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3 **Spectral response of *Eucalyptus saligna* under**
4 **water stress in southern Brazil**

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6 **Authors' contributions**

7 Author PAB initiated and conducted the field experiment. Authors LMM, KAS, ACS, GFSO,
8 EA, JJMJ, UNB and EAS managed and followed the field, collected and analyzed data,
9 wrote and edited the draft manuscript. All authors read and approved the final manuscript.

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13 **ABSTRACT**

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The present work aims to assess the effect of water stress on the reflectance emitted by leaves of *Eucalyptus saligna* individuals. The design was completely randomized and the study comprised 30 subjects who underwent 5 cycles of drought simulation, 45 days each. Five individuals were submitted to water deficit treatment and five were used as controls, remaining in adequate water conditions. The experiment and data collection were performed in the external facilities of the forest management laboratory of the Federal University of Santa Maria, Rio Grande do Sul, Brazil. Which it comprised the period from September 2014 to April 2015. Spectral information was collected from 24-month-old tree individuals in adequate water and water stress situations by means of FieldSpec@3 spectroradiometer. Subsequently, the spectral data for the electromagnetic spectrum range from 400 nm to 1700 nm were processed and analyzed. The resulting spectral behavior varied between water stress cycles. In the 450 nm wavelength range, the reflectances ranged from 3.8% to 7.4%, at 550 nm from 7.9% to 14% and at 650 nm from 4.8% to 8.8%. In the near infrared region, in the 900 nm to 1300 nm range, the reflectances ranged from 28% to 62%. The spectral response of *E. saligna* showed minimal differences when compared to healthy green vegetation, even though it was exposed to water deficit situations. From the information obtained, this research can be used as a parameter for comparative analysis between species belonging to the genus *Eucalyptus* sp.

Keywords: Reflectance; spectral signature; electromagnetic spectrum.

18 **1. INTRODUCTION**

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The analysis of data acquired through the interaction between the electromagnetic energy emitted by the sun and certain ground targets are key information on vegetation in a given area, for example, providing the basis for action planning to assist in the conservation, preservation and management different cultures [1,2]. The leaf is the most important plant organ in the process of absorption of electromagnetic radiation, being the estimation of the absorbed, transmitted and / or reflected energy achieved using different sensors [3].

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Using spectroradiometry, data are obtained from the spectral response of direct contact with the target, acquiring information about how the vegetation processes electromagnetic radiation, as well as the phenological state, canopy structure, among other factors [4,5].

31 Several studies have been performed in planted forests of *Eucalyptus* sp. using reflectance
32 for wood volume estimates [6,7,8,9]. However, it is noteworthy that the number of studies
33 that considered the phenological phases or effect of stress on reflectance is small compared
34 to those associated with dendrometry.

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36 Eucalypts species are cultivated for various purposes, such as renewable energy source,
37 medicinal use and pollution control [10,11]. The use of geoprocessing and remote sensing
38 technologies to monitor forest cover attributes, restoration and measurement is a must, and
39 this practice is increasingly used by researchers [12].

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41 The Australian *Eucalyptus* genus, although not unique to this country, belongs to the
42 Myrtaceae family and has about 740 species, 20 of which are widely planted worldwide
43 under different conditions [13,14]. The most used in Brazil are: *Eucalyptus grandis*,
44 *Eucalyptus saligna*, *Eucalyptus urophylla*, *Eucalyptus viminalis*, *E. grandis* and *E. urophylla*
45 hybrids and *Eucalyptus dunnii* [15,16].

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47 Brazilian Eucalypts plantations for commercial purposes aim at the production of raw
48 material for the production of charcoal, cellulose, paper, industrialized wood panels,
49 plywood, among other uses. The five regions of Brazil have cultivated areas, totaling over
50 5.1 million hectares [17].

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52 From this perspective, this study aimed to evaluate the effect of water stress on the
53 reflectance emitted by leaves of trees belonging to the species *E. saligna*, in order to
54 analyze the different spectral responses of individuals and, how this will affect homeostasis.

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57 2. MATERIAL AND METHODS

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59 2.1 Study Area

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61 The experiment was carried out in the external facilities of the forest management laboratory
62 of the Federal University of Santa Maria (UFSM), located in Santa Maria, Rio Grande do Sul
63 (Figure 1), with coordinates 29° 43' S and 53° 43' W. The climate of the region, according to
64 Köppen classification is Cfa type, subtropical with well distributed rainfall throughout the
65 year, with an average around 1700 mm and annual average temperature of 19.2° [18].

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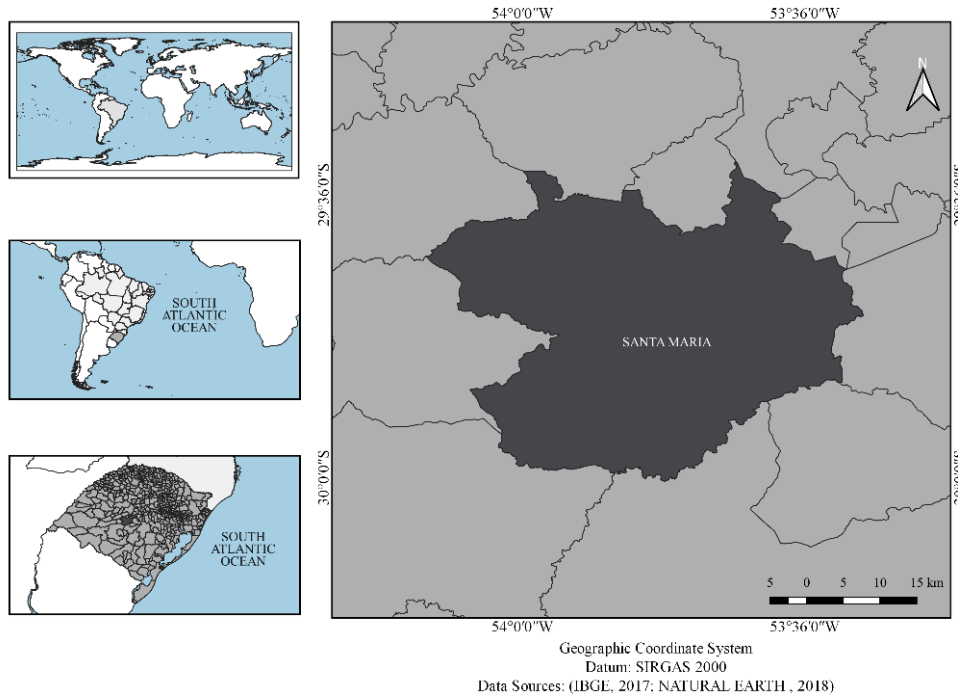


Figure 1: Location **Map of the municipality of Santa Maria-RS, Brazil.**

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2.2 Data Collect

The experiment and data collection were performed at the external premises of the UFSM forest management laboratory, where the spectral responses of 24-month-old *E. saligna* specimens were analyzed in appropriate water stress and water stress situations. The aforementioned individuals were placed in open-area pots. The trees were small in size (2 meters high) since they were under sandy soil, with nutrients' limitation and low rainfall.

The experimental design was completely randomized and the study included 30 individuals, who went through 45-day drought simulation cycles, starting in September 2014 until April 2015. Each drought cycle had five individuals in stress and five as stress. The latter remained under adequate water conditions. After the end of the first drought cycle, the plants that had been in deficit were maintained in adequate water condition, being equally monitored, but evaluated as an isolated group (called Post Cycle).

Using this approach, non-stressed trees were used for drought treatment application, allowing a homogeneous effect of drought effect for each cycle.

Following the described procedures, the spectral data were collected using the FieldSpec®3 RST 3ZC (Analytical Spectral Devices, Inc., USA) spectroradiometer, which operates in the spectral range of 350 to 2500 nm. The spectral range analyzed was between 400 and 1700 nm. Calibration was performed using a standard reference plate prior to measuring the reflectance value of the different species.

Two readings in young tissue (apical portion) and two in mature tissue were collected from each individual in order to better represent the species under study. The readings were taken between 11 am and 1 pm, at the period of greatest intensity of electromagnetic radiation on the target.

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99 2.3 Data Processing and Statistical Analysis

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101 The processing of the data was performed in software R Studio version 1.2.1335 and
102 Microsoft Excel, where were produced graphs expressing the variations of the spectral
103 behavior of the species and also the calculation of the arithmetic mean of the data of
104 reflectance collected from each individual per cycle and then proceeded to the analysis of
105 variance (ANOVA) followed by comparison means by the Tukey test at 5% significance.

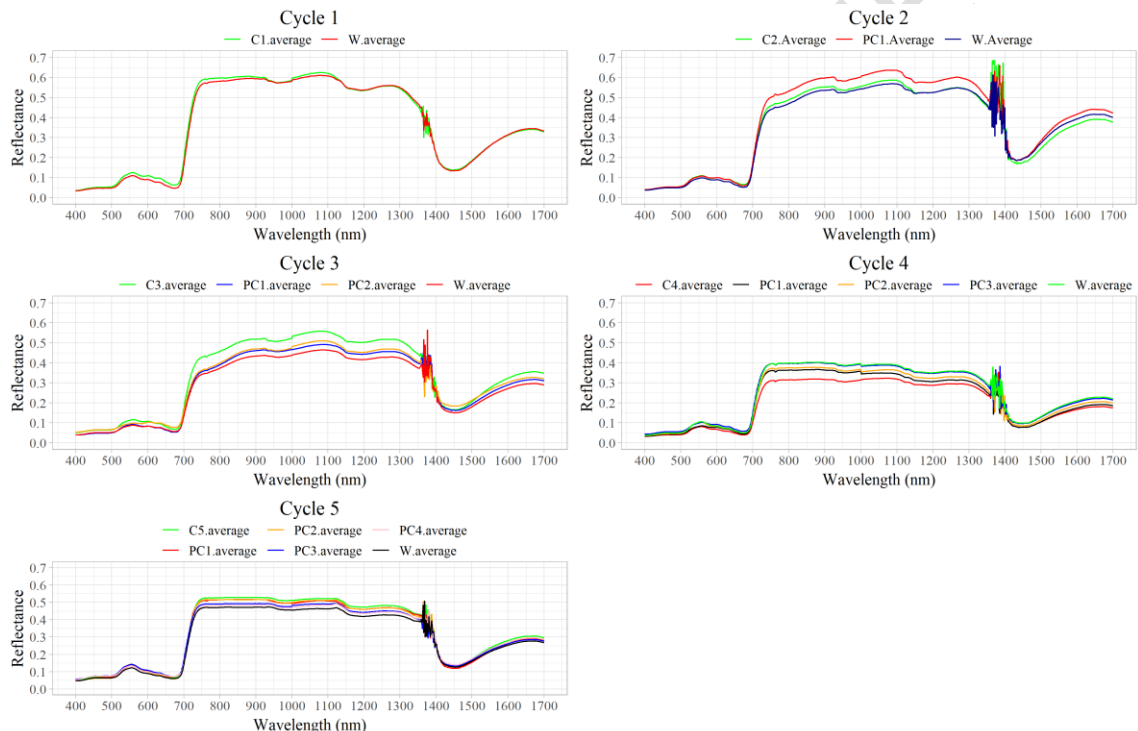
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107 3. RESULTS

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109 Below are the graphs showing the spectral signatures resulting from the different drought
110 simulation cycles, which serve as the basis for a better visualization of the statistical analysis
111 results soon after (Figure 2).

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114 **Figure 2: Graphs of the dry cycles.** W.average = Witness average; C1.average = cycle-1
115 average; C2.Average = cycle-2 average; C3.average = cycle-3; C4.average = cycle-4
116 average; C5.average = cycle-5 average; PC1.Average = average post-cycle-1; PC2.average
117 = average post-cycle 2; PC3.average = average post-cycle-3; PC4.average = average post-
118 cycle-4.

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120 In the 1st drought simulation cycle, which ran from September 1 to October 15, 2014, the
121 graphical interpretation of data on mean reflectance of treatments and controls followed the
122 same trend, as shown in Figure 2. Regarding the statistical analysis of the data, through
123 ANOVA it was verified that there was no significant difference at 5% probability level
124 between the averages of the treatments related to the aforementioned cycle and the
125 averages of the Witnesses, therefore, it was not necessary to perform the mean test.

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127 In the analysis of cycle 2, which occurred from October 16 to December 1, 2014, it can be
128 seen that individuals undergoing drought treatment in the previous cycle, here called Post-
129 cycle 1, obtained greater reflectance, especially in the region between 700 at 1400 nm, with
130 peaks greater than 0.6 between 1000 and 1100 nm.

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132 The results of this treatment differed significantly by Tukey test at 5% level of significance for
133 others (Table 1). The plants that were undergoing drought and control treatments were not
134 statistically different from each other in this cycle.

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Table 1. Tukey test results for cycle 2.

Factor	Average
Post-Cycle 1	0.40 a
Cycle 2	0.37 b
Witness	0.36 b

*Averages followed by same letter do not differ by Tukey test at 0.05 significance.

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139 In the analysis of Cycle 3, which occurred from December 1, 2014 to January 15, 2015,
140 we can see the treatment that went through the drought period with higher reflectance
141 results, in the range between 700 and 1400 nm, lower than those obtained by the best
142 treatment of the previous cycle, below 0.6.

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144 There was a significant difference between the reflectance values of plants that went
145 through drought in the other treatments. The Post-Cycle 1 and Post-Cycle 2 treatments did
146 not differ between themselves, obtaining lower reflectance than the others, with peaks
147 between 800 and 1300 nm, between 0.4 and 0.45 (Table 2).

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Table 2. Tukey test results for cycle 3.

Factor	Average
Cycle 3	0.34 a
Post Cycle 2	0.31 b
Post Cycle 1	0.30 b
Witness	0.28 c

*Averages followed by same letter do not differ by Tukey test at 0.05 significance.

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152 In cycle 4, held on January 16 to February 28, 2015, the reflectance of the individuals were
153 greater in ranges between 700 and 1300 nm, with a mean of post treatments 1, 2 and 3
154 being larger than the average reflectance of cycle 4.

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156 The analysis of variance showed a significant difference between the means of treatment
157 cycle 4, controls and cycle means of post-treatment 1, 2 and 3.

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159 The Tukey test indicated that the average of the treatment (Cycle 4) differ from the post-
160 treatment cycle 1 (pos1) and the post-cycle treatment 2 (post-cycle 2) at 0.05 significance.
161 The same was not true for post-cycle 3 (Table 3).

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Table 3. Tukey test results for cycle 4.

Factor	Average
Post-Cycle 1	0.32 a
Post-Cycle 2	0.32 a
Post-Cycle 3	0.31 ab
Cycle 4	0.31 ab
Witness	0.30 b

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**Averages followed by same letter do not differ by Tukey test at 0.05 significance.*

Cycle 5 was subjected to water scarcity from March 1 to April 15, 2015 (late summer to early autumn), obtaining higher results for reflectance in the range of 700 and 1300 nm of the spectrum.

Regarding the analysis of variance of cycle 5, there was not a significant difference between the means of this treatment, controls and post-treatment cycle. The Tukey test (Table 4) indicated that the average of the five individuals that were under water stress differed statistically from the control (normal water conditions), presenting the highest values. Only post-Cycle 4 differed statistically from cycle 5.

Table 4. Tukey test results for Cycle 5.

Factor	Average
Cycle 5	0.33 a
Post Cycle 2	0.32 ab
Post Cycle 1	0.31 ab
Post Cycle 3	0.31 abc
Post Cycle 4	0.31 bc
Witness	0.30 c

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**Averages followed by the same letter do not differ from each other by Tukey's 0.05 of significance.*

4. DISCUSSION

In the bands that make up the visible spectrum, there were variations in reflectances. At the wavelength of 450 nm the reflectance ranged from 3.8% to 7.4 % in 550 nm of 7.9% to 14% and at 650 nm of 4.8% to 8.8% (Table 5). These variations are the result of water changes in the leaves, which generate physical and biochemical changes in same, such as changes in the photosynthetic pigments, which makes it less sheet able to absorb electromagnetic radiation in this spectral region and this results in increased reflectance values [19]. Similarly to this research, Ribera-Fonseca et al. [20], with individuals of *Vaccinium corymbosum* to different water situations, obtained distinct spectral signatures [20]. Martins et al. submitted individuals of *Eucalyptus camaldulensis* and *Eucalyptus urophylla* to water deficit, verified

191 that they present alterations in photosynthesis, respiration, metabolism and absorption of
192 substances [21].

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Table 5. Reflectances in percentage of the different treatments.

Wavelength (nm)	Reflectances (%)		
	Cycles	Witness	Post-Cycle
450	3.8 to 6.7	4.5 to 6.3	4.5 to 7.4
550	7.9 to 13	8.5 to 12	8.2 to 14
650	4.8 to 8.1	5.9 to 6.6	5.5 to 8.8
900-1300	28 to 62	35 to 59	30 to 59
1445	8 to 17	9.8 and 18	7.7 to 19

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As the near-infrared region in the bands from 900 nm to 1300 nm, the reflectance ranged 28% to 62% (Table 5), and the spectral response of a healthy vegetation generally characterized by high reflectance, 40-60% [22].

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The variations in reflectances for this region of the electromagnetic spectrum are evidences of the internal reflection mechanism in the leaves, which is characterized as very intense, due to the spongy mesophyll structure, which is composed of cells and intracellular air spaces. When the amount of water in the leaf structure becomes high, there is a reduction in leaf reflectance. Water fills the air cavities forming a liquid medium inside the sheet. Thus, there is a decrease in the differences in the refractive index of the air and the hydrated cell wall, thus increasing its transmittance [23].

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In the mid-infrared, the reflectances of the treatments ranged from 7.7% to 19% in the 1445 nm spectrum (Table 5), which is one of the main bands that most interact with liquid water in the atmosphere. The variability in the reflectances presented is the result of the increase or reduction of the quantity of water in the leaves. For higher water contents, lower will be the mid-infrared reflectances. Conversely, as the moisture content of the leaves decreases, the mid-infrared reflectance increases substantially [24].

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Regarding the interactions with humidity, the spectral response of the individuals of the cycles was similar to those of *Magnolia grandiflora* trees, with moisture content of 50 and 75% [25]. It was also similar response spectral obtained of the experiment Strabeli in individuals of *E. saligna*, with water related content ranging from 68% to 83% [26]. Regarding the changes in reflectance, due to changes in water characteristics in individuals, these were not highly dissimilar to the properties of healthy vegetation, because as a function of humidity, they will only be substantial when the leaf turgor is less than 75% [27]. In this perspective, the cycle with the highest reflectance in relation to water content was 2nd, with values of 17% in the mid-infrared.

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5. CONCLUSION

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From this study, it was possible to understand that when subjecting individuals of the species *E. saligna* to water stress, they showed spectral behaviors that exposed regarding changes in the quantity of water in the cellular structures. There were variations in

229 absorptions and reflectances in the visible wavelength, which is a reflection of the
230 biochemical modifications of the leaves, thus affecting the photosynthesis process in the
231 trees. In addition, modifications occurred in the near and mid-infrared electromagnetic
232 spectrum ranges, where at 900 nm to 1300 nm the reduction in reflectances expressed
233 variations in the leaf structure of the spongy mesophyll. Already at 1445 nm, the increase in
234 reflectance is indicative of water stress.

235 The findings of this research can be user settings to as a benchmark for comparative
236 analysis among species of the genus *Eucalyptus* sp. Thus, it can be verified which species
237 has greater resistance to different drought cycles.

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UNDER PEER REVIEW

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REFERENCES

1. Moreira MA, Adami M, Rudorff BFT. Análise espectral e temporal da cultura do café em imagens Landsat. Pesquisa Agropecuária Brasileira. 2004; (39)3: 223-231. Portuguese.
2. Otanásio PN. Utilização de dados orbitais para avaliação da integridade das Áreas de Preservação Permanentes (APP) da região administrativa de Planaltina (DF). Trabalho de Conclusão de Curso (Bacharelado em Gestão Ambiental) - Universidade de Brasília, Planaltina, 45 p. 2014. Portuguese.
3. Käfer, PS, Rex FE, Santos M, Sebem E. Caracterização espectral e NDVI de espécies florestais das famílias Fabaceae, Myrtaceae, Rutaceae e Salicaceae. Enciclopédia Biosfera. 2016; 13(23): 262-275. Portuguese.
4. Ponzoni FJ, Rezende, ACP. Caracterização espectral de estágios sucessionais de vegetação secundária arbórea em Altamira (PA), através de dados orbitais. Revista Árvore. 2004; 28(4): 535-545. Portuguese.
5. Silva EA, Marangon GP, Dessbesell L, Morais WW, Lippert DB, Pereira RS. Caracterização espectral na reflectância de *Eucalyptus grandis*. Floresta. 2012; 42(2): 285-292. Portuguese.
6. Alba E, Marchesan J, Tramontina J, Mello E, Silva EA, Pereira RS. Uso de imagens de média resolução espacial para o monitoramento de dosséis de *Eucalyptus grandis*. Scientia agrarian. 2017; 18(4): 1-8. Portuguese.
7. Canavesi V, Ponzoni FJ, Valeriano MM. Estimativa de volume de madeira em plantios de *Eucalyptus* spp. utilizando dados hiperespectrais e dados topográficos. Revista Árvore. 2010; 34(3): 539-549. Portuguese.
8. Santos MM, Machado IES, Carvalho EV, Viola MR, Giongo M. Estimativa de parâmetros florestais em área de Cerrado a partir de imagens do sensor OLI Landsat 8. Floresta. 2017; 47(1): 75-84. Portuguese.
9. Macedo F, Sousa A, Gonçalves AC, Silva H, Rodrigues R. Estimativa do volume de madeira para *Eucalyptus* sp. com imagens de satélite de alta resolução espacial. Scientia Forestalis. 2017; 45(114): 237-247. Portuguese.
10. Goodger JQ, Senaratne SL, Van Der Peet P, Browning R, Williams SJ, Nicolle D, Woodrow IE. *Eucalyptus* subgenus *Eucalyptus* (Myrtaceae) trees are abundant sources of medicinal pinocembrin and related methylated flavanones. Industrial Crops and Products. 2019; 131: 166-172.
11. Zhao W, Zheng Z, Zhang J, Roger S, Luo X. Allelopathically inhibitory effects of eucalyptus extracts on the growth of *Microcystis aeruginosa*. Chemosphere. 2019; 225: 424-433.
12. Almeida DRA, Stark SC, Chazdon R, Nelson BW, Cesar RG, Meli P, Mendes AF.. The effectiveness of lidar remote sensing for monitoring forest cover attributes and landscape restoration. Forest ecology and management. 2019; 438: 34-43.
13. LIMA WP. Impacto ambiental do eucalipto. 2nd Ed. São Paulo: EDUSP; 1996. Portuguese.
14. Silva JAA. Potencialidades de florestas energéticas de *Eucalyptus* no Pólo Gesseiro do Araripe-Pernambuco. Anais da Academia Pernambucana de Ciência Agrônômica. 2013; 5: 301-319. Portuguese.
15. Grattapaglia D, Kirst M. *Eucalyptus* applied genomics: from gene sequences to breeding tools. New phytologist. 2008; 179(4): 911-929.
16. Pinto Júnior Je, Santarosa E, Goulart ICGR. Histórico do cultivo de eucalipto. In: Santarosa E, Penteado Junior JF, Goulart, ICGR, editors. Transferência de Tecnologia Florestal, Cultivo de eucalipto em propriedades rurais: diversificação da produção e renda. 1st ed. Brasília, DF: Embrapa; 2014. Portuguese.
17. ABRAF – Associação Brasileira de Produtores de Floresta Plantada. YearBook Statistical ABRAF – Base Year 2011. Brasília: ABRAF; 2012.

- 291 18. Buriol GA, Estefanel V, Swarowsky A, D'Avila RF, Heldwein AB. Homogeneidade e
292 estatísticas descritivas dos totais mensais e anuais de chuva de Santa Maria, Estado do
293 Rio Grande do Sul. Revista Brasileira de Recursos Hídricos. 2006; 11(4): 89-97.
294 Portuguese.
- 295 19. Ponzoni FJ, Shimabukuro YE, Kuplich TM. Sensoriamento remoto da vegetação. 2nd ed.
296 São Paulo: Oficina de Textos; 2012. Portuguese.
- 297 20. Ribera-Fonseca A, Jorquera-Fontena E, Castro M, Acevedo P, Parra JC, Reyes-Diaz M.
298 Exploring VIS/NIR reflectance indices for the estimation of water status in highbush
299 blueberry plants grown under full and deficit irrigation. Scientia Horticulturae. 2019; 256:
300 108557.
- 301 21. Martins GS, Freitas NC, Máximo WPF, Paiva LV. Gene expression in two contrasting
302 hybrid clones of *Eucalyptus camaldulensis* x *Eucalyptus urophylla* grown under water
303 deficit conditions. Journal of plant physiology. 2018; 229: 122-131.
- 304 22. Walter-Shea EA, Biehl LL. Measuring vegetation spectral properties. Remote Sensing
305 Reviews. 1990; 5(1): 179-205.
- 306 23. Moreira MA. Fundamentos do sensoriamento remoto e metodologias de aplicação. 1st
307 ed. São José dos Campos: Instituto Nacional de Pesquisas Espaciais (INPE), 2001.
308 Portuguese.
- 309 24. Jensen JR. Sensoriamento remoto do ambiente: uma perspectiva em recursos
310 terrestres. Tradução José Carlos Neves Epiphanyo (coord.) [et al]. São José dos
311 Campos: Parêntese; 2009. Portuguese.
- 312 25. Carter GA. Primary and secondary effects of water content on the spectral reflectance of
313 leaves. American Journal of Botany. 1991; 78(7): 916-924.
- 314 26. Strabeli TF. Resposta hiperespectral na determinação do conteúdo de água na folha em
315 diferentes espécies de *Eucalyptus* spp. 2016. 108 f. Dissertação (Mestrado em
316 Ciências) – Universidade de São Paulo, Piracicaba, 2016. Portuguese.
- 317 27. Bauer, ME; Vanderbilt, VC; Robinson, BF. Spectral properties of agricultural crops and
318 soils measured from space, aerial, field and laboratory sensors. In: Proceedings of the
319 XIV Congress of International Society for Photogrammetry, Hamburg, West Germany.
320 1980; 16: 56-73.