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Corylus avellana bark optical properties differ during and out of the vegetation season

Optične lastnosti skorje navadne leske se razlikujejo med vegetacijsko sezono in izven nje

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Abstract: We compared different bark traits of the common hazel (*Corylus avellana* L.) in four different months during the vegetation season (September, November) and out of the vegetation season (January, February) to get an insight into the changes in bark morphological, biochemical, and optical properties. Since bark of woody plants contains a layer of chlorenchyma, which can harvest transmitted light and perform photosynthesis, we also measured bark potential photochemical efficiency of photosystem II. The values of the latter parameter decreased during the research period, ranging from 0.77 in September to 0.22 in February. This could be attributed to low temperatures. The shapes of the bark reflectance curves were similar between the four samplings, with a peak in red and pronounced reflectance in the near-infrared spectrum. However, the level of reflectance differed between the four samplings. Regarding the time of the season, we obtained the most pronounced changes in the green, yellow, and red reflectance spectra. Light reflectance in these regions was positively related with chlorophyll *b* and carotenoid contents, while it was negatively related with anthocyanins and UV-B–absorbing substances. Transmittance spectra showed less variability between the four samplings. Regarding the studied pigments, the most pronounced changes were obtained for anthocyanin and UV-B–absorbing substances contents, which decreased in accordance with decreasing environmental temperatures. On the contrary, the level of photosynthetic pigments remained high, thus enabling undisturbed primary metabolism.

Keywords: bark, *Corylus avellana*, optical properties, pigments, potential photochemical efficiency of photosystem II (Fv/Fm)

Izvleček: Primerjali smo lastnosti skorje navadne leske (*Corylus avellana* L.) v štirih različnih mesecih med vegetacijskim obdobjem in izven vegetacijskega obdobja. Predvidevali smo, da se bodo biokemijske in posledično optične lastnosti skorje po odpadanju listov spremenile. Ker skorja lesnatih rastlin vsebuje tudi plast klorenhima, ki prestreza presevano svetlobo in vrši fotosintezo, smo na skorji merili tudi potencialno fotokemično učinkovitost fotosistema II. Izmerjene vrednosti slednjega parametra so se tekom raziskave zniževale, in sicer od 0,77 pri septembrskih vzorcih do 0,22 pri februarskih vzorcih, kar bi lahko pripisali nizkim temperaturam. Oblika krivulj odbojnosti sevanja skorje je bila med štirimi vzorčenji zelo podobna, z viškom v rdečem območju in veliko odbojnostjo v bližnjem infrardečem območju, medtem ko je bila raven odbojnosti med temi vzorčenji različna. Največje razlike v odbojnosti sevanja med vegetacijskim obdobjem in izven vegetacijskega obdobja smo zaznali v zelenem, rumenem in rdečem spektralnem območju. Odbojnost svetlobe v teh območjih je bila pozitivno povezana z vsebnostjo klorofila *b* in karotenoidov, negativno pa z antocianini in UV-B–absorbirajočimi snovmi. Presevni spektri so pokazali manjšo variabilnost. Primerjava različnih pigmentov je pokazala najbolj izrazite spremembe v vsebnosti antocianinov in UV-B–absorbirajočih snovi, ki so se manjšale skladno z zniževanjem okoljskih temperatur. Raven fotosinteznih pigmentov je skozi celotno obdobje ostala na visoki ravni in s tem omogočila nemoten primarni metabolizem.

Ključne besede: barvila, *Corylus avellana*, optične lastnosti, potencialna fotokemična učinkovitost fotosistema II (Fv/Fm), skorja

Introduction

Bark is a plant tissue outside the cambium layer, which can be found in stems, branches, and roots of woody plants, and has an important physiological and protective function (Martin and Crist 1970). It consists of the inner bark with secondary phloem and the outer bark or periderm, which is produced by the cork cambium (Romero 2014). Under the outer peridermal layers (rhytidomal), bark contains a layer of chlorenchyma that can harvest transmitted light and perform photosynthesis (Filippou et al. 2007, Wittmann and Pfanz 2008, 2014). The study of 24 different species revealed lower chlorophyll contents in twigs in comparison to leaves, but a higher chlorophyll/carotenoid ratio, indicating improved light harvesting (Levizou and Manetas 2007). Ultrastructural studies of bark and stem chloroplasts revealed that their features are typical for shade plants (Leong and Anderson 1984). However, in the case of *Eucalyptus nitens* bark exposed to high light conditions, the level of photosynthetic pigments was comparable to that of sun leaves (Tausz et al. 2005). Besides chlorophyll content, the level of bark photosynthesis also depends on other bark traits that shape bark optical properties. Light that reaches the plant tissue is a result of bark optical properties that comprise light reflectance, absorptance, and transmittance (Pilarski et al. 2008, Klančnik et al. 2015). It was shown that bark reflects light in the wavelength range between 700 and 2,000 nm that presents an optical window, in which light is reflected and transmitted in green plants (Henrion and Tributsch 2009). Transmittance of visible light through the periderm is low and depends on species and age of branches or stems (Aschan et al. 2001). Periderm absorbs a significant share of visible light, thus transmitting only 10–50% of ambient light (Manetas and Pfanz 2005). In the case of *Fagus sylvatica*, absorption of the bark decreased with increasing wavelength from about 92% at 400 nm to about 15% at 700 nm (Pilarski et al. 2008). Optical properties of a certain plant tissue are strongly affected by its overall biochemical properties (Klančnik et al., 2014a,b, 2016), primarily by the level of photosynthetic pigments. The level of photosynthetic pigments is changing due to varying environmental conditions and throughout the season (Larcher 2003). For example, in *Populus tremuloides* chlorophylls and carotenoids in bark showed the highest levels in late summer and the lowest levels in winter (Barr and Potter 1974). The variability in bark optical and biochemical properties also affects bark photosynthesis, which is enabled by CO_2 from the respiring living cells of xylem parenchyma, cambium, and phloem (Aschan and Pfanz 2003). Bark photosynthesis is especially important in deciduous trees out of the vegetation season, when plants are leafless. However, in the evergreen shrub *Myrica cerifera*, it was reported that bark photosynthesis increased carbon- and water-use efficiency, which contributed to the expansion of this species in the coastal environments (Vick and Young 2009). The level of photosynthesis in four-year-old stems of *Pinus monticola* and in young stems of *Betula pendula* was about 1 mmol m^{-2} s^{-1} (Cernusak and Marshall

2000, Wittmann et al. 2006), which is rather low in comparison to the level of photosynthesis, typical of leaves (Larcher 2003). Light-use efficiency of chlorenchyma may be estimated by measuring potential photochemical efficiency (Fv/Fm), which can also be used for monitoring changes in the physiological condition of bark in various trees (Alekseev et al. 2007). In *Pinus sylvestris* needles, Ivanov et al. (2006) detected a 65% reduction in Fv/Fm in winter, while in bark chlorenchyma, Fv/Fm remained relatively high.

In this study, we aimed to examine changes in the functional traits of bark in one-year-old twigs of the deciduous shrub *Corylus avellana* during and out of the vegetation season. We hypothesised that bark biochemical properties and consequently optical properties will change after the leaves will fall off. In addition, we also expected changes in Fv/Fm due to unfavourable temperatures in winter.

Materials and methods

Species and site description

The common hazel (*Corylus avellana* L.) is a 3–8 m high deciduous shrub, assigned to the vascular plant family Corylaceae, that thrives in open spaces at forest edges but is also used for hedgerows. It is native to Europe and western Asia. Monoecious flowers occur on bare branches in late winter to early spring before leaf development (Martinčič et al. 2007).

Plant samples were collected in open spaces in the area of Otočec (194 m a.s.l.; 45.8339 °N, 15.2202 °E). At each of the four samplings, one-year-old twigs were collected from ten randomly selected 1.5–2 m high plants. The twigs were processed on the day of sampling. The sampling and the analysis took place in September and November 2019, before the leaves fell off, and afterwards in January and February 2020, before the development of new leaves. Climate records on minimum daily air temperatures in the period from 1 September 2019 to 17 February 2020, obtained from the nearest meteorological station Novo mesto, are shown in Fig. 1.

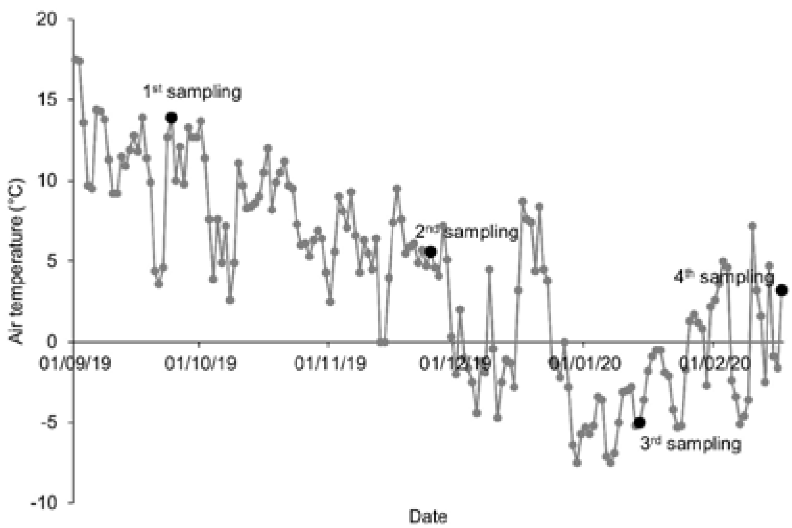

Figure 1: Dynamics of minimum daily air temperatures in the period 1 September 2019 – 17 February 2020 at the meteorological station Novo mesto, nearest to the sampling site.

Slika 1: Minimalne dnevne temperature zraka od 1. septembra 2019 do 17. februarja 2020, izmerjene na najbližji meteorološki postaji (Novo mesto).

Morphological properties

The analysis of bark thickness was carried out on transverse sections of vital one-year-old twigs. The thicknesses of secondary phloem and periderm were measured using a light microscope (CX41; Olympus, Tokyo, Japan) equipped with a digital camera (XC30; Olympus) and the CellSens software (Olympus).

Biochemical and physiological properties

Chlorophyll *a*, chlorophyll *b*, and carotenoid contents were determined for bark extracts according to the methodology described by Lichtenthaler and Buschmann (2001a,b). Acetone extracts' absorbances of ground bark samples were measured at three different wavelengths, namely 470 nm, 645 nm, and 662 nm, using a UV/VIS spectrometer (Lambda 25; Perkin-Elmer, Norwalk, CT, USA). Anthocyanin content was determined following the methodology of Drumm and Mohr (1978). Bark samples were ground, extracted in methanol:37% HCl = (99:1 (v/v)), centrifuged (4,000 rpm, 4 °C, 4 min) and then stored at 3–5 °C in the dark for 24 hours. Absorbances of the extracts were measured with a UV/VIS spectrometer at a wavelength of 530 nm. Total methanol-soluble UV-B–absorbing and UV-A–absorbing substances, which are a measure of total phenolics in ground bark samples, were extracted in a mixture of methanol, distilled water, and 37% HCl (79:20:1 (v/v)), according to the method described by Caldwell (1968). The samples were centrifuged (4,000 rpm, 10 °C, 10 min) and then scanned in the range from 280 nm to 319 nm for UV-B–absorbing substances, and in the range from 320 nm to 400 nm for UV-A–absorbing substances. The absorbance values were integrated for each UV region. All biochemical parameters are expressed per bark area. Fv/Fm was measured on fresh twigs as described by Schreiber et al. (1996), using a portable chlorophyll fluorometer (PAM-2100; Heinz Walz GmbH, Effeltrich, Bavaria, Germany). Before the measurement, twigs were kept in dark for 20 minutes.

Bark and leaf reflectance and transmittance

The optical properties of bark and leaves (when these were present) were determined in the laboratory on the day of sampling. Bark was carefully removed from the twigs with a razor blade. The measurements were performed in the range from 290 nm to 800 nm for reflectance and in the range from 290 nm to 800 nm for transmittance, using a portable spectrometer (Jaz Modular Optical Sensing Suite; Ocean Optics Inc., Dunedin, FL, USA; grating, #2; slit size, 25 μm) that was connected with an optical fibre (QP600-1-SR-BX; Ocean Optics Inc.) and an integrating sphere (ISP-30-6-R; Ocean Optics Inc.). The resolution of the measurements was ~1.3 nm. The reflectance spectrum was measured for the bark/leaf surface by illumination with a UV/VIS-near-infrared (NIR) light source (DH-2000; Ocean Optics, Inc.). We calibrated the spectrometer to 100% reflectance using a white reference panel with > 99% diffuse reflectance (Spectralon; Labsphere, North Sutton, NH, USA). In the case of the transmittance spectra, periderm surface was illuminated with a light source, while the integrating sphere that captured the transmitted light was positioned below the bark (or leaf). The incident angle of bark/leaf illumination was 90°. Prior to the measurement, we calibrated the spectrometer to 100% transmittance by light beam passing directly into the interior of the integrating sphere.

Statistical analysis

The significance of the differences between the four different samplings for each of the measured bark parameter was tested using one-way 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, and homogeneity of variance from the means was analysed using Levene's tests. The relationships between the physiological, biochemical, and morphological bark properties, and bark reflectance and transmittance were evaluated using Pearson's correlation analysis. IBM SPSS statistics 22.0 (IBM, Armonk, NY, United States) was used for these statistical analyses. The graphs were drawn in Microsoft Excel 2016 (Microsoft, Redmond, WA, USA). In addition, in the case of leaf reflectance and transmittance spectra, we performed Student's t-tests in Microsoft Excel 2016 to examine the differences between the first two samplings for each region of the leaf reflectance and leaf transmittance spectra separately, with significance accepted at $P \leq 0.05$.

Results

Bark and leaf optical properties

Bark reflectance spectra measurements revealed similar shapes, but different levels of light reflectance (Fig. 2). For all these measurements, we detected a peak in red and a pronounced increase in reflectance in NIR. We observed marked differences from violet to red between the autumn (September and November) and winter samples (January and February) (Tab. 1).

Bark transmittance spectra measurements revealed different shapes. The first peak was wider, ranging from green to red (Fig. 3). The autumn samples differed more than winter samples (Tab. 1). There was practically no transmittance at short wavelengths.

Leaf optical properties were measured in September, while the leaves were still green, and in November, when they turned yellow. This is clearly seen in the reflectance curves, which display lower values and a distinct peak in green in September, and much higher values with a very wide peak ranging from green to orange in November (Fig. 4). In September, the second peak only starts in NIR, while in November it already starts in red. The reflectance in short wavelengths did not differ significantly between the two samplings (Tab. 2).

In the case of leaf transmittance spectra, the differences between the two samplings were even more pronounced. When the leaves turned yellow, they became more transparent throughout the whole spectrum (Fig. 5). However, the differences in short wavelengths were not significant (Tab. 2). The shape of the leaf transmittance curves was very similar to those of the leaf reflectance spectra. The curve from September shows a distinct peak in green, while the one from November has a very wide peak, ranging from green to orange, as was the case in leaf reflectance spectra.

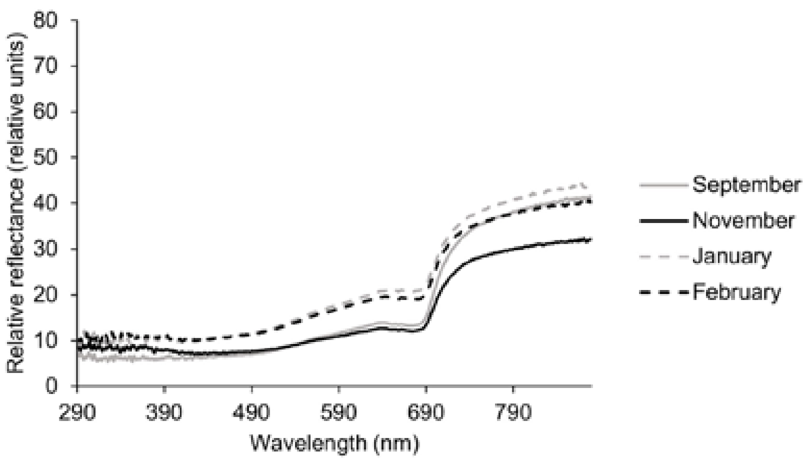

Figure 2: Mean relative bark reflectance (290 nm – 880 nm) for *Corylus avellana*, sampled in September, November, January, and February; the data were smoothed using moving averages with a period of five consecutive measurements (N = 10).

Slika 2: Povprečna relativna odbojnost skorje navadne leske med 290 in 880 nm ob vzorčenju v septembru, novembru, januarju in februarju; podatki so bili zglajeni z drsečimi povprečji iz vsakih sledečih petih zaporednih meritev (N = 10).

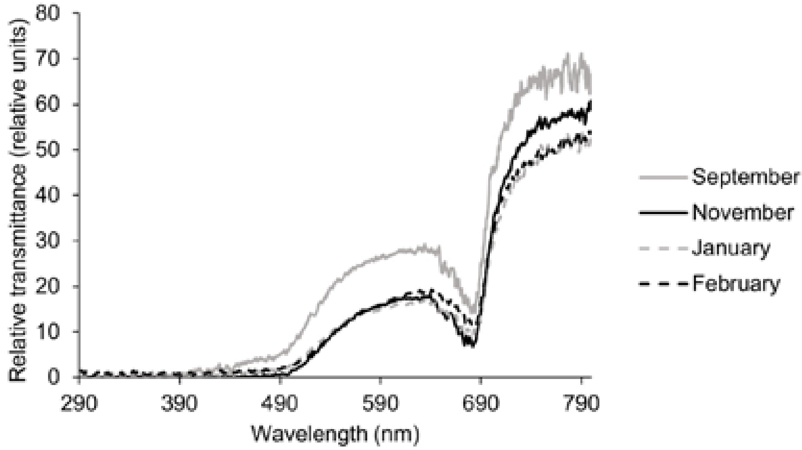

Figure 3: Mean relative bark transmittance (290 nm – 800 nm) for *Corylus avellana*, sampled in September, November, January, and February; the data were smoothed using moving averages with a period of five consecutive measurements (N = 10).

Slika 3: Povprečna relativna presevnost skorje navadne leske med 290 in 800 nm ob vzorčenju v septembru, novembru, januarju in februarju; podatki so bili zglajeni z drsečimi povprečji iz vsakih sledečih petih zaporednih meritev (N = 10).

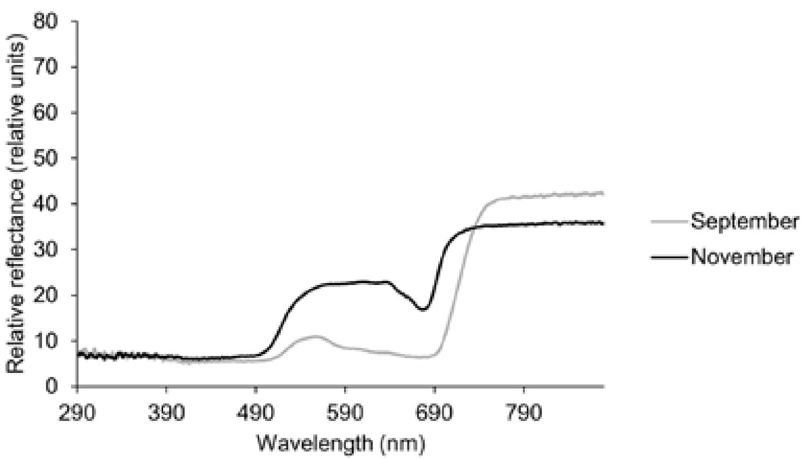

Figure 4: Mean relative leaf reflectance (290 nm – 880 nm) for *Corylus avellana*, sampled in September and November; the data were smoothed using moving averages with a period of five consecutive measurements (N = 10).

Slika 4: Povprečna relativna odbojnost listov navadne leske med 290 in 880 nm ob vzorčenju v septembru in novembru; podatki so bili zglajeni z drsečimi povprečji iz vsakih sledečih petih zaporednih meritev (N = 10).

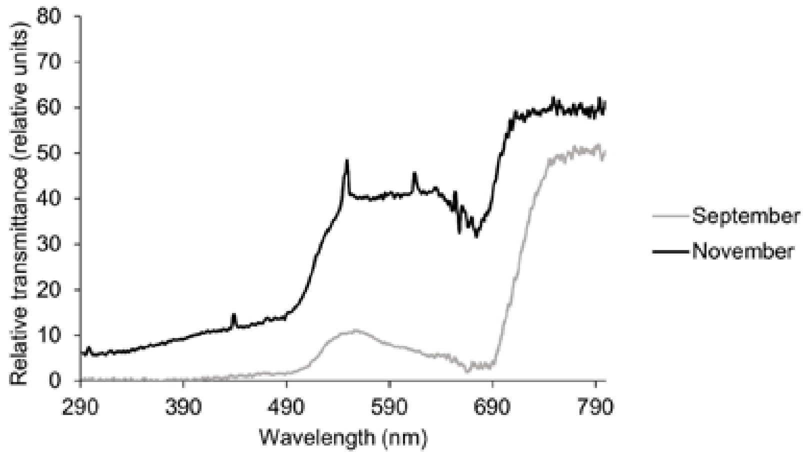

Figure 5: Mean relative leaf transmittance (290 nm – 800 nm) for *Corylus avellana*, sampled in September and November; the data were smoothed using moving averages with a period of five consecutive measurements (N = 10).

Slika 5: Povprečna relativna presevnost listov navadne leske med 290 in 800 nm ob vzorčenju v septembru in novembru; podatki so bili zglajeni z drsečimi povprečji iz vsakih sledečih petih zaporednih meritev (N = 10).

Besides optical properties, Tab. 1 also summarises physiological, biochemical, and morphological bark properties of *Corylus avellana* at different times of the year. There are some hints of changes in bark biochemical properties, however, most of them are not significant. There was an evident reduction in anthocyanins from September to February, and a decrease in photosynthetic pigments in November, while these showed an increase again towards spring. The level of UV-B–absorbing substances was somewhat higher in September and was later reduced. Small changes were also seen for bark morphological properties, which was expected. More pronounced changes were obtained when comparing Fv/Fm at different times of the year, which was decreasing along with decreasing temperatures. The values were optimal in September, while later on they revealed the presence of stress.

Table 1: Optical, physiological, biochemical, and morphological bark properties of *Corylus avellana*, sampled in September, November, January, and February.

Tabela 1: Optične, fiziološke, biokemijske in morfološke lastnosti skorje navadne leske ob vzorčenju v septembru, novembru, januarju in februarju.

Bark traits	Units	September	November	January	February
Optical: reflectance	au				
UV-B		6.33 ± 1.57^{a}	8.55 ± 1.96^{b}	10.54 ± 2.00^{c}	10.38 ± 1.97^{c}
UV-A		6.17 ± 1.18^{a}	8.21 ± 1.47^{b}	10.25 ± 1.74^{c}	10.80 ± 1.58^{c}
Violet		6.32 ± 1.43^{a}	7.25 ± 1.24^{a}	10.26 ± 2.08^{b}	10.20 ± 1.46^{b}
Blue		6.90 ± 1.91^{a}	7.53 ± 1.41^{a}	11.27 ± 2.53^{b}	11.15 ± 1.88^{b}
Green		9.22 ± 3.15^{a}	9.17 ± 2.18^{a}	14.65 ± 3.94^{b}	14.12 ± 2.74^{b}
Yellow		12.17 ± 3.65^{a}	11.37 ± 2.47^{a}	18.55 ± 4.22^{b}	17.62 ± 3.12^{b}
Red		14.24 ± 4.41^{a}	12.86 ± 3.18^{a}	21.31 ± 4.37^{b}	19.82 ± 4.13^{b}
NIR		36.74 ± 7.46^{b}	29.04 ± 7.06^{a}	39.80 ± 8.35^{b}	36.88 ± 6.96^{b}
Optical: transmittance	au				
UV-B		-0.13 ± 1.54^{a}	-0.18 ± 0.63^{a}	0.06 ± 0.68^{ab}	1.00 ± 2.29^{b}
UV-A		0.27 ± 1.20^{a}	-0.16 ± 0.50^{a}	0.08 ± 0.58^{a}	0.92 ± 2.31^{a}
Violet		1.83 ± 2.19^{b}	-0.02 ± 0.44^{a}	0.58 ± 0.94^{ab}	1.11 ± 2.47^{ab}
Blue		4.44 ± 3.81^{b}	0.48 ± 0.70^{a}	1.54 ± 1.63^{a}	1.75 ± 2.63^{a}
Green		18.30 ± 9.81^{b}	8.77 ± 6.04^{a}	9.46 ± 6.79^{a}	9.07 ± 5.87^{a}
Yellow		26.99 ± 7.48^{b}	16.50 ± 5.55^{a}	15.51 ± 7.06^{a}	17.03 ± 5.86^{a}
Red		24.83 ± 8.97^{b}	14.83 ± 7.99^{a}	14.94 ± 7.74^{a}	17.35 ± 7.46^{a}
NIR		62.71 ± 8.52^{b}	52.61 ± 12.11^{a}	46.48 ± 14.81^{a}	47.48 ± 10.31^{a}
Physiological					
Fv/Fm	au	0.768 ± 0.038^{d}	0.604 ± 0.077^{c}	0.316 ± 0.072^{b}	0.226 ± 0.050^{a}
Biochemical					
Chlorophyll *a*	mg cm^{-2}	0.012 ± 0.006^{b}	0.006 ± 0.002^{a}	0.012 ± 0.006^{b}	0.016 ± 0.003^{b}
Chlorophyll *b*	mg cm^{-2}	0.016 ± 0.005^{b}	0.009 ± 0.003^{a}	0.019 ± 0.009^{b}	0.020 ± 0.005^{b}
Carotenoids	mg cm^{-2}	0.004 ± 0.001^{ab}	0.002 ± 0.001^{a}	0.004 ± 0.001^{ab}	0.008 ± 0.009^{b}
Anthocyanins	au cm^{-2}	0.884 ± 0.378^{b}	0.672 ± 0.288^{ab}	0.649 ± 0.249^{ab}	0.480 ± 0.170^{a}
UV-B–AS	au cm^{-2}	6.076 ± 2.178^{b}	4.144 ± 1.108^{a}	4.587 ± 1.114^{a}	4.335 ± 0.588^{a}
UV-A–AS	au cm^{-2}	4.566 ± 1.590^{a}	3.495 ± 1.063^{a}	4.224 ± 1.516^{a}	4.131 ± 0.418^{a}
Morphological					
Periderm thickness	µm	68.56 ± 8.19^{ab}	63.14 ± 10.54^{ab}	72.28 ± 8.46^{b}	61.49 ± 7.99^{a}
Secondary phloem thickness	µm	103.85 ± 13.52^{b}	88.09 ± 11.95^{ab}	91.06 ± 12.53^{ab}	78.29 ± 17.40^{a}

Data are means ± SD (N = 10 for each column); different superscript letters within each row indicate significant differences ($P \leq 0.05$; Duncan tests); reflectance and transmittance spectra represent means within 5-nm intervals ($P \leq 0.05$, Duncan tests); au, arbitrary units; NIR, near-infrared; Fv/Fm, potential photochemical efficiency; UV-B–AS, UV-B–absorbing substances; UV-A–AS, UV-A–absorbing substances.

Table 2: Leaf reflectance and transmittance of *Corylus avellana* leaves, sampled in September and November.
Tabela 2: Odbojnost in presevnost listov navadne leske ob vzorčenju v septembru in novembru.

	Reflectance (au)		Transmittance (au)	
Colour region	**September**	**November**	**September**	**November**
UV-B	7.30 ± 1.62[a]	6.75 ± 1.53[b]	0.28 ± 0.66[a]	6.19 ± 17.16[a]
UV-A	6.60 ± 1.04[a]	6.75 ± 1.52[a]	0.24 ± 0.50[a]	7.90 ± 17.05[a]
Violet	5.45 ± 0.41[a]	6.24 ± 1.26[a]	0.80 ± 0.89[a]	11.36 ± 16.97[a]
Blue	5.64 ± 0.30[a]	6.76 ± 1.31[b]	1.61 ± 1.31[a]	13.80 ± 16.79[b]
Green	9.10 ± 2.09[a]	17.93 ± 6.67[b]	8.12 ± 5.47[a]	34.34 ± 18.42[b]
Yellow	8.28 ± 0.98[a]	22.75 ± 6.45[b]	7.43 ± 4.25[a]	41.34 ± 14.09[b]
Red	7.17 ± 0.93[a]	21.06 ± 8.06[b]	4.74 ± 3.53[a]	39.27 ± 15.35[b]
NIR	38.03 ± 8.10[a]	35.17 ± 9.01[a]	42.02 ± 13.59[a]	58.90 ± 10.48[b]

Data are means ± SD (N = 10 for each column); different superscript letters within each row indicate significant differences ($P \leq 0.05$; Student's t-tests); reflectance and transmittance spectra represent means within 5-nm intervals ($P \leq 0.05$; Student's t-tests); au, arbitrary units; NIR, near-infrared.

Pearson's correlation analysis between bark optical properties (reflectance and transmittance), and bark physiological, biochemical, and morphological properties revealed some strong relations (Tab. 3). The strongest negative correlation was obtained between bark reflectance in all regions of the spectrum except NIR, and Fv/Fm. Moderate to strong negative correlation was also obtained between bark reflectance and anthocyanins as well as UV-B–absorbing substances. As in the case of Fv/Fm, this relationship was significant across all regions of the spectrum, with the exception of the NIR region. There was also positive correlation between bark reflectance in green, yellow, and red, and carotenoids, and negative correlation between bark reflectance in short wavelengths (UV-B, UV-A, violet) and secondary phloem thickness.

We obtained less significant relations between bark transmittance spectra and bark physiological, biochemical, and morphological properties. All of these relations were positive.

Table 3: Pearson correlation coefficients between bark optical properties (reflectance and transmittance) and bark physiological, biochemical, and morphological properties for *Corylus avellana*; significant correlations are indicated in bold.

Tabela 3: Pearsonovi korelacijski koeficienti med optičnimi lastnostmi skorje (odbojnost in presevnost) ter fiziološkimi, biokemijskimi in morfološkimi lastnostmi skorje navadne leske; statistično značilne korelacije so označene s krepko pisavo.

Bark trait	Fv/Fm	Chl *a*	Chl *b*	Carotenoids	Anthocyanins	UV-B–AS	UV-A–AS	Periderm	Sec. phloem
Reflectance									
UV-B	**–0.80****	0.16	0.22	0.15	**–0.38***	**–0.41****	–0.19	0.14	**–0.34***
UV-A	**–0.81****	0.21	0.26	0.23	**–0.38***	**–0.43****	–0.21	0.07	**–0.40***
Violet	**–0.70****	0.26	0.29	0.28	**–0.34***	**–0.41****	–0.24	0.12	**–0.32***
Blue	**–0.65****	0.26	0.28	0.30	**–0.34***	**–0.41****	–0.26	0.12	–0.29
Green	**–0.59****	0.31	**0.33***	**0.32***	**–0.35***	**–0.39***	–0.25	0.11	–0.26
Yellow	**–0.57****	0.31	**0.34***	**0.35***	**–0.36***	**–0.36***	–0.23	0.15	–0.22
Red	**–0.56****	0.25	0.28	**0.34***	**–0.36***	**–0.35***	–0.24	0.20	–0.20
NIR	–0.23	0.34*	**0.34***	0.23	–0.07	–0.13	–0.10	0.24	0.13
Transmittance									
UV-B	–0.27	**0.34***	**0.39***	0.15	–0.14	0.05	0.05	–0.06	0.04
UV-A	–0.14	**0.34***	**0.38***	0.12	–0.07	0.13	0.07	–0.03	0.12
Violet	0.11	0.30	0.30	0.11	0.10	**0.36***	0.18	0.09	0.21
Blue	0.27	0.25	0.22	0.09	0.20	**0.39***	0.17	0.13	0.21
Green	**0.41****	0.16	0.14	0.07	0.29	0.28	0.10	0.08	0.13
Yellow	**0.37***	0.13	0.10	0.10	0.30	**0.33***	0.17	0.05	0.11
Red	0.25	0.18	0.13	0.15	0.30	**0.42****	0.27	0.10	0.11
NIR	**0.43****	0.07	0.06	0.05	**0.36***	0.27	0.14	0.03	0.19

*, $P \leq 0.05$; **, $P \leq 0.01$; NIR, near-infrared; Fv/Fm, potential photochemical efficiency; Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; UV-B–AS, UV-B–absorbing substances; UV-A–AS, UV-A–absorbing substances; Sec. phloem, secondary phloem.

Discussion

We compared different traits of *Corylus avellana* bark at different times of the year, during and out of the vegetation season. Bark of woody plants contains a layer of chlorenchyma that can harvest transmitted light and perform photosynthesis. During the vegetation season, the presence of leaves alters the environmental conditions affecting bark out of two reasons. One is changing of the quality and quantity of solar radiation reaching the bark due to filtering by leaves, which absorbs a significant share of radiation, as shown in the present study. The other reason is related with changes in temperature, which are more extreme out of the vegetation season (Středa et al. 2015). The period out of the vegetation season in temperate climates is related with low winter temperatures that may affect bark structure and function, as shown by the Fv/Fm measurements in our study. These were decreasing in accordance with decreasing minimum daily temperatures. Alekseev et al. (2007) also reported about the winter decrease of Fv/Fm activity and spring recovery, depending on climatic conditions of a particular year. In the case of bark of *Populus tremula*, Fv/Fm recovered only in late April, right before the start of the vegetation season (Solhaug and Haugen 1998).

Bark optical properties changed significantly during the studied period. Besides the time of the season, they also depend on twig age, which both determine their physical structure (Pilarski 1989, Kharouk et al. 1995, Pilarski et al. 2008) and their biochemical properties (Tokarz and Pilarski 2005), as also shown for different leaf types (Klančnik et al. 2014a, 2014b, 2016, Grašič et al. 2020). In the present study, bark spectral signatures (reflectance) revealed significant differences between the vegetation season and the period out of the vegetation season. The most pronounced differences were seen in the green, yellow, and red spectra. Light reflectance in these regions was positively related with chlorophyll *b* and carotenoid contents, while it was negatively related with anthocyanins and UV-B-absorbing substances. This is supported by previous studies, which showed that increased anthocyanin production in leaves enhanced the absorbance in the green and yellow regions (Neill and Gould 1999). Bark has optimised its reflection of incoming radiation in the range from 700 nm to 2,000 nm, which enables its temperature control (Henrion and Tributsch 2009). This was confirmed by high reflectance and transmittance in NIR in the present study with *C. avellana*. However, the relation between light transmittance and the measured parameters was less consistent. Thus, some additional parameters that were not included in the study might be involved. More pronounced changes were obtained in leaf optical properties in autumn. The leaf reflectance curves showed lower reflectance and a distinct green peak in September, and much higher reflectance and a very wide peak ranging from green to orange in November. The differences between the September and November samples were significant across the whole spectrum except for the UV-A, violet, and NIR regions. Leaves become much more transparent in November, especially in the range from 400 nm to 600 nm. These changes occur due to degradation of photosynthetic pigments and the persistence of other pigments, especially anthocyanins (Junker and Ensminger 2016).

Bark biochemistry changed only slightly during the studied period. The most pronounced changes were obtained for anthocyanins, which have multiple functions in bark. Besides affecting optical properties, anthocyanins exert beneficial effects on plant physiological processes, play a role in plant interactions with other organisms, for instance in repellence of herbivores and parasites, and provide the camouflage of plants against their background (Lev-Yadun and Gould 2008). The experiment with red maple revealed that higher anthocyanin levels in bark were induced by lower temperatures in winter (Sibley et al. 1999). This is contrary to our results, as we observed a decrease in anthocyanin levels with decreasing environmental temperatures (Fig. 1). This decrease was possibly due to lower photosynthetic activity, which was limited by low Fv/Fm under low temperatures. In white pine bark, the level of photosynthesis increased with increasing light intensity and bark temperature, maximum net photosynthesis being approximately 76% of dark respiration (Cernusak and Marshall 2000). We also observed a decrease in the production of UV-absorbing substances, which are various phenolic substances that present an efficient UV radiation filter (Rozema et al. 2002) and have a strong antioxidant and antibacterial effect, acting as radical scavengers and biocides (Pietarinen et al. 2006). Their production is an energetically demanding process (Germ et al. 2006). Therefore, the synthesis of these substances is the result of a trade-off between their production and investment of plants in primary metabolism. Conversely, the level of chlorophylls remained at a high level, which enabled undisturbed primary metabolism. These levels of chlorophylls corresponded to more than a half of the levels of these pigments in leaves of green plants (Grašič et al. 2019a, 2019b, 2020). In the study with aspen, bark was shown to contain 17–40% of the whole tree chlorophyll (Kharouk et al. 1995). The share of different photosynthetic pigments reflects the character of bark regarding light. A relatively low chlorophyll *a* to *b* ratio obtained in this study indicates a shady environment within the bark (Dale and Causton 1992). The contents of carotenoids in the bark of *C. avellana* were comparable with those in green leaves of some species (Grašič et al. 2019a, 2019b, 2020). In the study of Levizou et al. (2004), it was shown that carotenoid composition of the periderm in twigs does not fully reveal acclimation to shade. The physical environment within the twigs, especially hypoxia, seems to be more important than shade.

Summary

The common hazel (*Corylus avellana* L.) is a deciduous shrub thriving in open places at forest edges. One-year-old twigs from ten randomly selected plants in the area of Otočec (194 m a.s.l.; 45.8339 °N, 15.2202 °E) were sampled four times, in September and November 2019, and in January and February 2020. We compared different traits of *C. avellana* bark during and out of the vegetation season. We hypothesised that bark biochemical and consequently optical properties will change after the leaves will fall off, due to altered radiation environment. In addition, we also expected changes in potential photochemical efficiency of PS II (Fv/Fm) due to unfavourable temperature conditions in winter. Indeed, the Fv/Fm values were decreasing in accordance with decreasing minimum daily temperatures. Bark optical properties changed during the studied period. Spectral curves from different samplings revealed similar shapes, but different levels of light reflectance. For all the measurements, we detected a peak in red and pronounced reflectance in NIR. Regarding the time of the season, the most pronounced changes in reflectance were obtained in the green, yellow, and red spectra. Light reflectance in these regions was positively related with chlorophyll *b* and carotenoid contents, while it was negatively related with anthocyanins and UV–B-absorbing substances. Bark biochemistry showed only slight changes during the studied period. The most pronounced changes were obtained for anthocyanin contents, which have multiple functions in bark. We observed a decrease in anthocyanin contents with lowering environmental temperatures. The decrease in anthocyanin contents was possibly due to low photosynthetic activity on account of low Fv/Fm under low temperatures. In the period out the vegetation season, we also observed a decrease in the production of UV-absorbing substances, which are basically various phenolic substances. This decrease is possibly the result of a trade-off between the production of these substances and investment of plants in primary metabolism. On the contrary, the level of photosynthetic pigments remained at a high level, which enabled undisturbed primary metabolism. The levels of chlorophylls in the bark measured in this study corresponded to more than a half of the levels of these pigments in leaves of green plants. Relatively low chlorophyll *a* to *b* ratio obtained in this study indicates a shady environment within the bark. The contents of carotenoids in *C. avellana* bark were relatively high and comparable to the contents of these pigments in green leaves.

Povzetek

Navadna leska (*Corylus avellana* L.) je listopadni grm, ki uspeva na odprtih rastiščih ob robu gozda. Med septembrom in februarjem smo štirikrat vzorčili enoletne vejice desetih naključno izbranih rastlin na območju Otočca (194 m n. m.; 45,8339 °N, 15,2202 °E). Primerjali smo različne lastnosti skorje navadne leske med vegetacijskim obdobjem in izven vegetacijskega obdobja. Predvidevali smo, da se bodo po odpadanju listov zaradi spremenjenega sevalnega okolja spremenile biokemijske in posledično optične lastnosti skorje. Poleg tega smo pričakovali tudi spremembe v potencialni fotokemični učinkovitosti fotosistema II (Fv/Fm) zaradi neugodnih temperaturnih razmer pozimi. Vrednosti Fv/Fm so se zmanjševale z zniževanjem minimalne dnevne temperature. Optične lastnosti skorje so se v proučevanem obdobju spreminjale. Spektralne krivulje različnih vzorcev so imele podobne oblike, vendar različne stopnje odbojnosti svetlobe. Pri vseh meritvah smo zaznali vrh v rdečem območju in veliko odbojnost v bližnjem infrardečem območju. Primerjava vzorcev iz vegetacijskega obdobja in obdobja izven vegetacijske sezone je najbolj izrazite spremembe odbojnosti pokazala v zelenem, rumenem in rdečem delu spektra. Odbojnost svetlobe je bila v teh regijah pozitivno povezana z vsebnostjo klorofila *b* in karotenoidov, negativno pa z vsebnostjo antocianinov in UV–B-absorbirajočih snovi. Biokemijske lastnosti skorje so se v proučevanem obdobju le nekoliko spreminjale. Najbolj izrazite spremembe smo opazili v vsebnosti antocianinov, ki imajo v skorji več pomembnih funkcij. Zaznali smo skladno zmanjševanje vsebnosti antocianinov z zniževanjem okoljskih temperatur. To zmanjšanje je bilo verjetno posledica majhne fotosintezne aktivnosti, ki je bila omejena z majhno Fv/Fm pri nizkih temperaturah. V obdobju izven vegetacijske sezone smo opazili tudi zmanjšanje proizvodnje UV-absorbirajočih snovi, ki so v osnovi različne

fenolne snovi. To zmanjšanje je bilo verjetno posledica kompromisa med proizvodnjo teh snovi in vlaganjem asimilatov v primarni metabolizem. Nasprotno pa je raven fotosinteznih pigmentov ostala na visoki ravni, kar je omogočilo nemoten primarni metabolizem. Raven vsebnosti klorofilov v skorji je dosegla več kot polovico vsebnosti teh barvil v listih zelenih rastlin. Razmeroma nizko razmerje klorofil *a*/*b* kaže na senčno okolje znotraj skorje. Razmeroma velika vsebnost karotenoidov v skorji navadne leske je bila primerljiva z vsebnostjo teh barvil v zelenih listih.

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References

Alekseev, A. A., Matorin, D. N., Osipov, V. A., Venediktov, P. S., 2007. Investigation of the photosynthetic activity of bark phelloderm of arboreous plants using the fluorescent method. Moscow University Biological Sciences Bulletin, 62 (4), 164-170.

Aschan, G., Wittmann, C., Pfanz, H., 2001. Age-dependent bark photosynthesis of aspen twigs. Trees, 15, 431-437.

Aschan, G., Pfanz, H., 2003. Non-foliar photosynthesis − a strategy of additional carbon acquisition. Flora, 198 (2), 81-97.

Barr, M. L., Potter, L. D., 1974. Chlorophylls and carotenoids in aspen bark (*Populus tremuloides*). The Southwestern Naturalist, 19 (2), 147-154.

Caldwell, M. M., 1968. Solar ultraviolet radiation as an ecological factor for alpine plants. Ecological Monographs, 38 (3), 243-268.

Cernusak, L. A., Marshall, J. D., 2000. Photosynthetic refixation in branches of western white pine. Functional Ecology, 14 (3), 300-311.

Dale, M. P., Causton, D. R., 1992. Use of the chlorophyll *a*/*b* ratio as a bioassay for the light environment of a plant. Functional Ecology, 6 (2), 190-196.

Drumm, H., Mohr, H., 1978. The mode of interaction between blue (UV) light photoreceptor and phytochrome in anthocyanin formation of the *Sorghum* seedling. Photochemistry and Photobiology, 27 (2), 241-248.

Filippou, M., Fasseas, C., Karabourniotis, G., 2007. Photosynthetic characteristics of olive tree (*Olea europaea*) bark. Tree Physiology, 27 (7), 977-984.

Germ, M., Mazej, Z., Gaberščik, A., Trošt Sedej, T., 2006. The response of *Ceratophyllum demersum* L. and *Myriophyllum spicatum* L. to reduced, ambient, and enhanced ultraviolet-B radiation. Hydrobiologia, 170 (1), 47-51.

Grašič, M., Škoda, B., Golob, A., Vogel-Mikuš, K., Gaberščik, A., 2019a. Barley and spelt differ in leaf silicon content and other leaf traits. Biologia, 74, 929-939.

Grašič, M., Piberčnik, M., Zelnik, I., Abram, D., Gaberščik, A., 2019b. Invasive alien vines affect leaf traits of riparian woody vegetation. Water, 11, 2395.

Grašič, M., Sakovič, T., Abram, D., Vogel-Mikuš, K., Gaberščik, A., 2020. Do soil and leaf silicon content affect leaf functional traits in *Deschampsia caespitosa* from different habitats? Biologia Plantarum, 64, 234-243.

Henrion, W., Tributsch, H., 2009. Optical solar energy adaptations and radiative temperature control of green leaves and tree barks. Solar Energy Materials and Solar Cells, 93 (1), 98-107.

Ivanov, A. G., Krol, M., Sveshnikov, D., Malmberg, G., Gardeström, P., Hurry, V., Oquist, G., Huner, N. P., 2006. Characterization of the photosynthetic apparatus in cortical bark chlorenchyma of Scots pine. Planta, 223, 1165-1177.

Junker, L. V., Ensminger, I., 2016. Relationship between leaf optical properties, chlorophyll fluorescence and pigment changes in senescing *Acer saccharum* leaves. Tree Physiology, 36 (6), 694-711.

Kharouk, V. I., Middleton, E. M., Spencer, S. L., Rock, B. N., Williams, D. L. 1995. Aspen bark photosynthesis and its significance to remote sensing and carbon budget estimate in the boreal ecosystem. Water, Air, and Soil Pollution, 82, 483-497.

Klančnik, K., Pančić, M., Gaberščik, A., 2014a. Leaf optical properties in amphibious plant species are affected by multiple leaf traits. Hydrobiologia, 737, 121-130.

Klančnik, K., Vogel-Mikuš, K., Gaberščik, A., 2014b. Silicified structures affect leaf optical properties in grasses and sedge. Journal of Photochemistry and Photobiology B: Biology, 130, 1-10.

Klančnik, K., Zelnik, I., Gnezda, P., Gaberščik, A., 2015. Do reflectance spectra of different plant stands in wetland indicate species properties? In: Vymazal, J. (ed.): The Role of Natural and Constructed Wetlands in Nutrient Cycling and Retention on the Landscape. Springer, Cham, pp. 73-86.

Klančnik, K., Levpušček, M., Gaberščik, A., 2016. Variegation and red abaxial epidermis define the leaf optical properties of *Cyclamen purpurascens*. Flora, 224, 87-95.

Larcher, W., 2003. Physiological Plant Ecology: Ecophysiology and Stress Physiology of Functional Groups, 4th ed. Springer-Verlag, Berlin, 514 pp.

Leong, T.-Y., Anderson, J. M., 1984. Adaptation of the thylakoid membranes of pea chloroplasts to light intensities. I. Study on the distribution of chlorophyll–protein complexes. Photosynthesis Research, 5, 101-115.

Levizou, E., Petropoulou, Y., Manetas, Y., 2004. Carotenoid composition of peridermal twigs does not fully conform to a shade acclimation hypothesis. Photosynthetica, 42 (4), 591-596.

Levizou, E., Manetas, Y., 2007. Photosynthetic pigment contents in twigs of 24 woody species assessed by in-vivo reflectance spectroscopy indicate low chlorophyll levels but high carotenoid/ chlorophyll ratios. Environmental and Experimental Botany, 59 (3), 293-298.

Lev-Yadun, S., Gould, K., 2008. Role of anthocyanins in plant defence. In: Winefield, C., Davies, K., Gould, K. (eds.): Anthocyanins. Springer, New York, pp. 22-28.

Lichtenthaler, H. K., Buschmann, C., 2001a. Extraction of photosynthetic tissues: chlorophylls and carotenoids. Current Protocols in Food Analytical Chemistry, 1 (1), 165-170.

Lichtenthaler, H. K., Buschmann, C., 2001b. Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. Current Protocols in Food Analytical Chemistry, 1 (1), 171-178.

Manetas, Y., Pfanz, H., 2005. Spatial heterogeneity of light penetration through periderm and lenticels and concomitant patchy acclimation of corticular photosynthesis. Trees, 19, 409-414.

Martin, R. E., Crist, J. B., 1970. Elements of bark structure and terminology. Wood and Fiber Science, 3, 269-279.

Martinčič, A., Wraber, T., Jogan, N., Podobnik, A., Turk, B., Vreš, B., Ravnik, V., Frajman, B., Strgulc Krajšek, S., Trčak, B., Bačič, T., Fischer, M. A., Eler, K., Surina, B., 2007. Mala flora Slovenije. Ključ za določanje praprotnic in semenk, 4th ed. Tehniška založba Slovenije, Ljubljana, 967 pp.

Neill, S., Gould, K., 1999. Optical properties of leaves in relation to anthocyanin concentration and distribution. Canadian Journal of Botany, 77 (12), 1777-1782.

Pietarinen, S., Willför, S., Ahotupa, M., Hemming, J., Holmbom, B., 2006. Knotwood and bark extracts: strong antioxidants from waste materials. Journal of Wood Science, 2 (5), 436-444.

Pilarski, J., 1989. Optical properties of bark and leaves of *Syringa vulgaris* L.. Bulletin of the Polish Academy of Sciences: Biological sciences, 37, 253-269.

Pilarski, P., Tokarz, K., Kocurek, M., 2008. Optical properties of the cork of stems and trunks of beech (*Fagus sylvatica* L.). Polish Journal of Environmental Studies, 17 (5), 773-779.

Romero, C., 2014. Bark Structure and Functional Ecology. In: Cunningham, A. B., Campbell, B. M., Luckert, M. K. (eds.): Bark: Use, Management, and Commerce in Africa. Advances in Economic Botany, Vol. 17. New York Botanical Garden Press, New York, pp. 5-25.

Rozema, J., Björn, L. O., Bornman, J. F., Gaberščik, A., Hader, D. P., Trošt, T., Germ, M., Klisch, M., Gröniger, A., Sinha, R. P., Lebert, M., He, Y. Y., Buffoni-Hall, R., de Bakker, N. V. J., van de Staaij, J., Meijkamp, B. B., 2002. The role of UV-B radiation in aquatic and terrestrial ecosystems—an experimental and functional analysis of the evolution of UV-absorbing compounds. Journal of Photochemistry and Photobiology B: Biology, 66, 2-12.

Schreiber, U., Kühl, M., Klimant, I., Reising, H., 1996. Measurement of chlorophyll fluorescence within leaves using a modified PAM fluorometer with a fiber-optic microprobe. Photosynthesis Research, 47, 103-109.

Sibley, J. L., Ruter, J., Eakes, D. J., 1999. Bark anthocyanin levels differ with location in cultivars of red maple. HortScience, 34 (1), 137-139.

Solhaug, K., Haugen, J., 1998. Seasonal variation of photoinhibition of photosynthesis in bark from *Populus tremula* L.. Photosynthetica, 35, 411-417.

Středa, T., Litschmann, T., Středová, H., 2015. Relationship between tree bark surface temperature and selected meteorological elements. Contributions to Geophysics and Geodesy, 45 (4), 299-311.

Tausz, M., Warren, C. R., Adams, M. A., 2005. Is the bark of shining gum (*Eucalyptus nitens*) a sun or a shade leaf? Trees, 19, 415-421.

Tokarz, K., Pilarski, J., 2005. Optical properties and the content of photosynthetic pigments in the stems and leaves of the apple tree. Acta Physiologiae Plantarum, 27, 183-191.

Vick, J. K., Young, D. R., 2009. Corticular photosynthesis: a mechanism to enhance shrub expansion in coastal environments. Photosynthetica, 47 (1), 26-32.

Wittmann, C., Pfanz, H., 2008. General trait relationships in stems: a study on the performance and interrelationships of several functional and structural parameters involved in corticular photosynthesis. Physiologia Plantarum, 134 (4), 636-648.

Wittmann, C., Pfanz, H., Loreto, F., Centritto, M., Pietrini, F., Alessio, G., 2006. Stem CO_2 release under illumination: corticular photosynthesis, photorespiration or inhibition of mitochondrial respiration? Plant, Cell and Environment, 29 (6), 1149-1158.

Wittmann, C., Pfanz, H., 2014. Bark and woody tissue photosynthesis a means to avoid hypoxia or anoxia in developing stem tissues. Functional Plant Biology, 41 (9), 940-953.