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Anatomy, chlorophyll content and photosynthetic performance in current-year and previous-year Aleppo pine (*Pinus halepensis* Mill.) needles

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ABSTRACT:

Aleppo pine (*Pinus halepensis*) is a widespread Mediterranean woody species. Needles usually fall off the tree after the second year, which can be the reason for substantial crown defoliation under extreme environmental stress. The aim of the present investigation was to compare the anatomy, chlorophyll content and photosynthetic performance in current-year (CY) and previous-year (PY) Aleppo pine needles. Chlorophyll concentrations were determined spectrophotometrically, while photosynthetic performance was determined by measuring the increase in chlorophyll *a* fluorescence (the JIP test). The obtained results revealed that concentrations of chlorophylls *a* and *b* were almost twice as great in PY needles as in CY ones. The chlorophyll *a* to *b* ratio and values of the maximum quantum yield of photosystem II (Fv/Fm) showed no statistically significant difference between CY and PY needles. However, the performance index (PI_{ABS}) was significantly higher in CY needles compared to PY ones. Analysis of PI_{ABS} components revealed that CY needles had increased electron transport beyond the primary electron acceptor, Q_A^-, which was associated with a lower value of variable fluorescence at 2 ms (V_J) in comparison with PY needles. Also, some differences were observed in needle anatomy. Current-year needles had an increased proportion of mesophyll tissue, together with decreased proportions of vascular cylinder and resin duct areas. Also, many more starch grains were present in PY than in CY needles. Based on the presented results, it can be concluded that despite considerably lower chlorophyll content, CY needles exhibited photosynthetic performance better than that of older needles, which is an unusual phenomenon in woody species.

INTRODUCTION

Aleppo pine (*Pinus halepensis* Mill.) is a widespread Mediterranean woody species. Its natural and cultivated populations can be frequently found in the coastal area of the Adriatic Sea and Dalmatian islands, where it covers about 40000 ha of the land area. Although it was introduced in the Dalmatia region more than 1000 years ago, it is considered to be an autochthonous species that grows successfully in semi-arid and sub-humid areas with a Mediterranean climate, independently of the soil type (Prgin 2005). It is frequently grown as a horticultural species in urban and sub-urban areas, but is also used for afforestation of degraded karst areas, where it has a negative effect on existing Mediterranean shrubs (Bellot *et al.* 2004).

Usually, the needles of Aleppo pine fall off the tree after the second year. The significance of this lies in filling of grooves and holes in the landscape, which results in generation of humus and forest soil (JEDDI *et al.* 2009). However, substantial crown defoliation can occur under extreme environmental stress (CLAUSER *et al.* 1989), which contributes to a worsening of health conditions of the whole habitat. Numerous previous investigations of Aleppo pine revealed its sensitivity to different environmental factors alone or in combinations, –e.g., air pollutants such as ozone, SO_2 and NO_2 (VELISSARIOU *et al.* 1992; ANTTONEN *et al.* 1995; ELVIRA *et al.* 1998; KIVIMÄENPÄÄ *et al.* 2010); drought (GERANT *et al.* 1996; BORGHETTI *et al.* 1998; ALONSO *et al.* 2001; ATZMON *et al.* 2004; INCLÁN *et al.* 2005; ALEXOU 2013; TAIBI *et al.* 2018); temperature (FERNÁNDEZ *et al.* 2003; PUÉRTOLAS *et al.* 2005); surfactants (RICHARD *et al.* 1996); salt (ROCCO *et al.* 2013); cement dust (BAČIĆ *et al.* 1999); and other complex mixtures created by the release of various industrial pollutants (PASQUALINI *et al.* 2003; ROBLES *et al.* 2003).

The elongation and functional development of young conifer needles, mainly spruce [*Picea abies* (L.) H. Karst.], have been the topics of previous reports. Investigations of carbohydrate metabolism (HAMPP *et al.* 1994; EGGER *et al.* 1996) revealed that young spruce needles contain a large quantity of starch synthesised from sucrose, the level of which decreases during needle development. The increase in activity of enzymes that are crucial in starch metabolism starts as late as 50 to 70 days after the bud burst (EGGER & HAMMP 1996) and coincides with an increase in the levels of chlorophylls and carotenoids (LEPEDUŠ *et al.* 2003), as well as with development of the thylakoid system (SENSER *et al.* 1975). Apart from primary metabolism, the qualitative content and quantitative levels of secondary metabolites have also been investigated (STRACK *et al.* 1989; SLIMESTAD & HOSTETTMANN 1996; BILKOVA *et al.* 1999). These studies indicated the presence of more than 30 different phenolic compounds in young needles and further revealed that high levels of flavonols were present in June, while stilbenes and simple phenols prevailed in September. Besides performing a protective function, secondary metabolites are very important in the lignification process that takes place during needle elongation. The lignification process in young spruce needles is completed one month after the bud burst, which is associated with an increase in apoplast guaiacol peroxidase acidity and the appearance of new peroxidase isoforms (POLLE *et al.* 1994). GALLIANO *et al.* (1993) reported a decrease in the activity of cinnamyl alcohol dehydrogenase already in June, which indicates that there is co-regulation of enzymes involved in the production and polymerisation of monolignols during young needle development.

Since needles are the primary photosynthetic organs, the photosynthetic apparatus in them has also been the subject of previous investigation. Ultrastructural studies of spruce and pine needles (SENSER *et al.* 1975; SOIKELLI 1980) revealed seasonal variations in shape and organisation of chloroplasts during autumn and winter. Biochemical and physiological investigations showed that a functional photosystem II (PSII) is assembled already at a very early stage of needle development (LEPEDUŠ *et al.* 2005). However, a longer period of time (May to August) was needed for developing spruce needles to reach the level of chlorophyll, degree of expression of LHC II and Rubisco LSU proteins and positive values of net photosynthesis that can be attributed to mature needles (LEPEDUŠ *et al.* 2008). Such dynamics of biogenesis of the photosynthetic apparatus was closely associated with differentially regulated accumulation of preprotein translocation components of the plastids (FULGOSI *et al.* 2005). The present investigation represents a continuing manifestation of our broad scientific interest in the photosynthetic performance of conifer needles. Further, to the best of our knowledge, this is the first report showing better photosynthetic performance at the level of PSII electron transport in young developing Aleppo pine needles than in fully developed needles from the previous season.

The aim of the present investigation was to compare current-year (CY) and previous-year (PY) Aleppo pine needles with respect to their anatomy, chlorophyll content and photosynthetic performance, as well as to investigate differences in the regulatory mechanisms of photosynthesis. Based on preliminary investigations of ours carried out during the period from 2013 to 2015 (data not shown) that revealed discrepancies of chlorophyll content and photosynthetic performance, we hypothesised that, in spite of lower chlorophyll *a* and *b* content, CY needles might have more efficient photosynthetic electron transport in comparison with PY needles, as well as differentially regulated light absorption, photon trapping in the photosystem II (PSII) reaction center and processes of dissipation of excess excitation energy.

MATERIALS AND METHODS

Plant material. The material for this study was collected from about 40-year-old Aleppo pine (*Pinus halepensis* Mill.) trees growing in Primošten (Croatia) (43°36′25.12″ N, 15°55′13.71″ E) during June of 2016. Two distinct needle classes (current-year or CY needles and previous-year or PY needles) were collected separately from 10 healthy-looking trees. At the moment of sampling, CY needles were about 3 months old. Sampling was done in the morning (8:00 a.m).

Chlorophyll determination. Randomly selected current-year and previous-year needles from all trees were pooled together, cut into small pieces and ground into a fine powder using liquid nitrogen. Tissue powder was extracted using 100% acetone. Extraction was done in five replicates
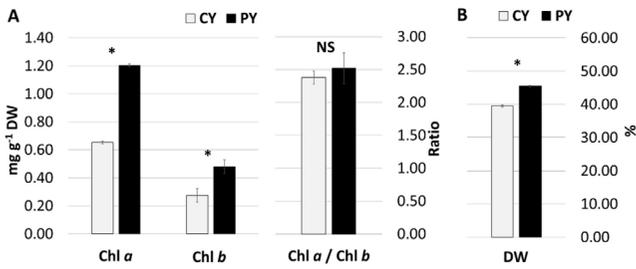
Fig. 1. Concentrations of chlorophylls *a* (Chl *a*) and *b* (Chl *b*) expressed as mg/g dry weight and the chlorophyll *a* to *b* ratio (Chl *a* / Chl *b*) (**A**); and percentage of dry weight - DW (**B**) in current-year (CY) and previous-year (PY) Aleppo pine (*Pinus halepensis* Mill.) needles. Significance at p (t) < 5% is marked by an asterisk (*). The columns represent mean values ± SD (n = 5).

for each needle class, respectively. The concentrations of chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) were determined spectrophotometrically using a Specord 40 instrument from Analytik (Jena, Germany) according to Lichtenthaler (1987). Pigment concentration was expressed as mg per g of dry weight (DW). Dry weight was determined after oven drying at 105°C for 24 hours and expressed as the percentage of fresh weight.

Measurement of chlorophyll *a* fluorescence. The kinetics of induction of chlorophyll *a* fluorescence was measured at ambient temperature using a Plant Efficiency Analyser (Handy-PEA, Hansatech, UK). Plant material was dark-adapted for 30 minutes before measurement. Measurements were performed *in situ* discretely on 10 attached needles from each class by exposing them to a pulse of saturating red light (3200 μmol m^{-2} s^{-1}, peak at 650 nm). The OJIP transients were recorded from 50 μs (F_0) to 1s (F_m). The obtained data were used to calculate the following JIP-test parameters: maximum quantum yield of photosystem II (Fv/Fm); the performance index (PI_{ABS}); density of reaction centres on chlorophyll basis (RC/ABS); the flux ratio of trapping per dissipation (TR_0/DI_0); electron transport beyond the primary electron acceptor, Q_A^- [$ET_0/(TR_0-ET_0)$]; and variable fluorescence at 2 ms (V_J) and 30 ms (V_I), respectively (Strasser *et al.* 2000).

Anatomical analyses. The material for light microscopic investigations was prepared as described previously (Lepeduš *et al.* 2001; Cesar *et al.* 2004). About 5-mm-long segments were cut from the middle of each needle and fixed for 24 hours at +4°C in 6% glutaraldehyde in 0.05 M phosphate buffer (pH = 6.8). The specimens were then dehydrated in 2-methoxyethanol, ethanol, n-propanol and n-butanol (two changes in each) and embedded in methacrylate resin (Historesin, Leica). Semi-thin (3 μm) sections were made using a rotary microtome (Leica RM2155) with glass knives and stained with 0.05% Toluidine Blue O in benzoate buffer (pH = 4.4). Lugol reagent was applied to demonstrate starch grains.

Statistical analysis. The obtained data were arranged in two groups according to the investigated needle classes and statistical evaluation was done by Student´s t-test using Statistica 8.0 software (StatSoft, Inc. 2007). Results are presented as means ± standard deviation of five replicates for pigment analysis, three replicates for dry weight, 80 replicates for fluorescence measurements and 90 replicates for anatomical measurements. The differences between compared samples (CY and PY needles) were considered significant at p < 0.05.

RESULTS AND DISCUSSION

Visual inspection of the investigated CY and PY needles revealed no symptoms of yellowing or any other visual damage. Current-year needles were much brighter than PY needles, which was in accordance with measured chlorophyll concentrations. Concentrations of chlorophylls *a* (Chl *a*) and *b* (Chl *b*) in CY and PY Aleppo pine needles are shown in Fig 1. Previous-year needles had about two times greater values of both Chl *a* and Chl *b* concentrations (1.203 and 0.653 mg/g DW, respectively) in comparison with CY needles (0.481 and 0.275 mg/g DW, respectively). Consequently, the chlorophyll *a* to *b* ratio (Chl *a* / Chl *b*) was not significantly different between CY and PY needles (Fig. 1). Elvira *et al.* (1998) reported seasonal fluctuation of total chlorophyll levels and the chlorophyll *a* to *b* ratio, with a marked reduction during the summer months in both previous- and current-year needles of Aleppo pine. The values for the summer months reported in their investigation were similar to the values obtained in our investigation (Fig. 1). Decreased levels of both chlorophyll *a* and chlorophyll *b* in current-year needles in relation to previous-year needles of Norway spruce [*Picea abies* (L.) H. Karst.] were also previously reported in mid-June (Lepeduš *et al.* 2008), with no difference in values of the chlorophyll *a* to *b* ratio between them. Thus, this seems to be a somewhat universal pattern of chlorophyll accumulation dynamics during the development of conifer needles. Such relations between the investigated CY and PY Allepo pine needles were in fact expected, since the CY needles were still elongating and accordingly did not exhibit the anatomical and physiological features evident in mature PY needles. Since needles of both categories were collected on the side of the tree that was directly sun-exposed and without any shading, accommodation to long-term irradiance gradients through the canopy can be excluded as the reason for such differences of chlorophyll content between young and still developing CY and mature PY needles. Likely, the observed decrease of pigment content in CY needles served as protective mechanism against possible photo damage that might occur due to high levels of irradiation in June. Such a conclusion agrees with our previous investigations of different woody species (Lepeduš *et al.* 2011; Mlinarić *et al.* 2017), where we observed similar relations between

A

B
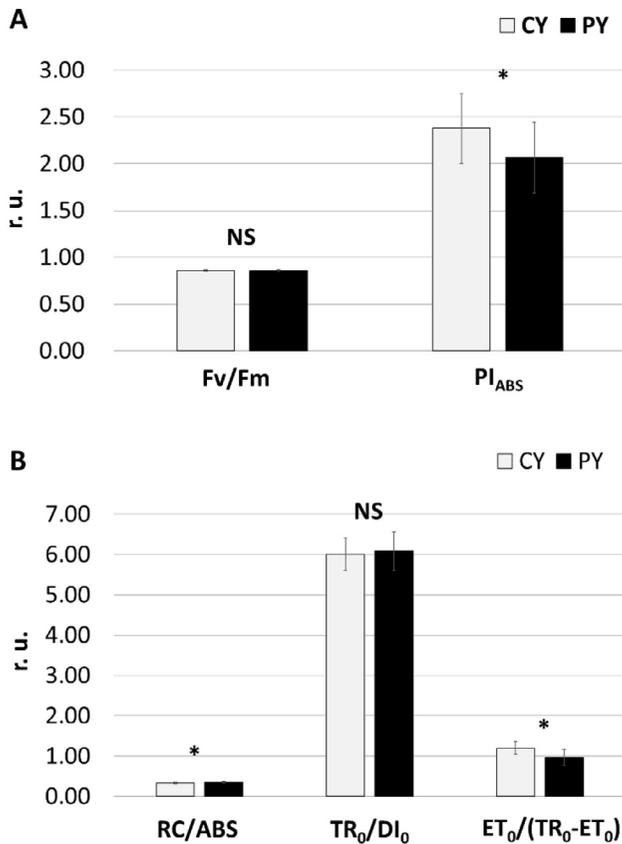
Fig. 2. Maximum quantum yield of photosystem II (Fv/Fm) and performance index (PI_{ABS}) (**A**) and density of reaction centres on chlorophyll basis (RC/ABS), the flux ratio of trapping per dissipation (TR_0/DI_0) and electron transport beyond the primary electron acceptor, Q_A^-, [$ET_0/(TR_0-ET_0)$] (**B**) in current-year (CY) and previous-year (PY) Aleppo pine (*Pinus halepensis* Mill.) needles (expressed in relative units; r.u.). Significance at p (t) < 5% is marked by asterisk (*). The columns represent mean values ± SD (n =80).

A

B

Fig. 3. The O-P normalised and native (small chart) chlorophyll *a* fluorescence kinetics (OJIP transients) (**A**) and variable fluorescence at 2 ms (V_J) and 30 ms (V_I) (**B**) in current-year (CY) and previous-year (PY) Aleppo pine (*Pinus halepensis* Mill.) needles (expressed in relative units; r.u.). Significance at p (t) < 5 % is marked by an asterisk (*). The columns represent mean values ± SD (n =80).

young developing and mature leaves with respect to chlorophyll accumulation. There we reported that lower chlorophyll content is correlated with accumulation of photosynthetically essential proteins, but also with activity of important antioxidative enzymes.

Values of the chlorophyll *a* to *b* ratio appear to be very important for maintaining the physiological function of needles by means of ensuring efficient photosynthesis, since its levels are closely linked with the accumulation of light-harvesting proteins of PSII (LHCII) (Mathis & Burkey 1989; Tanaka & Tanaka 2000; Sato *et al.* 2015) and in that way are directly involved in regulating the primary photochemistry of PSII. Values of the maximum quantum yield of PSII (Fv/Fm) were identical (0.86) in both CY and PY needles (Fig. 2A), which indicates that both needle classes had a fully developed and functional PSII (Schrebier *et al.* 1994). Since the primary photochemistry of PSII reflects the absorption and trapping of energy fluxes, it was clear that these processes function well already in young CY needles. However, values of the performance

index (PI_{ABS}) revealed a significant difference between CY (2.38 r. u.) and PY (2.06 r. u.) needles (Fig. 2A). The PI_{ABS} parameter is considered as a much better indicator of plant vitality assessed on the basis of photosynthesis since it takes into account the directing of excitation energy to photosynthetic electron transport and the dissipation of excess excitation energy as heat, in addition to absorption and trapping of excitation energy (Tsimilli-Michael *et al.* 2000; van Heerden *et al.* 2007). Analysis of PI_{ABS} components also revealed some differences between CY and PY needles (Fig. 2B). The density of reaction centres on chlorophyll basis (RC/ABS) was slightly higher in PY (0.35 r. u.) than in CY (0.33 r. u.) needles. The flux ratio of trapping per dissipation (TR_0/DI_0) appeared to be not significantly different between CY (6.01 r. u.) and PY (6.09 r. u.) needles. The most prominent difference was observed in values of electron transport beyond the primary electron acceptor, Q_A^-, [$ET_0/(TR_0-ET_0)$]: 1.20 r. u. in CY and 0.96 r. u. in PY needles. These data indicate that the main reason for better photosynthetic performance of
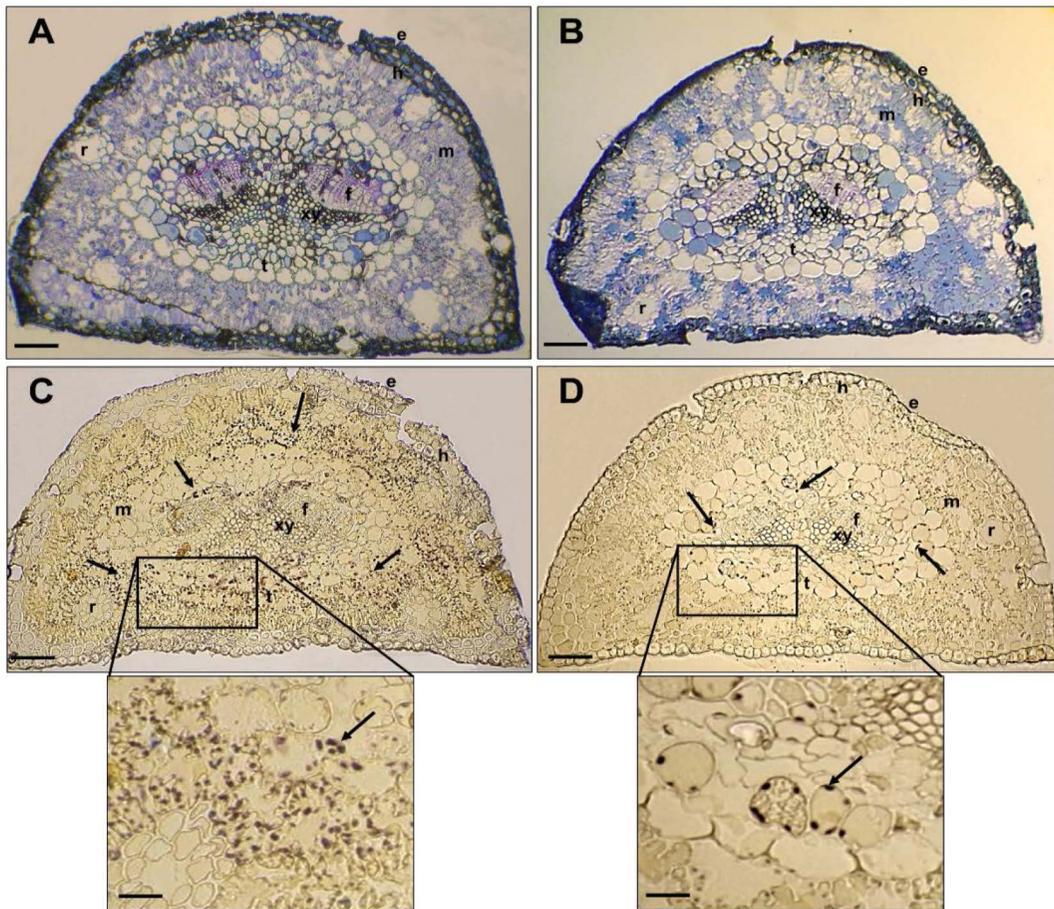
Fig. 4. Cross sections of Aleppo pine (*Pinus halepensis* Mill.) previous-year (**A**, **C**) and current-year (**B**, **D**) needles. Cross sections (**A**, **B**) were stained with 0.05% Toluidine Blue. Cross sections (**C**, **D**) were stained with Lugol solution to detect starch granule distribution (arrows); e - epidermis, h -hypodermis, m - mesophyll, xy - xylem, f - phloem, t - transfusion tissue, r - resin duct, Bar = 200 μm, insert bar =15 μm.

CY over PY needles is associated with their better electron transport capability beyond Q_A^-.

When dark-adapted needles were irradiated with a pulse of saturating light, the characteristic (STRASSER & STRASSER 1995) polyphasic chlorophyll *a* fluorescence transient (an OJIP-shaped curve) drawn on a logarithmic time-scale was observed in both CY and PY needles (Fig. 3A, small chart). The O-P normalisation revealed differences in the O-J step (Fig. 3A), which resulted in a higher value of variable fluorescence at 2 ms (V_J) in PY (0.51 r. u.) compared to CY (0.46 r. u.) needles, while variable fluorescence at 30 ms (V_I) showed no significant difference (0.87 r. u. in PY and in 0.86 r. u. in CY needles, respectively) (Fig. 3B). The increase in the J step and V_J value observed in PY needles suggests the accumulation of reduced plastoquinones (Q_A^-, $Q_A^-Q_B$ and $Q_A^-Q_B^-$) due to restricted re-oxidation of Q_A^- (STRASSER *et al.* 2004). This would explain the better capability of CY needles for more efficient use of trapped light energy in electron transport beyond Q_A^- (Fig. 3B). Since there were no observed differences between CY and PY needles with respect to

shape of the J-I and I-P steps in the OJIP curves (Fig. 3A), it can be concluded that reduction of the $ET_0/(TR_0-ET_0)$ parameter in PY needles was influenced neither by the accumulation of Q_B-nonreducing PSII reaction centres ($Q_A^-Q_B^{2-}$) (GOVINDJEE 2004) nor by reduction of electron carriers on the acceptor side of PSI (KALAJI *et al.* 2017).

Microscopic analysis of needle cross-sections (Fig. 4) showed differences of section area between CY and PY needles (PY needles had on average 1.3 times greater area than CY needles). Also, it can be seen that xylem and phloem elements as well as the transfusion tissue were better developed in PY than in CY needles (Fig. 4A, B). As shown in Fig. 5, statistically significant differences were found between PY and CY needles with respect to the areas of mesophyll tissue, resin ducts and vascular cylinder in relation to the total cross-section area. However, there was no difference in area of the epidermis and hypodermis between CY and PY needles. The percentage of mesophyll tissue area was 48.33 and 49.97% of total cross section area in PY and CY needles, respectively (Fig. 5A). Resin duct area was 0.36 and 0.30% of total cross section
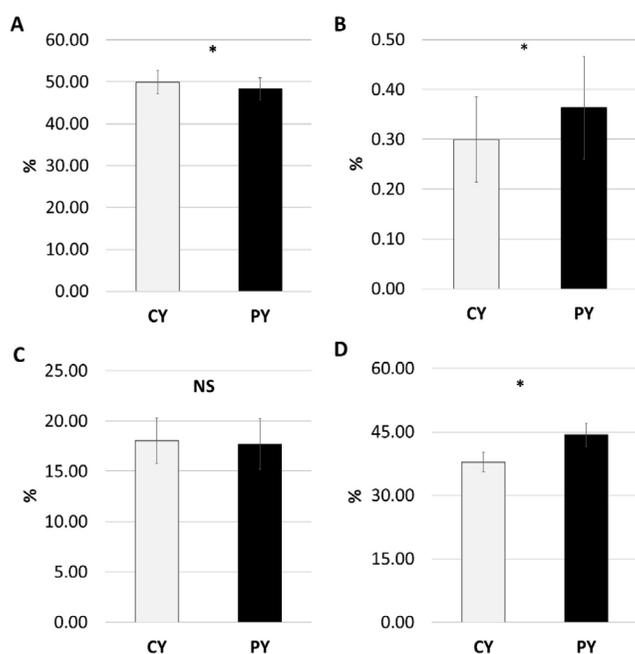
Fig. 5. Mean values of the percentage of cross sections occupied by mesophyll (**A**), resin ducts (**B**), epidermis and hypodermis (**C**) and endodermal transfusion parenchyma with two vascular bundles (**D**) in current-year (CY) and previous-year (PY) Aleppo pine (*Pinus halepensis* Mill.) needles. Significance at p (t) < 5 % is marked by an asterisk (*). The columns represent mean values ± SD (n = 90).

area in PY and CY needles, respectively (Fig. 5B). The percentage of area of the epidermis and hypodermis was 17.71 and 18.06% of total cross section area in PY and CY needles, respectively (Fig. 5C). The percentage of vascular cylinder area was 44.32% of total cross section area in PY and 37.90% in CY needles (Fig. 5D). Conifer needle anatomy is influenced by both, developmental and environmental factors (Niinemets *et al.* 2007). Since needles are the key photosynthetic organs in conifers, it is expected that their main anatomical features would correspond to light-harvesting demands. The enlarged mesophyll fraction in CY needles (Fig. 5A) indicated their photosynthetic competence, which corresponded to their better photosynthetic capability in comparison with PY needles (Fig. 2B).

Developmentally related changes of needle anatomy (greater percentage of mechanical tissues in PY needles), also known as heteroblasty (Zoth *et al.* 2011), would likely have arisen to meet environmental demands during the autumn and winter periods, which are associated with decreased light interception (Niinemets 2010). Also, it is well known that low temperatures in combination with even small amounts of light might have damaging effects on PSII that decreased its functionality. Our results concerning shape of the OJIP curves, as well as data on the V_J parameter (Fig. 3), indicate that PY needles modified their photosynthetic performance in the sense of decreased photosynthetic electron transport

(Fig. 2). Another prominent anatomical difference between CY and PY needles that is closely associated with photosynthetic competence was in the accumulation and appearance of starch. Many more starch grains were present in PY than in CY needles (Fig. 4C, D). Different patterns of accumulation of starch grains were also seen: their highest density in PY needles was in the mesophyll tissue, while in CY needles it was in the transfusion tissue. Such differences of starch accumulation are considered to be a consequence of disturbed source to sink transport of sugars (Lemoine *et al.* 2013). The normal daily dynamics of starch accumulation, where starch accumulates during the daytime and degrades at night, is regulated by circadian clock genes (Kim *et al.* 2017). The growth processes in young CY needles likely demand intensive carbon allocation from the mesophyll to other sink tissues. This is the probable reason why starch was lacking in mesophyll cells in the morning, when needles were collected (Fig. 4D). Since PY needles have finished their growth and development in the sense of elongation and tissue formation, they might have reduced needs for assimilates, a circumstance which caused decreased starch degradation at night. Although the homogeneous starch accumulation in the morning that was observed in PY needles (Fig. 4C) might arise as a consequence of different environmental factors such as Mg and K deficiency (Fink 1999), here it was most likely due to the somewhat difficult phloem loading associated with needle age (Turgeon 2006). Such source to sink relations of carbohydrate metabolites in photosynthetic organs are associated with regulation of photosynthetic efficiency (Roitsch 1999), which was also observed in PY needles, where increased starch accumulation was linked with decreased photosynthetic efficiency (Figs. 2, 4).

CONCLUSION

Based on the presented results, it can be concluded that despite their considerably lower chlorophyll content, young developing CY needles had better photosynthetic performance and a higher percentage of mesophyll tissue than in older PY needles. Lower chlorophyll content forced them to adapt their light-harvesting demands to the need for efficient photosynthetic utilisation of absorbed light energy, which is essential for elongation of needles and growth and development of tissues. Analysis of JIP test parameters showed that the main factor making this possible was better utilisation of absorbed and trapped light energy in CY needles due to their more competent photosynthetic electron transport than in PY needles. On the other hand, though PY needles retained efficient photosynthetic performance, it was lower than in CY needles. Likely, the main regulatory pathway here was photosynthetic down-regulation due to negative feed-back by starch accumulation, which lowered the electron transport rate in PY needles. Since they showed no difference from CY needles with respect to values of the

flux ratio of trapping per dissipation (TR_0/DI_0), it can be concluded that considerably increased chlorophyll content in PY did not disturb primary PSII photochemistry owing to the high irradiation present in June.

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REZIME

Anatomija, sadržaj hlorofila i fotosintetska uspešnost ovosezonskih i prošlosezonskih iglica alepskog bora (*Pinus halepensis* Mill.)

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Alepski bor (*Pinus halepensis* Mill.) široko je rasprostranjena mediteranska drvenasta vrsta. Iglice najčešće otpadaju nakon druge godine, što bi mogao biti mogući uzrok značajnog ogoljenja krošnje u uslovima ekstremnog sredinskog stresa. Cilj ovog istraživanja je bio uporediti anatomiju, sadržaj hlorofila i fotosintetsku uspešnost ovosezonskih (CY) i prošlosezonskih iglica (PY) alepskog bora. Koncentracije hlorofila određene su spektrofotometrijski, dok je fotosintetska uspešnost određena merenjem porasta fluorescencije hlorofila *a* (JIP test). Rezultati su pokazali da su koncentracije hlorofila *a* i *b* skoro dvostruko povišene u prošlosezonskim u poređenju sa ovosezonskim iglicama. Vrednosti odnosa hlorofila *a* i *b* te maksimalnog prinosa kvanta fotosistema II (Fv/Fm) nisu pokazale statistički značajne razlike između istraživanih kategorija iglica. Međutim, indeks fotosintetske uspešnosti (PI_{ABS}) bio je značajno povišen u ovosezonskim u odnosu na prošlosezonske iglice. Analiza komponenata parametra PI_{ABS} pokazala je kako su ovosezonske iglice imale povišen transport elektrona iza primarnog akceptora, Q_A^-, što je bilo povezano sa sniženom vrednosti varijabilne fluorescencije na 2 ms (V_J) u poređenju sa prošlosezonskim iglicama. Takođe, utvrđene su neke anatomske razlike između istraživanih iglica. Ovosezonske iglice imale su povišen udeo površine mezofila na preseku iglice te snižene udele površina vaskularnog cilindra i smolenica. Prošlosezonske iglice sadržavale su izrazito više skroba nego li ovosezonske iglice. Na temelju dobijenih rezultata može se zaključiti da usprkos izrazito nižem sadržaju hlorofila ovosezonske iglice pokazuju znatno bolju fotosintetsku uspešnost od starijih (prošlosezonskih) iglica, što predstavlja neobičan fenomen kod drvenastih vrsta.

Ključne reči: alepski bor, fluorescencija hlorofila *a*, anatomija iglica, razviće iglica, *Pinus halepensis*, fotosinteza