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**ORIGINAL ARTICLE**

# Heat-stress response of photosystem II in five ecologically important tree species of European temperate forests

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## Abstract

Heat negatively affects photosynthesis, thus tree growth and eventually survivability. The light-dependent photosynthesis reactions are considered highly heat sensitive; therefore, we studied the impact of supra-optimal temperatures on photosystem II (PSII) in five important tree species (*Abies alba, Fagus sylvatica, Picea abies, Pinus sylvestris* and *Quercus petraea* agg.). We simulated short-term heat stress at different levels and assessed PSII thermotolerance via fluorescence parameters derived from the OKJIP transient. To capture the seasonal changes in PSII thermostability and the stress tolerance index, the measurements were performed twice: at the beginning of the vegetation season before the natural heat events occurred and during the peak of the vegetation season. Moreover, the content of photosynthesis-related pigments was analysed. We confirmed the relationship between the appearance of the K-step at the OKJIP curve and the concentrations of photosynthesis-related pigments. We found that broadleaved tree species showed overall higher PSII thermostability and had a higher content of pigments than coniferous species. The most heat-tolerant species was *Q. petraea* agg., which presented enhanced PSII thermotolerance after heat events. *F. sylvatica* responded by a decrease in PSII thermostability during the vegetation season, though its thermostability was still higher than that of the coniferous species and was comparable to that of *Q. petraea* agg. The PSII thermotolerance of *P. abies* was relatively high compared to that of the other coniferous species on both dates, but its response was inconsistent across the assessed parameters. Contrary, the PSII thermostability of *A. alba* and *P. sylvestris* was generally low on both measurement dates.

**Keywords** Assimilatory pigments · Interspecific variability · OKJIP test · Thermostability · Thermotolerance of PSII

## Introduction

Sessile oak (*Quercus petraea* Mattusch., Liebl.), Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* L., H. Karst), silver fir (*Abies alba* Mill.) and European beech (*Fagus sylvatica* L.) are among the ecologically and economically most important tree species that constitute the main core of temperate forests in Central Europe (San-Miguel-Ayanz et al. 2016; Buras and Menzel 2019). However, ongoing climate change will exert strong evolutionary pressure during the upcoming decades, affecting the growth, vitality, biomass production, and survival of tree species, which can eventually severely threaten ecological stability and production of European forest ecosystems (Kalaji et al. 2016; Bauhus et al. 2017; Krstic et al. 2018; Matkala et al. 2021).

According to climate change scenarios, the likelihood of temperature extremes will greatly increase. Until now, the mean annual temperatures have risen 1 °C above the long-term average, and the frequency of heat waves has already increased significantly on monthly to seasonal time scales (Coumou and Robinson 2013; Teskey et al. 2015). Susceptibility to high temperatures can therefore be a limiting factor for the distribution of plant species and may lead to a shift in

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species distribution and/or a change in species composition in these ecosystems.

Heat stress induces changes in individual plants at biochemical and physiological levels, which are then reflected at the ecosystem level. For these reasons, the study of physiological processes can be used to explain the responses of trees to heat events and the effect of climate change on forest ecosystems. Physiological responses to stress factors can be divided into three reactions. The first is the primary physiological response, which is an immediate reaction of the plant to short-term stress. Another is acclimation, which is an adjustment to longer-lasting changes in natural conditions by modification of the physiological processes (Kao and Chang 2001; Brestic et al. 2012; Brestič and Živčák 2013). The last type of stress response is genetic-dependent adaptation, which is induced by natural selection driven by obvious environmental differences between habitats. Local adaptation affects changes in genetic structure and lasts a long time – over several generations. Hence, the response of plants to surrounding conditions is conditioned by their adaptive and acclimation potential (Kao and Chang 2001; Brestič and Živčák 2013; Konôpková et al. 2018; Kurjak et al. 2019).

One of the most heat-sensitive physiological mechanisms in plants is photosynthesis, of which PSII is the most thermosensitive component (Ghouil et al. 2003; Wahid 2007; Mathur et al. 2014). Heat stress negatively affects photosynthetic processes ranging from suppression to destruction, manifested in the following effects: reduction in $CO_2$ assimilation because of the inhibition of RuBisCO activase, disintegration of chlorophyll molecules, increase in the fluidity of thylakoid membranes, disorganization of enzymes, denaturation of the main D1 and D2 proteins, separation of the reaction centres from PSII, and/or damage to the electron transport chain and oxygen-evolving complex (Froux et al. 2004; Godoy et al. 2011; Brestič and Živčák 2013; Kalaji et al. 2016; Guha et al. 2018).

The fast kinetics of chlorophyll *a* fluorescence is one of the most effective methods to assess the status of PSII under heat stress. For intact plants, the fluorescence induction curve (FIC) has a typical shape with four distinguishable bands: O, J, I, and P. However, overheating causes significant changes in the FIC, with several indicators of heat damage: (i) the value of the O-band, which represents the minimal fluorescence ($F_0$), abruptly increases, reflecting an accumulation of reduced quinone ($Q_A$) as a result of the nonuniform distribution of the energy flux from reaction centres (RCs) or the inhibition of electron transport from $Q_A$ to $Q_B$ (Kouřil et al. 2004; Strasser et al. 2004); (ii) the value of the P-band, which represents the maximal fluorescence (Fm), decreases as a result of denaturation of chlorophyll proteins (Yamane et al. 1997); (iii) an additional K-band occurs on the FIC at the time of 3 ms, which reflects irreversible damage of PSII caused by structural and biochemical alterations to antenna complexes, slowing of the electron flux from pheophytin to $Q_A$ or disruption of the oxygen-evolving complex (Lazár et al. 1999; Stirbet et al. 2014); and (iv) the overall photochemical performance of PSII decreases as a result of these changes. High temperatures are also often related to precipitation deficits, which can alter their negative effects ( Coumou and Robinson 2013; Teskey et al. 2015; Ionita et al. 2020).

Higher plants have developed various ways to protect the photosynthetic apparatus, especially its membranes, against heat-induced damage. The accumulation of zeaxanthin, increase in heat-shock protein synthesis, abscisic acid accumulation and synthesis of isoprenes are the most important mechanisms (Havaux et al. 1991; Sharkey and Zhang 2010; Mathur et al. 2014). All of these features can vary between different species as well as between different populations as a result of both long-term adaptations and short-term acclimation to increasing temperatures (Berry and Bjorkman 1980; Havaux 1993) and may indicate their different response abilities.

According to Hansen et al. (2002), light-demanding species have higher energy utilizing capacity than do light-intolerant species. On the other hand, light-demanding species have upregulated antioxidant capacity as a protective mechanism against oxidative stress, resulting in increased tolerance to high light intensity and to overheating. Therefore, we hypothesize that, compared with the shade-tolerant species *A. alba* and *F. sylvatica*, *P. sylvestris* and *Q. petraea* agg., which are light-demanding species, have higher concentrations of photosynthesis-related pigments and high PSII thermostability at the start of the growing season. Moreover, we assume that light-demanding species can enhance their PSII thermostability after natural heat events because of their higher acclimation capacity resulting from the generally high intensity and heterogeneity of irradiation levels in their natural habitats. We expected *P. abies* to show a mediocre but plastic response, as it is generally shade-tolerant, but it can also grow as a solitary tree on full sun-exposed sites; in addition, young trees are especially able to adapt to high irradiation well.

## Material and Methods

### Study area and plant material

This research was conducted in the western part of the Carpathian Mountains in Slovakia (48°45′10″N, 19°1′39″E, 728 m a.s.l.). The study area is situated in the temperate climatic zone with a temperate Central European climate; the average annual air temperature is 6.7 °C, and the total annual precipitation is 962 mm.

The five most common tree species of Central Europe were selected for assess their thermostability of PSII:

*A. alba*, *F. sylvatica*, *P. abies* and *P. sylvestris*, *Q. petraea* agg. (Table 1). Six adult individuals of each tree species growing within one square kilometre were selected and marked. Their mature leaves and needles were collected in the morning from the unshaded parts of tree crowns from the same height of approximately 8 m. The samples were enclosed in plastic bags immediately after sampling to prevent desiccation. To capture differences in PSII thermostability during the vegetation season, sampling was performed on two dates: early summer before the trees were exposed to natural stress (21 June 2018, henceforth referred to as "non-stress conditions") and peak summer (7 August 2018, henceforth referred to as "mild stress conditions").

## Meteorological data

Daily average air temperature and daily precipitation during the vegetation season were obtained from an automatic meteorological station (EMS Brno, Czech Republic) located in an open space within the area of sampling. The monthly values of the parameters were calculated from continually measured climatic data. An assessment of the weather anomalies, i.e., a comparison of air temperature and precipitation with long-term averages calculated from Kremnické Bane (758 m n. m., weather station of the Slovak hydrometeorological institute SHMÚ from the period 1987–2016) during the year of the experiment, was performed following the methods of the SHMÚ (Lapin et al. 1988).

## Measurements of PSII thermostability

The heat stress was simulated by using a WNE22 temperature-controlled water bath (Memmert, Germany) with samples enclosed in Erlenmeyer flasks. The flasks were immersed in the water bath and exposed to the specific temperature for 30 min. Temperatures ranging from 32 °C to 50 °C (steps of approximately 2 °C) were used. The first changes in the photochemical efficiency of silver fir and European beech were observed at temperatures above 38 °C (Konôpková et al. 2018; Kurjak et al. 2019); therefore, 32 °C was considered a non-stressing temperature within a short-term stress event.

**Table 1** Optimal altitude and climatic characteristics of the tested tree species (Pagan 1999)

| Species | Climate optimum | | |
|---|---|---|---|
| | Altitude [m a.s.l.] | Mean daily temperature [°C] | Annual precipitation [mm] |
| *P. sylvestris* | 150−400 | 7.5−9.0 | 400−500 |
| *Q. petraea* agg. | 150−500 | 6.5−8.5 | 500−700 |
| *F. sylvatica* | 400−800 | 5.0−7.0 | 800−900 |
| *A. alba* | 500−1000 | 4.5−6.5 | 900−1000 |
| *P. abies* | 560−1350 | 2.0−4.0 | 900−1500 |

After exposure to heat stress, the samples were dark adapted using leaf clips for 30 min. The samples were then illuminated by a one-second-long saturation pulse of high intensity (3500 µmol $m^{-2}$ $s^{-1}$), and the fast kinetics of chlorophyll *a* fluorescence was measured using a Handy PEA fluorimeter (Hansatech Instruments, Ltd., United Kingdom). The chlorophyll *a* fluorescence was recorded every 10 µs to obtain a polyphasic fluorescence curve with a characteristic OJIP shape. The occurrence of the K-band at 300 µs of the OJIP curve is characteristic of heat-stressed plants. The data were processed using Biolyzer 5 HP software (Laboratory of Bioenergetics, University of Geneva, Switzerland) to calculate the JIP test parameters (Strasser et al. 2004). Measurements were repeated twice for each individual and averaged. The performance of the photosynthetic apparatus after exposure to heat stress was determined based on the following chlorophyll *a* fluorescence parameters (Strasser et al. 2000):

- $\mathbf{F_0}$ – basal fluorescence of chlorophyll *a* in dark-adapted leaves, recorded for 50 µs,
- $\mathbf{F_M}$ – maximal fluorescence of chlorophyll *a* in dark-adapted leaves,
- $\mathbf{F_V}$ – variable fluorescence, $F_V = F_M - F_0$,
- $\mathbf{F_V/F_M}$ – maximum quantum yield of photochemical efficiency of PSII, the ratio of variable and maximal fluorescence, $F_V/F_M = (F_0 - F_M)/F_M$,
- **RC/ABS** – number of active reaction centres in PSII,
- **PI** – performance index of the photochemical activity (based on absorption),
- $\mathbf{W_K}$ – parameter derived from the OKJIP curve representing variable fluorescence within 300 µs (K-band) and reflecting heat-induced irreversible damage to PSII.

The parameters derived from the basic fluorescence parameters expressing PSII thermostability (Froux et al. 2004; Konôpková et al. 2018) were as follows:

- $\mathbf{T_{15(Fv/Fm)}}$ – the temperature at which $F_V/F_M$ decreases by 15% from the maximal value measured at the non-stressing temperature of 32 °C,
- $\mathbf{T_{15(PI)}}$ – the temperature when the performance index (PI) decreases by 15% from the maximal value measured at the non-stressing temperature,
- $\mathbf{T_{15(RC/ABS)}}$ – the temperature when the number of active reaction centres decreases by 15% from the maximal value measured at the non-stressing temperature,
- $\mathbf{T_C}$ – the critical temperature of PSII stability; the temperature at which $F_0$ abruptly increases,
- $\mathbf{T_{Wk}}$ – the critical temperature at which the variable fluorescence at the K-band abruptly increases.

A stress tolerance index (STI) was calculated for each thermostability parameter as follows:

$STI = \frac{y_s \times y_p}{(\overline{y_p})^2}$ (Fernandez 1992),where $y_p$ is the measured value for the trait under non-stress conditions (June), $y_s$ is the measured value for the trait under stress conditions (August), and $\overline{y_p}$ is the mean trait value of all measured variants under non-stress conditions.

### Photosynthesis-related pigments

The content of the photosynthesis-related pigments was determined using a spectrophotometric method for pigment separation. The pigment extraction was performed with an organic aqueous solvent composed of 80% acetone. Freeze-dried leaves or needles (0.20 g) were homogenized in 20 ml of 80% acetone with washed sea sand and $MgCO_3$. After homogenization, the solution was filtered twice, and the absorbance at different wavelengths (470 nm, 646.8 nm, 663.2 nm, and 750 nm) was recorded by a SPECORD 200 spectrophotometer (Analytik Jena AG, Germany). To determine the concentrations of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl $a+b$), and total carotenoids (Car $x+c$), the following equations, based on specific coefficients of absorption for the aqueous solvent of 80% acetone, were used (Lichtenthaler 1987):

$$\mathrm{Chl}_a = 12.25\ \mathrm{A}_{663.2} - 2.79\ \mathrm{A}_{646.8}$$

$$\mathrm{Chl}_b = 21.50\ \mathrm{A}_{646.8} - 5.10\ \mathrm{A}_{663.2}$$

$$\mathrm{Chl}_a + b = 7.15\ \mathrm{A}_{663.2} + 18.71\ \mathrm{A}_{646.8}$$

$$\mathrm{Car}x + c = 1000\ \mathrm{A}_{470} - 1.82\ \mathrm{Chl}a - 85.02\ \mathrm{Chl}b$$

The content of the pigments was ultimately calculated per gram of dry weight.

### Statistical analyses

Statistical analyses were performed using R (R Core Team, Austria) and XLSTAT (Addinsoft, USA) software.

To obtain the seasonal climatic trends, the climatic data, consisting of temperatures and precipitation during the growing season of 2018, were first transformed using Fourier transformation. The homogeneity of the temperature data between two measurement days was tested using Pettit's nonparametric test.

For the fluorescence data, the normality of distribution was first tested by the Shapiro-Wilk test, which revealed that some parameters needed to be transformed. Further, the homoscedasticity of species was examined by Bartlett's test. The differences among species and measurement days were assessed using two-way ANOVA. We assessed the relationships between measured physiological traits. Moreover, based on the single linkage Euclidean distances of the STI, the species could be aggregated into distinguishable groups.

## Results

### Climate analysis

The climate characteristics of average daily temperatures and precipitation were measured during the summer (May—August) of 2018 (Fig. 1). The total precipitation was below the long-term averages in all evaluated months, except May. The precipitation was unevenly distributed during the study period due to frequent local precipitation and storms. With respect to average daily air temperature, May and June were slightly above average, July was average, and August was extremely above average. Pettit's test identified two distinguishable periods (Fig. 1) during the summer of 2018, with a significant change in daily average temperatures on 19 July. The first period (1 May—19 July) was determined as a "colder and wetter period", with more frequent precipitation events with higher daily precipitation totals. The second period, characterized by elevated daily average temperatures and less frequent precipitation, lasted from 19 July to 31 August. Only 12.6 mm of precipitation fell from the start of the second period to the day of measurement (4th of August); therefore, this period was determined to be a "warmer and drier period" (Fig. 1).

### Thermotolerance analysis

The average values of critical temperatures for each parameter, species and date are listed in Table 2 along with the results of the analysis of variance. The analysis confirmed the significant interspecific differences in all assessed parameters of PSII thermotolerance, except $T_{15(RC/ABS)}$, which differed just marginally between species. Overall thermostability and acclimation effects for each species were expressed by stress tolerance indices (STI) in a heat map (Fig. 2). Values of STI greater than one represents improvement of corresponding physiological trait after the stress period. If the physiological trait was negatively influenced by stress the value of STI is lower than one. The broadleaved species show overall higher thermotolerance of PSII than did the coniferous species, and this differentiation was also confirmed by cluster analysis. The coniferous and broadleaved species could be separated into two distinguished groups, where the conifers were further divided into two subgroups. *A. alba* and *P. sylvestris* were aggregated together and represented the least thermotolerant species, and the separation of *P. abies* is a result of its moderate PSII thermotolerance.

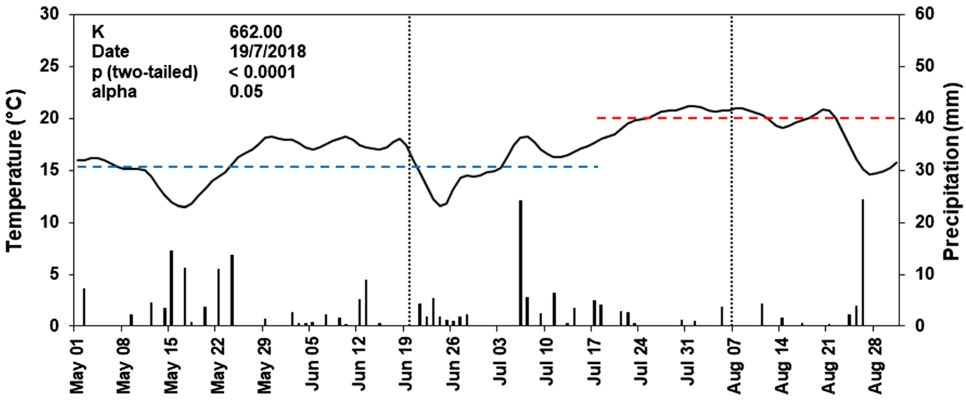

**Fig. 1** Daily temperature trend after Fourier transformation and daily precipitation at the study site. The dashed blue and red lines represent two significantly different temperature periods based on Pettit's test. The dotted vertical lines represent the measurement dates

**Table 2** The results of an analysis of variance of the parameters of PSII thermostability are presented (Df, degrees of freedom and significance of F tests ***, P < 0.001; **, P < 0.01; *, P < 0.05; ·, P < 0.1), including the species means and standard deviations for both dates (before and after natural heat stress occurrence)

| Analysis of Variance | | | | | | Mean ± SD | |
|---|---|---|---|---|---|---|---|
| Source of variance | Df | F | P | | Species | non-stressed | stressed |
| $T_{15(PI)}$ | | | | | | | |
| Species | 4 | 4.112 | 0.00384 | ** | *F. sylavica* | 37.58 ± 2.15 | 36.81 ± 3.12 |
| Date | 1 | 3.517 | 0.06341 | · | *P. sylvestris* | 36.64 ± 1.61 | 33.96 ± 1.53 |
| Species*Date | 4 | 2.63 | 0.03821 | * | *Q. petraea* agg. | 36.57 ± 2.66 | 37.67 ± 1.45 |
| Residuals | 109 | | | | *A. alba* | 36.94 ± 1.52 | 36.85 ± 2.05 |
| | | | | | *P. abies* | 38.16 ± 1.07 | 36.97 ± 2.09 |
| $T_{15(RC/ABS)}$ | | | | | | | |
| Species | 4 | 2.04 | 0.094 | · | *F. sylavica* | 38.96 ± 1.42 | 37.53 ± 2.89 |
| Date | 1 | 1.738 | 0.1903 | | *P. sylvestris* | 37.94 ± 1.67 | 35.85 ± 1.96 |
| Species*Date | 4 | 2.214 | 0.0723 | · | *Q. petraea* agg. | 37.5 ± 2.32 | 38.88 ± 2.05 |
| Residuals | 107 | | | | *A. alba* | 37.43 ± 1.84 | 37.19 ± 2.31 |
| | | | | | *P. abies* | 38.52 ± 0.93 | 38.06 ± 2.41 |
| $T_{15(Fv/Fm)}$ | | | | | | | |
| Species | 4 | 7.333 | 2.91E-05 | *** | *F. sylavica* | 51.04 ± 3.44 | 48.19 ± 2.07 |
| Date | 1 | 0.623 | 0.4318 | | *P. sylvestris* | 47.4 ± 2.76 | 47.23 ± 2.02 |
| Species*Date | 4 | 4.311 | 0.00283 | ** | *Q. petraea* agg. | 47.72 ± 0.82 | 48.31 ± 0.74 |
| Residuals | 108 | | | | *A. alba* | 47.11 ± 0.94 | 46.24 ± 1.47 |
| | | | | | *P. abies* | 47.41 ± 0.56 | 49.08 ± 1.8 |
| $T_C$ | | | | | | | |
| Species | 4 | 3.054 | 0.0199 | * | *F. sylavica* | 43.79 ± 1.47 | 43.62 ± 0.77 |
| Date | 1 | 1.257 | 0.2646 | | *P. sylvestris* | 41.65 ± 1.8 | 42.28 ± 1.82 |
| Species*Date | 4 | 0.726 | 0.5762 | | *Q. petraea* agg. | 44.09 ± 0.73 | 43.96 ± 0.9 |
| Residuals | 109 | | | | *A. alba* | 43.37 ± 0.77 | 41.07 ± 5.75 |
| | | | | | *P. abies* | 44.37 ± 1.78 | 42.92 ± 3.97 |
| $T_{Wk}$ | | | | | | | |
| Species | 4 | 8.188 | 8.22E-06 | *** | *F. sylavica* | 40.05 ± 1.01 | 41.23 ± 2.46 |
| Date | 1 | 0.963 | 0.329 | | *P. sylvestris* | 38.92 ± 1.42 | 40.46 ± 1.19 |
| Species*Date | 4 | 0.859 | 0.491 | | *Q. petraea* agg. | 42.07 ± 1.57 | 41.7 ± 1.64 |
| Residuals | 109 | | | | *A. alba* | 39.3 ± 0.84 | 39.21 ± 1.03 |
| | | | | | *P. abies* | 38.42 ± 5.05 | 38.21 ± 2.47 |

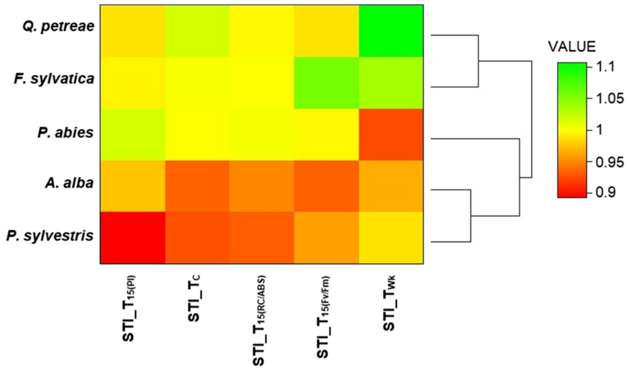

**Fig. 2** Heatmap and cluster analysis of stress tolerance indices (STIs). The values at the red end of the spectrum represent low stress tolerance, and values at the green end of the spectrum represent high stress tolerance

Acclimation during the mild stress period was confirmed by species × date interactions, which were observed for two parameters, $T_{15(PI)}$ and $T_{15(Fv/Fm)}$, suggesting the divergence of species responses to changing conditions within the tested period. The PSII thermostability of *Q. petraea* increased for both of these parameters during the growing season. Thus, *Q. petraea* showed the highest ability to acclimate to mild heat stress occurring during the vegetation season to prevent heat-related damage at the PSII level. *F. sylvatica* showed a decline in $T_{15(PI)}$, $T_{15(Fv/Fm)}$ and $T_{15(RC/ABS)}$ after the mild stress period. However, despite the decline, it maintained an overall high level of thermostability comparable to that of *Q. petraea* agg. Compared with *A. alba* and *P. sylvestris*, *P. abies* showed overall higher critical temperatures on both dates, but its response during the season was not consistent across the assessed parameters. *A. alba* and *P. sylvestris* showed a decrease or non-significant changes between the two measurement days for all evaluated parameters and maintained an overall low thermostability of PSII.

## Photosynthesis-related pigment analysis

The content of the photosynthesis-related pigments was measured on both dates. Significant differences between species for all assessed parameters were observed (Suppl. 1). Compared with coniferous species, broadleaved species showed approximately two times higher contents of chlorophyll and carotenoids (Fig. 3). Differences between dates were mostly non-significant for the tested species. We found a significant decrease only in the concentration of total chlorophyll for *P. abies* and for both chlorophyll and carotenoids in the case of *P. sylvestris*. We found positive relationships between the contents of chlorophyll and carotenoids and the thermotolerance parameter $T_{Wk}$ (Fig. 4), and differences between broadleaved and coniferous species were also apparent.

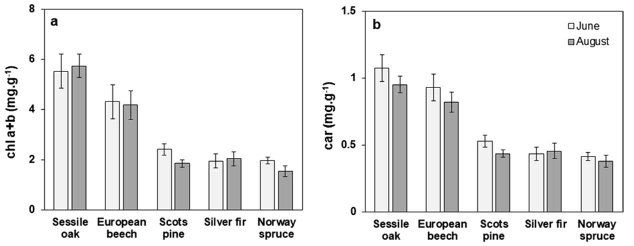

**Fig. 3** Means ± 95% confidence intervals of concentrations of chlorophyll (**a**) and carotenoids (**b**)

## Discussion

Several studies have documented the variability of heat tolerance among seedlings in different broadleaved tree species or Mediterranean conifers (Dreyer et al. 2001; Froux et al. 2004). Nevertheless, little is known about the interspecific variability of the most important tree species of the temperate zone. Analysis of several PSII parameters under simulated heat stress revealed that thermostability thresholds differ in sensitivity. The rankings of the sensitivity from the lowest (most sensitive) to the highest (most robust) average temperature are as follows: $T_{15(PI),}$ $T_{15(RC/ABS),}$ $T_{Wk}$, $T_C$, and $T_{15(Fv/Fm)}$. Individual parameters express the effect of heat on subsequent processes of PSII photochemistry, and their relative sensitivity to heat stress corresponds to the general consensus (Brestič et al. 2012; Kalaji et al. 2016; Konôpková et al. 2018). Overall, the performance index was the most responsive to heat. This index is considered one of the most sensitive parameters of PSII efficiency influenced by many environmental factors, as it consists of three independent parameters reflecting PSII photochemistry (Strasser et al. 2000; Živčák et al. 2008; Stirbet et al. 2014; Bussotti et al. 2019).

All parameters except $T_{15(RC/ABS)}$ showed significant differences between species. The main hypothesis of this study was that there are significant differences in PSII thermostability between light-demanding species (*P. sylvestris, Q. petraea* agg.) and shade-tolerant species (*A. alba, F. sylvatica*), where light-demanding species are hypothesized to show higher PSII thermostability than are shade-tolerant species (*P. abies* represents a mix of sun-shade plant archetypes and thus belongs to neither group). This was driven by assumptions that light-demanding species are adapted to higher irradiance influx with higher energy-utilizing capacity and upregulated antioxidant capacity (Hansen et al. 2002; Vieira et al. 2021); thus, these species may also have higher thermostability of PSII. Our results cannot confirm this hypothesis, as *P. sylvestris* showed the lowest overall thermostability, which even decreased after the heat period with low precipitation. Our observations that *P. sylvestris* has lower PSII thermostability than other observed species

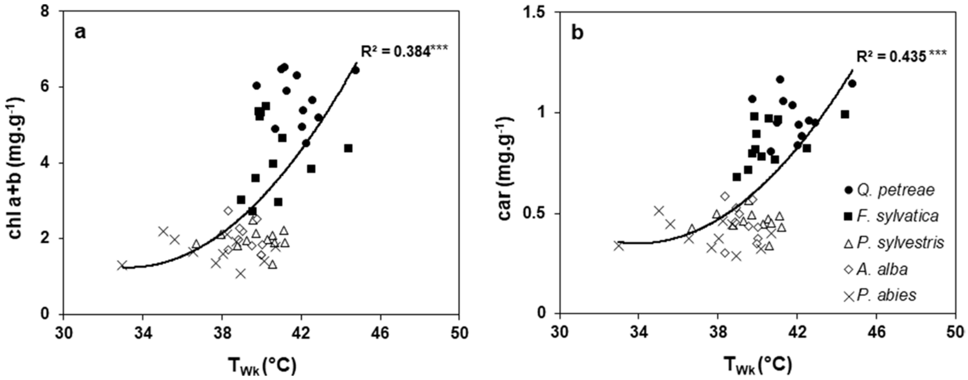

**Fig. 4** Relationships between the concentrations of chlorophyll (**a**) and carotenoids (**b**) and the critical temperature at which variable fluorescence at the K-band abruptly increases

except *A. alba* were unanticipated and are worth further investigation. Recent paper focused on temperature curves of Fv/Fm revealed that $T_{50}$ (temperature inducing 50% decline in Fv/Fm) for *P. sylvestris* and *P. nigra J. F. Arnold* was significantly lower than for *A. alba* and *P. abies* (Kunert et al. 2021). As most of the relevant physiological studies have focused on low-temperature treatment (James et al. 1994; Ottander et al. 1995; Sveschnikov et al. 2006), there is a lack of comparable studies; thus, we explored intraspecific differences in PSII under heat stress among species from the same genus. *Pinus halepensis* Mill. had higher PSII photochemical efficiencies and quenching than *Quercus ilex* in the normal physiological temperature range. On the other hand, *Q. ilex* showed a higher thermotolerance than *P. halepensis* for all the measured fluorescence parameters (Méthy et al. 1997; Savi et al. 2015). This suggests that the genus *Pinus* favours increased growth and productivity over thermotolerance. However, Kurjak et al. (2019) confirmed the significantly higher thermostability of beech growing in a relatively warm plot compared to a relatively cold plot. It is possible that the defensive mechanisms of *P. sylvestris* were not fully exacerbated under natural mild heat stress occurred.

Cluster analysis based on stress tolerance indices divided the studied species into two groups: broadleaves and conifers. We can see similar differentiation if we compare the thermostability of coniferous species (*Cupressus dupreziana subsp. atlantica* (Gaussen) Silba, C. semperviren*s* L., *Pinus halepensis, P. nigra* Arn.) according to Froux et al. (2004) with broadleaved species (*Acer pseudoplatanus* L., *Betula verrucosa* Roth, *Fraxinus excelsior* L., *F. sylvatica, Q. petraea* agg., *Q. robur*) according to Dryer et al. (2001), where broadleaves have significantly higher PSII thermostability than conifers. Overall, the higher PSII thermostability of broadleaved species could be a beneficial trait under future scenarios with warmer climates.

One of the factors that enables broadleaves to achieve higher thermostability might be a higher concentration of chlorophyll and carotenoids. Statistical differences in chlorophyll $a+b$ and carotenoid $x+c$ concentrations reveal another differentiation between broadleaves and conifers. Similar results were found by Li et al. (2018), who compared the content of pigments between different plant species, communities and functional groups. Those authors observed that, in comparison to the studied coniferous species, broadleaved species had an overall higher content of chlorophyll pigments. Chlorophyll accumulation is often associated with the heat-stress response. Studies performed on mutants (Chen et al. 2006a, b) showed that disruption of the ability to accumulate chlorophyll leads to relatively low basal thermotolerance and a decreased ability to develop higher thermotolerance under heat stress. Higher carotenoid content enables plants to enhance their thermotolerance by quenching reactive oxygen species (Young 1991) and by preserving membrane integrity (Havaux 1998). We found a significant positive correlation between pigment concentration and the critical temperature at which variable fluorescence at the K-band abruptly increases ($T_{Wk}$). The K-step indicates negative effects of modification of LHCs and inhibition of electron transport from pheophytin to $Q_A$ and may be associated with dissociation of the oxygen-evolving complex (Strasser 1997; Lazar et al. 1999; Stirbet et al. 2018). The observed correlation might thus reflect the positive stabilization effect of pigments on vulnerable parts affected by the K-band increase. We can also observe partitioning of broadleaves and conifers within this relationship.

Our second hypothesis was that light-demanding species have a higher range of acclimation than shade-tolerant species (Van de Peer et al. 2017; Gianoli and Escobedo 2021). The effect of mild stress on the acclimation of PSII thermostability was reflected in the species × date interaction only for critical temperatures $T_{15(PI)}$ and $T_{15(Fv/Fm)}$. As expected, *Q. petraea* agg. showed higher $T_{15(PI)}$ and $T_{15(Fv/Fm)}$ after the mild stress period, and *F. sylvatica* responded by a decrease in PSII thermostability during the vegetation season. However, its thermostability was still higher than that of coniferous tree species and was comparable to that of *Q. petraea* agg. Surprisingly, relatively low critical temperatures were found for *P. sylvestris* after mild stress, rejecting our hypothesis.

We confirmed a significant negative effect of stress period on pigment concentrations for *P. sylvestris* and *P. abies*. This could lead to a decrease in the thermostability of *P. sylvestris*; however, we did not see such a pattern for *P. abies*. The effect of leaf/needle development is expected to enhance thermostability as assimilatory organs mature. This effect has been described in physiological research on both herbaceous and tree species. The PSII thermostability of *P. abies* increases with the age of the needles due to the increased thermal stability of thylakoid membranes (Karlický et al. 2016). Compared with young leaves, mature leaves of *Coffea arabica* L. show higher heat tolerance of photochemistry and cell membrane stability (Marias et al. 2017). A study by Jiang et al. (2006) suggested that the enhanced thermostability of PSII might be associated with improved stability of the oxygen-evolving complex (OEC) to heat stress during leaf development. In addition to the leaf development effect, an effect of seasonality was also found, while *P. nigra, P. halepensis, C. sempervirens* and *C. atlantica* seedlings showed the highest PSII thermostability during summer (Froux et al. 2004). We assume that the decline in PSII thermostability of *P. sylvestris* is thus caused not by the effect of leaf development or seasonality (which should be positive) but rather by the negative effect of the mild stress period experienced by the assessed trees. This lack of acclimation response and overall low PSII thermostability may be limiting factors of *P. sylvestris* and *A. alba* under upcoming more frequent and more severe heat waves (Breshears et al. 2021). It is important to explore the phenotypic plasticity and the mechanisms underlying the recovery process in the future, which may differ among species, possibly compensating for the disadvantages found.

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**Data availability** The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

**Code availability** not applicable.

## Declarations

**Conflicts of interest/Competing interests** The authors declare that they have no conflict of interest.

**Ethics approval** The authors declare that the research meets ethical standards applicable to the research discipline.

**Consent to participate** All authors approved the participation.

**Consent for publication** All authors agree with the contents of the manuscript and gave final approval for publication.

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