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Research Article

The role of light in the adaptation of *Thymus praecox* Opiz subsp. *praecox* for diverse habitat conditions

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Abstract

The light decides about the course and efficiency of photochemical processes, being an important component of the surrounding environment shaping the plant composition in specific conditions. *Thymus praecox* subsp. *praecox* belongs to endangered taxa due to preferences for open habitats, which as a result of natural succession are exposed to overgrowth and shading with forest-scrub vegetation. In this study, an attempt was made to check the physiological activity of creeping thyme in changing environmental conditions prevailing on isolated stands in the Ojców National Park (Southern Poland). The increase in fresh and dry mass and the percentage of water in plants were determined, the content of chlorophyll *a* and *b* was measured, the intensity of chlorophyll *a* fluorescence was examined and the degree of electrolytes leakage through cell membranes was checked. The main aim was to determining the optimal habitat conditions for this taxon, which could help protect it. Based on the conducted research it was found, among others higher mass increase in plants from a sunny stand. Regardless of the measurement period, an increase in chlorophyll *a* and *b* content and a higher degree of cell membranes destabilisation in plants from a partially shaded stand was observed. The obtained results show, that *T. praecox* subsp. *praecox* characterises small habitat flexibility - even partial shade is not a convenient habitat for it. To optimal development, this subspecies requires conditions with high light availability. Creeping thyme is a heliophilic and thermophilic taxa and the sunny stands are optimal for it.

Keywords: morphological plasticity; Ojców National Park; physiological activity; protected species; Southern Poland; *Thymus praecox* subsp. *praecox*

Introduction

During the growing season, solar radiation reaching the Earth's surface changes significantly (Théry, 2001; Yang *et al.*, 2018b). The photosynthetic apparatus of plants, due to different light conditions, has built defense mechanisms against the effects of different photosynthetically active radiation (PAR) (Carvalho *et al.*, 2011; Bayat *et al.*, 2018). Plants have created physical and endogenous barriers absorbing or dispersing excess solar radiation (e.g. Smith, 1982; Steyn *et al.*, 2002; Albert *et al.*, 2009). The morphological plasticity of plants

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belonging to the same species is illustrated by the fact that the same set of genes can create different phenotypes when exposed to different environmental factors. Therefore, morphological plasticity is assigned a key role in the adaptation of plants to changing environmental conditions (Frank *et al.*, 2013). Specifying the species requirements for the environment, i.e. defining its ecological niche or available habitat (Harper, 1981), is particularly important for endangered taxa, such as, e.g. creeping thyme *Thymus praecox* Opiz. subsp. *praecox*.

Figure 1. A blooming specimen of creeping thyme *Thymus praecox* Opiz subsp. *praecox* - Ojców, June 2018 (Photo A. Sołtys-Lelek)

Thymus praecox subsp. *praecox* is one from 8 included taxa of *Thymus praecox* Opiz from sect. Serpyllum (Euro+Med 2006-) with islet occurrence in Europe (Meusel *et al.*, 1978; Duchoň, 2012). In Poland, it grows only in southern part, in Ojców National Park - ONP (N - 19°46'55,979"E 50°15'4,086"N; E - 19°51'11,998"E 50°10'29,894"N; W - 19°46'9,501"E 50°12'55,254"N; S - 19°50'47,379"E 50°10'13,017"N) in the Prądnik Valley (Dolina Prądnika), between Ojców and Prądnik Korzkiewski, and in the central and upper parts of the Sąspowska Valley (Dolina Sąspowska). Here, the rock turf *Festucetum pallentis* (Kozł. 1928) Kornaś 1950 (Biderman and Bąba, 2001) is the habitat of *T. praecox* subsp. *praecox*.

T. praecox is a semi-shrub with creeping stems that usually have barren tips and with laterally growing flowering branchlets. It has stiff, blade-like leaves; the lateral nerves on the underside of the leaves are quite thick, and the highest pair connects to the main nerve into a short thickened marginal nerve (Rutkowski, 2004). Flowering shoots of this species are evenly hairy, and leaves on the flowering branchlets become gradually larger towards the top (Figure 1). Leafed branchlets grow from terminal or lateral buds located on various older parts of the plant, often under the soil surface. In ONP it forms small tufts of individuals, usually vegetative reproducing (Biderman, 1991). The taxon has been entered in the "Polish Red Book of Plants" with the status CR - critically endangered (Biderman and Bąba, 2001), and it is in the "Red list of vascular plants in Poland" with the status E - threatened of extinction, critically endangered (Zarzycki and Szeląg, 2006). In Poland, the species is under strict legal protection and requires active conservation measures (item 1.388 according to RMS, 2014 - Rozporządzenie Ministra Środowiska z dnia 9 października 2014 r. w sprawie ochrony gatunkowej roślin Dz. U. 16 października 2014 r. Poz. 1409). The succession of forest and shrub vegetation, and the ensuing shading of sites, is a factor that threatens its survival. There are few publications on this taxon in the literature. They concern cytological, genetic and biochemical issues, some of its subspecies (Trela-Sawicka, 1972; Mártonfi and Mártonfiová, 1996; Avci, 2011; Karbstein *et al.*, 2019).

The aim of this study was to compare the physiological activity of *Thymus praecox* subsp. *praecox* specimens growing in different habitat conditions, on sunny and partially shaded stands. Attempts were made to determine the effect of light intensity on: the fresh and dry mass and the water content of shoots (*i*), the chlorophyll *a* and *b* content (*ii*), the chlorophyll *a* fluorescence intensity (*iii*) and the degree of destabilisation of plant cell membranes (*iv*). These explorations aimed at understanding physiology in general and at determining the optimal habitat conditions for this rare and endangered taxon, what could help protect it in the future.

Materials and Methods

Plant material

Thymus praecox subsp. *praecox* shoots were collected *in situ* from the natural habitat of the Ojców National Park (permission DLP-III-4102-661/47342/13/MD) on three measurement dates: beginning of species vegetation (May), optimum vegetation (June) and end of vegetation (July) 2018, from two stands: SP - Maidens' Rocks (Skały Panieńskie) and K - beneath Krukowski's Rock (Skała Krukowskiego) (Figure 2).

Research stands

In order to characterise the designated test stands, the following measurements were taken: photosynthetically active radiation intensity (PAR) (light intensity meter: model 189, Li-Cor, Inc, Lincoln, USA), ambient temperature and relative humidity (digital hygrometer: PWT 221, Elmetron, Poland). Stand 1 - Maidens' Rocks (SP) was characterised by higher light intensity, higher temperature and lower air humidity, compared to the stand 2 - Krukowski's Rock (K) (Table 1).

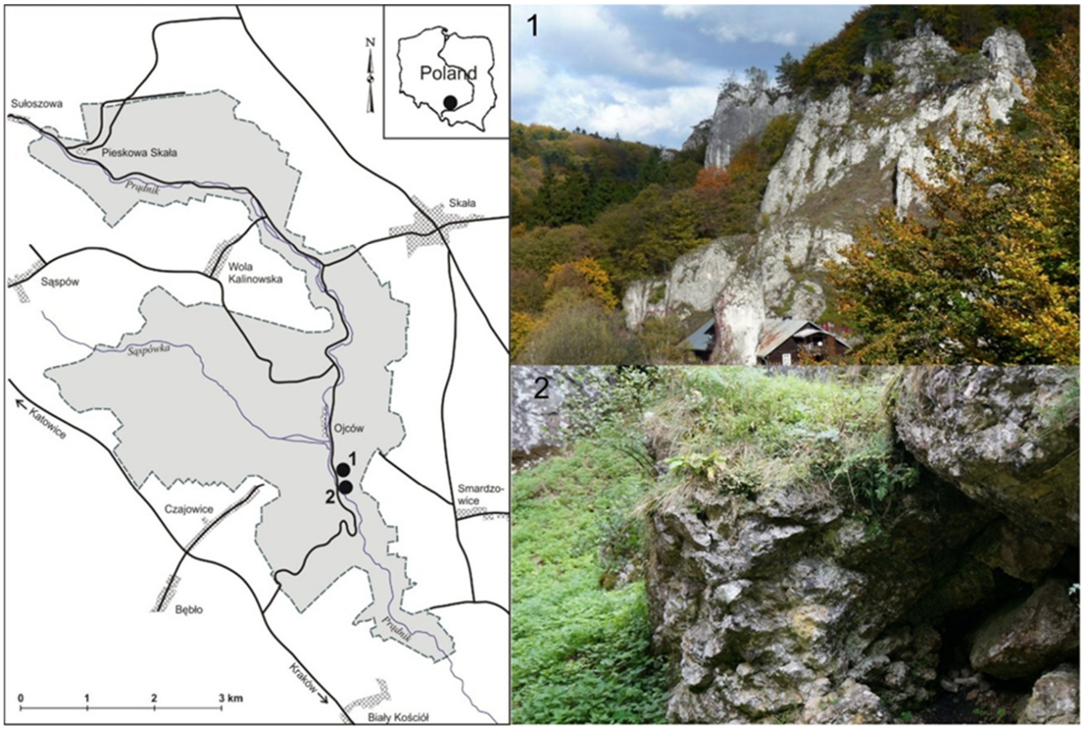

Figure 2. Location of research stands with *Thymus praecox* Opiz subsp. *praecox* in Ojców National Park (Southern Poland); 1 - Maidens' Rocks (Skały Panieńskie), 2 - Krukowski's Rock (Skała Krukowskiego) (Photo A. Sołtys-Lelek)

Table 1. Environmental parameters: light intensity (L), temperature (T) and relative humid (RH) from SP - Maidens' Rocks, K - beneath Krukowski's Rock in the Ojców National Park

Parameters	V		VI		VII	
	SP	K	SP	K	SP	K
L (μmol m^{-2} s^{-1})	284.0 a	147.5 b	295.2 a	150.9 b	256.0 a	132.9 b
T (°C)	20.3 a	19.5 b	30.7 a	28.4 b	18.9 a	16.5 b
RH (%)	42.73 b	53.91 a	40.01 b	63.89 a	50.23 b	62.59 a

Note: months: V, VI, VII; a, b, c - values differ significantly according to Tukey test at $p \leq 0.05$

Granulometry of soil samples in the 80 nm - 2 mm measuring range (Fritsch laser particle size meter) were also analysed. The percentage of granular fractions was determined by the Bouyoucos-Casagrande areometric method in Prószyński's modification (PN-ISO 11277: 2005, 2005). The soils were classified according to USDA standards (Polskie Towarzystwo Gleboznawcze, 2009). The soil granulometric parameters of the above-mentioned stands are presented in Table 2.

On the studied stands occur initial rendzinas - soils developed on limestone bedrock, with the profile structure type A-AC-Cca. They are characterised by very thin humus level (A), which was created with the participation of herbaceous plant remains. The analysed stands do not differ significantly in terms of granulometric composition. In both cases sand, especially fine-grained, dominates. The dust fraction and clay have a scant share, and their genesis is associated with the roots of plants occurred in throughout the solum. The analysis was carried out at the humus level (A), and the presence of this fraction increases the soil's water capacity and provides better ecological conditions for xerothermic grasslands.

Table 2. Basic granulometric parameters of soil in natural sites from which *Thymus praecox* Opiz subsp. *praecox* plant material was sampled in the Ojców National Park; SP - Maidens' Rocks, K - beneath Krukowski's Rock

Profile	Silt				Sand				PTG 2008 with standard USDA
	Clay	Fine	Coarse	Very fine	Fine	Medium	Coarse	Very coarse	
SP	2	11	13	19	30	20	5	0	ls
	2	15	18	20	23	19	4	0	sl
K	2	13	10	13	21	13	25	3	ls
	3	15	22	22	21	14	4	0	sl

Note: PTG - particle size distribution and textural classes of soils and mineral materials - classification of polish society of soil science 2008; ls - loamy sand, sl - sandy loam; analysis was performed using the Fritsch laser particle sizer; measurement range: 80 nm - 2 mm, mean = 5

Fresh and dry mass and tissue water content

The plant material was weighed on a scale (Radwag WPS 210, Poland), and dried in a dryer (Wamed SUP-100, Poland) for 48 hours at 105 °C to analyse the dry mass. Based on the obtained masses, the tissue water content was determined according to Black and Pritchard (2002).

Chlorophyll content

The chlorophyll content (Chl *a*, *b*, *a* + *b* and *a*/*b*) in *T. praecox* subsp. *praecox* leaves was determined according to Barnes *et al.* (1992). Fresh plant material was extracted in 3 ml dimethyl sulfoxide (SIGMA-Aldrich) at 65 °C for 12 h. The absorbance of chlorophyll *a* and *b* was determined at wavelengths: λ = 665 and 648 nm, using the Aqarius 9500 spectrophotometer (Cecil Instruments, Cambridge, United Kingdom).

Chlorophyll a fluorescence

Chlorophyll *a* fluorescence of *T. praecox* subsp. *praecox* leaves was measured using a FMS1 fluorimeter (Hansatech, Norfolk, United Kingdom). In order to quenching the light phase of photosynthesis, the leaves were acclimatised to the dark for 30 minutes using clips, and then they were exposed to excitation light at 1000 $\mu mol \times m^{-2} \times s^{-1}$, during 1 s. Based on the measurements performed values of minimum fluorescence (F_0) (Baker and Rosenquist, 2004), maximum fluorescence (F_m), variable fluorescence (F_v) and maximum photochemical PSII efficiency ($F_v/F_m = (F_m-F_0) / F_m$) (Björkmann and Demmig, 1987) were determined. In addition, a photochemical activity of photosystem II (PSII) was imaged using a FluorCam FC 800C (Photon Systems Instruments, Czech Republic). After earlier acclimatisation to darkness according to Lichtenthaler *et al.* (2004) method the following parameters were analysed: the stationary fluorescence (F_t), the maximum photochemical efficiency of PSII (QY_{max}), the non-photochemical quenching (NPQ) and the vitality of PSII (Rfd).

Electrolyte leakage

The degree of destabilisation of cell membranes of *T. praecox* subsp. *praecox* specimens was examined according to Barabasz-Krasny *et al.* (2018). Single thyme shoots were placed in polypropylene vials with 10 ml distilled water and shaken for 3 h on a shaker (Labnet, Rocker, USA) to determine the outflow of electrolytes from live cells (E1). Then the plant material was frozen at -70 °C. After 24 h the shaking procedure was repeated to the total electrolyte leakage from dead cells (E2) was measured. The percentage of electrolyte leakage (EL) was calculated according to the formula: EL = (E1/E2)×100%.

Statistical analysis

The experiment was carried out in 2 independent series of 5 repetitions. The significance of differences between mean values (± SD) were analysed by the ANOVA / MANOVA parametric test using the post hoc Tukey test (HSD) ($p \leq 0.05$) in Statistica 13.0 for Windows.

Results

Fresh, dry mass and water content

In the case of measurements of fresh and dry mass of *T. praecox* subsp. *praecox* shoots, the highest increase in masses was found in plants growing in the sunny stand, in relation to plants growing in the partially shaded stand. The tissue water content, in the first and second period of measurement dates (May and July), was higher in plants growing on the Krukowski's Rock (K) stand. While, in the second measurement date (June), a higher water content was observed in *T. praecox* subsp. *praecox* from Maidens' Rocks (SP) stand (Table 3).

Table 3. Fresh, dry mass (g) and tissue water content (TWC) of *Thymus praecox* Opiz subsp. *praecox* collected from SP - Maidens' Rocks, K - beneath Krukowski's Rock in the Ojców National Park

Parameters	V		VI		VII	
	SP	K	SP	K	SP	K
Fresh mass (g)	0.141 a	0.122 a	0.125 a	0.043 b	0.030 a	0.022 a
Dry mass (g)	0.048 a	0.038 b	0.035 a	0.014 b	0.010 a	0.006 b
TWC (au)	0.0066 b	0.0069 a	0.0072 a	0.0068 b	0.0067 b	0.0073 a

Note: months: V, VI, VII; a, b, c - values differ significantly according to Tukey test at $p \leq 0.05$

Chlorophyll content

In *Thymus praecox* subsp. *praecox* leaves significant differences in the chlorophyll *a* and *b* content between plants growing in the sunny (SP) and partially shaded (K) stands were found (Figure 3). The chlorophyll *a* content was lower in May, compared to the content of this pigment in June and July. Regardless of the measurement date, the chlorophyll *a* content was lower in thymus plants growing in the sunny stands, in relation to the plants from the partially shaded stands. The highest chlorophyll *a* content in *T. praecox* subsp. *praecox* plants from Krukowski's Rock stands during the optimal vegetation (June) was found. In June and July, the chlorophyll *b* concentration was higher in plants growing on the Krukowski's Rock (K) stand. At the Maidens' Rocks (SP) stand, the chlorophyll *b* content was the highest in May. In the other two periods of the study, the chlorophyll *b* content in leaves was significantly lowest.

The total chlorophyll *a* and *b* content was higher in plants growing on the Krukowski's Rock (K) stand, regardless of the vegetation period. In the first measurement period (May), the lowest chlorophyll *a* + *b* concentration, in plants growing on the Maidens' Rocks (S) stand, was observed. In June, the highest total chlorophyll content, in plants growing in the partially shaded stand, was found. The chlorophyll *a*/*b* ratio varied depending on the measurement period. Regardless of the light intensity at the studied stands, along with the growth and development of thyme plants, an increase in the value of this parameter was demonstrated.

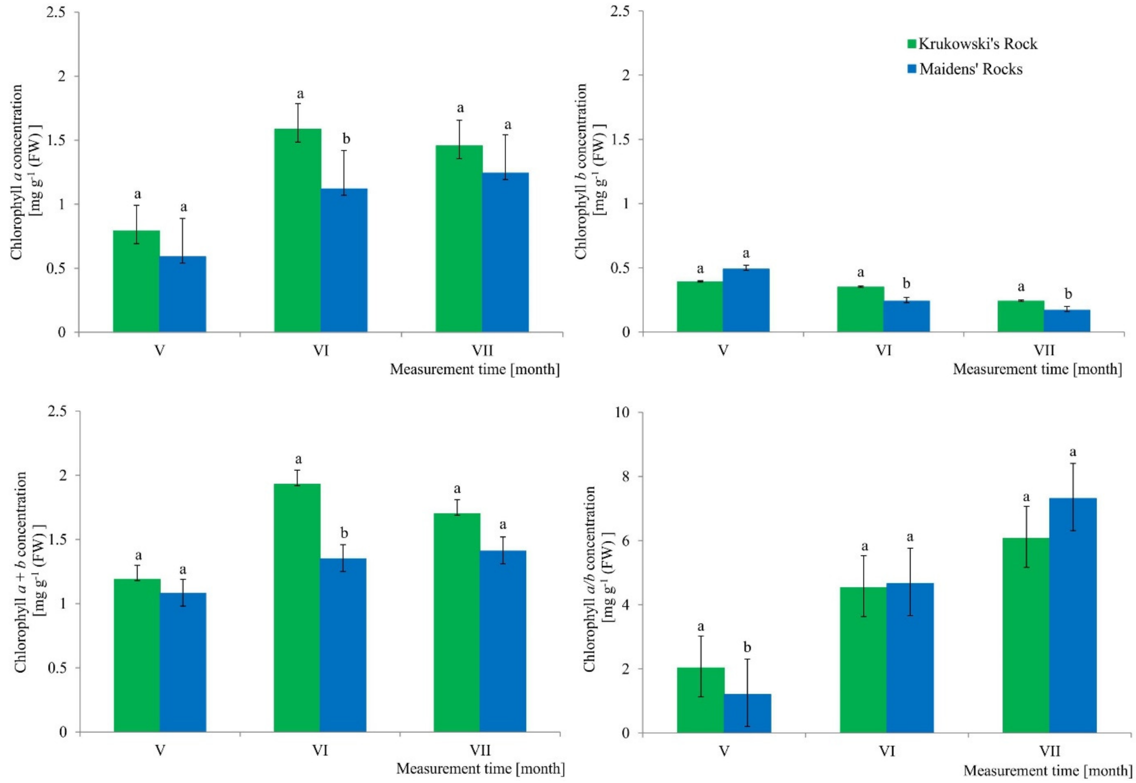

Figure 3. Chlorophyll content of *Thymus praecox* Opiz subsp. *praecox* collected from Maidens' Rocks, beneath Krukowski's Rock in the Ojców National Park in different time (months: V, VI, VII)

Note: a, b, c - values differ significantly according to Tukey test at $p \leq 0.05$

Chlorophyll a fluorescence

Based on the chlorophyll *a* fluorescence measurement, a various effect of light intensity on the photosynthetic activity of *T. praecox* subsp. *praecox* was observed (Table 4).

In May, the minimum fluorescence (F_0), maximum fluorescence (F_m) and variable fluorescence (F_v) values were significantly higher in plants growing in the partially shaded stand, compared to the values of these parameters in plants from the sunny stand. In the other two measurement dates, the increase of the fluorescence parameters values was significantly higher in *T. praecox* subsp. *praecox* from the sunny stand, compared to the plants from the shady stand. Regardless of the vegetation period of plants, the maximum photochemical PSII efficiency (F_v/F_m) values were significantly higher in plants growing in the sunny stand, compared to plants from the partially shaded stand.

Table 4. Intensities of chlorophyll *a* fluorescence parameters in leaves of *Thymus praecox* Opiz subsp. *praecox* collected from SP- Maidens' Rocks, K - beneath Krukowski's Rock in the Ojców National Park in different time (months: V, VI, VII)

Parameters	V		VI		VII	
	SP	K	SP	K	SP	K
F_0	125.4 b	141.8 b	171.8 a	123.0 b	183.2 a	150.6 ab
F_m	827.2 ab	1003.6 a	1241.0 a	521.8 b	1262.4 a	1135.2 a
F_v	761.8 b	801.8 b	1069.2 a	482.8 c	1079.2 a	1004.6 a
F_v/F_m	0.852 a	0.847 a	0.861 a	0.820 a	0.854 a	0.862 a

Note: a, b, c - values differ significantly according to Tukey test at $p \leq 0.05$; F_0 - minimum fluorescence, F_m - maximum fluorescence, F_v - variable fluorescence, F_v/F_m - maximum photochemical PSII efficiency

At the first measurement period (May), the stationary fluorescence (F_t) values were higher in plants from the Krukowski's Rock (K), compared to *T. praecox* subsp. *praecox* from the Maidens' Rocks (SP) (Figure 4). The highest F_t values were observed in June, both on the one and second stands with *T. praecox*. In July, the plants from Maidens' Rocks (SP) had slightly higher F_t values than plants growing on Krukowski's Rock (K) stand. QY_{max}, in the first and the third measurement period (May and July), had lower values in relation to the plants from the second measurement period (June). In May, higher activity of plants growing on Maiden' Rocks (SP) was observed, and in July on Krukowski's Rock (K). In June, QY_{max} values in *T. praecox* leaves were very similar in both stands. NPQ and Rfd in the first measurement period (May) reached higher values in thymus plants growing on the Maidens' Rocks (SP). In the second measurement period (June), similar values of these parameters were revealed. The lowest NPQ and Rfd values were observed for plants from the Krukowski's Rock (K) on the third measurement period (July).

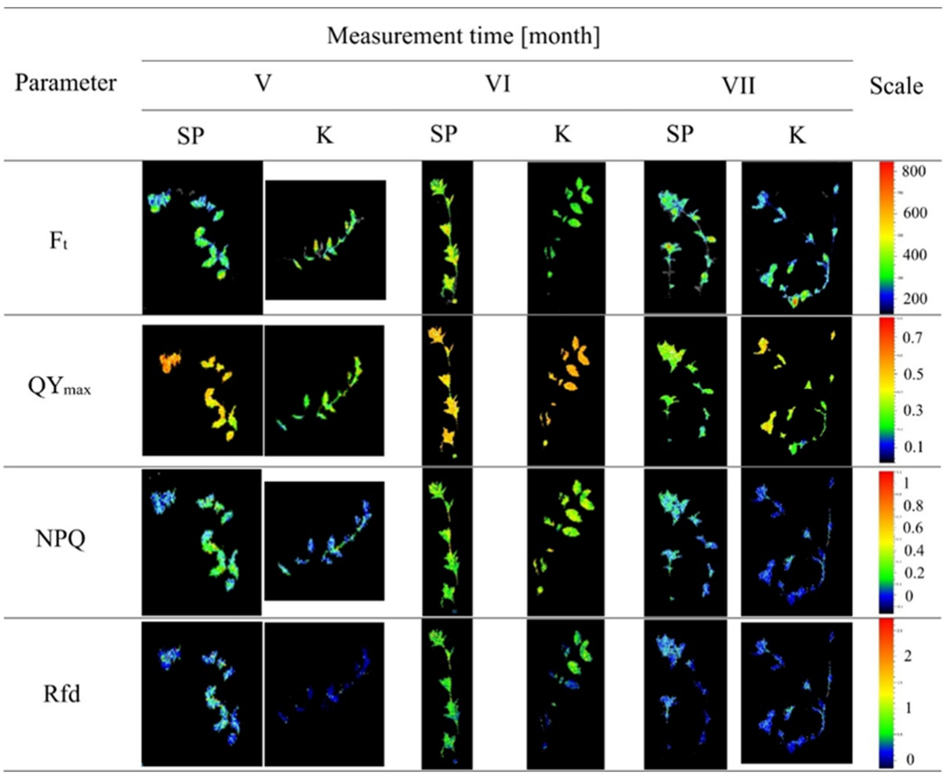

Figure 4. Imaging of chlorophyll *a* fluorescence parameters (F_t - the stationary fluorescence, QY_{max} - the maximum photochemical efficiency of PSII, NPQ - the non-photochemical quenching, Rfd - the vitality of PSII) in leaves of *Thymus praecox* Opiz subsp. *praecox* collected from SP - Maidens' Rocks, K - beneath Krukowski's Rock in the Ojców National Park in different time (months: V, VI, VII)

Electrolyte leakage

Based on the measurements of electrolyte leakage through cell membranes from *T. praecox* subsp. *praecox* shoots, a significant increase in water and ion management destabilisation was observed in plants from the sunny stand only in May. In June and July, the electrolyte leakage was not only lower by half, compared to the May measurements, but definitely higher in plants growing in the partially shaded stand (Figure 5).

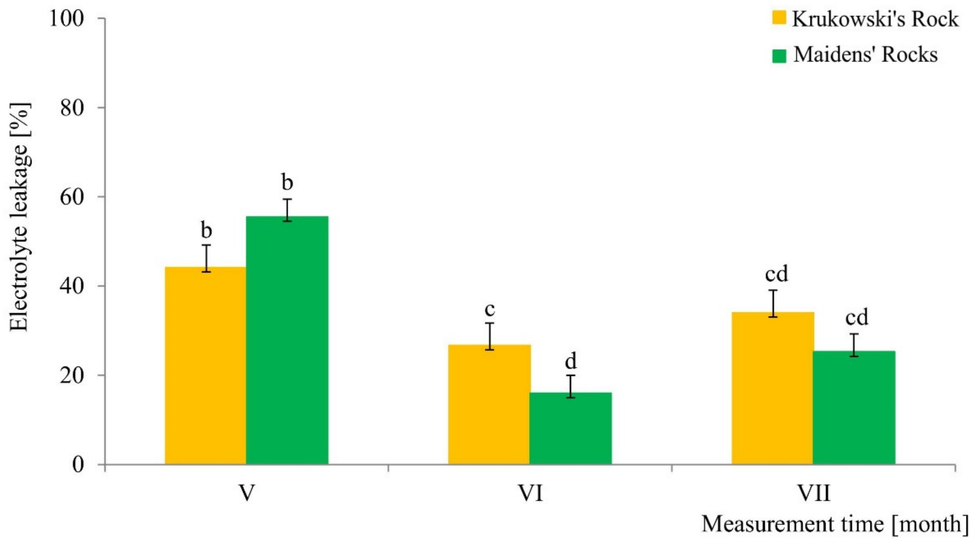

Figure 5. Electrolyte leakage of *Thymus praecox* Opiz subsp. *praecox* collected from Maidens' Rocks, beneath Krukowski's Rock in the Ojców National Park in different time (months: V, VI, VII)
Note: a, b, c - values differ significantly according to Tukey test at $p \leq 0.05$

Discussion

The spectral composition and intensity of light reaching the plants have a fundamental impact on their growth and development (Tyagi and Gaur, 2003; Valladares and Niinemets, 2008). They decide not only about the course and efficiency of photochemical processes, but also contain information about the surrounding environment and allow the best adaptation to natural conditions (Pilarski *et al.*, 2012). The quantity and quality of sunlight reaching the plants in shady stands allows maximum use of low radiation intensities in the long-term range. At stands with direct access to light, it enables the use of blue and near red radiation. Thus, it leads to the development of a photosynthetic apparatus best adapted to specific environmental conditions (Lichtenthaler *et al.*, 1981; Yamamoto *et al.*, 2008). Light capture depends on the angle of illumination, leaf anatomy and other morphological and physiological traits (Gutschick, 1999; Pearcy and Way, 2012). Few studies indicate how leaf features and trees crown architecture effect on the point of light compensation for whole plants (Baltzer and Thomas, 2007).

In the Ojców National Park *Thymus praecox* subsp. *praecox* occurs, among others on the Maidens' Rocks (SP) located in the south-eastern part of the Park and under the Krukowski's Rock (K) in the north-eastern part of the ONP, where differences in the light intensity, temperature and air humidity were demonstrated (Table 1). These differences are also confirmed by other parameters given from these stands, e.g. the annual total radiation (annual irradiation) - on the Maidens' Rocks (SP) stand it is 3.500-3.600 MJ m^{-2}, and under Krukowski's Rock (K) stand it is estimated at 3.400-3.500 MJ·m^{-2} (Wojkowski and Caputa, 2009). However, it is worth noting that these values are not diametrically different, which confirms the strict attachment of this species to xerothermic habitats. Soil preferences are an additional premise for its - in both stands *T. praecox* occurs on sandy rendzinas (Table 2), rich in calcium (Pawłowski, 1967). Therefore, in the analysed stands *T. praecox* subsp. *praecox* belongs to thermophilic species, typical for xerothermic grasslands, which is why the availability of the right quantity of light is a factor so important for its survival in the natural conditions.

The diversity of morphological and physiological features of *T. praecox* subsp. *praecox* plants growing in different lighting conditions provides information on their degree of acclimatisation in a diverse habitat. Plant morphology depends not only on endogenous growth processes, but primarily on environmental factors

(Barthélémy and Caraglio, 2007). The analysis of *T. praecox* plant masses showed that significantly higher values of these parameters were for plants from the sunny stand (SP), regardless of the measurement period (Table 3). In the literature have already been observed similar relationships. For example, Zang *et al.* (2003) and Mielke and Schaffer (2010) found a decreased in biomass production of plants growing at low light intensity. Low radiation levels lead to an increase in leaf surface area and plant height. Meanwhile, the high radiation intensity is associated with an increase in leaf thickness, by increasing the number of cell layers, or by developing palisade layer and parenchyma tissue. These types of modifications help prevent damage caused by excessive quantity of light reaching the leaves (Matos *et al.*, 2009).

Excess light reduces the content of photosynthetic pigments, which are responsible for its absorption (Wittmann *et al.*, 2001; Pilarski *et al.*, 2012). These reactions are the result of acclimatisation strategies developed by plants for specific conditions (Yang *et al.*, 2007; Mielke and Schaffer, 2010; Możdżeń *et al.*, 2014; Możdżeń, 2019). Acclimatisation involves a change in metabolic processes (including light collection and CO_2 capture), ranging from adjusting leaf morphology to changes in the stoichiometry of the photosynthetic apparatus (Wright *et al.*, 2004; Kono and Terashima, 2014, 2016; Zheng *et al.*, 2014). One of the defence strategies of plants is a significant increase in the number of photosynthetic pigments that act as antennas, absorbing the required light energy (Chen, 2007). They show a higher efficiency of light absorption per unit of leaf biomass, enabling the plant to achieve better carbon balance, while limiting light (Enriquez and Sand-Jensen, 2003; Li and Kubota, 2009). In *T. praecox* subsp. *praecox* studies, the chlorophyll *a* content decreased with increasing of light exposure. The chlorophyll *b* content, which is responsible for transmitting light energy during photosynthesis and obtaining more energy for its effective use, was lower in thymus plants from the sunny stand (SP) (Figure 3). These types of changes contribute to a reduction in the rate of electron transport in the light phase of photosynthesis and an increase in the likelihood of photoinhibition (Maxwell and Johnson, 2000; Miyata *et al.*, 2015).

The low light intensity causes to reduce the thickness of the palisade layer and the parenchyma leaves. As a result, it reduces the photosynthetic activity of plants by reducing energy transport from PSII to PSI (Yao *et al.*, 2017; Yang *et al.*, 2018a; Możdżeń, 2019). One of the main factors in the regulation of photosynthesis and plant response to environmental conditions is chlorophyll *a* fluorescence (Dai *et al.*, 2009). It is used to measure the activity of photosystem II (PSII) and photo-inhibition by determining the potential quantum efficiency under light and shadow (Rascher *et al.*, 2010). *T. praecox* subsp. *praecox* studies showed that plants growing in the sunny stand were characterised by higher PSII photosynthetic activity (Table 4). Despite the high efficiency of excitation energy transfer between pigments in PSII (F_0) antennas, changes in the number of reduced electron acceptors in PSII (F_m) were observed. These types of changes contribute to the dissipation of excitation energy in the form of heat (F_v), and lead to disturbances in the splitting of water, adversely affecting on the maximum photochemical efficiency of plants (F_v/F_m) (Krause and Weis, 1991; Guidi *et al.*, 2007). Other limiting factors may be the dissociation of LHCII proteins from the cortical part of PSII or degradation of the D1 protein and inactivation of reaction centres in PSII (Havaux, 1993; Rintamaki *et al.*, 1995). One of the mechanisms to deal with adverse environmental conditions is to limit the use of excitation energy and transport speed.

The heliophilic nature of *Thymus* species is confirmed by QY_{max} values, which in the sunny stand (SP) were clearly higher than in the shaded stand (K) (Figure 4). These types of plant responses are most likely related to leaf structure and high energy radiation absorption (Van Rensen and Vredenberg, 2011). Plants growing in shadow have a relatively low ability to transport electrons in photosynthesis and to bind CO_2 (Lichtenthaler and Babani, 2004; Schumann *et al.*, 2017). Changes in the F_v/F_m and QY_{max} values effect on the non-photochemical quenching (NPQ) activity. They are generally associated with the relative speed of electron transport and result from the dissipation of excitation energy in the heat form (Maxwell and Johnson, 2000; Proctor and Smirnoff, 2000). According to Proctor (2005) NPQ increases with increasing radiation intensity. However, plants to minimize PSII damage, depending on the species, development phase and stand of occurrence, still shape photo-protective mechanisms and multistage repair cycles (Kirchhoff, 2014). One example of regulating the quantity of light reaching the plants is rearrangement of the position of chloroplasts in the cell (Augustynowicz and Gabryś, 1999). In the case of the Rfd parameter in *T. praecox* subsp. *praecox* studies, in plants from the sunny stand an increase of its value was observed. This type of response indicates increased photosynthetic activity and no disorder of CO_2 assimilation (Lichtenthaler and Rinderle, 1988; Schumann *et al.*, 2017).

Water and ion management plays a significant role in plant response to environmental factors. It is connected, among others with the electrolytes leakage through cell membranes, which are dynamic structures and support numerous biochemical and biophysical reactions. Water-ion balance disorders mainly consist in changing metabolic processes, lowering enzymatic activity, reducing photosynthesis and changing fluidity of membranes, especially in the lipid layer (Campos *et al.*, 2003; Demidchik *et al.*, 2014). These types of changes are often associated with increased membrane permeability, effect on membranes integrity and cell division under stress. In extreme cases, they are irreversible and lead to cell death. The study shows that in *T. praecox* subsp. *praecox* growing in the sunny stand, the electrolytes leakage was significantly lower than in plants growing in the partially shaded stand (Figure 5). Once again, this is related to the fact that this species is a thermophilic taxon and the sunny stands are optimal for it for growth and development. Changes in the electrolyte leakage may be due to membrane damage. However, it should be remembered that the response of plants to stress depends not only on the species, variety, but also on the type and amount of stress factors acting on them (Prasch and Sonnewald, 2015; Możdżeń, 2019).

A thorough knowledge of issues related to the physiological adaptation of *T. praecox* subsp. *praecox* and the functioning of its photosynthetic apparatus positively progresses towards the possibility of growing this species in controlled conditions. This may be an additional safeguard for the existence of its endangered populations in the natural environment. However, at the current stage of knowledge about this species, the most important seems to be ensuring adequate light availability through active protection measures to stop overgrowth of rock complexes. The removal of trees and shrubs and their offshoots that could shadow *T. praecox* subsp. *praecox* populations may provide conditions preferred by this species at its existing stands. The intensity of light is undoubtedly one of the most important environmental factors, deciding not only about the intensity, but also about the possibility of the life processes of this species, and as studies have shown here, it is not a taxa showing high habitat flexibility - even partial shade is not a convenient habitat for it.

Conclusions

These studies confirm the fact that *T. praecox* subsp. *praecox* is an extremely heliophilic taxa and the sunny stands are optimal for it. It is closely related to this type of habitat, characterised by low environmental flexibility - relatively small changes in the availability of light negatively affect its growth and development.
(*i*) Productivity of fresh and dry mass of *Thymus praecox* subsp. *praecox* specimens from the sunny stand was higher regardless of the progress of the growing season, compared to plants from the partially shaded stand; however, the water content in tissues was generally lower in the sunny stand. (*ii*) The chlorophyll *a* and *b* content decreased with increasing light exposure and was significantly lower in the sunny stand, regardless of the progress of the growing season. (*iii*) in May, the minimum (F_0), maximum (F_m) and variable (F_v) fluorescence values were significantly higher in plants in the partially shaded stand; in June and July values of these parameters were higher in thymus plants growing in the sunny stand. (*iv*) The electrolyte leakage through cell membranes of *T. praecox* subsp. *praecox* shoots only in May was higher in plants growing in the sunny stand; in June and July, the outflow of electrolytes was not only lower by half, but definitely lower than for plants growing in the partially shaded stand.

Authors' Contributions

Research concept and design: BB-K and AS-L. Acquisition and/or assembly of data: BB-K and KM. Data analysis and interpretation: BB-K and KM. Drafting the article: BB-K, KM and IT. Critical revision: BB-K, KM and IT. Final approval: BB-K, KM, IT and AS-L. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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