Spectral features associated with nitrogen, phosphorus, and potassium deficiencies in *Eucalyptus saligna* seedling leaves

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Abstract. Nitrogen (N), phosphorous (P) and potassium (K) deficiency symptoms were induced in *Eucalyptus saligna* seedlings. Leaves of these seedlings were measured radiometricaly in order to characterize spectrally the symptoms. It was used the SPECTRON SE-590, linked to a LICOR integrating sphere, running at 0.4 to 0.09 μm spectral range. The results indicated that only the symptoms related with K deficiency could be spectrally detected in the visible region. The time lag from the beginning of the experiment was fundamental to characterize the symptoms, due to the synergism of the symptoms related with mineral deficiencies. The SPECTRON SE-590 fine spectral resolution proved unnecessary to spectrally characterize the symptoms.

1. Introduction

The area forested with *Eucalyptus* in Brazil is estimated to be approximately 4.5 million ha. Most of this area is located in regions characterized by water deficit and low fertility soils. Deficiencies of nitrogen (N), phosphorus (P), and potassium (K) in reforested soils are very common, and result in significant losses of productivity (usually between 20 and 50%).

The identification of soil nutrient deficiencies is usually carried out through the analysis of the soil and of the plant itself. Nonetheless, this process can be expensive and time consuming, depending on the extent of the area to be evaluated. Hence, there is a demand for the development of new techniques to overcome the limitations of the methods presently in use.

The application of remote sensing techniques to study and/or evaluate stress in vegetation is not new and is often reported in the literature. Frequently, the satellite's spatial, spectral and temporal resolutions are regarded as the limiting factors to detect changes in the radiance reflected by the vegetation. Disperati (1991) presented

a brief overview on the use of remote sensing techniques to study forest diseases and insect damages, including several papers which corroborate the mentioned limitations.

Meyers (1983) reported some results obtained *in lab* by authors which addressed the issue of changes in the spectral reflectance of leaves when subject to different concentrations of salts and nutrients. Morphological studies indicated that plants cultivated in saline environments display a more developed palisade parenchyma, a smaller number of chloroplasts, and a smaller amount of chlorophyll; additionally, they are characterized by smaller inter-cellular spaces and present a smaller number of stomata per unity of area. These specific characteristics are responsible for changes in the reflectance of the stressed vegetation. A comparison between the radiometric measurements of healthy leaves and of leaves subject to high levels of salinity indicated a reduction in reflectance and an increase in transmitance in the visible region in the latter. Additionally, the stressed leaves displayed a more compact cellular arrangement than those not stressed, resulting in a lower reflectance in the infrared region.

Thomas *et al.* (1971) pointed out that there exists a dependency relation between the concentration of nitrogen and the concentrations of chlorophyll-*a* and -*b*. Since the concentrations of these chlorophyll affect the spectral reflectance of leaves in the visible region, then both the presence and the amount of nitrogen can be assessed through the analysis of radiometric data. Gholz *et al.* (1996) carried out *in lab* radiometric measurements on *Pinus* sp. needles, indicating the existence of a relationship between the nitrogen concentration and the reflectance in the green region (0.45 to 0.52 μm). That relationship has been analysed using linear regression models. Card *et al.* (1988) have studied the correlation between chemical concentration and reflectance values measured from leaves. They concluded that chemical concentrations might be predicted from spectra using linear regression models.

The objective of the present study is to evaluate spectrally the deficiency symptoms of N, P and K on *Eucalyptus saligna* leaves, exploring the limitations and potentials of *in lab* radiometric measurements to achieve this aim.

2. Material and methods

2.1. *Induction of nutritional deficiencies symptoms*

Samples from a red-yellow latosol, alic, medium texture, were collected in the soil layer 0–20 cm deep in an area of 'cerrado' vegetation in Mogi-Guaçu, São Paulo State. The soil samples were left to dry out at shadow, then sieved (grid of 2 mm), homogenized and characterized both physically and chemically. The soil texture was: sand = 620 g kg^{-1}, silt = 20 g kg^{-1}, and clay = 360 g kg^{-1}. The original chemical characteristics of the soil, following methodology suggested by Raij *et al.* (1987) were: organic matter = 55 g kg^{-1}, pH in $CaCl_2$ 0.01 mol L^{-11} (1:2.5) = 3.9, P-resin = 4 mg dm^{-3}, K = 0.6 mmol dm^{-3}, Ca = 0 mmol dm^{-3}, Mg = 2 mmol dm^{-3}, H + Al = 50 mmol dm^{-3}, base saturation = 20% and Aluminium saturation = 56%.

Increasing doses of N, P and K were applied to soil sub-samples to get different levels of nutrients availability. The doses of N, P, and K applied were: 0, 50, 100, and 200 mg kg^{-1} soil of N; 0, 50, 100, and 200 mg kg^{-1} soil of P; and 0, 25, 50, and 100 mg kg^{-1} soil of K. The sources of N, P, and K used were ammonia sulphate; monophosphate of sodium and potassium, and potassium chlorite, respectively, all p.a. These sources were homogenized with the soil samples. These different doses will also be referred to as treatments.

One seedling of *Eucalyptus saligna* was cultivated in 2 kg capacity plastic pot for all treatments. The pots were watered with distilled water, keeping as close as possible the water availability at field capacity.

After 45 days from planting, all treatments received a solution of micronutrients (4 mg Zn, 1 mg B, 1.5 mg Fe, 1 mg Mn, 1 mg Cu, and 0.1 mg Mo kg^{-1} soil). From this date on, at every 10 days until the end of the experiment, all pots levels received 25 mg N kg^{-1} in a solution of ammonia sulphate (p.a.), except those which received increasing doses of N.

The study was carried out in a green house for 175 days. The twelve treatments (four doses for each N, P, and K) were repeated ten times and displayed in a randomized block design experiment. To ease identification, each experimental unit was assigned a code of the type nutrient-dose-repetition. Hence, code N50-3 was associated with the third repetition of the sub-sample that received 50 mg N kg^{-1} soil.

2.2. *Determination of the minimum number of leaves for radiometric measurements*

The first signs of N and K deficiencies appeared 110 days after the installation of the experiment. Despite the absence of P deficiency, radiometric data were collected, to assess the Coefficients of Variation (CVs) associated with the Directional-Hemispherical Reflectance Factors (DHRs) for each treatment. Four spectral bands were used (band 1: 0.45 to 0.52 μm; band 2: 0.52 to 0.60 μm; band 3: 0.63 to 0.69 μm; and band 4: 0.76 to 0.90 μm) to estimate the minimum number of leaves to be radiometricaly measured by means of equation:

$$n_{ij} = \frac{t^{2*}_{\alpha/2} CV_i^2}{E} \tag{1}$$

where n_{ij} is the estimated number of leaves to be radiometrically measured in band i and treatment j ($i=1, ..., 4$; $j=1, ..., 6$); $t_{\alpha/2}$ is the tabulated t-value at $\alpha=0.05$ level of significance; CV_i is the coefficient of variation of band i ($i=1, ..., 4$). E is the maximum error, assumed here to be 10%.

The values from 1 to 4 for subscript j refer to the levels of deficiency, as described in §2; levels 5 and 6 refer to two strata: a lower stratum, consisting of old leaves; and an upper stratum, consisting of new leaves. From each vase of nitrogen deficiency induction, a total of six leaves were collected: three from the top third and three from the bottom third. A similar procedure was adopted for plants considered to potassium deficiency induction. However, in this case, twelve leaves were sampled, due to the larger amount of leaves that presented this kind of deficiency. The computations were carried out for each treatment individually. A set of leaves, which did not present any deficiency symptoms, was also used as a control treatment.

All sampled leaves were placed in plastic bags and immediately taken to the laboratory for radiometric measurements. Data from each leaf (upper side) were collected in the range 0.4 to 1.1 μm, using a spectroradiometer SPECTRON SE-590 linked to a LICOR integrating sphere. At the beginning and at the end of every six measurements, the radiance reflected from the integrating sphere was measured. These values were used to calculate the DHRs for each treatment which, in turn, were used for the computation of the mean and the standard deviation for each spectral band and treatment, to be used in equation (1). The minimum number of leaves to be sampled was based on the largest estimated value of n_{ij}, independent of the treatment.

2.3. *Radiometric measurements*

Five radiometric data collection campaigns were carried out, corresponding to different phenological stages from the beginning of the experiment (day 0). The campaigns, numbered I to, were set at days 116, 117, 140, 162, and 175, respectively. Between campaigns I and II (one day apart) it was not expected phenological changes, thus they could be considered as one campaign as well, but they were considered as two different campaigns for organizational purpose. At each campaign, a different number of leaves was sampled, always satisfying the minimum number determined (n_{ij}) using equation (1).

At each campaign, the radiometric measurements followed the procedure described in §2. A One Way Analysis of Variance (ANOVA) was used to test the existence of significant differences between the mean spectral responses for each treatment.

The data collected during Campaign I were subject to ANOVA tests for each one of the 256 spectral bands in the range 0.4 to 1.1 μm. The objective was to verify the existence of any particular spectral characteristic that could be associated with mineral deficiency symptoms. In addition, ANOVA tests were also applied to the DHRs values for the four spectral bands considered. Regardless of the procedure, each leaf, for each treatment, was assumed to be a repetition for the randomized block design experiment. The tests took into account the differences in sample size, and were carried out for each stratum individually.

Whenever an ANOVA test indicated the existence of significant differences between the DHR means, a Tuckey test was used to identify the significantly different means at 0.05 level of significance. The ANOVA tests were carried out for each band separately.

2.4. *Relationship between the nutrients concentration and the spectral bands*

In order to study the existence of a possible relationship between the concentrations of N, P and K, and the DHR value for each spectral band, the concentration of each one of these elements in vases selected according to the deficiencies symptoms presented during Campaign V were measured. Ten, fourteen, and twenty-nine repetitions of the simulated N, P, and K deficiencies were selected, respectively.

The green material used for the radiometric measurements was dried at 65°C until constant weight. The dry material was mineralized using the nitroperchloric digestion, as suggested by Sarruge and Haag (1974). The determination of the macronutrients concentrations also followed the methodology suggested by these authors.

Files compatible with specific worksheets were organized for each treatment. The files contained the N, P, and K concentrations and the mean DHR values for each of the four spectral bands. The coefficient of correlation between the N, P, and K concentration values and the DHR values was calculated for each of the bands. Graphs were also produced to visualize the type of the relationship between variables, and the goodness-of-fit of the adjustments.

Additionally, multiple regression equations were derived, assuming the DHR as the dependent variable and the nutrient's concentration as independent variables. Transformations of the concentrations were sometimes introduced in the regression analysis, as independent variables. The transformations considered were: N/P; N/K; P/K; *Ln* (N); *Ln*(P); *Ln*(K); *Ln*(N)/*Ln*(P); *Ln*(N)/*Ln*(K); *Ln*(P)/*LN*(K);

*SQRT(*N*); SQRT(*P*);* and *SQRT*(K), where *Ln* and *SQRT* stand for Natural Logarithm and Square Root, respectively.

3. Results and discussion

3.1. *Minimum number of leaves for radiometric measurements*

Table 1 presents the values of the CVs and the minimum number of leaves to be sampled for radiometric measurements. From table 1, it can be observed that the largest value of n, as defined by equation (1), was 10. table 2 presents the actual number of leaves used for radiometric measurements for each treatment and campaign. The numbers presented in table 2 refer to each stratum. Hence, nine leaves were collected for treatment N, for each stratum (upper and lower), during Campaign I.

3.2. *Mean DHRs values*

The results from the ANOVA tests carried out for the 256 bands (§2.1) were consistently significant at 5% for all treatments, at the larger bands (blue, green, red, and near-infrared). Hence, the mean DHR values were used for each of the spectral bands considered.

The Appendix displays the plots of the mean DHR values for each treatment and campaign, considering only the old leaves because the young ones presented similar behaviour. From these graphs it can be readily seen that the DHRs values followed a similar pattern for all campaigns. In the visible region, the DHR values associated with treatments K and P were in general lower than those for the other treatments, especially in the green region (band 2). This can be possibly related to the deficiencies symptoms associated with these two elements. According to Dell

Table 1. Coefficients of Variation (CV s) and minimum number of leaves to be radiometrically measured for each spectral band.

	Band 1		Band 2		Band 3		Band 4	
Treatment	*CV*	*n*	*CV*	*n*	*CV*	*n*	*CV*	*n*
N—LS	10.8	5	11.4	5	12.1	6	1.07	1
N—US	0.7	1	3.12	1	2.4	1	1.04	1
K—LS	4.0	1	11.6	5	6.9	2	4.14	1
K—US	2.9	1	7.8	2	1.4	1	2.52	1
K—LS	0.7	1	7.0	2	1.47	1	0.22	1
K—US	13.2	7	15.9	10	15.03	9	2.01	1

US = Upper Stratum; LS = Lower Stratum.

Table 2. Number of leaves radiometrically measured at each Campaign.

Campaign	N	P	K	Control
I	09		36	09
II	09		36	09
III	21	27	57	09
IV	15	27	36	06
V	15	24	48	06

et al. (1995), the P deficiency, especially in older leaves, is characterized by the presence of small red patches with small white or brown necroses in their center. The symptoms related to the K deficiency in *Eucalyptus* sp., according to these authors, are not as consistent as for N. However, in general, they are characterized by red patches along all leaf, the presence of wrinkles at the leaf borders, followed by necroses. Similar symptoms were reported by Gonçalves (1995), in a study more appropriate to the conditions in Brazil.

The DHR values in the near-infrared region did not display significant trends during the first two campaigns. However, during Campaigns III, IV and V, the DHR values for the N treatment were slightly smaller than those for the other treatments, especially at the lower stratum. The sequence, from lower to higher values of DHR, was associated to treatments N, K, P, and Control, respectively. A more comprehensive study at this region would require the analysis of transversal cuts of leaves, to assess possible variations in their internal structure. This was not carried out at this study. Most of the literature on mineral deficiencies symptoms in vegetation is based on the visual analysis of the leaves and of the plant, when stressed.

3.3. *Analysis of variance for the mean DHR Values, per spectral band and campaign*

An ANOVA test was carried out for the mean DHR values obtained for each of the 256 bands assessed during Campaign I. Since significant and non-significant differences were found between the mean values associated with the narrow bands (256) comprised within the larger bands (4) defined in this study, the ANOVA tests were then carried out exclusively for these four spectral regions. The values of the statistic 'F' obtained for each band, treatment, campaign, and stratum, are presented in table 3.

The results in table 3 indicate that most of the significant differences concentrate in the visible region, for both strata. In the near-infrared region, significant differences were observed only during the first two campaigns, suggesting that the DHR values were little affected by deficiencies in either N, P, or K. Another observation from table 3 is the consistent decrease on the significant 'F' values at the last two campaigns. This can be possibly explained by the generalization of the symptoms amongst the different treatments, inhibiting the characteristic symptoms associated with a specific deficiency (synergism).

Tuckey's test was applied in all cases where the ANOVA test led to the rejection of the hypotheses of equality of means at 5% level of significance, to identify the significantly different means. The results are presented in table 4. The means that have assigned a same letter (a, b or c), are not significantly different at 5% level of significance. Hence, at the lower stratum, band 1, campaign I, the C and N means

Table 3. ANOVA 'F' values obtained for each band, treatment, campaign, and stratum.

Campaign	LS	US	LS	US	LS	US	LS	US
I	7.46*	0.20^{ns}	52.58*	22.6*	13.54*	3.45*	5.87*	14.06*
II	7.14*	2.2^{ns}	104.2*	32.16*	33.16*	6.69*	0.68^{ns}	3.63*
III	14.03*	11.02*	19.26*	27.7*	44.46*	3.34*	2.25^{ns}	1.71^{ns}
IV	4.84*	1.75^{ns}	6.67*	4.47*	8.08*	2.16^{ns}	2.24^{ns}	0.53^{ns}
V	6.27*	2.09^{ns}	15.68*	6.31*	5.41*	7.36*	0.85^{ns}	0.41^{ns}

*Significant at 5% level of significance.
US = Upper Stratum; LS = Lower Stratum.

Table 4. Results of the Tuckey's test.

	Band 1		Band 2		Band 3		Band 4	
	LS	US	LS	US	LS	US	LS	US
I	C 0.04761 a		N 0.09088 a	N 0.08979 a	C 0.05107 a	N 0.04754 a	N 0.37123 a	C 0.38388 a
	N 0.04735 a		C 0.08597 a	C 0.07904 a	N 0.05106 a	C 0.04456 a	C 0.36893 ab	N 0.37186 b
	K 0.04307 b		K 0.05944 b	K 0.06506 b	K 0.04262 b	K 0.04297 b	K 0.35846 b	K 0.36448 b
II	N 0.04839 a		N 0.10006 a	N 0.08969 a	C 0.06808 a	N 0.04818 a		C 0.37723 a
	C 0.04704 ab		C 0.09295 a	C 0.08273 a	N 0.05713 b	C 0.04569 a		N 0.37379 a
	K 0.04231 b		K 0.05565 b	K 0.06338 b	K 0.04335 c	K 0.04706 b		K 0.36729 b
III	N 0.05360 a	C 0.05304 a	C 0.09919 a	C 0.09716 a	N 0.07151 a	P 0.05952 a		
	C 0.05301 a	N 0.05064 a	N 0.09032 a	N 0.09665 ab	C 0.05802 b	N 0.05782 ab		
	P 0.04703 b	P 0.04984 a	P 0.07460 b	P 0.08510 ab	P 0.05720 b	C 0.05401 b		
	K 0.04674 b	K 0.04588 b	K 0.06955 b	K 0.06156 c	K 0.04781 c	K 0.04706 b		
IV	C 0.06130 a		C 0.11608 a		N 0.07079 a			
	N 0.05632 ab		N 0.11276 a		C 0.06551 ab			
	K 0.05494 ab		K 0.08808 b		K 0.05564 ab			
	P 0.04845 b		P 0.08784 b		P 0.05374 b			
V	C 0.06130 a		C 0.11608 a	C 0.1161 a	N 0.06965 a	N 0.07414 a		
	N 0.05488 a		N 0.11149 a	N 0.10417 a	C 0.06551 ab	C 0.05777 ab		
	K 0.05026 ab		K 0.07749 b	P 0.08456 ab	K 0.0575 abc	K 0.05488 b		
	P 0.04444 b		P 0.06554 b	K 0.07634 b	P 0.0530 bc	P 0.05481 b		

US = Upper Stratum; LS = Lower Stratum.

were not significantly different, but they were different from the K mean; at the lower stratum, band 4, campaign I, means N and C were equal, means C and K were equal, but means N and K were significantly different.

The results in table 4 indicate that the mean DHR values for treatments K and P were significantly lower in the visible region than those for the other treatments, in most bands and campaigns. The means of the control and the N treatment could only be discriminated in the following situations: (*a*) in band 4 during the first campaign and at the upper stratum; and (*b*) in band 3 during the second and third campaigns. The means for treatments P and K could only be discriminated in bands 2 at the upper stratum and band 3 at the lower stratum, during the third campaign.

Since the symptoms displayed by the plants in the last two campaigns can be (at least visually) associated with different or multiple mineral deficiencies, the DHR mean values for the different treatments are more similar, leading to the acceptance of the hypothesis of equality of means. This observation suggests that the best period to spectrally discriminate the mineral deficiencies on leaves is comprised between 140 and 162 days from the beginning of the experiment.

3.4. *Relationship between the DHR values and the nutrient concentration*

Table 5 displays the results from the chemical analysis of the leaves, whose DHR values were used for regression analysis. According to Gonçalves (1995), the normal concentrations of N, P and K in adult plants of the genera *Eucalyptus* sp. are 13.6 to 18.0 $g\,kg^{-1}$; 0.9 to 1.3 $g\,kg^{-1}$; and 9.0 to 13.0 $g\,kg^{-1}$, respectively. From table 5 it can be observed that the nitrogen concentrations varied from 6.0 to 8.1 $g\,mg^{-1}$, thus indicating levels of deficiency. The remaining treatments presented concentrations slightly inferior to those considered adequate.

For the P treatment specifically, the concentrations of N ranged from 6.2 to 11.2 $g\,kg^{-1}$, (indicating some deficiency of this element); the concentrations of P

Table 5. Concentrations of N, P and K in the treatments whose leaves were subject to chemical analysis.

Treatment	N $g\,kg^{-1}$	P $g\,kg^{-1}$	K $g\,kg^{-1}$	Treat	N $g\,kg^{-1}$	P $g\,kg^{-1}$	K $g\,kg^{-1}$	Treat	N $g\,kg^{-1}$	P $g\,kg^{-1}$	K $g\,kg^{-1}$
N0 2	6.8	0.9	13.5	P150 5	7.1	0.8	3.3	K25 1	11.8	1.5	5.5
N0 9	6.6	0.8	8.8	P150 4	6.9	1.0	3.9	K0 3	14.1	1.0	2.4
N50 10	6.7	0.7	13.8	P0 10	9.8	0.6	4.6	K25 5	11.3	1.0	3.6
N0 4	6.6	1.0	9.6	P0 3	9.8	0.5	7.0	K25 2	7.4	0.9	3.2
N0 7	7.4	0.9	10.2	P0 9	10.9	0.6	2.1	K50 4	13.2	0.9	5.2
N0 6	7.6	1.4	8.6	P75 7	7.6	0.8	3.8	K0 5	15.9	1.0	1.4
N0 5	6.0	1.1	7.6	P75 5	8.0	0.9	5.0	K0 7	14.6	0.8	2.2
N50 9	7.6	0.9	8.0	P75 9	7.6	1.0	4.8	K50 7	15.7	1.1	4.3
N50 2	7.8	0.9	8.2	P0 1	9.8	0.6	5.4	K25 6	12.4	1.5	4.6
N50 8	8.1	1.0	10.8	P300 2	6.2	1.6	3.6	K0 4	15.8	1.0	1.8
				P75 6	7.1	1.0	2.4	K0 1	15.7	1.0	1.8
				P0 5	11.2	0.6	7.4	K25 9	13.0	1.4	3.8
				P150 10	7.8	0.9	4.2	K50 6	7.7	0.7	7.8
				P75 1	8.8	0.9	4.4	K25 7	11.9	1.0	3.8
								K0 8	11.7	1.0	2.6
								K50 3	12.4	0.8	3.0
								K25 4	10.6	1.3	2.9
								K25 8	12.1	1.0	2.4
								K0 10	16.4	1.1	1.6
								K50 9	7.7	0.8	5.2
								K100 4	7.4	0.9	5.2
								K50 1	13.0	0.8	3.2
								K50 8	10.2	0.7	3.2
								K100 5	9.5	0.8	5.2
								K100 0	12.3	1.0	4.2
								K100 8	8.8	0.9	5.0
								K0 9	14.4	1.0	2.6
								K25 10	12.0	1.0	2.9
								K25 10	12.1	0.8	5.5

ranged from 2.1 to 7.4 $g\,kg^{-1}$ (indicating an expected deficiency of this element); and the concentrations of K ranged from 2.1 to 7.4 $g\,kg^{-1}$ (characterizing an expressive deficiency of this element).

The K treatments presented concentrations of N ranging from 7.4 to 16.4 $g\,kg^{-1}$ (no deficiency); concentrations of P ranging from 0.7 to 1.5 g kg (indicating adequate concentrations of this element); and concentrations of K ranging from 1.4 to 7.8 $g\,kg^{-1}$ (characterizing the induced deficiency of this element). Table 6 presents the coefficients of correlation between the mean DHR values for each of the three sets of deficiency simulations

The shadowed columns indicate the main coefficients of correlation for each simulation. It can be observed that in most cases, the largest values of the coefficient of correlation are associated with the columns representing the simulated deficiency, suggesting the efficiency of the simulations. This is better characterized by the correlation coefficients associated with the K concentrations, which are low for the N and P simulations, and high for the specific K simulation. Figure 1 displays the diagrams relating the concentrations and the radiometric measurements at each band.

It can be seen from the diagrams in figure 1 that the concentration of nitrogen and the DHR values for the four bands present an inverse relation in all graphs

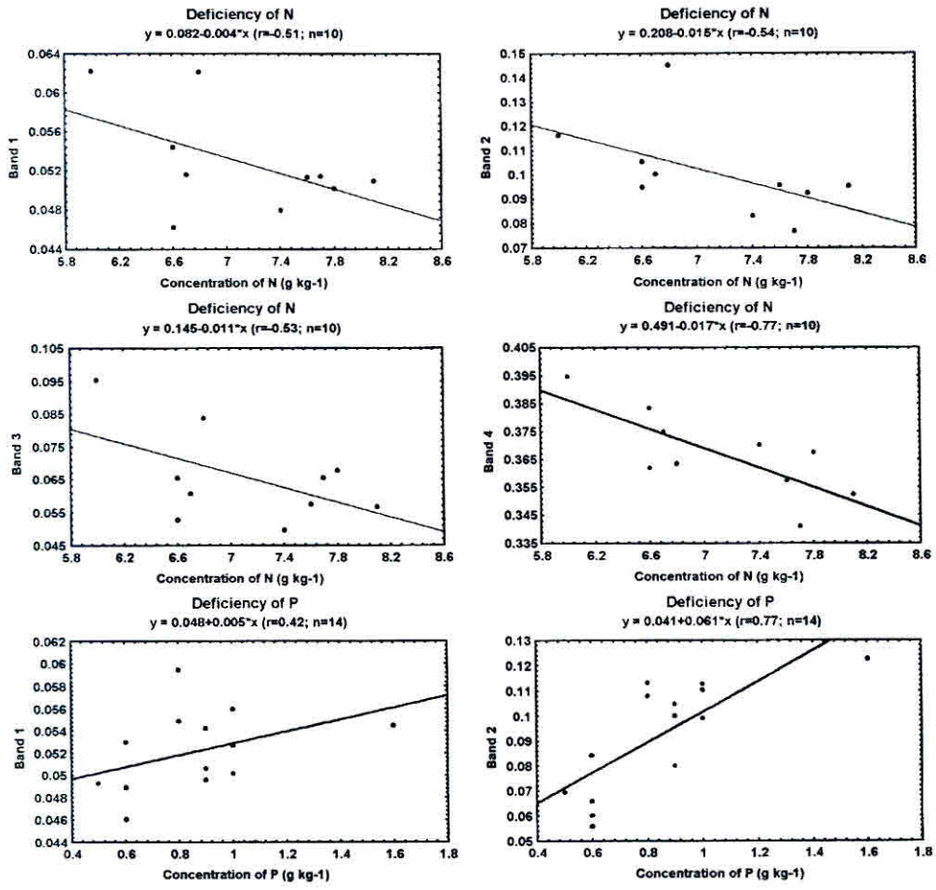
Table 6. Coefficients of correlation between the mean DHR values for each of the three sets of deficiency simulations.

Simulated N deficiency	N	P	K
Band 1	−0.51	0.49	0.19
Band 2	−0.54	0.29	0.47
Band 3	−0.53	0.45	0.03
Band 4	−0.77	0.31	0.00
Simulated P deficiency	**N**	**P**	**K**
Band 1	−0.62	0.42	−0.04
Band 2	−0.88	0.77	−0.23
Band 3	−0.18	0.34	0.04
Band 4	−0.65	0.33	−0.34
Simulated K deficiency	**N**	**P**	**K**
Band 1	−0.18	0.08	0.36
Band 2	−0.32	0.11	0.40
Band 3	−0.43	0.12	0.50
Band 4	−0.18	0.23	0.37

associated with the simulated N deficiency. This result is expected for the first three bands (visible region), since Nitrogen is related with the protein synthesis which promote the photosynthetic process. According to Devlin (1969), the nitrogen deficiency **disturbs** the metabolic function of the chlorophyll, which is the photosynthetic element responsible for the absorption of the electromagnetic radiation and transformation in vital energy for the plant. Hence, a decrease of the reflectance from the plants, in these bands, is expected when the concentration of nitrogen is increased. The decrease of the DHR values with increased concentration of Nitrogen, in the infrared region, may be explained by a possible compaction on the internal structure of the leaves. Unfortunately, this could not be observed in the present work.

The diagrams associated with the P and K deficiencies indicate that the DHR values increased with increased concentrations of these elements, in all bands. According to Devlin (1969), Phosphorus is one of the components of the nucleic acids and enzymes, besides being fundamental for the tissues composition. As for Potassium, Devlin (1969) points out its fundamental role as activator of the enzymes responsible for the carbohydrates metabolism, as well as in the apical dominance. Hence, these elements participate in both the photosynthetic process and in the tissues composition. The visual symptoms described by Gonçalves (1995) for P and K deficiencies were observed in all leaves sampled in the present study. These were characterized by increased darkening of the foliar limbo, followed by the presence of red patches. These patches were distributed in the entire foliar area, in the case of P, and in smaller patches which were followed by the necroses of the leaves borders, in the case of K. Table 7 presents the multiple regression equations determined for each spectral band.

For N and P, higher values of r were obtained for the multiple linear regression equations than for the simple regression presented in the diagrams displayed in figure 1. This suggests that the effect of the nutrient's concentration on the DHR values can be better explained jointly than isolated. For K, this multiple effect was not as evident, since the values of r in the simple linear regression were higher than



those for the multiple regressions. This result is in accordance with the statistical analysis which indicated significant differences between the DHR values obtained from the simulated K deficiencies.

4. Conclusions

Considering only the differences between DHR values as references to evaluate N, P and K deficiencies in *Eucalyptus saligna* seedling leaves, the symptoms related with the K deficiencies were easier detected using spectral data in the visible region. The time lag from the beginning of the experiment was fundamental to achieve that result, due to the synergism of the symptoms related with mineral deficiencies which became more apparent at the latest stages of the experiment

Through the CV values it was clear the influence of N in the relationship between the nutrients concentrations and DHR. This influence was stronger in band 2 (green region) and band 4 (near-infrared region) and it can also be observed in CV values of the P treatments that presented a slight deficiency of N (table 6). In spite of this influence of N deficiency in all treatments, P concentrations presented a positive correlation with DHR values.

The results from simple and multiple regression indicated that the DHR values

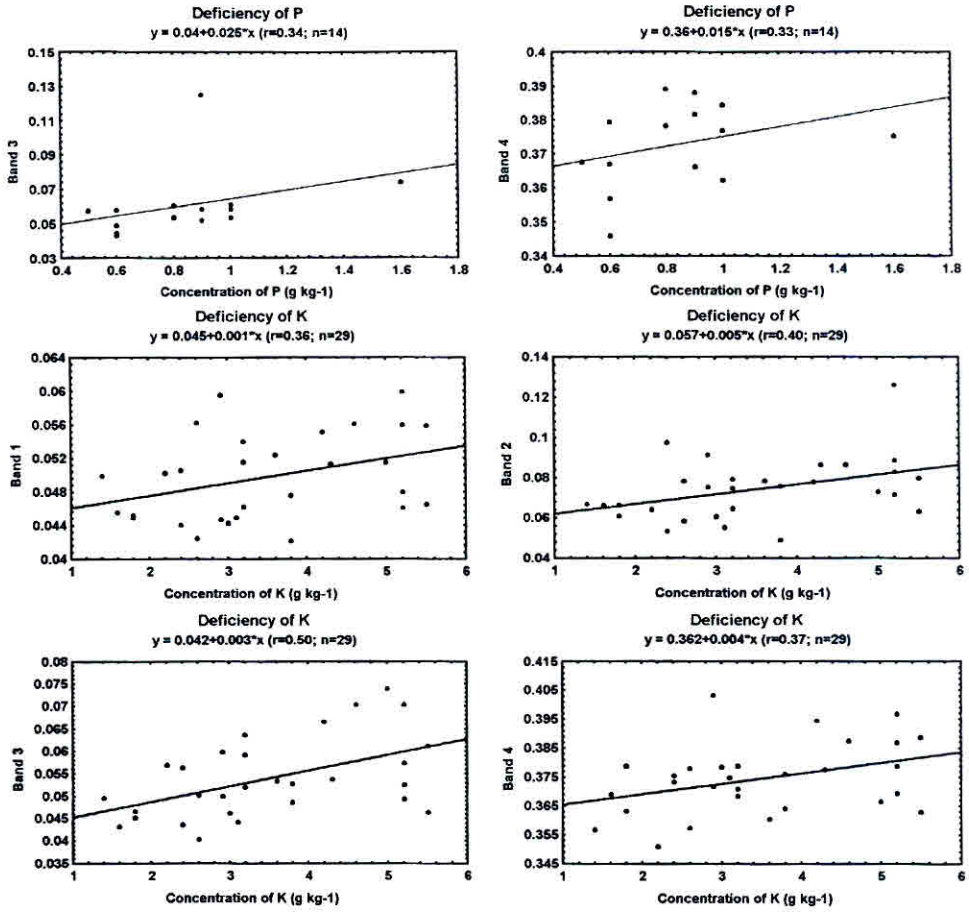


Figure 1. Diagrams illustrating the relationship between the nutrients concentration at each spectral band considered.

can be better estimated using a combination of the variables (at least of those studied here) than the variables isolated.

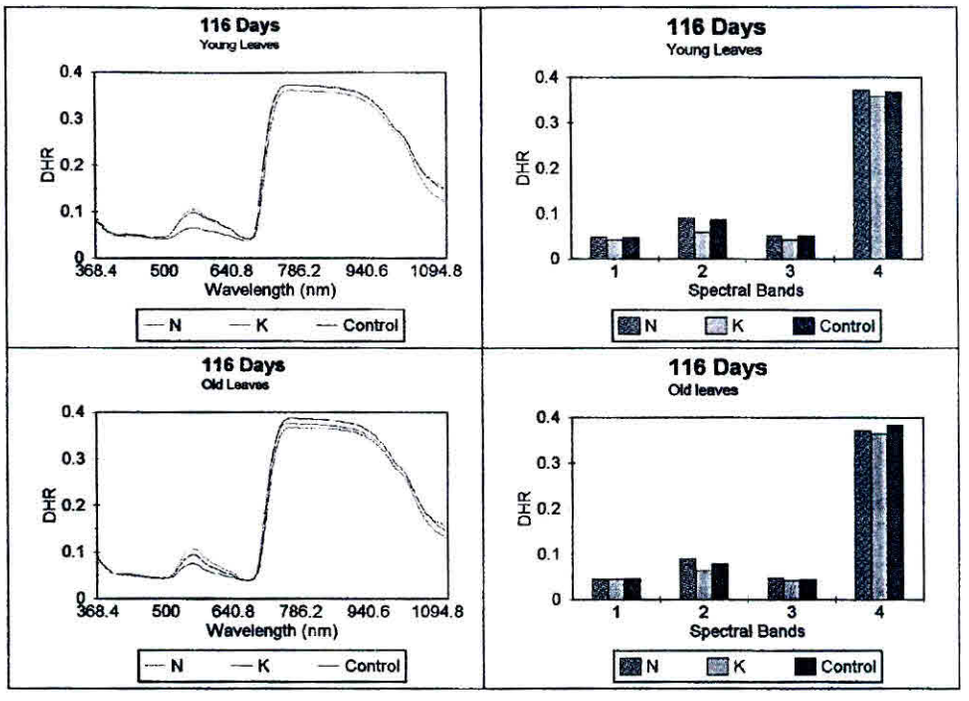
The SPECTRON SE 590 fine resolution proved unnecessary to spectrally characterize the deficiency symptoms pretended, since the results did not indicate any particular spectral feature associated with the mineral deficiencies simulated in this study. In future works, it could be considered some statistical techniques like that utilized by Card *et al.* (1988) and Williams *et al.* (1983) in data processing, which include stepwise multiple linear regression and rationing of derivatives at specific wavelengths. Flack and Chang (1987) have mentioned that the first one (stepwise multiple linear regression) has a potential to identify independent variables that are merely noise in the model. Card *et al.* (1988) have showed that technique works quite well as long as the number of sample spectra is sufficiently large.

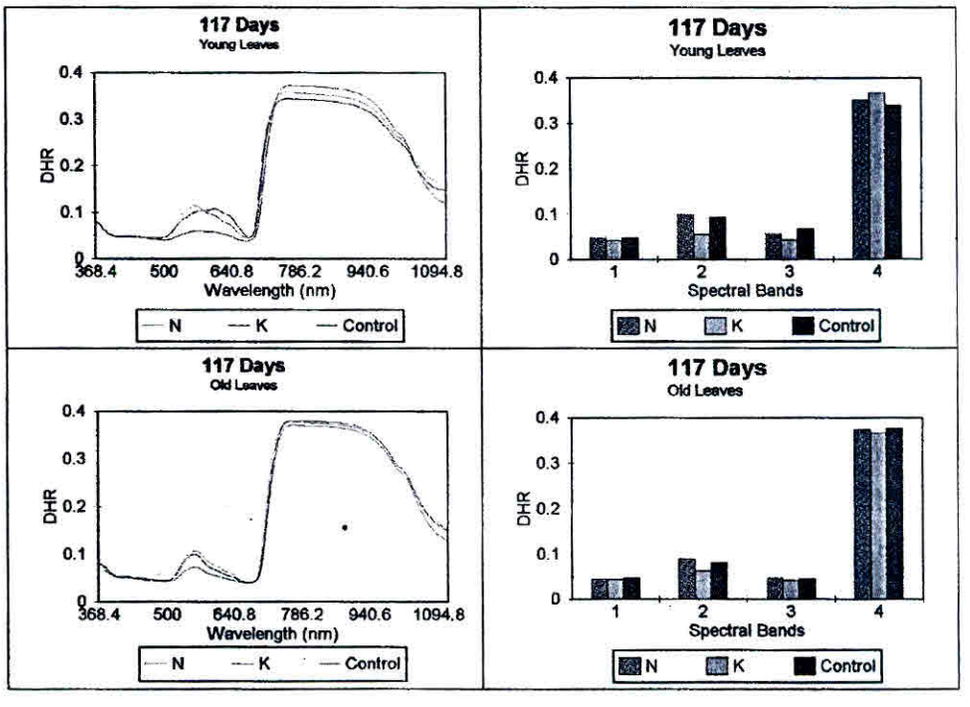
Finally, it could be tested the use of some reflectance transformations that have been proposed with different degrees of success, like $\log 1/\rho$ (where ρ is the reflectance value) that was used by Card *et al.* (1988) and provided good results.

Table 7. Multiple regression equations used to estimate the DHR values from the nutrient's concentration.

Nutrient	Band	Equations	R^2 Adj	n	Signif p
N	1	$FRH_1 = -0.355592 - 0.069174\ N + 0.253976\ P + 0.024663\ K + 0.481766\ Ln(N) - 0.235736\ Ln(K) - 0.405212\ Ln(P)/Ln(K)$	0.988	10	<0.00110
	2	$FRH_2 = 15.1819 + 6.7232\ Ln(P) - 2.2175\ Ln(K) + 0.0026\ Ln(N)/Ln(P) - 14.4424\ SQRT(P) + 1.4107\ SQRT(K)$	0.849	10	<0.0141
	3	$FRH_3 = 0.844545 + 0.07862\ P + 0.063005\ K - 0.648747\ Ln(K)$	0.731	10	<0.0038
	4	$FRH_4 = 1.25049 + 0.03545\ N - 1.20262\ P - 0.42827\ N/K + 3.63229\ P/K + 0.6858\ Ln(P)$	0.820	10	<0.0156
P	1	$FRH_1 = 9.59408 + 1.73963\ P + 0.60952\ K + 1.64266\ Ln(P) + 3.4767\ Ln(K) + 0.00024\ Ln(N)/Ln(P) + 0.21181\ Ln(N)/Ln(K) + 0.18051\ Ln(P)/Ln(K) - 0.12108\ SQRT(N) - 7.10831\ SQRT(P) - 5.70195\ SQRT(K)$	0.989	14	<0.00101
	2	$FRH_2 = 11.6874 + 3.7631\ P + 0.0251\ K + 3.7262\ Ln(P) + 0.0476\ Ln(N)/Ln(K) - 0.1441\ SQRT(N) - 15.1288\ SQRT(P)$	0.939	14	<0.00007
	3	$FRH_3 = -68.734 - 14.979\ N + 5.37\ P + 3.515\ K - 123.062\ Ln(N) + 7.049\ Ln(P) + 15.571\ Ln(K) + 0.002\ Ln(N)/Ln(P) + 0.432\ Ln(N)/Ln(K) + 172.014\ SQRT(N) - 24.972\ SQRT(P) - 29.323\ SQRT(K)$	0.942	14	<0.04769
	4	$FRH_4 = 9.0178 + 4.4923\ P + 0.5869\ K + 0.0498\ N/P - 1.3526\ N/K - 10.6462\ P/K - 0.0013\ Ln(N)/Ln(P) + 2.8554\ Ln(N)/Ln(K) + 2.3064\ Ln(P)/Ln(K) - 6.0839\ SQRT(P) - 4.3679\ SQRT(K)$	0.941	14	<0.01373
K	1	$FRH_1 = 0.135020 + 0.07953\ N/K - 0.125450\ P/K + 0.037394\ Ln(P) - 0.021891\ SQRT(N)$	0.110	30	<0.18011
	2	$FRH_2 = 30.8709 + 0.0994\ N + 9.3495\ P + 9.9802\ Ln(P) - 0.7459\ SQRT(N) - 38.755\ SQRT(P)$	0.160	30	<0.13881
	3	$FRH_3 = 0.03109 + 0.012164\ SQRT(K)$	0.206	30	<0.01355
	4	$FRH_4 = 0.389721 - 0.041813\ P/K + 0.030555\ Ln(P)/Ln(K)$	0.176	30	<0.04509

Appendix


116 Days
Young Leaves
DHR
0.4
0.3
0.2
0.1
0
368.4 500 640.8 786.2 940.6 1094.8
Wavelength (nm)
N K Control
116 Days
Young Leaves
DHR
0.4
0.3
0.2
0.1
0
1 2 3 4
Spectral Bands
N K Control
116 Days
Old Leaves
DHR
0.4
0.3
0.2
0.1
0
368.4 500 640.8 786.2 940.6 1094.8
Wavelength (nm)
N K Control
116 Days
Old leaves
DHR
0.4
0.3
0.2
0.1
0
1 2 3 4
Spectral Bands
N K Control


117 Days
Young Leaves
DHR
0.4
0.3
0.2
0.1
0
368.4 500 640.8 786.2 940.6 1094.8
Wavelength (nm)
N K Control
117 Days
Young Leaves
DHR
0.4
0.3
0.2
0.1
0
1 2 3 4
Spectral Bands
N K Control
117 Days
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368.4 500 640.8 786.2 940.6 1094.8
Wavelength (nm)
N K Control
117 Days
Old Leaves
DHR
0.4
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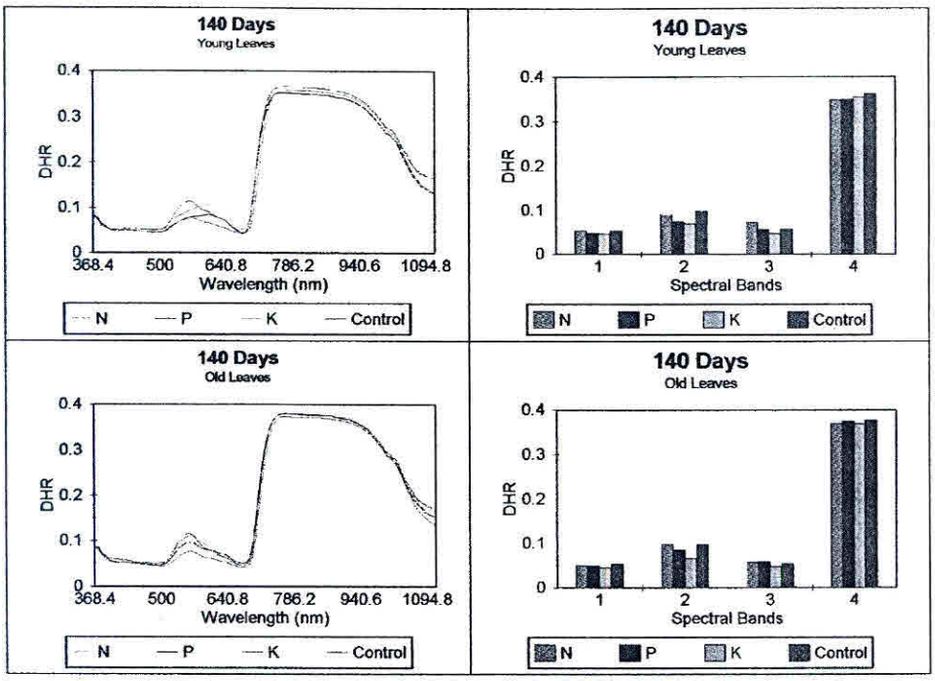
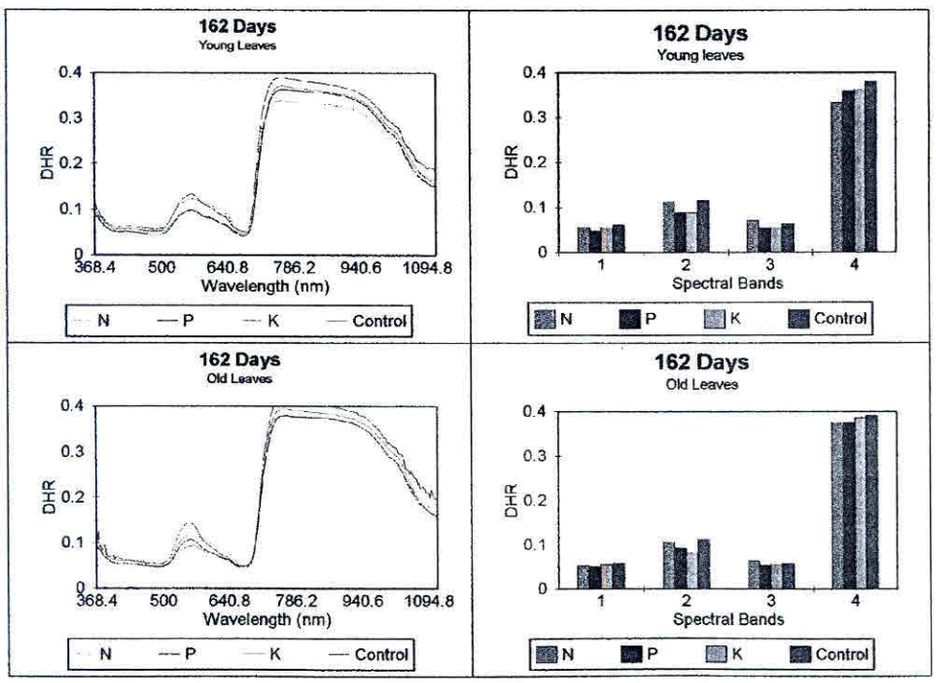
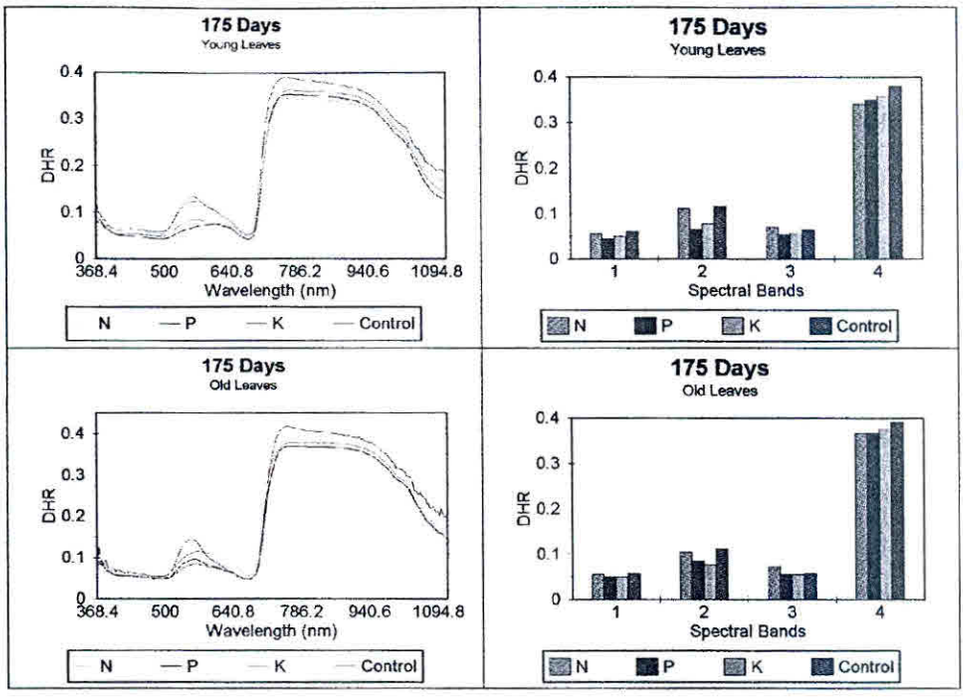
1 2 3 4
Spectral Bands
N K Control


140 Days
Young Leaves
DHR
0.4
0.3
0.2
0.1
0
368.4 500 640.8 786.2 940.6 1094.8
Wavelength (nm)
N P K Control
140 Days
Young Leaves
DHR
0.4
0.3
0.2
0.1
0
1 2 3 4
Spectral Bands
N P K Control
140 Days
Old Leaves
DHR
0.4
0.3
0.2
0.1
0
368.4 500 640.8 786.2 940.6 1094.8
Wavelength (nm)
N P K Control
140 Days
Old Leaves
DHR
0.4
0.3
0.2
0.1
0
1 2 3 4
Spectral Bands
N P K Control


162 Days
Young Leaves
DHR
0.4
0.3
0.2
0.1
0
368.4 500 640.8 786.2 940.6 1094.8
Wavelength (nm)
N P K Control
162 Days
Young leaves
DHR
0.4
0.3
0.2
0.1
0
1 2 3 4
Spectral Bands
N P K Control
162 Days
Old Leaves
DHR
0.4
0.3
0.2
0.1
0
368.4 500 640.8 786.2 940.6 1094.8
Wavelength (nm)
N P K Control
162 Days
Old Leaves
DHR
0.4
0.3
0.2
0.1
0
1 2 3 4
Spectral Bands
N P K Control



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