-20 %, 20-40 % and 40-60 %). Plants of espinheira-santa and yerba mate were monitored for development and their marketable vegetative parts were harvested, processed and aqueous extracts were prepared for the analysis of presence of secondary compounds. The yerba mate showed the highest production of marketable biomass under high cover. By contrast, the biosynthesis of the secondary compound, theobromine, was greater in the lowest cover, while chlorogenic acid and caffeine were higher in the cover (40-60 %). Espinheira-santa showed no difference in either development or yield among the assessment treatments and, the production of phenols was lower in the cover above 40 %.

Keywords*:* Yerba mate; Espinheira-santa; Photosynthetically active radiation; Ultraviolet radiation

Resumo

As regiões meio-oeste do Estado de Santa Catarina possui grande diversidade de essências florestais com aplicação medicinal, no entanto, muitos desses recursos são mal explorados ou explorados de forma extrativista. Esta pesquisa teve como objetivo avaliar os efeitos da cobertura do dossel em sistemas agroflorestais no desenvolvimento e produção de compostos secundários em espinheira-santa (*Maytenus ilicifolia*) e erva-mate (*Ilex paraguariensis*). Duas áreas experimentais foram plantadas seguindo o mesmo espaçamento e arranjo de plantas. Blocos experimentais foram estabelecidos pela poda das plantas pioneiras, com três níveis variáveis de cobertura (0-20 %, 20-40 % e 40-60 %). As plantas de espinheira-santa e erva mate foram monitoradas quanto ao desenvolvimento e as partes vegetativas comercializáveis colhidas, processadas e preparados extratos aquosos para análise de compostos secundários. A erva-mate mostrou a maior produção de biomassa comercializável sob maior cobertura. Por outro lado, a biossíntese do composto secundário teobromina foi maior na cobertura 0-20 %, enquanto o ácido clorogênico e a cafeína foram maiores na cobertura 40-60 %. Espinheira-santa não mostrou diferença no desenvolvimento ou produção entre os tratamentos e a produção de fenóis foi menor em cobertura acima de 40 %.

Palavras-chave: Erva-mate; Espinheira-santa; Radiação fotossinteticamente ativa; Radiação ultravioleta

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Introduction

The increase in the demand for yerba mate (*Ilex paraguariensis*) has piqued the interest of many farmers (BARBOSA *et al.*, 2015). The consumption increase of yerba mate is related to its anti-oxidant, antioxidant and lipolytic action (BRACESCO *et al.*, 2011). These effects are due to the presence of phenols, flavonoids, caffeine, theobromine, theophylline and tannins (BRANDÃO *et al.*, 2013).

Another forest essence of interest is espinheira-santa (*Maytenus ilicifolia*), particularly after the Ministry of Health started, in 2007, a program for inclusion of herbal medicines on the list of medicines supplied by the Unified Health System (SUS) (CARVALHO *et al.*, 2008).

Espinheira-santa is usually used in the treatment of digestive disorders, nausea, heartburn and gastric mucosa (SANTOS-OLIVEIRA *et al.*, 2009), these effects are mainly related to the presence of triterpenes (LEITE *et al.*, 2010) and polyphenols (XAVIER; D'ANGELO, 1996).

Intercropped production systems, particularly agroforestry systems (AFS), have been highlighted by Eibl *et al.* (2000) as the most profitable and efficient systems for the production of yerba mate. In this scenario, one important issue arises: what is the optimal cover intensity for yerba mate and espinheira-santa, which combines high production of marketable biomass as well as the appropriate levels of secondary compounds?

Therefore, the aim of the present study is to identify the influence of canopy cover on the vegetative development, on the commercial production of biomass and on the dynamics of secondary compounds, with nutraceutical and pharmacological purposes, for yerba mate and espinheira-santa.

Material and methods

The experiments were carried out in the period from August 2013 to January 2017 in two agroforestry systems in the municipalities of Fraiburgo, SC state and Lebon Régis, also in SC state, with different characteristics (Table 1). Average rainfall of the study areas is 1,500 mm/year and annual average temperature is 15.3°C (TRABAQUINI; VIEIRA, 2017). The climate is classified as Cfb - temperate climate, mild summer, evenly distributed rains, no dry season, according to Köppen classification described by Alvares *et al.* (2013).

Table 1 – Experimental areas description.

Tabela 1 – Descrição das áreas experimentais.

Characteristics	Fraiburgo	Lebon Régis
Geographic coordinates	27°01'43"S, 50°56'32"W	25°57'43" S, 50°49'44" W
Site name	Chácara Refazenda	Sítio Butiá Verde
Planted area (ha)	2.2	3
Altitude (m)	1,094	964
Relief	Plain	Southwest-facing slope
Soil aspect	Deep with few stones	Presence of stones and gravel
Previous use	Apples	Maize/Soybean
Regeneration time	6 years	4 years
Base saturation (V %)	88.5	36.6
Description of the vegetation before planting	*Mimosa scabrella* *Ocotea puberula* *Hovenia dulcis* *Solanum mauritianum* *Eucalyptus grandis*	*Baccharis spicata* *Baccharis dracunculifolia Baccharis uncinella* *Mimosa scabrella* *Ocotea puberula*

The AFS were implemented in the winter of 2013. The seedlings of yerba mate and espinheira-santa were evenly intercropped in the planting rows in a 5x1 ratio, respectively. The rows were spaced at 2.5 m, and the distance between plants was 1.5 m, which resulted in a density per hectare of 2,222 plants of yerba mate and 444 plants of espinheira-santa.

The seedlings of yerba mate were purchased at APROMATE - Association of Yerba Mate Producers from Machadinho, RS state. All the seeds originated from a biclonal offspring called Cambona 4 (NUNES *et al.*, 2015). Thus, a common origin of the plants could be established and phenotypic variability could thus be reduced. For planting purposes, seedlings with a height of 15 ± 2 cm were selected. The seedlings of espinheira-santa selected for planting was 23 ± 2 cm.

In the first year, original tree vegetation was preserved with a view to reducing the effect of frost. For this reason, the treatments with different levels of cover were not set up until August, 2014.

To monitor the plant development in the first year, five rows (spaced at 20 meters) were selected, in which eight plants of *Maytenus ilicifolia* and eight of *Ilex paraguariensis* were marked. Spacing was at least three meters, with a total of 40 plants of each species. Plant height was assessed with a graduated tape measure and shoot diameter, with a pachymeter (Starrett Brasil, MEBT-8/200 model 125), in the months of January and August 2014.

In the second year, between the months of August and October 2014, experimental blocks were set up using a randomized block design with three treatments and six replicates. The minimum distance between blocks was 10 meters and between plots, 5 meters. Each plot was 10 m wide and 20 m long. The following treatments were randomly selected in the plots: 0-20 %, 20-40 % and 40-60 % of canopy cover.

To achieve the established level of cover, the pioneer plants were cut or pruned until the desired cover intensity was visually achieved. The correct level of cover was measured with photographs of the canopy above the plots. The images were treated with the software HemiView (Delta-T Devices Ltd), and the cover percentage was determined on four distinct points of each plot using a methodology adapted from Jonckheere *et al.* (2004). The rate of cover of plots was reassessed in the month of April 2015 through new photos of the canopy.

After the pioneer trees were pruned and cut, five yerba mate plants and three espinheira-santa plants (with at least three-meter spacing) were selected per plot and then marked. In the second year of studies, these were the plants used for assessment of the parameters of development (height and shoot diameter) by using the same methodology described previously. The assessments were performed in the summer and winter of 2015 and summer of 2016. On the latter date, assessments were also made on the influence of treatments on the interception of incident solar radiation, in the range of 300 to 750 nm. Five measurements were made per plot using a Li-cor Inc. spectro-radiometer model LI-1800 (Table 2).

The data on height and shoot diameter underwent analysis of variance ANOVA ($p<0.05$), using the statistical software SAS (CARY, 2011). The means were compared by the Duncan's test.

The differences in the radiation profile between the areas are due to the morphological characteristics of the plants in the canopy (SCHLEPPI; PAQUETTE, 2017). Many of the plants responsible for shading in the Lebon Regis area are of the genus *Baccharis* (Table 1), characterized by Beretta *et al.* (2008) as narrow and lanceolate.

Table 2 – Conditions observed in each experimental area of the percentage of interception of ultraviolet (UV) radiation, photosynthetically active radiation (PAR) and red to far-red (F:FR) ratio in three different cover classes in agroforestry systems.

Tabela 2 – Condições observadas em cada área experimental quanto ao percentual de interceptação das radiações ultravioleta (UV), fotossinteticamente ativa (PAR) e relação vermelho: vermelho-distante (V:VD) nas três diferentes classes de cobertura em sistemas agroflorestais.

Location: Fraiburgo				
Radiation	**λ**	**0-20 %**	**20-40 %**	**40-60 %**
UV	300-390 nm	65.9 %	87.4 %	95.6 %
PAR	400-700 nm	57.0 %	84.7 %	95.9 %
F:FR ratio		2.20	2.05	1.40
Location: Lebon Régis				
UV	300-390 nm	71.4 %	82.1 %	95.4 %
PAR	400-700 nm	63.0 %	77.3 %	94.8 %
F:FR ratio		2.15	1.85	1.40

The light and shade regime were more influenced by factors such as wind and sun position, thus allowing the entry of many beams of radiation that intersect the canopy without being intercepted, forming a mosaic of shade and light (Figure 1). In the experiment in Fraiburgo, the cover in this treatment has proved to be more compact.

The harvest was performed of the same plants marked for monitoring of development, as described previously. The plants of yerba mate were harvested twice. The first harvest was performed in August 2015, where all the branches were cut at 20 cm from the ground. The second harvest was performed in August 2016. For extract preparation and analysis of secondary compounds, only the material of the second harvest was used.

The espinheira-santa plants were harvested only in the month of October 2016 by cutting half of the leaf area. The removed branches were pruned at 20 cm above the ground (MARIOT; BARBIERI, 2006).

The samples, one of yerba mate and one of espinheira-santa for each plot, were weighed in a precision scale, crushed in a mechanical mill and separated a sample of 30 grams, which has been dried in an oven at a temperature of 40°C until constant weight.

The aqueous extracts of the samples were prepared by using three grams of the dried material of espinheira-santa plus 150 mL of distilled water at 90°C (BRASIL, 2016). The extracts of *Ilex paraguariensis* were prepared by weighing 35 grams of yerba mate and adding distilled water at 80°C (HEINRICHS; CARNEIRO, 2001), which were then cooled to ambient temperature. The extracts were filtered in filter paper, stored in 30 mL amber glass vials and then frozen.

The alkaloids theobromine, chlorogenic acid and caffeine, belonging to the group methylxanthine, were the compounds which underwent quantitative assessment in the yerba mate samples. In the samples of espinheira-santa, total phenol content and the compounds epigalocatechin, catechin, epicatechin, epicatechin gallate, ferulic acid and naringin were analyzed individually.

Figure 1 – Images on the left show the light/shadow pattern of plots of the 20-40 % cover treatment in the experiment of Lebon Régis, where the canopy is composed of plants of the genus *Baccharis*. The photograph of the canopy is shown below; the right side shows the light/shadow patterns and the photograph of the canopy of Fraiburgo experiment for the same treatment.

Figura 1 – Imagens à esquerda mostrando o padrão sombra/luz das parcelas do tratamento 20-40 % de cobertura no experimento de Lebon Régis, com dossel composto por plantas do gênero *Baccharis*. Fotografia do dossel abaixo; ao lado direito os padrões de sombra/luz e fotografia do dossel do experimento de Fraiburgo para o mesmo tratamento.

Source: Authors (2019)

These compounds were selected based on the availability of analytical standards in the Brazilian market. The analyses were performed on a HP 1100 high-performance liquid chromatographer, with a Lichrospher RP_{18} columm (5 μm) fitted with a 272 nm UV detector and quaternary pump system. The analyses of yerba mate followed the methodology adapted from Berté (2011); for espinheira-santa, that of Morelli (2011).

The compounds were identified according to their order of elution and by comparing their retention times with those of their pure standards. Quantification was performed with the external standardization method, through the correlation of the area (mAU*s) of the peak of the compound with the standard curve realized with each assessed standard.

The quantification of total phenols present in the samples of espinheira-santa was performed using the method proposed by Singleton *et al.* (1999).

All content analyses were performed in triplicate for each experimental plot, and the replicates were used to calculate the average concentrations of the compounds. The data underwent analysis of variance ANOVA ($p<0.05$) with the statistical program SAS (CARY, 2011) and the means were compared by Duncan's test.

Results and discussion

Yerba mate

The data on height and shoot diameter of yerba mate plants show that there were no differences among the treatments in the area of Fraiburgo (Table 3). In the area of Lebon Régis, the treatment of 0-20 % cover showed plants with the greatest height when compared with the intermediate treatment (20-40 %), which did not differ from the treatment of 40-60 % cover. This behavior differs from the results reported by several authors (DA SILVA *et al.*, 2007; MAZUCHOWSKI *et al.*, 2007), which identified the greatest heights in seedlings exposed to intense shading. In these studies, the observed behavior is justified by a phenomenon known as Shade-avoidance Syndrome (SAS), as described by Ballaré (2014), when plants are exposed to shading conditions based on the response of photoreceptors, a series of reactions start and they result in the transcription of growth-promoting hormones.

Table 3 – Mean ± standard deviation of height (cm) and shoot diameter ± standard deviations (cm) of yerba mate (*Ilex paraguariensis*) plants in the first three years of development in agroforestry systems under three classes of canopy cover (0-20 %, 20-40 % and 40-60 %).

Tabela 3 – Média da altura ± desvio padrão (cm) e diâmetro do coleto ± desvio padrão (cm) de plantas de erva-mate (*Ilex paraguariensis)* nos três primeiros anos de desenvolvimento em sistemas agroflorestais submetidas a três classes de cobertura de dossel (0-20 %, 20-40 % e 40-60 %).

AFS Fraiburgo			**January**		**August**		
2014 (preliminary)	**Height (cm)**		**16.94 ± 4.94**		**42.33 ± 18.45**		
	0-20 %		20-40 %		40-60 %		CV %
January 2015	106.25 ± 22.02	ns	113.17 ± 11.40		101.60 ± 18.45		13.,11
January 2016	82.19 ± 30.49	ns	92.96 ± 43.59		92.23 ± 22.88		39.48

AFS Lebon Régis			January		August		
2014 (preliminary)	Height (cm)		17.65 ± 6.12		23.27 ± 10.97		
	0-20 %		20-40 %		40-60 %		CV %
January 2015	71.62 ± 37.77	ns	71.56 ± 36.95		73.29 ± 35.11		38.81
January 2016	101.30 ± 38.58	a*	78.03 ± 15.19	b	92.10 ± 42.31	ab	13.60

AFS Fraiburgo			January		August		
2014 (preliminary)	Shoot diameter (cm)		0.34 ± 0.05		0.65 ± 0.14		
	0-20 %		20-40 %		40-60 %		CV %
January 2015	1.40 ± 0.25	ns	1.36 ± 0.16		1.37 ± 0.23		12.,02
January 2016	1.87 ± 0.24	ns	2.00 ± 0.38		2.01 ± 0.25		13.73

AFS Lebon Régis			January		August		
2014 (preliminary)	Shoot diameter (cm)		0.40 ± 0.08		0.53 ± 0.15		
	0-20 %		20-40 %		40-60 %		CV %
January 2015	1.07 ± 0.29	ns	1.07 ± 0.32		1.14 ± 0.28		24.29
January 2016	1.87 ± 0.45	ns	1.60 ± 0.29		1.87 ± 0.63		24.15

Where: * Means followed by the same letter do not differ by the Duncan's test ($p<0.05$). ns = non significant.

A hypothesis to explain the differences in height observed in the treatments in the area of Lebon Régis takes into account the characteristics of existing cover in the 20-40 % treatment, as previously discussed (Figure 1). Under these conditions, the cells experience a regime of radiation in which high rates of photosynthesis are not possible because of shadow on the plants. At the same time, many beams of light can cross the canopy, hence preventing the expression of the SAS that expresses itself after a certain time of exposure in the shade. Salter *et al.* (2003) demonstrated that the cells of *Arabidopsis* initiate the expression of the gene *ATHB-2*, one of those responsible for SAS, at 60 min after the beginning of the exposure to shade (low levels of R:FR); as soon as the cell starts to receive direct radiation (high levels of R:FR), the entire process is interrupted.

The yields found in the harvest are shown in detail in Table 4. In the region of Lebon Régis, no difference was found among the treatments, while in Fraiburgo, the marketable production of biomass recorded in the second harvest was higher in the 40-60 % treatment when compared with the treatment with lower levels of cover. The increase in productivity of yerba mate in more shaded environments is often reported (POLETTO *et al.*, 2010).

Table 4 – Mean ± standard deviation of production of marketable biomass (grams) per plant of yerba mate (*Ilex paraguariensis*) in the first two seasons in agroforestry systems under three cover classes (0-20 %, 20-40 % and 40-60 %).

Tabela 4 – Média ± desvio padrão da produção de biomassa comercial (gramas) por planta de erva-mate (*Ilex paraguariensis)* nas duas primeiras safras em sistemas agroflorestais submetidas a três classes de cobertura (0-20 %, 20-40 % e 40-60 %).

	Harvest	0-20 %				20-40 %				40-60 %				CV %
Fraiburgo	August 2015	75.87	±	38.38	ns	75.83	±	13.55		80.33	±	19.97		31,91
	August 2016	103.67	±	78.38	b*	123.67	±	29.99	ab	169.17	±	116.01	a	56,15
Lebon Régis	August 2015	54.33	±	36.84	ns	41.93	±	41.55		43.07	±	22.55		71,08
	August 2016	126.83	±	63.51	ns	100.80	±	75.94		110.00	±	72.40		61,13

Where: * Means followed by the same letter do not differ by the Duncan's test ($p<0,05$). ns = non significant.

Westphalen (2016), found that yerba mate plants that developed in environments with 40 % less solar radiation, showed higher production of marketable biomass, compared with full sunlight exposure. This behavior is characteristic of shade-tolerant species that redirect resources for the root growth, in an attempt to minimize the loss of water through transpiration (POORTER; NAGEL, 2000; RODE *et al.*, 2015).

The production of secondary compounds was influenced by the treatments (Table 5). In the area of Fraiburgo, the theobromine concentration was higher in the treatment with the greatest cover when compared with the treatment with intermediate cover, with no difference in the treatment with the lowest cover (0-20 %). In the area of Lebon Régis, the theobromine content in the 0-20 % treatment was higher than in the others. The biosynthesis of alkaloids from the group of xanthines can be influenced by shading (DARTORA *et al.*, 2011).

To evaluate the effect of 75 % artificial cover on the theobromine content of yerba mate plants, Girardi (2010) found that in harvests carried out either in the winter or in the summer, shaded plants showed higher theobromine content than those kept in full sunlight.

The concentration of the chlorogenic acid was influenced by the treatments only in the area of Fraiburgo. The content found in the treatment with the lowest cover (0-20 %) was lower than in the other treatments. These data differ from the values found by Dartora *et al.* (2011), in which the concentration of chlorogenic acid in aqueous extracts of shaded plants was 10,650 ± 380 μg mL^{-1} while in plants in full sunlight, such content was 14,420 ± 530 μg mL^{-1}.

Table 5 – Mean ± standard deviation of the concentration of the compounds chlorogenic acid, theobromine and caffeine in µg mL^{-1} present in aqueous extracts of yerba mate (*Ilex paraguariensis*) under three cover classes (0-20 %, 20-40 % and 40-60 %).

Tabela 5 – Média ± desvio padrão da concentração dos compostos teobromina, ácido clorogênico e cafeína em µg mL^{-1} presentes em extratos aquosos de erva-mate (*Ilex paraguariensis)* submetidas a três classes de cobertura (0-20 %, 20-40 % e 40-60 %).

		Cover classes							
Compounds		**0-20 %**			**20-40 %**		**40-60 %**		**CV %**
				Fraiburgo					
Teobromine	µg.mL^{-1}	103.48 ± 42.57	*	ab	93.11 ± 6.75	b	134.76 ± 51.18	a	24.05
Chlorogenic acid	µg.mL^{-1}	17.64 ± 2.41	*	b	20.94 ± 1.68	a	21.55 ± 2.74	a	4.56
Caffeine	µg.mL^{-1}	352.79 ± 69.11	*	b	460.58 ± 142.93	b	607.47 ± 218.74	a	18.86
				Lebon Régis					
Teobromine	µg mL^{-1}	232.67 ± 50.08	*	a	175.08 ± 74.64	b	159.55 ± 81.29	b	19.01
Chlorogenic acid	µg mL^{-1}	32.78 ± 5.58	ns		34.78 ± 3.78		31.05 ± 1.97		9.63
Caffeine	µg mL^{-1}	586.55 ± 128.04	*	b	749.11 ± 245.58	a	394.64 ± 107.56	c	16.85

Where: * Means followed by the same letter do not differ by the Duncan's test ($p<0.05$). ns = non significant.

Caffeine presented higher concentrations in the treatment with the highest cover rates (40-60 %) in the experiment in Fraiburgo. By contrast, in the area of Lebon Régis, the 40-60 % treatment showed the lowest levels of caffeine, followed by the 0-20 % cover treatment and the intermediate treatment (20-40 %), which showed significant differences in the caffeine concentration among themselves. The increase of caffeine levels in shaded herbs was found in other studies (MACHADO *et al.*, 2007; GIRARDI, 2010).

When Dartora *et al.* (2011) analyzed the levels of xanthines in aqueous extracts of plants kept in full sunlight and in the shade at four different locations in the southern region of Brazil, they found that the concentrations of theobromine and caffeine were higher in plants kept under open sky. For the extracts obtained from dehydrated leaves, theobromine content ranged from 2,590 ± 640 µg mL^{-1} to 4,600 ± 250 µg mL^{-1} and caffeine content, from 9,480 ± 720 µg mL^{-1} to 13,850 ± 670 µg mL^{-1}. In both regions, the contents of xanthines were higher in plants kept under cover of more than 50% in comparison with those kept in open sky. Rakocevic *et al.* (2006) found that shaded plants showed higher levels of caffeine (1.68 g 100 g^{-1}) than those kept in full sunlight (1.34 g 100 g^{-1}), while the levels of theobromine were lower in the shade (0.18 g 100 g^{-1}) than for unshaded plants (0.77 g 100 g^{-1}).

The effect of radiation in the production of alkaloids is due to the action of ultraviolet rays (UV) on plant cell (SCHREINER *et al.*, 2012). Coelho *et al.* (2007) emphasize that moderate levels of shading do not cause large variations in the levels of xanthines and biomass accumulation in yerba mate. However, intense rates of shade can impair the plant development and the production of secondary compounds.

Espinheira-santa

The assessments of height and shoot diameter of espinheira-santa (*Maytenus ilicifolia*) do not indicate a significant difference among the treatments tested in the two experimental areas (Table 6). It should be noted that the attacks observed in various plants during the spring of 2015 by caterpillars, possibly belonging to the family Crambidae, caused considerable losses in some treatments, and even led to a reduction in plant height in the evaluation of January 2006.

Table 6 – Mean height ± standard deviation (cm) and shoot diameter ± standard deviations (cm) of espinheira-santa (*Maytenus ilicifolia*) plants in the first three years of development in agroforestry systems under three shading classes (0-20 %, 20-40 % and 40-60 %).

Tabela 6 – Média da altura ± desvio padrão (cm) e diâmetro do coleto ± desvio padrão (cm) de plantas de espinheira-santa (*Maytenus ilicifolia)* nos três primeiros anos de desenvolvimento em sistemas agroflorestais submetidas a três classes de sombreamento (0-20 %, 20-40 % e 40-60 %).

AFS Fraiburgo			**January**	**August**	
2014 (preliminary)	**Height (cm)**		**29.07 ± 5.35**	**45.49 ± 16.76**	
	0-20 %		20-40 %	40-60 %	CV %
January, 2015	61.28 ± 25.60	ns	63.01 ± 11.92	58.66 ± 7.29	27.01
August, 2015	70.26 ± 23.09	ns	69.73 ± 19.78	68.56 ± 25.13	30.79
January, 2016	57.58 ± 16.23	ns	78.69 ± 19.83	70.03 ± 22.08	28.28
August, 2016	85.99 ± 27.66	ns	86.92 ± 21.49	77.54 ± 21.40	31.94
AFS Lebon Régis			January	August	
2014 (preliminary)	Height (cm)		57.12 ± 13.00	59.10 ± 13.01	
	0-20 %		20-40 %	40-60 %	CV %
January, 2015	68.77 ± 17.60	ns	66.63 ± 18.79	69.10 ± 18.35	22.77
August, 2015	74.87 ± 27.36	ns	81.06 ± 16.30	84.54 ± 10.01	23.16
January, 2016	87.51 ± 39.02	ns	77.59 ± 25.49	87.81 ± 38.62	30.53
August, 2016	93.50 ± 37.39	ns	85.73 ± 17.29	100.81 ± 29.04	29.68
AFS Fraiburgo			January	August	
2014 (preliminary)	Shoot diameter (cm)		0.33 ± 0.05	0.58 ± 0.17	
	0-20 %		20-40 %	40-60 %	CV %
January, 2015	0.24 ± 0.20	ns	0.13 ± 0.10	0.15 ± 0.09	28.21
August, 2015	1.00 ± 0.25	ns	0.86 ± 0.18	0.95 ± 0.27	24.58
January, 2016	1.09 ± 0.37	ns	1.27 ± 0.24	1.09 ± 0.25	26.81
August, 2016	1.18 ± 0.31	ns	1.28 ± 0.22	1.18 ± 0.30	26.98
AFS Lebon Régis			January	August	
2014 (preliminary)	Shoot diameter (cm)		0.63 ± 0.12	0.66 ± 0.11	
	0-20 %		20-40 %	40-60 %	CV %
January, 2015	0.17 ± 0.15	ns	0.25 ± 0.20	0.17 ± 0.11	19.12
August, 2015	1.14 ± 0.31	ns	1.06 ± 0.16	1.07 ± 0.18	19.85
January, 2016	1.53 ± 0.31	ns	1.38 ± 0.30	1.61 ± 0.22	20.75
August, 2016	1.53 ± 0.45	ns	1.37 ± 0.29	1.67 ± 0.46	26.69

Where: ns = non significant.

Studies conducted in a greenhouse by Benedetti *et al.* (2009) indicated an increase in the plant height of *Maytenus ilicifolia* by 44 cm in the period of 11 months. In another study conducted in a municipality in Santa Catarina state called São Bento do Sul, Hanisch *et al.* (2013) evaluated the plant height of espinheira-santa in three AFS areas, and they found mean growth of 36 cm in the first two years. In the present study, the mean gain in height in the same period was 60.5 cm for the area of Fraiburgo and 70.3 cm for Lebon Régis.

Maytenus ilicifolia stands out for its plasticity as far as the demand for light is concerned (RADOMSKI; BULL, 2010). It can adapt to both full sunlight and diffuse light, a condition that was also found in the present experiments.

The production of marketable biomass of *Maytenus ilicifolia* (Table 7) followed the same trend of the data for plant development. There were no significant differences among the treatments. Similar data were found by Boeger *et al.* (2009) when assessing the production of fresh mass of espinheira-santa seedlings intercropped with two other tree species.

Table 7 – Mean ± standard deviation of production of marketable biomass (grams) per plant of espinheira-santa (*Maytenus ilicifolia*) in the first harvest in agroforestry systems under three cover classes (0-20 %, 20-40 % and 40-60 %).

Tabela 7 – Média ± desvio padrão da produção de biomassa comercial (gramas) por planta de espinheira-santa (*Maytenus ilicifolia)* na primeira safra em sistemas agroflorestais submetidas a três classes de cobertura (0-20 %, 20-40 % e 40-60 %).

Location	0-20 %	20-40 %	40-60 %	CV %
Fraiburgo	24.44 ± 15.04 ns	25.00 ± 11.09	24.47 ± 13.47	64.54
Lebon Régis	13.61 ± 5.31 ns	15.28 ± 5.68	15.56 ± 6.12	42.41

Where: ns = non significant.

Radomski & Bull (2010) reported that adult plants respond to increased light availability by producing a greater amount of specific mass.

The concentration of phenols identified in the aqueous extracts of espinheira-santa were influenced by the different levels of cover which were tested (Table 8). In the experiment of Fraiburgo, the treatments with 0-20 % and 20-40 % cover showed higher concentrations of phenols that the treatment with the highest cover (40-60 %). In the area Lebon Régis, the treatments with 0-20 % and 20-40 % cover do not differ from each other, but they were higher than the 40-60 % treatment.

These data corroborate those of other studies which also identified a positive influence of radiation on the production of phenols (RADOMSKI; BULL, 2010; ROCHA *et al.*, 2014). The increase in the biosynthesis of phenols as a strategy of defense against the harmful effects of ultraviolet rays is described for other species (CARBONELL-BEJERANO *et al.*, 2014; FORMICA-OLIVEIRA *et al.*, 2017), as a result of their antioxidant effect on cell structures exposed to UV radiation (BETA *et al.*, 2017).

When some phenolic compounds present in the samples are assessed separately, it can be seen that naringin presented concentrations of 0.64 ± 0.63 μg mL^{-1} in the 20-40 % cover, thus higher than the content found in the 40-60 % treatment; neither of them was different from the treatment with 0-20 % cover.

In the experiment of Lebon Régis, the only compound that showed significant changes with variation of cover was the ferulic acid. In the treatment with the greatest cover (40-60 %), its content was lower than the contents found in the 0-20 % and 20-40 % treatments.

Table 8 – Mean ± standard deviation of total phenols concentration in mg.mL^{-1} and of compounds naringin, catechin, epigallocatechin, epicatechin, epigallocatechin gallate and ferulic acid in µg.mL^{-1} present in aqueous extracts of espinheira-santa (*Maytenus ilicifolia)* plants under three cover classes (0-20 %, 20-40 % and 40-60 %).

Tabela 8 – Média ± desvio padrão da concentração total de fenois em mg.mL^{-1} e dos compostos naringina, catequina, epigalocatequina, epicatequina, galato de epigalocatequina e ácido ferúlico em µg.mL^{-1} presentes em extratos aquosos de plantas de espinheira-santa (*Maytenus ilicifolia*) em plantas sob três classes de cobertura (0-20 %, 20-40 % e 40-60 %).

		Fraiburgo								
Compounds		**0-20 %**			**20-40 %**		**40-60 %**		**CV %**
Phenols	mg mL^{-1}	0.80 ± 0.22	*	a	0.81 ± 0.19	a	0.69 ± 0.11	b	1.89
Naringin		0.40 ± 0.68	*	ab	0.64 ± 0.63	a	0.10 ± 0.24	b	20.48
Catechin		2.15 ± 3.86	ns		2.86 ± 2.32		0.99 ± 1.79		35.82
Epigallocatechin	µg mL^{-1}	0.23 ± 0.38	ns		0.13 ± 0.15		0.03 ± 0.06		14.55
Epicatechin		10.04 ± 18.96	ns		9.65 ± 6.95		3.58 ± 6.35		52.46
Epigallocatechin gallate		1.07 ± 1.50	*	b	4.30 ± 2.74	a	3.35 ± 2.86	a	13.27
Ferulic acid		10.02 ± 4.98	ns		12.31 ± 7.55		12.32 ± 0.82		8.57
		Lebon Régis							
Compounds		0-20 %			20-40 %		40-60 %		CV %
Phenols	mg mL^{-1}	1.51 ± 0.85	*	a	1.35 ± 0.56	ab	1.17 ± 0.31	b	5.95
Naringin		0.07 ± 0.20	ns		0.00 ± 0.00		0.00 ± 0.00		9.71
Catechin		1.00 ± 2.10	ns		0.25 ± 0.22		0.20 ± 0.28		33.99
Epigallocatechin	µg mL^{-1}	0.05 ± 0.14	ns		0.00 ± 0.00		0.00 ± 0.00		6.88
Epicatechin		4.97 ± 10.07	ns		0.86 ± 1.02		0.32 ± 0.36		61.85
Epigallocatechin gallate		1.01 ± 0.25	ns		1.01 ± 1.50		1.36 ± 2.57		16.39
Ferulic acid		12.17 ± 1.65	*	a	12.43 ± 0.66	a	9.42 ± 5.15	b	1.68

Where: *Means followed by the same letter do not differ by Duncan's test ($p<0.05$) ns = non significant.

In Fraiburgo epigallocatechin gallate presented lower concentrations in the treatment with 0-20 % cover when compared with the treatment with higher rates of cover. The other tannins of the group showed no changes in response to treatments, this condition may be another effect of the plasticity of the species previously discussed. When analyzing the presence of the compound epicatechin in 12 samples of *Maytenus ilicifolia* harvested in different regions of the state of Paraná, Beltrame *et al.* (2012) found a wide variation in the content of the compound, from 250 µg mL^{-1} to traits identified in some samples being evaluated. These observed variations may be originated from the genetic variation of the sampled plants.

Conclusions

The biosynthesis of caffeine and chlorogenic acid in yerba mate is favored in environments with more than 20 % of cover, in this condition, it was observed a higher production of marketable biomass in one of the experimental areas.

There was a positive effect of shading on the parameters of productivity of marketable biomass and production of secondary compounds in yerba mate. Some of the parameters evaluated were favored by higher rates of cover, while others by intermediate cover (20-40 %). Adjustments of light and the shadow intensity in agroforestry plantations with yerba mate should take into account the type of application of the harvested product.

For *Maytenus ilicifolia*, the development and marketable biomass are not affected by the change in cover rates, the content of the tannin epigallocatechin gallate is favored by shading above 20 % and the biosynthesis of total phenols, specifically of the compound naringin, is reduced by shadowing rates above 40 %.

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