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Bark spectral signatures of one-year-old twigs of different shrubs mainly depend on their biochemical traits

Spektralni podpisi skorje enoletnih vejic različnih grmovnih vrst so večinoma odvisni od biokemijskih lastnosti skorje

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Abstract: The interaction of bark with light depends on the optical properties of the bark, which are important for plant energy balance especially out of the vegetation season. Light reflected from bark also represents a kind of "bark spectral signature" that may be species-specific. This study examines some morphological, biochemical, and physiological traits that may affect the reflectance of the bark of 1-year-old twigs of different shrubs: *Corylus avellana*, *Rosa canina*, *Ligustrum vulgare*, *Sambucus nigra*, *Cornus sericea* var. *flaviramea*, and *Viburnum lantana*. High variability was seen across these species for morphological, biochemical, and physiological traits, except for photochemical efficiency of photosystem II. The bark spectral signatures differed significantly across these species. The reflectance peak for *C. sericea* var. *flaviramea* was in red, for *C. avellana* in green, and the other species showed a wide peak from green to red light. Redundancy analysis revealed that UV-B–absorbing substances and anthocyanin content, along with outer and inner bark thickness, together explained 61% of the reflectance spectra variability. Outer bark thickness was important for reflectance in UV, violet, and blue, while anthocyanins were important for reflectance in green and yellow. Chlorophyll *b* was negatively related to the reflectance of visible light. This study revealed great importance of biochemical traits for explaining bark reflectance. Differences in "bark spectral signatures" enable differentiation across species out of the vegetation season.

Keywords: bark, pigments, reflectance, UV-B–absorbing substances, woody plants

Izvleček: Interakcija skorje s svetlobo je odvisna od optičnih lastnosti skorje, ki so pomembne za energijsko ravnovesje rastlin zlasti izven vegetacijske sezone. Svetloba, ki se odbije od skorje, predstavlja tudi nekakšen "spektralni podpis skorje", ki je lahko vrstno specifičen. V tej raziskavi smo proučili nekatere morfološke, biokemijske in fiziološke lastnosti, ki lahko vplivajo na odbojnost skorje enoletnih vejic različnih grmovnih vrst: *Corylus avellana*, *Rosa canina*, *Ligustrum vulgare*, *Sambucus nigra*, *Cornus sericea* var. *flaviramea* in *Viburnum lantana*. Opazili smo veliko variabilnost med proučevanimi vrstami glede njihovih morfoloških, biokemijskih in fizioloških lastnosti, razen fotokemične učinkovitosti fotosistema II. Med izbranimi vrstami so se

značilno razlikovali tudi spektralni podpisi skorje. Vrh odbojnosti pri vrsti *C. sericea* var. *flaviramea* je bil v rdečem, pri *C. avellana* v zelenem, druge vrste pa so pokazale širok vrh, ki se je raztezal od zelenega do rdečega spektralnega območja. Redundančna analiza je pokazala, da vsebnost UV-B–absorbirajočih snovi in antocianinov skupaj z debelino zunanje in notranje skorje skupaj pojasnjujejo 61 % variabilnosti odbojnih spektrov. Debelina zunanje skorje je bila pomembna za odbojnost v UV, vijoličnem in modrem spektralnem območju, vsebnost antocianinov pa za odbojnost v zelenem in rumenem spektralnem območju. Klorofil *b* je bil negativno povezan z odbojnostjo vidnega dela spektra. Ta raziskava je pokazala velik pomen biokemijskih lastnosti za razlago odbojnosti skorje. Razlike v "spektralnih podpisih skorje" omogočajo razlikovanje med vrstami izven vegetacijske sezone.

Ključne besede: lesnate rastline, odbojnost, pigmenti, skorja, UV-B–absorbirajoče snovi

Introduction

Bark comprises tissues outside the vascular cambium, and consists of the inner bark, which contains secondary phloem, and the outer bark or periderm, produced by the cork cambium (Romero 2014). Bark protects stems against physical disturbances (e.g., fire) and environmental factors (e.g., extreme temperatures, drought), and it has high repair potential (Romero et al. 2009). In addition, the outer bark is an effective barrier against pollutants (Pfanz 1999), and enables transport of assimilates and water (Poorter et al. 2014). Bark physiological and biochemical traits vary significantly across species, and these specific bark traits define the bark interactions with the abiotic and biotic environments (Larcher 2003).

The surface structure of bark determines the composition and structure of epiphytic communities (Tewari et al. 2009). In various forest types, the suitability of bark for epiphytic lichen and bryophyte colonisation increases with increasing bark roughness (Black and Harper 1979). Ferrenberg and Mitton (2014) demonstrated that smooth bark limits the attacks of insects specialised in attacking tree stems. Bark may also contain high concentrations of flavonoids, which are known to have multiple functions (Carrillo-Parra et al. 2012). These substances protect plants from different biotic and abiotic stresses and act as filters for UV radiation, signal molecules, detoxifying agents, and antimicrobial compounds (Samanta et al. 2011).

The bark chlorenchyma is located under the rhytidomal or outer peridermal layers; it can harvest transmitted light and use the stem internal CO_2 for photosynthesis (Pfanz et al. 2002, Wittmann and Pfanz 2008, 2014). The transmission of light through the periderm is low and depends on the species and age of the branches or stems (Aschan et al. 2001). A study by Wittmann and Pfanz (2016) revealed that transmittance of photosynthetically active radiation (PAR) through the periderm of young stems varied between 8.5% and 42.0%, while PAR transmittance through the total bark ranged from 2.2% to 6.2%.

The fixation of CO_2 via photosynthesis reduces internal carbon dioxide losses and thus contributes to a favourable carbon balance (Filippou et al. 2007, Eyles et al. 2009). Wittmann and Pfanz (2008) showed that photosynthesis of the bark of young stems can replace 60% to 90% of respiratory carbon losses. Assimilation of CO_2 in stems has several advantages over photosynthesis in leaves, as stems lose less water and contain high concentrations of CO_2, which reduce the level of photorespiration in stems (Damesin 2003). In young stems, respiration and the photosynthetic rate are generally higher than in older stems (Wittmann and Pfanz 2008). The amount of light that can reach different stem tissues affects plastid differentiation, and thus also their photosynthetic properties (Wittmann and Pfanz 2016). As branches grow older, the bark and the peridermal layer become thicker, and consequently the amount of chlorophyll and photosynthetic activity decrease (Pilarski 1999, Wittmann et al. 2001). Bark photosynthesis does not only have an important role in maintaining the carbon balance, but it also improves the oxygen content and enables undisturbed respiration of

metabolically active tissues (Wittmann and Pfanz 2014). Green stems with poorly developed periderm have functional stomata and can assimilate large amounts of carbon, especially during the period when the plants are leafless (Filippou et al. 2007). Also, in spite of the low light levels in winter, bark photosynthetic activity and respiration contribute to a more favourable carbon balance from late autumn to early spring, when photosynthetic activity in evergreen organs is reduced (Wittmann and Pfanz 2007) or is absent in deciduous species.

The light regime within the stem affects the differentiation of plastids and thus affects the photosynthetic properties of individual tissues in the stem (Wittmann and Pfanz 2016). Light penetration depends on the optical properties of the bark. Light reflected from bark represents a kind of "bark spectral signature" that is species-specific and can reveal the physiological and biochemical properties of the bark (Tokarz and Pilarski 2005, Levizou and Manetas 2007). In deciduous species, the bark spectral signatures enable differentiation among woody species out of the vegetation season using remote sensing (Atkilt Girma et al. 2013), and host discrimination by bark and timber beetles (Campbell and Borden 2005). Bark optical properties also contribute to the bark energy balance and prevent overheating (Henrion and Tributsch 2009).

In the present study, we examined different plant traits that affect bark reflectance of 1-year-old twigs of different woody species: *Corylus avellana*, *Rosa canina*, *Ligustrum vulgare*, *Sambucus nigra*, *Cornus sericea* var. *flaviramea*, and *Viburnum lantana*. We hypothesised that the bark biochemical traits and the bark thickness have important roles in shaping the bark reflectance. To show this, we analysed different pigments and UV-absorbing substances, and measured the inner and outer bark thicknesses, and the bark reflectance spectra.

Materials and methods

Plant species

The species studied were the woody plants *Corylus avellana* L. (Betulaceae), *Rosa canina* L. (Rosaceae), *Ligustrum vulgare* L. (Oleaceae), *Sambucus nigra* L. (Adoxaceae), *Cornus sericea* var. *flaviramea* L. (Cornaceae), and *Viburnum lantana* L. (Caprifoliaceae). All of these species are shrubs that are native to Europe, except for *C. sericea*, which is native to North America. However, the cultivar *C. sericea* var. *flaviramea* was developed in Europe. The studied species are all deciduous, with the exception of *L. vulgare*, which is a semi-evergreen or deciduous shrub that is frequently grown as a hedge. *C. avellana* is an important component of hedgerows. *R. canina* is a climbing shrub that has stems, which are covered with small, sharp, hooked prickles. *S. nigra* is also very common in hedgerows and scrubland, and it is also widely grown as an ornamental shrub or small tree. *C. sericea* var. *flaviramea* is widely grown as an ornamental plant that grows best in moderate warmth in sunny places, although it can also tolerate shade. *V. lantana* is a tree-like shrub that is common along waysides and is also grown as an ornamental plant.

One-year-old and about 0.5 cm thick twigs from 10 plants of each species were sampled from open places in Ljubljana (46°3'N; 14°28'E) and Golnik (46°19'N; 14°19'E), and were processed on the day of sampling. The sampling and analysis took place before the development of leaves, from February to the end of March.

Morphological properties

Analysis of bark thickness was carried out on transverse sections of vital 1-year-old twigs. The measurements included the thickness of the inner and outer bark (periderm). The measurements were performed using a light microscope (CX41; Olympus, Tokyo, Japan) equipped with a digital camera (XC30, Olympus) and the CellSens software (Olympus).

Biochemical properties

The chlorophyll *a*, chlorophyll *b*, and carotenoid contents were determined for bark extracts according to Lichtenthaler and Buschmann (2001a, b), with absorbance measured at 470 nm, 645 nm, and 662 nm using a UV/VIS spectrometer (Lambda 25; Perkin-Elmer, Norwalk, CT, USA). Antho-

cyanin content was determined on bark extracts as described by Drumm and Mohr (1978), with absorbance measured at 530 nm. Total methanol-soluble UV-B–absorbing and UV-A–absorbing substances (as a measure of total phenolics) were also extracted from fresh twigs, according to Caldwell (1968), with absorbance measured from 280 nm to 319 nm, and from 320 nm to 400 nm, respectively. The extinction values were integrated for each UV region. The biochemical parameters are expressed per bark area.

Physiological properties

Chlorophyll fluorescence was measured using a portable chlorophyll fluorometer (PAM-2100; Heinz Walz GmbH, Effeltrich, Bavaria, Germany). The potential and effective photochemical efficiency of photosystem (PS) II (Fv/Fm and Fq'/Fm', respectively) were evaluated according to Schreiber et al. (1996), with the measurements performed on fresh twigs. Prior to the Fv/Fm measurements, twigs were kept in the dark for 20 minutes. Fq'/Fm' measurements were performed at daylight at >1000 μmol m^{-2} s^{-1}, with temperatures ranging from 10 to 15 °C. Fq'/Fm' was calculated using equation 1,

$$\mathrm{Fq'/Fm'} = \frac{Fm' - F'}{Fm'} \quad (1)$$

where Fm' is maximum fluorescence from dark- or light-adapted leaf, respectively (PS II centers closed), and F' is fluorescence emission from dark- or light-adapted leaf, respectively (Brestic and Zivcak 2013).

Potential photochemical efficiency (Fv/Fm) was calculated using the equation 2,

$$Fv/Fm = 1 - \frac{Fo}{Fm} \quad (2)$$

where Fo is minimal fluorescence from dark-adapted leaf (PS II centres open), and Fm is maximum fluorescence from dark- or light-adapted leaf, respectively (PS II centres closed) (Brestic and Zivcak 2013).

Optical properties

Bark reflectance was determined in the laboratory on the day of sampling. The bark was carefully removed from the twigs. The reflectance spectrum was measured from 290 nm to 800 nm, at a resolution of ~1.3 nm, using a portable spectrometer (Jaz Modular Optical Sensing Suite; Ocean Optics, Inc., Dunedin, FL, USA; grating, #2; slit size, 25 μm) with an optical fibre (QP600-1-SR-BX, Ocean Optics, Inc.) and an integrating sphere (ISP-30-6-R, Ocean Optics, Inc.). The reflectance spectrum was measured for the bark surface by illumination with a UV/VIS-near infrared (NIR) light source (DH-2000; Ocean Optics, Inc.). The spectrometer was calibrated to 100% reflectance using a white reference panel with >99% diffuse reflectance (Spectralon, Labsphere, North Sutton, NH, USA). More details on the procedure for measurement of optical properties can be found in Klančnik et al. (2015).

Statistical analysis

Statistical analysis was carried out in IBM SPSS statistics 22.0 using one-way multivariate analysis of variance followed by Duncan's post-hoc multiple comparison tests. Prior to this analysis, normal distributions of the data were tested using Shapiro-Wilk tests in Past 3.14. We performed redundancy analysis (RDA) using CANOCO for Windows 4.5 programme package to determine whether variations in the reflectance spectra were related to morphological and biochemical parameters (ter Braak and Šmilauer 2002). The spectra were divided into spectral bands (i.e., UV-B, UV-A, violet, blue, green, yellow, red, and NIR) to obtain more detailed information about the correlations between explanatory and response variables. Forward selection of the explanatory variables was used to avoid co-linearity between the variables (Lepš and Šmilauer 2003). The level of significance was accepted at $p \leq 0.05$. Non-significant explanatory variables were excluded from further analysis. All of the variables in the RDA were standardised to eliminate the influence of magnitude differences between scales and units.

Results

In the comparison of the biochemical traits, the greatest variability was seen for anthocyanin content (Tab. 1). The chlorophyll *a* to *b* ratio varied a lot across the species. The bark of *C. sericea* var. *flaviramea* differed the most from the other species, with the highest total chlorophylls, UV-absorbing substances, and anthocyanins, but not carotenoids. The peridermal layers had different thicknesses in the species studied. *V. lantana* had the thickest outer bark, while for *S. nigra* the inner bark was the thickest. *R. canina* and *C. sericea* var. *flaviramea* still had the epidermal layer and a very thin layer of periderm. Fv/Fm was high in the majority of species, however, Fq'/Fm' was low and did not differ across the species.

The bark spectral signatures were very variable. The bark reflectance differed the most in the visible part of the spectrum. *C. sericea* var. *flaviramea* had high reflectance in the UV-B and NIR regions, and low reflectance in the visible region (Fig. 1). Its reflectance spectra had distinct peaks in the red region. However, the reflectance peak of other species was wide, and ranged from green to red, with the exception of *C. avellana*, which had a peak in the green region. The reflectance of *V. lantana* gradually increased along the spectra, with a slight depression in the red edge.

Table 1: Selected bark biochemical and physiological traits, along with bark reflectance and thicknesses for the different woody species examined in this study.
Tabela 1: Izbrane biokemijske in fiziološke lastnosti ter odbojnost in debelina skorje različnih lesnatih vrst, obravnavanih v tej raziskavi.

Traits	Units	Data measures according to species					
		Corylus avellana	*Rosa canina*	*Ligustrum vulgare*	*Sambucus nigra*	*Cornus sericea*	*Viburnum lantana*
Biochemical							
Chlorophyll *a*	mg cm^{-2}	0.012 ±0.001^{bc}	0.022 ±0.002^{a}	0.010 ±0.002^{b}	0.016 ±0.002^{bc}	0.019 ±0.012^{abc}	0.014 ±0.009^{bc}
Chlorophyll *b*	mg cm^{-2}	0.006 ±0.001^{a}	0.013 ±0.001^{bc}	0.007 ±0.003^{ab}	0.011 ±0.003^{b}	0.029 ±0.023^{c}	0.017 ±0.014^{bc}
Carotenoids	mg cm^{-2}	0.005 ±0.000^{ac}	0.008 ±0.001^{b}	0.003 ±0.001^{a}	0.005 ±0.001^{c}	0.006 ±0.003^{abc}	0.006 ±0.001^{bc}
Anthocyanins	au cm^{-2}	0.44 ±0.03^{a}	0.84 ±0.17^{bc}	0.23 ±0.07^{a}	0.63 ±0.05^{b}	1.48 ±0.53^{c}	1.12 ±0.28^{c}
UV-B–AS	au cm^{-2}	4.90 ±0.45^{a}	9.64 ±0.93^{bc}	7.69 ±1.11^{ab}	10.64 ±1.43^{c}	23.10 ±4.32^{d}	12.27 ±1.42^{c}
UV-A–AS	au cm^{-2}	4.09 ±0.37^{a}	9.11 ±0.89^{bc}	7.45 ±1.13^{ab}	8.94 ±1.01^{bc}	7.69 ±1.41^{b}	10.89 ±1.76^{c}
Bark thickness							
Inner bark	µm	84 ±7^{a}	163 ±20^{c}	173 ±18^{ac}	210 ±17^{b}	137 ±25^{c}	169 ±40^{bc}
Outer bark$^{\#}$	µm	64 ±8^{a}	33 ±1^{b}	92 ±5^{a}	165 ±10^{c}	37 ±3^{b}	284 ±32^{c}
Physiological							
Photochemical efficiency of PS II							
Fq'/Fm'	au	0.12 ±0.05^{a}	0.12 ±0.07^{a}	0.14 ±0.11^{a}	0.15 ±0.04^{a}	0.11 ±0.03^{a}	0.14 ±0.03^{a}
Fv/Fm	au	0.36 ±0.07^{ac}	0.62 ±0.03^{b}	0.65 ±0.06^{abd}	0.71 ±0.03^{d}	0.48 ±0.05^{c}	0.69 ±0.05^{d}
Reflectance	%						
UV-B		4.3 ±3.4^{abd}	8.8 ±1.1^{b}	4.9 ±2.2^{a}	10.0 ±1.7^{bc}	11.7 ±0.6^{c}	0.3 ±1.8^{d}
UV-A		6.6 ±2.2^{ab}	9.3 ±1.0^{ab}	7.7 ±1.2^{a}	13.6 ±1.8^{c}	9.2 ±0.5^{b}	0.1 ±2.0^{c}
Violet		14.1 ±8.7^{ab}	12.6 ±7.3^{a}	11.2 ±2.8^{a}	13.8 ±2.3^{a}	8.1 ±0.4^{b}	3.6 ±2.0^{c}
Blue		16.8 ±8.9^{ab}	14.3 ±7.9^{a}	14.2 ±3.0^{a}	15.0 ±2.3^{a}	7.6 ±0.4^{c}	7.2 ±3.6^{bc}
Green		23.5 ±7.4^{a}	20.2 ±7.4^{a}	23.5 ±3.6^{a}	25.5 ±3.7^{a}	7.3 ±0.5^{b}	11.1 ±4.3^{b}
Yellow		26.5 ±6.7^{ab}	21.8 ±6.7^{ab}	24.5 ±3.4^{a}	23.3 ±3.2^{a}	10.9 ±1.2^{c}	15.5 ±4.9^{bc}
Red		25.9 ±6.7^{a}	20.3 ±6.6^{a}	21.8 ±3.0^{a}	19.2 ±2.8^{a}	13.0 ±1.4^{b}	19.1 ±5.4^{ab}
NIR		49.0 ±7.5^{ab}	56.4 ±4.9^{a}	56.8 ±1.9^{a}	55.7 ±6.2^{a}	67.4 ±1.7^{c}	43.8 ±7.5^{b}

Data are means ±SD (n = 5-10 for each species); different superscript letters within each row indicate significant differences ($p \leq 0.05$; Tukey tests); reflectance spectra represent means within 5-nm intervals ($p \leq 0.05$, Tukey tests). Abbreviations: au, arbitrary unit; Fq'/Fm', effective photochemical efficiency; Fv/Fm, potential photochemical efficiency; UV-B–AS, UV-B–absorbing substances; UV-A–AS, UV-A–absorbing substances; $^{\#}$epidermal layer with periderm.

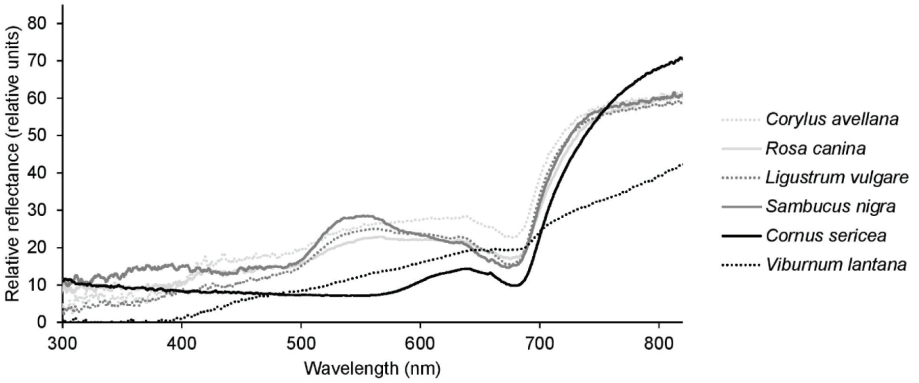

Figure 1: Relative reflectance spectra of bark of the different shrub species.
Slika 1: Relativni odbojni spektri skorje različnih grmovnih vrst.

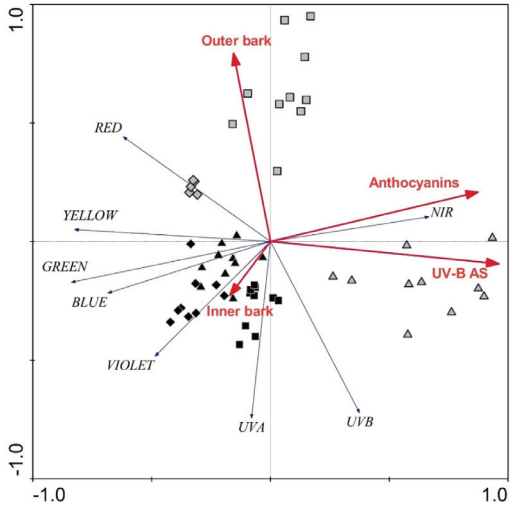

Figure 2: The strength of the associations between the bark properties, in terms of inner and outer bark thicknesses, anthocyanins, UV-B–absorbing substances (UV-B–AS), and different regions of the reflectance spectra. Grey diamonds, *Corylus avellana*; black squares, *Rosa canina*; black diamonds, *Ligustrum vulgare*; black triangles, *Sambucus nigra*; grey triangles, *Cornus sericea* var. *flaviramea*; grey squares, *Viburnum lantana*. The plot was generated using redundancy analysis.

Slika 2: Moč povezav med lastnostmi skorje, in sicer med debelino notranje in zunanje skorje, antocianini, UV-B–absorbirajočimi snovmi (UV-B–AS) ter različnimi območji odbojnih spektrov. Sivi diamanti, *Corylus avellana*; črni kvadrati, *Rosa canina*; črni diamanti, *Ligustrum vulgare*; črni trikotniki, *Sambucus nigra*; sivi trikotniki, *Cornus sericea* var. *flaviramea*; sivi kvadrati, *Viburnum lantana*. Graf je bil narejen v okviru redundančne analize.

RDA revealed negative relationships between the biochemistry and the reflectance in the visible region of the spectrum (Fig. 2). Here, UV-B–absorbing substances alone explained 36% of the variability of the bark reflectance spectra, anthocyanins alone explained 31%, while periderm and chlorophyll *b* alone explained 15% each. When considered together, UV-B–absorbing substances explained 38% of the variability ($p = 0.001$), and the outer bark thickness, inner bark thickness, and anthocyanin content explained an additional 12% ($p = 0.001$), 7% ($p = 0.001$), and 4% ($p = 0.009$) of the variability, respectively. With the measured parameters, as much as 61% of the reflectance spectra variability was explained.

In the explanation of the different parts of the reflectance spectra redundancy analyses, outer bark thickness was seen to have a crucial role in explaining the reflectance in the UV, violet, and blue regions. Furthermore, anthocyanins were important to explaining the reflectance in the green and yellow regions, while the UV-B–absorbing substances explained most of the reflectance in the red and NIR regions (Tab. 2).

Discussion

In general, high variability was seen across the species for the morphological and biochemical traits, with less variability for the physiological traits. One of the important traits was the amount of photosynthetic pigments, which are responsible for bark photosynthesis (Pfanz 1999). The stems of woody plants usually contain much lower amounts of photosynthetic pigments than the leaves. However, in young stems with a thin layer of cork, the pigment content can be similar to that in leaves (Kocurek and Pilarski 2012). This was also the case in the present study, as we measured

Table 2: Explained variance of the different reflectance spectra regions for the woody species according to the morphological and biochemical parameters, as obtained by redundancy analysis.
Tabela 2: Pojasnjena varianca različnih spektralnih območij odbojnih spektrov za lesnate vrste glede na morfološke in biokemijske parametre, ugotovljena s pomočjo redundančne analize.

Spectral region	Bark trait	Explained variance (%)	p
UV	Outer bark thickness	35	0.001
	Inner bark	9	0.002
	UV-A–AS	4	0.040
	Anthocyanins	4	0.031
Violet, blue	Outer bark thickness	39	0.001
	UV-A–AS	6	0.030
Green, yellow	Anthocyanins	48	0.001
	Inner bark thickness	13	0.001
	Carotenoids	8	0.002
	Outer bark thickness	5	0.010
	Chlorophyll *b*	4	0.032
	Chlorophyll *a*	3	0.020
Red, NIR	UV-B–AS	40	0.002
	Outer bark thickness	7	0.010
	Inner bark thickness	7	0.012

Abbreviations: p, p-value; UV-B–AS, UV-B–absorbing substances; UV-A–AS, UV-A–absorbing substances.

comparable amounts of photosynthetic and other pigments per area, as seen for the leaves of different herbaceous species (Grašič et al. 2019a, b, c). The bark usually contains 90% of the total photosynthetic pigments, while the remaining 10% is located in the deeper layers of the woody stem (Tokarz and Pilarski 2005). The main reason for this distribution is that the formation of bark reduces the intensity of radiation that reaches the chlorophyll layer and affects the content of photosynthetic pigments in the inner tissues (Pilarski et al. 2008). In the species studied here, there were differences across the levels of chlorophyll *a*, and even more pronounced differences for chlorophyll *b*, which resulted in variable chlorophyll *a*/*b* ratios that ranged from 2.00 in *C. avellana* to 0.65 in *C. sericea* var. *flaviramea*. According to these ratios, we cannot generally speak about a shady character of the bark inner environment. Shady character can only be confirmed for two species in this study, namely *C. sericea* var. *flaviramea* and *V. lantana*, with chlorophyll *a*/*b* ratios below 1. Such ratios reveal higher contents of the accessory pigment chlorophyll *b* (in comparison to the main pigment chlorophyll *a*), which is needed to increase light-use efficiency in shady environments (Larcher 2003).

The bark of *C. sericea* var. *flaviramea* was the thinnest, but it had the highest total chlorophylls, UV-absorbing substances, and anthocyanins, as was also evident from the noticeably red colour of the bark. In *V. lantana*, anthocyanin content was almost the same as in *C. sericea* var. *flaviramea*, even though its bark colour was completely different, as also revealed from the reflectance spectra. The colour of anthocyanins varies according to the vacuolar pH, and therefore it cannot be implicitly assumed that non-red tissues lack anthocyanins (Gould et al. 2000). Anthocyanins have multiple functions in plants. They can protect shade-adapted chloroplasts from exposure to high light intensity (Gould et al. 2000), and act as chemical repellents and visual signals. Therefore, they are also important in plant and animal interactions (Carrillo-Parra et al. 2012). Some anthocyanins also have significant antiviral, antibacterial, and fungicidal activities (Lev-Yadun and Gould 2008), which provides an advantage for plants with high anthocyanin levels. Their levels can also increase due to low temperatures, which was the case for a container-grown red maple in Georgia (Sibley et al. 1999). The anthocyanin production is related to the redox potential of the plastoquinone pool in the photosynthetic electron transport chain that regulates the anthocyanin biosynthesis genes (Kumar Das et al. 2011). In the study with green and red stems of *C. stolonifera*, Gould et al. (2010) showed high attenuation of PAR by anthocyanins, especially of green and yellow light, and pronounced differences in photoprotective potential and anthocyanin concentrations in the red stems compared to the green stems. *C. sericea* var. *flaviramea* showed the highest reflectance in the UV and NIR regions, and the lowest reflectance in the visible regions. The reflectance peak of other species was wide, and ranged from green to red, except for *C. avellana*, which had a peak in the green region. The reflectance of *V. lantana* gradually increased along the spectra, with a slight depression in the red edge. These reflectances were related to bark morphology and biochemistry, as shown by RDA. Anthocyanins act as effective light screening substances (Kumar Das et al. 2011), as also seen in the present study. Negative relationship was seen between the anthocyanins and the reflected light especially in the green, yellow, and blue regions. Their importance was also shown by RDA performed with specific regions of the spectrum, where anthocyanins explained 48% of the green and yellow reflectance spectra variability. In addition to anthocyanins, stems can contain high levels of other products of the phenylpropanoid pathway. The accumulation of UV-absorbing substances represents a primary mechanism by which plants can acclimatise to changing UV environments, including altered UV-B (Bornman et al. 2015). These phenolic substances strongly absorb in the UV region, while they reflect light of longer wavelengths. However, when a variety of vegetable crop plants were examined for tolerance to UV, it was shown that the levels of chlorophyll and UV-absorbing substances did not correlate directly with their sensitivity (Smith et al. 2000). The reflectance in the NIR region was explained by the UV-absorbing substances. The study of Kokaly and Skidmore (2015) showed that reflectance of different phenolic compounds in plants increases with increasing wavelength and reaches its peak at around 1600 nm. We obtained positive relationship between the amount

of UV-absorbing substances and the reflectance spectrum in the NIR region.

The presence of photosynthesis in green stems and in the bark of woody stems has been reported for different tree species (Pfanz 1999). Green stems without a well-developed periderm and with abundant, functional stomata can carry out photosynthesis (Nilsen 1995). The lack of stomata on woody stems results in the accumulation of respiratory CO_2, which supports CO_2-rich and O_2-poor environments (Mancuso and Marras 2003). CO_2 might also diffuse into the chlorenchyma through the lenticels (Eyles et al. 2009). The highest photosynthetic activity takes place in the outer bark, which contains higher amounts of photosynthetic pigments compared to the inner bark, which has less chlorophyll and carotenoids, and mainly serves for storage of assimilates that are produced in the outer bark or in the leaves (Pilarski 1999). The photochemical efficiency of PS II in the bark has been reported to be much lower than in the leaves (Manetas and Yiotis 2009). The Fv/Fm measured in the present study ranged from relatively low in *C. avellana* (0.36) to relatively high in *S. nigra* (0.71). These efficiencies reveal the presence of strong and mild permanent stress, respectively. The mean for the photosynthetic apparatus for undisturbed functioning is close to 0.83 (Björkman and Demmig 1987). Fq'/Fm' was low and did not differ across these species (means from 0.11 to 0.15), which showed strong transient stress that was possibly the consequence of the low temperatures during the sampling period. Solhaug and Haugen (1998) reported that the lowest photochemical efficiency of PS II in bark was detected during the winter period, due to the low temperatures. The chlorophyll fluorescence might also reflect the wood structural changes, such as changes in the bark chlorenchyma (Johnstone et al. 2014). High Fv/Fm along with efficient uptake of internal CO_2 might support the maintenance of a favourable carbon balance in the plant, especially during winter (Ivanov et al. 2006).

Conclusions

The parameters that affected bark reflectance of 1-year-old twigs varied across the studied species. The present results revealed high importance of twig biochemistry for light reflectance. However, these relations may not be directly transferable to the plant level, since they depend on twig age and also on environmental conditions that may significantly alter plant traits. Therefore, additional research is needed to study this relation in twigs of different age, and in different times of the season, which is especially important in the case of deciduous species. Nevertheless, bark of the species examined here shows different spectral signatures, which enables differentiation across species out of the vegetation season.

Summary

Bark comprises tissues outside the vascular cambium, and consists of the inner bark, which contains secondary phloem, and the outer bark or periderm. Bark protects the stem from physical disturbances, environmental factors and pollutants, and enables transport of assimilates and water. The physiological and biochemical traits of bark vary greatly between species. This study examined factors that affect bark reflectance of 1-year-old twigs of different shrub species: *Corylus avellana*, *Rosa canina*, *Ligustrum vulgare*, *Sambucus nigra*, *Cornus sericea* var. *flaviramea*, and *Viburnum lantana*. We hypothesised that bark biochemical properties and bark thickness play an important role in shaping bark reflectance and thus also the so-called "bark spectral signatures". We analysed contents of various pigments and UV-absorbing substances in the bark and measured bark thickness and bark reflectance spectra. Bark morphological and biochemical properties showed great variability between the species. The bark of *C. sericea* var. *flaviramea* differed the most from the other species, showing the highest amounts of total chlorophyll, UV-absorbing substances, and anthocyanins. The peridermal layer was developed differently in the studied species. *V. lantana* had the thickest outer bark, while the inner bark was thickest for *S. nigra*. *R. canina* and *C. sericea*

var. *flaviramea* had an epidermis and a very thin peridermal layer. Photosynthetic pigments in the bark allow the process of photosynthesis, which mostly takes place in the outer bark. Outer bark contains higher amounts of pigments compared to the inner bark, which serves primarily as an assimilate storage. Fv/Fm was relatively high in most of the studied species, while Fq'/Fm' was relatively low and did not differ between the species. Redundancy analysis revealed associations between bark biochemical properties and reflectance in the visible part of the spectrum. UV-B–absorbing substances explained 38% of the variability of the reflectance spectra ($p = 0.001$), outer bark thickness explained 12% ($p = 0.001$), inner bark thickness 7% ($p = 0.001$), and anthocyanins explained an additional 4% ($p = 0.009$) of the variability. Therefore, these parameters together explained as much as 61% of the variability of the reflectance spectra. Bark of the studied species showed different spectral signatures resulting from different bark physiological and biochemical properties. This allows us to differentiate between species out of the vegetation season.

Povzetek

Skorja zajema tkiva zunaj vaskularnega kambija in je sestavljena iz notranje skorje, ki vsebuje sekundarni floem, ter zunanje skorje ali periderma. Skorja ščiti steblo pred fizičnimi motnjami, okoljskimi dejavniki in onesnaževali ter omogoča transport asimilatov in vode. Fiziološke in biokemijske lastnosti skorje se med vrstami močno razlikujejo. V raziskavi smo proučili dejavnike, ki vplivajo na odbojnost skorje enoletnih vejic različnih grmovnih vrst: *Corylus avellana*, *Rosa canina*, *Ligustrum vulgare*, *Sambucus nigra*, *Cornus sericea* var. *flaviramea* in *Viburnum lantana*. Predvidevali smo, da imajo biokemijske lastnosti in debelina skorje pomembno vlogo pri oblikovanju odbojnosti oziroma tako imenovanih "spektralnih podpisov" skorje. Analizirali smo vsebnost različnih pigmentov in UV-absorbirajočih snovi v skorji ter izmerili debelino skorje in njene odbojne spektre. Morfološke in biokemijske lastnosti skorje so pokazale veliko variabilnost med vrstami. Najbolj se je od ostalih vrst razlikovala skorja vrste *C. sericea* var. *flaviramea*, ki je vsebovala največ skupnih klorofilov, UV-absorbirajočih snovi in antocianinov. Proučevane vrste so imele različno razvito peridermalno plast. Najdebelejšo zunanjo skorjo je imela vrsta *V. lantana*, medtem ko je bila notranja skorja najdebelejša pri vrsti *S. nigra*. Vrsti *R. canina* in *C. sericea* var. *flaviramea* sta imeli prisotno povrhnjico in zelo tanko plast periderma. Fotosintezni pigmenti v skorji omogočajo proces fotosinteze, ki večinoma poteka v zunanji skorji. Ta vsebuje več pigmentov v primerjavi z notranjo skorjo, ki je namenjena predvsem shranjevanju asimilatov. Potencialna fotokemična učinkovitost FS II je bila pri večini proučevanih vrst razmeroma visoka, dejanska fotokemična učinkovitost FS II pa relativno nizka in se ni razlikovala med vrstami. Redundančna analiza je razkrila povezave med biokemijskimi lastnostmi in odbojnostjo skorje v vidnem delu spektra. UV-B–absorbirajoče snovi so razložile 38 % variabilnosti odbojnih spektrov ($p = 0{,}001$), debelina zunanje skorje 12 % ($p = 0{,}001$), debelina notranje skorje 7 % ($p = 0{,}001$), vsebnost antocianinov pa še dodatne 4 % ($p = 0{,}009$) variabilnosti. Omenjeni parametri so skupaj torej pojasnili kar 61 % variabilnosti odbojnih spektrov. Skorja proučevanih vrst je pokazala različne spektralne podpise, ki so posledica različnih fizioloških in biokemijskih lastnosti skorje. To nam omogoča razlikovanje med vrstami izven vegetacijske sezone.

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