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Seasonal and needle age-related variations in the biochemical characteristics of *Pinus nigra* subsp. *pallasiana* (Lamb.) Holmboe

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Abstract

Deligöz A., Bayar E., Genç M., Karatepe Y., Kırdar E., Cankara F.G. (2018): Seasonal and needle age-related variations in the biochemical characteristics of *Pinus nigra* subsp. *pallasiana* (Lamb.) Holmboe. J. For. Sci., 64: 379–386.

Variations in the photosynthetic pigments and total carbohydrate contents of needles of different age classes (current-year, 1-year-old, 2-year-old and 3-year-old) of *Pinus nigra* subsp. *pallasiana* (Lambert) Holmboe trees in a young natural stand were investigated during the growing season. In current-year needles, total carbohydrate content was lower during June and July when the needle growth continued than in older age classes, but it was similar to other age classes in the months of August to October. Seasonal patterns of variations in total carbohydrate content were almost similar in 1-, 2-, and 3-year-old needles. Chlorophyll and carotenoid contents increased from May to June, remained relatively constant or declined slightly during summer and autumn in 1-, 2-, and 3-year-old needles. In October, the pigment content was highest in 1-year-old needles, and lowest in 3-year-old needles. Our study indicated that total carbohydrate and pigment contents were affected by needle age classes and seasons.

Keywords: chlorophyll; carotenoid; tree; black pine; soluble sugar

Plant development, growth, and productivity are closely related to photosynthesis (Silkina, Vinokurova 2009). Photosynthesis is the process by which atmospheric CO_2 is transformed into sugars using sunlight as an energy source and water as an electron donor (Olascoaga et al. 2014). In higher plants, light absorption is provided by chlorophylls and carotenoids (Güneş, Inal 1995). Thus, it is emphasized that chlorophyll content of a forest canopy can be an indicator in the measurement of physiological state such as photosynthetic capacity, developmental stage, productivity and stress (Curran et al. 1990). Differentiations in chlorophyll amount directly affect the intensity of photosynthesis and carbohydrates produced in plants (Kutbay, Kilinç 1992). Carotenoids, on the other hand, both join in photosynthesis by absorbing light energy at a definite wavelength and transferring it to chlorophyll and protect the photosynthetic apparatus from excess light – photoprotection (Sandmann et al. 2006; Ashraf, Harris 2013). Differences in the concentration of carotenoids in many plants are also related to daily and seasonal changes in external factors (Matysiak 2001). Chlorophyll concentration in needles may change according to species (Gond et al. 1999), provenance within species

(Junker et al. 2017), time of the year (Bowling et al. 2018) and growing seasons (Jach, Ceulemans 2000). It is also emphasized that chlorophyll content in the conifers may increase or decrease related to the needle position in the canopy as well as all the plant or individual needle age (Silkina, Vinokurova 2009). And also needle morphology, anatomy, chemical composition, and photosynthesis can change with tree age (Räim et al. 2012).

Carbohydrates have a highly significant effect on the relationship between photosynthesis and biomass (Mandre et al. 2002). It is detected that the amount and variety of carbohydrates found in plants differ in various plant organs and conditions all throughout the growing season. As a result, it is emphasized that plants show different behaviours in events such as development and growth, productivity and quality, resistance to cold (Köksal et al. 2001). Moreover, it is also reported that carbohydrate content in the leaves may change according to the location in the canopy (Mandre et al. 2002). Morphology and biochemistry of needles are related to age (Yan et al. 2012).

This study was conducted to determine the effect of the needle age of Anatolian black pine which has an important place in Turkey's forestry, on total carbohydrate content, and pigment (chlorophyll a, chlorophyll b, total chlorophyll and carotenoid) content. Changes in total carbohydrate content and pigment content of needle samples of different age in the growing season were also examined in this study.

MATERIAL AND METHODS

Study area. The research was conducted in a natural stand located in Burdur-Aziziye region, Turkey (37°24'N, 30°11'E). The average altitude of the study area is 1,350 m and the main aspect of the study area is northeast. In the area, the bedrock is composed of claystone and the texture is clay loam. In the region, mean annual air temperature is 12°C and mean annual precipitation is 437 mm (Karatepe et al. 2014). In the period of study, meteorological data were obtained from a meteorological station located in the study area. Daily mean air temperature (°C) and relative humidity (%) data recorded at the meteorological station in the study area are shown in Fig. 1.

Plant material. Studies were conducted on trees of Anatolian black pine (*Pinus nigra* subsp. *pallasiana* (Lambert) Holmboe), approximately 23 years of age and growing in a natural stand. Ten trees within the stand were chosen for study. It was preferred to have healthy, strong and tall trees with smooth, well-formed, unbroken or undamaged crowns. Trees were located within 1,000 m^2 area. During the 2015 growing season, one shoot sample from the south side of the middle part of each tree canopy was taken by pruning poles in monthly periods from May to October. The shoot samples were placed in zipper bags and held in portable cooler boxes during the transport to the laboratory. Current-year needles, 1-, 2- and 3-year-old needle samples were collected from the shoot samples at the laboratory.

Pigment analysis. Chlorophyll content was determined by Arnon (1949) method. The composite samples of each needle age class were obtained by mixing equal numbers of needles (sixteen fascicles) taken from each of the 10 trees. All needles were cut into small pieces with scissors. About 100 mg composite samples of fresh current, 1-, 2- and 3-year-old needles were taken for analysis. Measurements were performed in three replications of each needle age class. Needles were homogenized in 10 ml of 80% acetone, mixed about 10–15 s in the vortex and the supernatant was used for the estimation of pigments. The absorbance of the supernatant (*A*) was read at 450, 645 and 663 nm wavelengths using a

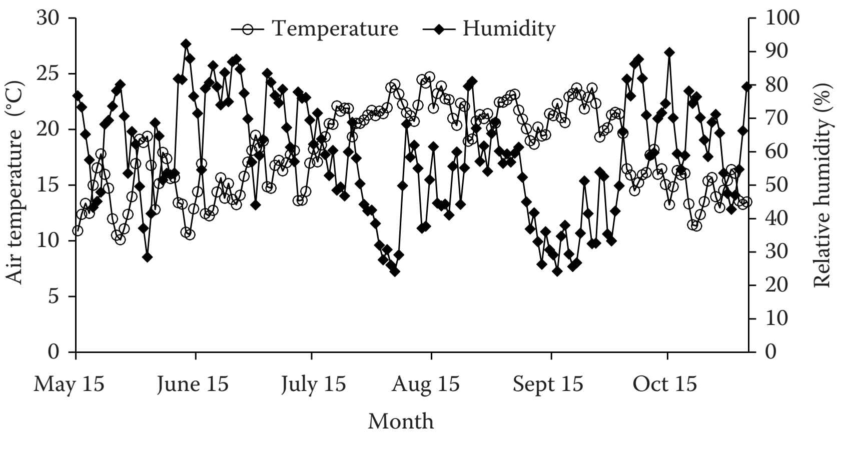

Fig. 1. Daily mean air temperature (°C) and relative humidity (%) for the sampling period in the study area

spectrophotometer (T80+UV/VIS spectrophotometer; PG Instruments, UK). The following equations (Eqs 1–4) published by Arnon (1949) were used to calculate chlorophyll and carotenoid content:

$$\text{Chlorophyll a} = 12.7(A_{663}) - 2.69(A_{645}) \tag{1}$$

$$\text{Chlorophyll b} = 22.9(A_{645}) - 4.68(A_{663}) \tag{2}$$

$$\text{Total chlorophyll} = 20.2(A_{645}) + 8.02(A_{663}) \tag{3}$$

$$\text{Carotenoid} = 4.07(A_{450}) - [(0.0435 \times \text{Chlorophyll a}) + (0.367 \times \text{Chlorophyll b})] \tag{4}$$

Total carbohydrate content analysis. Determination of the total carbohydrate content from different needle age classes was carried out in three replications. Needle samples were dried at 65°C for 48 h and then they were ground using a coffee grinder. 100 mg sample was incubated in 10 ml of 80% ethanol for 24 h and then centrifuged at 6,000 rpm for 10 min. 0.05 ml sample and 1 ml of 5% phenol solution and 5 ml H_2SO_4 were added to each tube and mixed in the vortex. After keeping them at room temperature for an hour, total carbohydrate content ($mg \cdot g^{-1}$) was determined by spectrophotometer at a 490 nm wavelength according to DuBois et al. (1956), using the phenol-sulphuric acid method and glucose as standard.

Data analysis. Total carbohydrate content and photosynthetic pigments were subjected to analysis of variance procedures using the SPSS statistical package software (Version 20.0, 2011). If there was a significant difference between the parameters measured, means were compared using Duncan's multiple range test at 0.05 level.

RESULTS

Total carbohydrate content of current-year, 1-, 2-, and 3-year-old needles of Anatolian black pine trees during the growing season is given in Fig. 2. In the current-year, 1-, 2-, and 3-year-old needles, the season had a significant effect on total carbohydrate content (all $P < 0.05$; Fig. 2). Total carbohydrate content in Anatolian black pine varied from 33.60 $mg \cdot g^{-1}$ DW (dry weight) to 78.26 $mg \cdot g^{-1}$ DW in current-year needles, 75.74–90.81 $mg \cdot g^{-1}$ DW in 1-year-old needles, 62.49–93.28 $mg \cdot g^{-1}$ DW in 2-year-old needles, and 70.06–100.25 $mg \cdot g^{-1}$ DW in 3-year-old needles. Total carbohydrate content had the lowest value in current-year needles in June and started to increase from July onwards, and remained relatively stable until October. In 1- and 2-year-old needle samples, total carbohydrate content which was high in May, decreased in June, showed an increase in July and August, and after the decrease in September it increased in October again. In 3-year-old needle samples, a decrease was observed in June, and it was increased from July and reached the highest value in August and then showed a decrease until October again. Generally, the lowest total carbohydrate content was detected in June in all needle age classes.

In this study, significant effects of needle age on total carbohydrate content were determined on some sampling dates ($P < 0.05$; Fig. 2). Current-year needles had the lower value of total carbohydrate content than the other age classes in June and July. In general, there were no significant differences between 1-, 2-, and 3-year-old needles in total carbohydrate content. In August and October, however, the differences between needle age classes in total

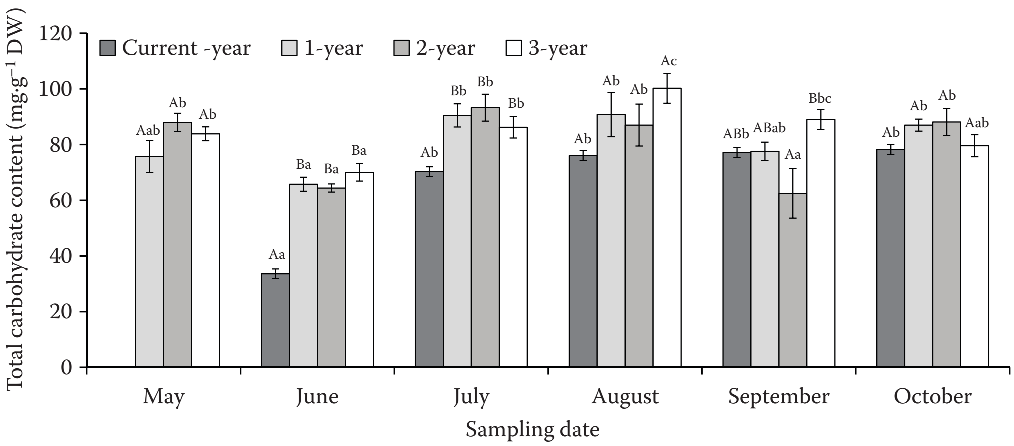

Fig. 2. Age effects (current-year, 1-, 2-, 3-year needles) on seasonal changes in total carbohydrate content ($mg \cdot g^{-1}$ DW, mean ± SE, number of samples = 4) of Anatolian black pine trees

Different capital letters indicate a significant difference between needles of different age classes, and different lower-case letters indicate a significant difference between months ($P < 0.05$), there were no current-year needles available in May carbohydrate content were not found statistically significant (Fig. 2).

In June, current-year needles had lower chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids than in the other months (Tables 1–4). Chlorophyll a was low in 1-, 2- and 3-year-old needles in May and high in June, August, September and October (Table 1). Moreover, there was not a signif-

Table 1. Chlorophyll a (mg·g^{-1} fresh weight, mean ± SE, number of samples = 4) in needles of different age classes from Anatolian black pine trees

	Current-year	1-year	2-year	3-year	*P*-value
May	no needles*	0.51 ± 0.01^{aA}	0.39 ± 0.06^{aA}	0.35 ± 0.08^{aA}	ns
June	0.37 ± 0.02^{aA}	0.81 ± 0.03^{bB}	0.72 ± 0.11^{bB}	0.76 ± 0.07^{bB}	< 0.05
August	0.76 ± 0.00^{bA}	0.83 ± 0.02^{bcB}	0.82 ± 0.02^{bB}	0.74 ± 0.02^{bA}	< 0.05
September	0.75 ± 0.01^{bA}	0.81 ± 0.02^{bA}	0.83 ± 0.02^{bA}	0.80 ± 0.00^{bA}	ns
October	0.79 ± 0.02^{bAB}	0.89 ± 0.00^{cC}	0.83 ± 0.01^{bB}	0.75 ± 0.02^{bA}	< 0.05
P-value	< 0.05	< 0.05	< 0.05	< 0.05	

*no current-year needles available in May; different capital letters indicate a significant difference between needles of different age classes, and different lower-case letters indicate a significant difference between months ($P < 0.05$), ns – not significant

Table 2. Chlorophyll b (mg·g^{-1} fresh weight, mean ± SE, number of samples = 4) in needles of different age classes from Anatolian black pine trees

	Current-year	1-year	2-year	3-year	*P*-value
May	no needles*	0.26 ± 0.02^{aA}	0.18 ± 0.01^{aA}	0.18 ± 0.04^{aA}	ns
June	0.15 ± 0.01^{aA}	0.33 ± 0.01^{bB}	0.33 ± 0.02^{bB}	0.32 ± 0.03^{bB}	< 0.05
August	0.30 ± 0.01^{bAB}	0.32 ± 0.01^{bA}	0.30 ± 0.00^{bAB}	0.28 ± 0.01^{bA}	< 0.05
September	0.31 ± 0.01^{bA}	0.33 ± 0.01^{bA}	0.34 ± 0.01^{bA}	0.32 ± 0.00^{bA}	ns
October	0.32 ± 0.01^{bB}	0.34 ± 0.00^{bB}	0.32 ± 0.01^{bB}	0.28 ± 0.01^{bA}	< 0.05
P-value	< 0.05	< 0.05	< 0.05	< 0.05	

*no current-year needles available in May; different capital letters indicate a significant difference between needles of different age classes, and different lower-case letters indicate a significant difference between months ($P < 0.05$), ns – not significant

Table 3. Total chlorophyll (mg·g^{-1} fresh weight, mean ± SE, number of samples = 4) in needles of different age classes from Anatolian black pine trees

	Current-year	1-year	2-year	3-year	*P*-value
May	no needles*	0.77 ± 0.03^{aA}	0.56 ± 0.06^{aA}	0.54 ± 0.13^{aA}	ns
June	0.52 ± 0.03^{aA}	1.14 ± 0.04^{bB}	1.05 ± 0.13^{bB}	1.08 ± 0.10^{bB}	< 0.05
August	1.06 ± 0.01^{bAB}	1.15 ± 0.04^{bC}	1.13 ± 0.02^{bBC}	1.01 ± 0.02^{bA}	< 0.05
September	1.06 ± 0.01^{bA}	1.14 ± 0.03^{bA}	1.17 ± 0.03^{bA}	1.12 ± 0.00^{bA}	ns
October	1.11 ± 0.04^{bB}	1.23 ± 0.01^{bC}	1.15 ± 0.02^{bB}	1.02 ± 0.02^{bA}	< 0.05
P-value	< 0.05	< 0.05	< 0.05	< 0.05	

*no current-year needles available in May; different capital letters indicate a significant difference between needles of different age classes, and different lower-case letters indicate a significant difference between months ($P < 0.05$), ns – not significant

Table 4. Carotenoid contents (mg·g^{-1} fresh weight, mean ± SE, number of samples = 4) in needles of different age classes from Anatolian black pine trees

	Current-year	1-year	2-year	3-year	*P*-value
May	no needles*	0.22 ± 0.01^{aA}	0.17 ± 0.02^{aA}	0.18 ± 0.03^{aA}	ns
June	0.15 ± 0.01^{aA}	0.28 ± 0.01^{bcB}	0.28 ± 0.01^{bcB}	0.27 ± 0.02^{bB}	< 0.05
August	0.24 ± 0.00^{bAB}	0.26 ± 0.01^{bB}	0.25 ± 0.00^{bB}	0.23 ± 0.00^{abA}	< 0.05
September	0.26 ± 0.00^{cA}	0.28 ± 0.01^{cB}	0.29 ± 0.01^{cB}	0.28 ± 0.00^{bAB}	< 0.05
October	0.27 ± 0.01^{cB}	0.30 ± 0.00^{cC}	0.28 ± 0.00^{bcB}	0.25 ± 0.00^{bA}	< 0.05
P-value	< 0.05	< 0.05	< 0.05	< 0.05	

*no current-year needles available in May; different capital letters indicate a significant difference between needles of different age classes, and different lower-case letters indicate a significant difference between months ($P < 0.05$), ns – not significant

icant difference in chlorophyll a from June to October. Similar results were determined in chlorophyll b and total chlorophyll, too (Tables 2 and 3). In general, carotenoid content was rather lower in May than in the other months. In 1-year-old needles, the lowest carotenoid content was determined in May, and the highest carotenoid content was determined in September and October (Table 4).

A significant effect of needle age on pigment content was found on some sampling dates ($P < 0.05$; Tables 1–4). There was not a difference between 1-, 2-, and 3-year-old needles in terms of chlorophyll and carotenoid in May. On the other hand, in June, chlorophyll and carotenoid contents in current-year needles were lower than in the other age classes. In October, the lowest chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents were determined in 3-year-old needles and the highest chlorophyll and carotenoid contents were determined in 1-year-old needles. From August onwards, chlorophyll content in current-year needles reached almost the same level as in the other age classes of needles (Tables 1–4).

DISCUSSION

In the current-year, 1-, 2-, and 3-year-old needles, total carbohydrate content was significantly affected by season. In the current-year, 1-, 2-, and 3-year-old needles, seasonal patterns of total carbohydrate content may be related to seasonal changes in phenological and physiological activities. The lowest total carbohydrate content in all needle age classes was determined in June (spring). It corresponds to the time when trees were actively undergoing shoot elongation. The decrease from May to June in soluble carbohydrates in previous year's needles may result from translocation into current-year needles and from elongation of the axis of new shoots (Mandre et al. 2002). Because in evergreen conifer, the carbohydrates and photosynthesis from the previous year's needles support shoot growth until the current-year needles develop (Hansen, Beck 1994). In this study, the increases observed after June show that the accumulation of total carbohydrate content was conducted slowly in all age classes. Schaberg et al. (2000) found that sugar concentrations peaked in winter and were at their lowest values in spring for *Picea rubens* Sargent. Vaz et al. (2010) found that leaf concentrations of total soluble sugars were lower in summer than in autumn for both species *Quercus ilex* Linnaeus and *Quercus suber* Linnaeus. Oleksyn et al. (2000) reported that soluble carbohydrate concentrations in 1-year-old needles were lowest in spring and summer, highest in autumn and winter in *Pinus sylvestris* Linnaeus. Total soluble sugars in plants increase during the onset of cold acclimation (Sakai, Larcher 1987) and these sugars may play a role in cold tolerance (Wong et al. 2003). Soluble sugars are highly sensitive to environmental stresses and under abiotic stresses, such as drought, cold or salinity soluble sugar concentrations also increased (Rosa et al. 2009; Sami et al. 2016). On the other hand, an accumulation of carbohydrates in leaves is also considered as an earlier response to Mg deficiency (Farhat et al. 2016). Thus, in this study, an increase or decrease of total carbohydrate content may be related to internal and environmental conditions such as light, water or temperature.

In *Pinus cembra* Linnaeus trees, significant effects of season and tissue type were detected in total soluble sugar content (Gruber et al. 2011). In this study, significant effects of needle age on total carbohydrate content were determined on some sampling dates. Total carbohydrate content in current-year needles in June and July was lower than in needles of the other age classes. A similar result was also seen in *Pinus koraiensis* Siebold & Zuccarini (Yan et al. 2012). In *P. sylvestris* populations, soluble carbohydrate concentration in current-year needles was determined to be lower than in 1- and 2-year-old needles (Oleksyn et al. 2000). Li et al. (2001) found out that in July when current-year needles were not ripe yet, soluble sugar, starch and non-structural carbohydrate (NSC) concentrations in current-year needles were lower than in 1-year-old needles in *P. cembra* trees. This difference disappeared at the end of the growth season. In the same study, it was stated that the significance of the differences in total NSC concentration in the same tissue (needle or branch segments) from different age classes could be related to their light environment, different tissue dry matter density and activity. In this study, there was not a significant difference in total carbohydrate content between 1-, 2-, and 3-year-old needles. At the end of the season, there was not a significant difference in total carbohydrate content between all the needle age classes, either. Soluble sugar content in 1-, 2-, and 3-year-old needles collected soon after the bud break was found to be similar in Scotch pine, too (Fischer, Höll 1991).

Seasonal changes in photosynthetic pigments are under the control of the daily light period (Vogg et al. 1998). Uvalle Sauceda et al. (2007) reported that chlorophyll and carotenoid contents were significantly different between years, seasons and between plants within years and season. During the year a seasonal fluctuation was determined in chlorophyll levels in *Pinus halepensis* Miller needles (Elvira et al. 1998). In this study, chlorophyll and carotenoid contents were minimum in May, while they were found higher but stable in summer and autumn. Similarly, chlorophyll and carotenoid contents were stated to be at a maximum level in August and to reduce in spring in *P. sylvestris* and *Picea abies* (Linnaeus) H. Karsten, too (Linder 1972). Chlorophyll content in one-year-old *P. sylvestris* needles under natural climatic conditions exhibited a seasonal change with low levels during winter and high levels during summer (Vogg et al. 1998). Again, chlorophyll content in *Picea sitchensis* (von Bongard) Carrière was maximum in summer (Lewandowska, Jarvis 1977). Weng et al. (2005) reported that the chlorophyll concentration in needles of Taiwan spruce was higher in summer and lower in winter. A decrease in chlorophyll in the period from April to July during the growing season and an increase from July to October were determined in *Juniperus virginiana* Linnaeus (Brett, Singer 1973).

Significant effects of needle age on chlorophyll and carotenoid amount were detected in some sampling periods like in total carbohydrate content. Pigment content in current-year needles was lower than in older needles in June. Chlorophyll a and total chlorophyll concentration was affected by needle age in *Pinus ponderosa* P. & C. Lawson seedlings (Houpis et al. 1988). It was detected that in *P. abies*, chlorophyll a, chlorophyll b, total chlorophyll, and total carotenoid concentrations were lower in 1-year-old needles than in 2- and 3-year-old needles in August and no difference in these pigments existed between 2- and 3-year-old needles (Soukupová et al. 2000). Similarly, no significant difference in pigment content was found between 1-, 2-, and 3-year-old needles in May, June, and September in this study, either. Moreover, generally, the highest pigment content was determined in 1-year-old needles in October. Pigment content (chlorophyll a, chlorophyll b, and total chlorophyll, carotenoid) in 1-year-old needles was found to be higher than in the current-year needles in *Abies alba* Miller, too (Bačić et al. 2003).

CONCLUSIONS

The total carbohydrate and chlorophyll content indicated variations as to season and needle age classes. The difference in total carbohydrate content between needle ages revealed itself in current-year needles in June and July. While total carbohydrate content in current-year needles was at lower levels than in older needle age classes especially in June and July when the needle growth continued, total carbohydrate content in current-year needles was generally similar to that of needles of other age classes from August to October. Between fully developed and physiologically ripened 1-, 2-, and 3-year-old needles there was not a significant difference in terms of total carbohydrate content. The pigment contents of all needle age classes were similar in September. It showed that chlorophyll a, total chlorophyll and carotenoid content of 1-, 2-, and 3-year-old needles tended to decrease with the increasing age of needles only in October. These obtained results will light the way of ecologists and physiologists in understanding the whole tree physiology and in estimating productivity at an individual and ecosystem level.

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