Spectral Response of *Eucalyptus saligna* under Water Stress in Southern Brazil

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**Authors’ contributions**

This work was carried out in collaboration among all authors. Author PAB initiated and conducted the field experiment. Authors LDMM, KADS, ACDS, GFDSO, EA, JMJ, UDNB and EAS managed and followed the field, collected and analyzed data, wrote and edited the draft manuscript. All authors read and approved the final manuscript.

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**ABSTRACT**

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.

1. INTRODUCTION

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].

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].

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

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

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

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

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

2. MATERIALS AND METHODS

2.1 Study Area

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

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.

2.3 Data Processing and Statistical Analysis

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

3. RESULTS

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

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

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

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

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

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

### Table 1. Tukey test results for cycle 2

<table>
<thead>
<tr>
<th>Factor</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-cycle 1</td>
<td>0.40 a</td>
</tr>
<tr>
<td>Cycle 2</td>
<td>0.37 b</td>
</tr>
<tr>
<td>Witness</td>
<td>0.36 b</td>
</tr>
</tbody>
</table>

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

![Fig. 2. Graphs of the dry cycles](image)

_W.average = Witness average; C1.average = cycle-1 average; C2.Average = cycle-2 average; C3.average = cycle-3; C4.average = cycle-4 average; C5.average = cycle-5 average; PC1.Average = average post-cycle-1; PC2.average = average post-cycle 2; PC3.average = average post-cycle-3; PC4.average = average post-cycle-4_
Table 2. Tukey test results for cycle 3

<table>
<thead>
<tr>
<th>Factor</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle 3</td>
<td>0.34 a</td>
</tr>
<tr>
<td>Post cycle 2</td>
<td>0.31 b</td>
</tr>
<tr>
<td>Post cycle 1</td>
<td>0.30 b</td>
</tr>
<tr>
<td>Witness</td>
<td>0.28 c</td>
</tr>
</tbody>
</table>

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

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

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

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

Table 3. Tukey test results for cycle 4

<table>
<thead>
<tr>
<th>Factor</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post cycle 1</td>
<td>0.32 a</td>
</tr>
<tr>
<td>Post cycle 2</td>
<td>0.32 a</td>
</tr>
<tr>
<td>Post cycle 3</td>
<td>0.31 ab</td>
</tr>
<tr>
<td>Cycle 4</td>
<td>0.31 ab</td>
</tr>
<tr>
<td>Witness</td>
<td>0.30 b</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Factor</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle 5</td>
<td>0.33 a</td>
</tr>
<tr>
<td>Post cycle 2</td>
<td>0.32 ab</td>
</tr>
<tr>
<td>Post cycle 1</td>
<td>0.31 ab</td>
</tr>
<tr>
<td>Post cycle 3</td>
<td>0.31 abc</td>
</tr>
<tr>
<td>Post cycle 4</td>
<td>0.31 bc</td>
</tr>
<tr>
<td>Witness</td>
<td>0.30 c</td>
</tr>
</tbody>
</table>

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

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 the by high reflectance, 40-60% [22].

Table 5. Reflectances in percentage of the different treatments

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Cycles</th>
<th>Reflectances (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>450</td>
<td>3.8 to 6.7</td>
<td>4.5 to 6.3</td>
</tr>
<tr>
<td>550</td>
<td>7.9 to 13</td>
<td>8.5 to 12</td>
</tr>
<tr>
<td>650</td>
<td>4.8 to 8.1</td>
<td>5.9 to 6.6</td>
</tr>
<tr>
<td>900-1300</td>
<td>28 to 62</td>
<td>35 to 59</td>
</tr>
<tr>
<td>1445</td>
<td>8 to 17</td>
<td>9.8 and 18</td>
</tr>
</tbody>
</table>
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].

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].

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.

5. CONCLUSION

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

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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