SPECTROMETRIC MEASUREMENTS OF REFLECTED RADIATION IN ECOLOGY RESEARCH

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ABSTRACT. In recent years, remote sensing proved to be very effective for ecological and conservation biological applications. Spectral remote sensing generates a remarkable array of ecologically valuable measurements for detecting natural and human-induced changes in the terrestrial ecosystems. In this study hyperspectral remote sensing method based on reflectance measurements in the visible and near infrared spectral ranges was applied for assessing the influence of some adverse natural changes in the environment (enhanced UV-B radiation and acid rain) on the physiological state of deciduous trees (species *Paulownia tomentosa*). Reflected radiation from the leaves was recorded on the seventh and fourteenth days after the adverse treatments by means of a portable fiber-optics spectrometer in the spectral range 350-1100 nm with a spectral resolution of 1.5 nm. Statistical and first derivative analyses and five narrow-band vegetation indices (mNDVI – modified Normalized Difference Vegetation Index, f_D - Disease index, SR –Simple Ratio, PRI – Photochemical Reflectance Index, TCARI - Transformed Chlorophyll Absorption Reflectance Index) were used to evaluate the changes in the spectral reflectance of the investigated tree groups. Indices mNDVI and SR performed best for discrimination between different treatments. Shift of the first derivative maxima on the reflected spectra to the lower wavelenghts was observed for all treatments which indicated the presence of the changes in the physiological state of the paulownia trees against the control.

Keywords: Remote sensing, hyperspectral leaf reflectance, vegetation indices, ecology

СПЕКТРОМЕТРИЧНИ ИЗМЕРВАНИЯ НА ОТРАЗЕНА РАДИАЦИЯ ЗА ЦЕЛИТЕ НА ЕКОЛОГИЯТА

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РЕЗЮМЕ. През последните години се оказа, че дистанционните изследвания са много ефективни за екологични и природозащитни биологични приложения. Спектралните дистанционни изследвания генерират изключително разнообразие от екологично ценни измервания за откриване на природни и предизвикани от човека промени в земните екосистеми. В това проучване хиперспектрален метод за дистанционни изследвания, основан на измерване на отразена радиация във видимата и близката инфрачервена спектрални области, е приложен за изследване на влиянието на някои неблагоприятни природни промени в околната среда (повишена UV-B радиация и киселинен дъжд) върху физиологичното състояние на широколистни дървета (вид *Paulownia tomentosa*). Отразената от листата радиация е регистрирана с портативен спектрометър с гъвкав световод в спектралния диапазон 350 -1100 nm със спектрална разделителна способност 1.5 nm. Статистически анализ, анализ на първа производна и пет теснолентови вегетационни индекси (mNDVI, f_D, SR, PRI, TCARI) са използвани за оценка на измененията в отразената от изследваните групи дървета радиация. Най-добри резултати за разграничаване на различните третирания по спектрални данни дават вегетационните индекси mNDVI and SR. Установено е отместване на максимумите на първите производни на отражателните спекри към по-ниските дължини на вълните за всички третирания, което е индикатор за настъпили изменения във физиологичното състояние на растенията, спрямо това на контролните.

Ключови думи: Дистанционни изследвания, хиперспектрална отразена радиация, вегетационни индекси, екология

Introduction

In recent years, remote sensing (RS) proved to be very effective for ecological and conservation biological applications. The efforts to predict the consequences of ecosystem function changes, both natural and human induced, on the regional and global distributions and abundance of species should be a high research priority. Further, the available RS tools and analysis techniques are ever evolving, and new capabilities continue to emerge, including sensors with improved spatial and spectral resolution (Medina et al., 2013; Rumpf et al., 2010). But the potential contribution of RS to study ecology in general, wildlife and more specifically ecosystem management, has yet to be realized.

RS generates a remarkable array of ecologically valuable measurements, which includes the details of habitats (land cover classification) and their biophysical properties (integrated ecosystem measurements) as well as the capacity to detect natural and human-induced changes within and across landscapes (change detection) (Wang et al., 2010).

The importance of environmental protection, climatic change, and nature preservation called for an intensive research of vegetation cover on global and regional scale by spectral remote sensing methods (Kerr and Ostrovsky, 2003). Monitoring of vegetation is essential for understanding dynamic biosphere processes. These are, to a large extent, formed by global photosynthetic energy transformation, which includes carbon dioxide assimilation and oxygen release by vegetation, and plays a role in maintaining the water balance (Veroustraete et al., 2002; Govender et al., 2009).

The reflectance spectrometry is a rapidly developing new branch of RS and field technologies covering the acquisition of reflectance spectra in the optical wavelength range of 0.4-2.5 μm, their processing and interpretation, which are, thus, suitable for investigation of the spectral behaviour of natural resources, such as soil, natural vegetation cover, waters, etc., (Aggarwal, 2008; Usha and Bhupinder, 2013). Spectrometry is based on the principle that molecules have specific frequencies at which they rotate or vibrate correspondingly with discrete energy levels (Myneni and Ross, 1991). Hence, depending on its molecular components, different materials will present essentially different spectral behaviour. The whole set of absorption features in the wavelength range constitutes a spectral pattern or so-called "spectral signature" of the materials.

Because of the importance of photosynthetic function, leaf optical properties have been the subject of hundreds of studies since the middle of the last century. Most papers focused on the spectral properties of leaves (reflectance and transmittance) which were used to estimate their biochemical content (Chl - chlorophyll, water, dry matter, etc.) and their anatomical structure (Carter and Spiering, 2002). Many other studies explore hyperspectral data applications in agriculture, environment, forestry and ecology (Krezhova, 2011; Usha and Bhupinder, 2013).

Over the past decade, researchers have studied also various spectral vegetation indices (SVIs) to detect different vegetation diseases and stresses. A large number of SVIs have been developed for estimation of leaf structure and pigment content (Mahlein et al., 2013). By calculating ratios of several narrow-bands at different ranges of the spectrum, SVIs result in a reduction of data dimension, which is also useful in effective data analysis for stress and disease discrimination (Delalieux et al., 2009).

Our investigations are aimed to show the efficiency of remote sensing method, measurements of leaf spectral reflectance in the visible (VIS) and near infrared (NIR) spectral ranges, for assessing the influence of some adverse natural changes in the environment such as enhanced UV-B radiation and acid rain, the effect of growth regulator MEIA and its application in combination with acid rain and UV-B on the physiological state of young deciduous trees (species *Paulownia tomentosa*).

Materials and methods

Plant material

The investigations were conducted with three months old paulownia seedlings grown as soil cultures in a growth chamber under controlled conditions (12 h light /12 h dark photoperiod, photon flux density of 90 μmol $m^{-2}sec^{-1}$, humidity 60-70%, and temperature 25±1°C). The plants were divided into six groups. The first group included healthy (control) trees. The trees from the second and third groups were treated with acid rain and enhanced UV-B radiation. The plants from the forth group were sprayed with 1 mM MEIA (beta-monomethyl ester of itaconic acid). The fifth and sixth groups of trees were sprayed with MEIA and after a day they were treated with the two natural stresses – (MEIA+acid rain) and (MEIA+UV-B).

Paulownia is ideal tree species for a forestation, improvement and restoration of contaminated with heavy metals and harmful substances soils and poor soils. Consuming these substances, the trees released them from the soil. After falling of the leaves, reaching 75 centimetres in diameter, they not only fertilized, but structured soil with natural humus. Paulownia also clears the air of harmful gases that are often with unacceptably high concentrations, especially in large industrial cities. Paulownia absorbs ten times more carbon dioxide than any tree species, releasing large amounts of oxygen (El-Showk and El-Showk, 2003).

Leaf spectral reflectance

Green vegetation species all have unique spectral features which are influenced by the leaf surface, internal architecture, life cycle, biochemical composition (pigments, water, nitrogen, minerals, etc.) and their health (Smith, 2001; Gitelson et al., 2003). The property used to quantify the spectral signatures is called spectral reflectance, a function described by the ratio of the intensity of reflected light to the illuminated light for each wavelength in VIS (0.4–0.7 μm), NIR (0.7-1.2 μm), and short-wave infrared (SWIR, 0.95-2.5 μm) spectral ranges, eq. 1:

$$R(\lambda) = L_r(\beta,\lambda)/L_i(\alpha,\lambda). \qquad (1)$$

Spectral reflectance R(λ) characterizes the portion $L_r(\lambda)$ of incident energy $L_i(\lambda)$ that is reflected by a surface at a given wavelength λ. Reflectance may be further qualified by parameters such as the angle of incidence α, and the angle of reflection β.

The typical leaf features influencing the spectral reflectance in three main optical spectral regions are: leaf pigments, cell structure, and water content. Figure 1 shows an example of the spectral reflectance curves for coniferous and broadleaf species, respectively, in the optical spectrum from 0.4 to 2.5 μm. Photons of the VIS wavelengths are strongly absorbed by foliar pigments. Chl is the main pigment that absorbs blue (400 to 495 nm) and red (620 to 700 nm) light, and transfer the absorbed energy into the photosynthetic electron chain (Sims and Gamon, 2002). The values of the reflectance in this portion of the electromagnetic spectrum are low. Leaf Chl content is closely related to plant stress and diseases (Carter and Knapp, 2001; Krezhova et al., 2012). When plants undergo environmental stresses, leaf Chl content is observed to decline. This results in an increase in the reflectance and transmittance over the green and red spectral ranges.

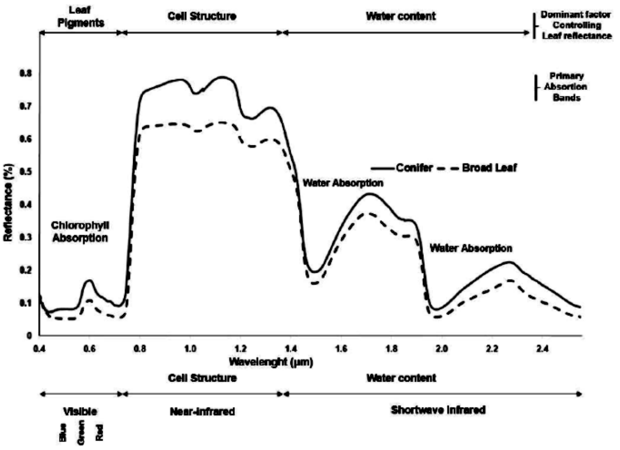

Fig. 1. Spectral reflectance curves of a coniferous and a broadleaf species in the optical spectrum from 0.4 to 2.5 µm

The reflectance for healthy vegetation increases dramaticly in the spectral range 0.68-0.72 µm (red edge position). In the NIR range (0.72-0.85 µm) the values of the reflectance are highest and the magnitude depends on leaf development and cell structure (Hyperspectral remote…, 2009). In the SWIR range strong absorption bands around 1.45, 1.95 and 2.50 µm appear and the reflectance is mainly determined by the leaf tissue and water content (Pettorelli et al., 2014).

Spectrometric measurements

Spectral reflectance was collected using a portable fiber-optic spectrometer USB2000 (Ocean Optics) in the spectral range 350-1100 nm at a spectral resolution (halfwidth) of 1.5 nm. The used detector is a high-sensitivity 2048-element CCD array. The measurements were carried out on an experimental setup in laboratory. The light signal from the object studied is guided to the entrance lens of the spectrometer by a fiber-optic cable directed perpendicular to the measured surface. As a source of light, a halogen lamp providing homogeneous illumination of measured leaf surfaces was used. In the beginning of each measurement the emitted spectrum of the source was registered from a diffuse reflectance standard. The spectral reflectance characteristics (SRC) of the investigated plants were determined as the ratio between the reflected from leaves radiation and this one reflected from the standard.

Data analyses

The Student's t-test was applied for determination of the statistically significance of the differences between the sets of the values of the reflectance spectra of healthy (control) and treated leaves. Statistical analyses were performed in four most informative for the reflectance curves of green plants spectral ranges: green (520-580 nm, maximal reflectivity of green vegetation), red (640-690 nm, maximal chlorophyll absorption), red edge (690-720 nm, maximal slope of the reflectance spectra) and NIR (720-770 nm). The statistical significance of the differences between SRC of control and infected plants was examined in ten wavelength bands (λ_1 = 475.22 nm, λ_2 = 489.37 nm, λ_3 = 524.29 nm, λ_4 = 539.65 nm, λ_5 = 552.82 nm, λ_6 = 667.33 nm, λ_7 = 703.56 nm, λ_8 = 719.31 nm, λ_9 = 724.31 nm, and λ_{10} = 758.39 nm) suggested to be disposed uniformly over the investigated ranges (Krezhova et al., 2005). First derivatives of the sets of reflectance data were calculated for assessing the red edge positions of the curves as an indicator for plant physiological status. Five narrowband vegetation indices were calculated for assesment of the changes of the physiological state of the Paulownia leaves:

- mNDVI – modified Normalized Difference Vegetation Index - an index of plant "greenness" or leaf chlorophyll content

$$\mathrm{mNDVI} = \frac{R_{NIR} - R_{Red}}{R_{NIR} + R_{Red}}, \qquad (2)$$

where: NIR = 750 nm, red = 705 nm;

- f_D - Disease Index (specific for individual study)

$$f_D = \frac{R_{500}}{R_{500} - R_{570}}; \qquad (3)$$

- PRI - Photochemical Reflectance Index – an index of photosynthetic efficiency

$$\mathrm{PRI} = \frac{R_{531} - R_{570}}{R_{531} + R_{570}}; \qquad (4)$$

- SR - Simple Ratio index- plant stress status

$$\mathrm{SR} = \frac{R_{690}}{R_{760}}; \qquad (5)$$

- Transformed Chlorophyll Absorption Reflectance Index (TCARI)

$$3\left[(R_{700} - R_{670}) - 0{,}2(R_{700} - R_{550})\frac{R_{700}}{R_{670}}\right]. \qquad (6)$$

Results and discussion

The averaged (over 25 pixels) SRCs of control and treated Paulownia leaves, measured on the seventh day after the treatments, are shown in Fig. 2. SRCs of the five treated tree groups differed against the control in all investigated spectral range (450-850 nm). In the green and red ranges (520 to 690 nm) the SRCs values are higher than the control excepting the case of enhanced UV-B radiation. This is due to decreasing of the Chl content that results in the expression of other leaf pigments such as carotenes and xanthophyll, causing a broadening of the green reflectance peak at 550 nm and an increasing of the reflectance.

In NIR range the SRC values of treated groups are lower than the control. These differences appeared as result of the changes that occurred in leaf cell structure and water content. In the case of acid rain treatment the differences are visibly largest. The leaves sprayed with MEIA looked visually like healthy leaves and their SRC are close to the control. The SRCs of leaf groups under combined treatments (MEIA+Acid rain and MEIA+UV-B) are more close to the control SRC than in the cases of single treatment.

Statistical significance of the differences between SRCs of control and treated leaves was established by means of theStudent's t-test. P-values and the sets of means are shown in Table 1. In the first column of the table R_i/R_{ic} (i = 1,…,10) designates that the data sets of spectral reflectance of the infected (R_i) and the control (R_{ic}) leaves are compared at the ten above listed wavelengths. SRC of the leaves sprayed with acid rain differed statistically significant against the control in seven of the investigated wavelengths while for the case of MEIA+Acid rain - in five wavelengths. SRC of the treated with MEIA leaves differed statistically significant in four wavelengths in spectral ranges 520-690 nm because of its stimulating growth action. For case UV-B radiation the statistically significant differences found are less (six wavelengths) than in case of acid rain. For MEIA+UV-B treatment the significant differences are four.

Table 1.
Means and p-values of the Student's t-test of SRC pairs of control and treated Paulownia leaves

Pairs	Control	Acid rain		MEIA+Acid rain		UV-B		MEIA+UV-B		MEIA	
compared	mean	p	mean	p	mean	p	mean	p	mean	p	mean
R_1/R_{1c}	6.68	ns	7.57	ns	7.94	ns	7.85	ns	7.59	ns	7.28
R_2/R_{2c}	6.83	ns	7.80	ns	8.28	ns	8.17	ns	7.81	ns	7.46
R_3/R_{3c}	18.91	***	15.11	ns	21.56	**	18.07	ns	12.17	ns	16.51
R_4/R_{4c}	25.18	***	18.89	***	28.26	*	15.00	ns	15.86	**	20.33
R_5/R_{5c}	26.44	***	19.68	***	29.96	ns	15.63	ns	16.58	***	21.12
R_6/R_{6c}	6.62	ns	6.93	ns	7.16	***	6.03	***	5.98	**	6.95
R_7/R_{7c}	29.76	**	22.31	ns	32.63	ns	17.74	***	19.67	*	23.74
R_8/R_{8c}	56.02	*	37.14	***	57.49	**	29.81	ns	38.46	ns	36.19
R_9/R_{9c}	63.20	**	40.58	**	63.58	**	32.77	**	43.43	ns	38.84
R_{10}/R_{10c}	78.67	***	47.68	***	74.50	***	38.85	**	54.26	ns	43.75

*ns – no statistical significance; * - $p<0.05$; ** - $p<0.01$; *** - $p<0.001$*

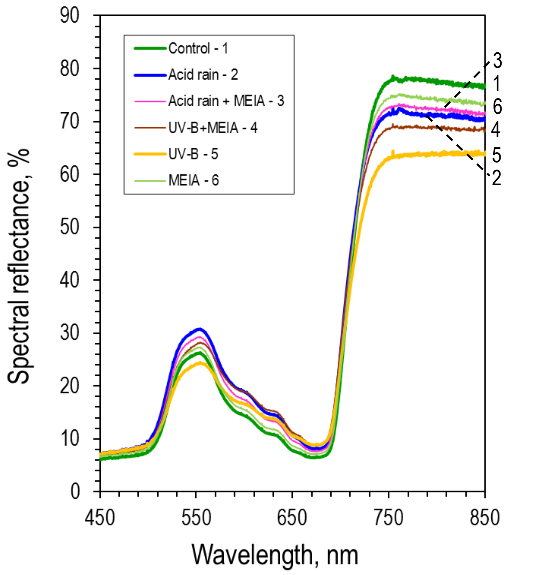

Fig. 2. Averaged SRC of Paulownia leaves: control and treated with acid rain, UV-B radiation, MEIA and their combinations on the 7th day after the treatment

First derivatives of the averaged SRCs of six leaf groups were calculated and analyses were performed to evaluate the red edge position of the SRCs. Figure 3 shows the maxima of the first derivatives of SRC of all the investigated groups. The maximal values of the main peaks of the derivatives of the five groups of treated leaves are shifted to the lower wavelengths with respect to the control. A minor difference (1 nm) is observed in the case of treatment with MEIA. For the cases (Acid rain) and (MEIA+Acid rain) the differences are about 3 nm and 1.5 nm, respectively, which reveals the favourable effect of MEIA for reduction of the stress action. In the case of UV-B the observed shift is about 2.5 nm, while the effect of the combined treatment (MEIA+UV-B) produced a shift of about 5 nm. On the 7th day after the UV-B treatment the leaves were thinner and with yellow spots.

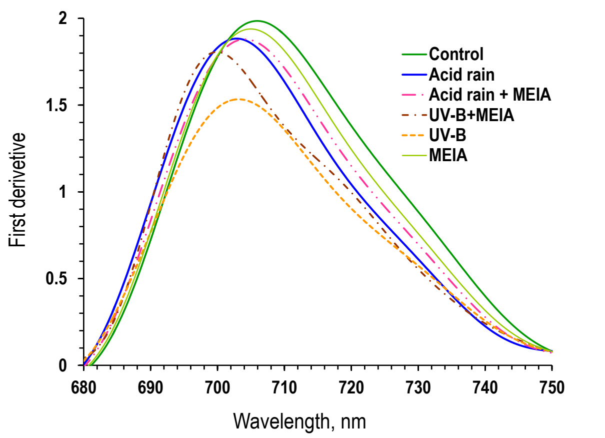

Fig. 3. Maximum of the first derivatives on SRC of control and treated with acid rain, UV-B radiation, MEIA and their combinations on the 7th day after the treatment

The averaged (over 23 pixels) SRCs of control and treated Paulownia leaves, measured on the 14th day after the treatment, are shown in Fig. 4. The five treatments lead to similar trend in the leaf spectral behaviour as on the 7th day. However, in the case of Acid rain the differences become much more expressed but (MEIA+Acid rain) action brings the SRC again more close to the control.

The results after applying the Student's t-test for sets of spectral data measured on the 14th day after the treatments are given in Table 2. The number of statistically significant differences for individual treatments is increased indicating deepening of the stress. The positive MEIA action on the plant status is confirmed. For cases (MEIA+Acid rain) and (MEIA+UV-B) the number of statistically significant differences decreased due to the growth regulator.

Table 2.
Means and p-values of the Student's t-test of SRC pairs of control and and treated Paulownia leaves

Pairs compared	Control mean	Acid rain		MEIA+Acid rain		UV-B		MEIA+UV-B		MEIA	
		p	mean	p	mean	p	mean	p	mean	p	mean
R_1/R_{1c}	5.07	***	4.20	ns	5.00	***	3.99	***	3.15	ns	3.86
R_2/R_{2c}	5.52	***	4.95	ns	5.98	*	4.91	ns	4.07	***	4.82
R_3/R_{3c}	15.04	**	15.92	**	18.76	ns	15.07	**	13.67	ns	15.64
R_4/R_{4c}	19.64	**	20.62	**	23.18	**	18.74	ns	17.39	ns	19.24
R_5/R_{5c}	20.57	*	21.58	*	24.20	*	19.54	ns	18.18	ns	20.69
R_6/R_{6c}	5.59	*	5.28	*	6.77	ns	5.56	ns	4.65	ns	5.24
R_7/R_{7c}	23.60	**	24.30	*	27.41	ns	22.18	**	20.79	ns	23.41
R_8/R_{8c}	44.20	ns	43.15	**	42.32	***	37.00	***	35.52	***	39.50
R_9/R_{9c}	49.00	***	47.18	*	45.31	***	40.08	***	38.46	***	42.867
R_{10}/R_{10c}	58.32	**	54.33	***	50.27	***	46.13	***	43.79	***	49.96

*ns – no statistical significance; * - p<0.05; ** - p<0.01; *** - p<0.001*

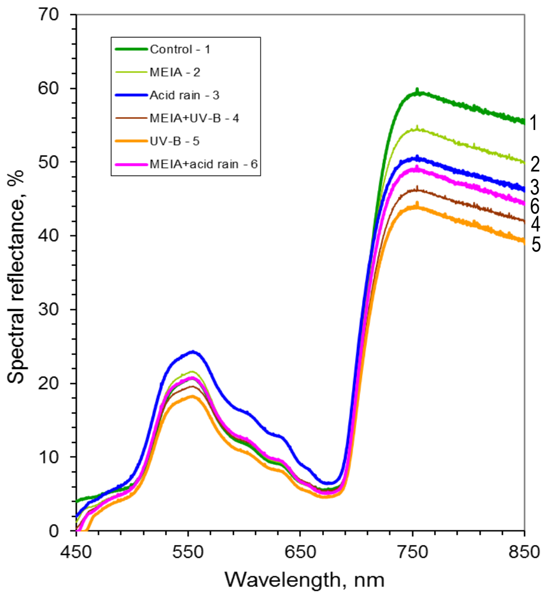

Fig. 4. Averaged SRC of Paulownia leaves: control and treated with acid rain, UV-B radiation, MEIA and their combinations on the 14th day after the treatment

Figure 5 shows the maxima of the first derivatives of the averaged SRCs of healthy and treated Paulownia leaves on the 14th day. For all cases the maxima are shifted to lower wavelengths with respect to the control. The largest difference is for the case Acid rain (about 5.5 nm). For the combination (MEIA+Acid rain) this difference is lower (approx. 3 nm). Similarly, the combination MEIA+UV-B brings down the shift of the maximum to about 2.5 nm from about 3 nm for the single treatment. This is in favour to the conclusion for the MEIA action to suppress the effects of single stresses.

Five narrow-band vegetation indices: mNDVI, f_D, PRI, SR and TCARI were computed (equations 2 to 6) for each set of spectral data. Table 3 shows the mean values of indices and statistical significance of differences against the control. The SVI data sets were processed by the method of analysis of variance for estimation of the significance of differences between variant means at levels $p < 0.05$, 0.01, 0.001 depending on the data dispersion. The best results for separation of the healthy from the exposed to natural stresses Paulownia leaves gave SVIs mNDVI and SR. For mNDVI mean values for treated groups decreased in comparison with the values of the control group while for SR – increased. In both cases the differences are statistically significant.

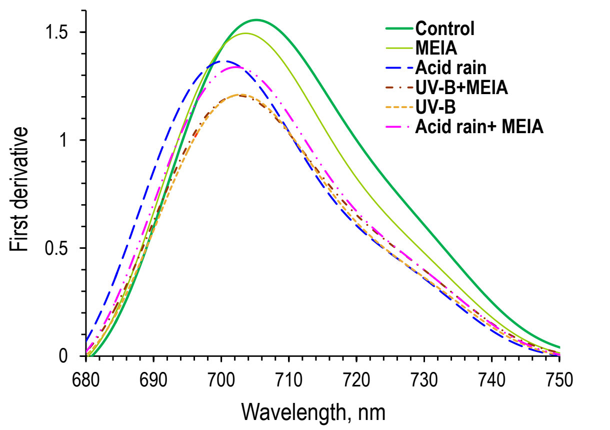

Fig. 5. Maximum of the first derivatives on SRC of control and treated with acid rain, UV-B radiation, MEIA and their combinations on the 14th day after the treatment

Conclusions

Remote sensing method, hyperspectral measurements of leaf spectral reflectance in the VIS and NIR spectral ranges, was applied for assessing the influence of some adverse natural changes in the environment such as enhanced UV-B radiation and acid rain as well as the effect of growth regulator MEIA and its application in combination with acid rain and UV-B on the physiological state of young deciduous trees (species *Paulownia tomentosa*). Statistical and first derivative analyses of the reflectance data and five narrowband vegetation indices (mNDVI, f_D, SR, PRI, TCARI) were implemented to investigate the spectral behaviour of Paulownia trees. The results prove that the spectral reflectance characteristics of the investigated plants gave best discrimination between the leaf groups in accordance with their physiological state.

Table 3.
Values of five narrow-band vegetation indices calculated in this study

Paulownia leaves	mNDVI	f_D	PRI	SR	TCARI	mNDVI	f_D	PRI	SR	TCARI
	on the 7th day after treatment					on the 14th day after treatment				
Control	0.356	0.26	0.044	0.115	55.42	0.369	0.269	0.038	0.128	43.39
Acid rain	0.309 ***	0.271 ns	0.027 ***	0.164 ***	63.81 ***	0.258 ***	0.262 ns	0.019 ***	0.208 ***	51.47 *
MEIA+acid rain	0.339 ***	0.269 ns	0.026 ***	0.149 ***	60.00 ns	0.362 ns	0.274 ns	0.024 ns	0.133 ns	56.57 ***
UV-B	0.306 ***	0.291 **	-0.001 ***	0.194 ***	49.36 *	0.304 ***	0.252 ***	0.037 ns	0.160 ***	39.32 ***
MEIA+UV-B	0.291 ***	0.266 ns	-0.007***	0.191 ***	57.04 ns	0.305 ***	0.273 ns	0.031 ***	0.178 ***	40.70 ns
MEIA	0.376 ns	0.277 ns	0.048 ns	0.124 *	57.11 ns	0.323 ***	0.249 ***	0.038 ns	0.142 *	47.00 *

ns – no statistical significance; * - $p<0.05$; ** - $p<0.01$; *** - $p<0.001$

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