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Selected physiological processes of *Betula ×oycoviensis* Besser in different habitat conditions of the Ojcowska Valley (Southern Poland)

Abstract

For years, the Ojców birch *Betula ×oycoviensis* Besser was considered an endemic species. However, it has turned out that apart from southern Poland it also occurs in other European locations. Nevertheless, it is a rare taxon that is vulnerable to extinction. The experiment aimed to learn about the functioning of the photosynthetic apparatus of Ojców birch and the condition of individuals of this species occurring at two stands in the Ojców National Park with different levels of sunlight. These studies have shown that this birch is a heliophilous taxon and that not very large changes in light intensity do not cause significant environmental stress. However, they induce differences in the content of chlorophyll pigments.

Keywords: birch, chlorophyll, FluorCam, fluorescence, rare species

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Introduction

Plants have developed mechanisms that allow them to adapt to changing light intensity conditions throughout the plant, within the organ, and at the cellular and molecular level (Karpinski et al., 1999; Mullineaux, Karpinski, 2002). Light energy not only determines the synthesis of organic compounds during photosynthesis, but also influences the growth and development of plants in the process of photomorphogenesis (Kraepiel et al., 2001; Możdżeń et al., 2014). Throughout the life cycle, light influences seed germination, stem growth, leaf development, chloroplast formation, chlorophyll synthesis, as well as flowering, fruiting, and aging (Woźny, Jerzy, 2004; Zhang et al., 2013; Muneer et al., 2014; O'Carrigana et al., 2014; Allahverdiyeva et al., 2015). The receivers of light signals are specialised photomorphogenetic pigments: phytochrome, cryptochrome, and receptors of ultraviolet. Thanks to them, plants receive information about quantitative and qualitative changes in the composition of the light spectrum and the time of its exposure. Depending on light conditions and other environmental factors, plants receive varying amounts of light. Absorption of appropriate light causes the induction or inhibition of intracellular signalling pathways that mediate the plant response to a given light source (Frankowski et al., 2001; Liu et al., 2011). All of these complex relationships are extremely important for the functioning of plants and therefore entire ecosystems.

One of the more interesting tree species in the Ojców National Park (ONP) in southern Poland is the Ojców birch *Betula ×oycoviensis* Besser. The species is of hybrid origin, whose parent forms are the silver birch *B. pendula* Roth and the Szafer's birch *B. szaferi* Jent.-Szaf. ex Stasz. It was first found in 1805 in Hamernia near Ojców by Willibald Besser and was described by him in 1809 (Besser, 1809). This *locus classicus* is currently an enclave of the ONP. Initially it was considered an endemic species. However, as a result of later research for this species, its geographical distribution has become better known. As it has turned out, it grows outside southern Poland, also in scattered locations in the Southern part of the Scandinavian Peninsula – in Denmark, Sweden, in Czechia, near Lviv in Ukraine, Kuybyshev in Russia, Austria, and Romania (Staszkiewicz, 2001; Buriánek et al., 2014; Baláš et al., 2016). Some researchers consider it a subspecies of silver birch (*B. pendula* Roth subsp. *oycoviensis* (Besser) Á. et D. Löve). It is a tree growing up to 20 m in height. Morphologically, it is very similar to the common silver birch (Staszkiewicz, 2001; Kříž, 2003). However, it differs significantly from it, among other things, in the number of leaves on a short shoot. Their number in the Ojców birch ranges from 3 to 7, while in the silver birch it is only 1 or 2 (Fig. 1 – Appendix 1). It blooms abundantly in April or May and very early in the 2nd or 3rd year of life.

In Poland, the Ojców birch is a very rare and endangered taxon, that is why it has been included in the *Polish Red Data Book of Plants*… (Zarzycki et al., 2014). It used to grow in nine locations but now it is meet in only five. Its population is estimated at about 250 individuals. The shading of its localities poses a substantial threat to the species of population. It grows best in full sunlight. In the shadow, its localities quickly disappear (Vítámvás et al., 2020). Such a situation occurred in the case of one of the parent forms of this species – Szafer's birch, whose occurrence in the wild has not been documented in Poland since 2001.

The aim of the research undertaken here was to analyse selected physiological parameters (electrolyte leakage from birch leaf cells, chlorophyll *a* and *b* content, chlorophyll *a* fluorescence imaging) related to the functioning of the photosynthetic apparatus of *Betula ×oycoviensis* Besser and the general condition of individuals of this species occurring in different light conditions at two stands in the ONP (Southern Poland).

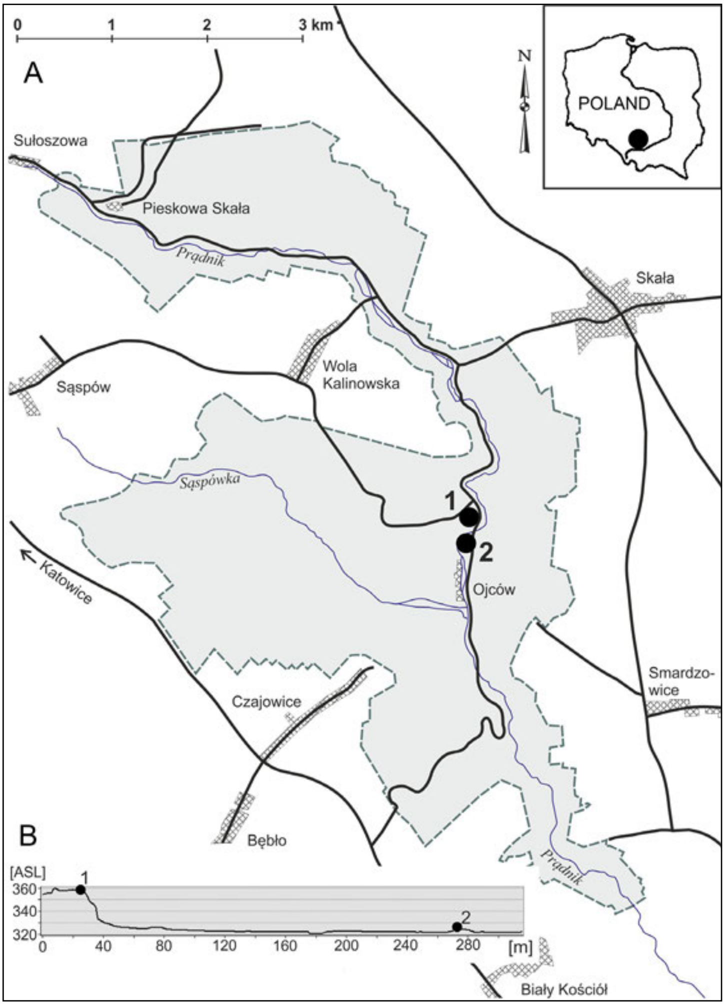


Fig. 2. Collection stands of plant material from leaves of *Betula ×oycoviensis* Besser.; A – boundaries of the Ojców National Park; B – cross-section profile of the Ojców Valley: 1 – Góra Zamkowa, 2 – Park Zamkowy in the Ojców National Park (Southern Poland)

Materials and methods

The plant material consisted of fragments of two-year-old shoots with leaves of the Ojców birch (*Betula ×oycoviensis*), collected from two stands (Fig. 2):

1) Góra Zamkowa (50°12'42.56"N 19°49'49.76"E) in Ojców (360 m above sea level), with relative sunlight ranging from 126 to 135% (Michalik, 1983) – Fig 3A;
2) Park Zamkowy (50°12'34.62"N 19°49'42.20"E), located at an altitude of approx. 324 m above sea level, with relative sunlight ranging from 106 to 115% (Michalik, 1983) – Fig. 3B.

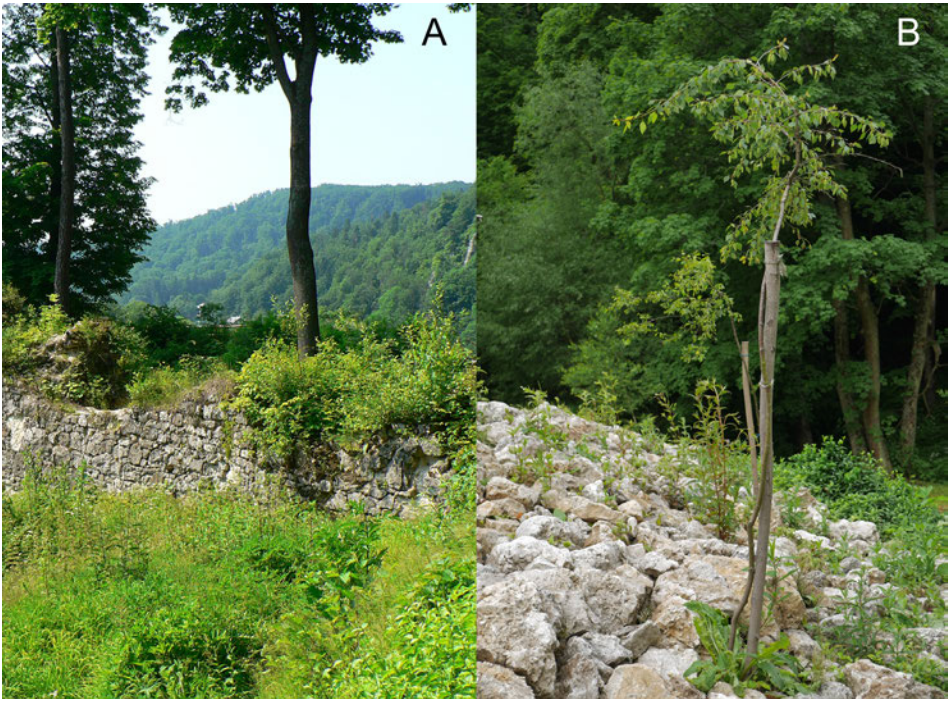


Fig. 3. Research stands in Ojców National Park: A – Góra Zamkowa, B – Park Zamkowy (Photo. A. Sołtys-Lelek)

The electrolyte leakage from birch leaf cells (*B. ×oycoviensis*) growing in the study area was determined using a multifunctional device (*CX-701 Elmetron*, Zabrze, Poland). Each leaf of Ojców birch was placed separately in test falcons filled with 30 ml of deionised water with a conductivity of 0.05 μS.cm^{-1}. The falcons were then shaken for 3 h on a shaker (*Rocker Labnet International*, New York, USA) and on a Votrex (*Biomix BVX-10*, Blizne Jasińskiego, Poland). After this time, the electrical conductivity of the diffusates (L_Z) was measured. After this measurement, the plant material was frozen at –80°C in a freezer (*Scanvac CoolSafeTM PRO 55-4*, Lynge, Denmark) to destroy cells. The material was then thawed and subjected to the same procedure as above. In the next step, total tissue electrolyte content (L_M) was measured. The measurements were performed in 10 repetitions for each of the tested objects. The percentage of electrolyte leakage from plasma membranes was calculated using the formula:

$$EL = (L_Z / L_M) \cdot 100\%.$$

EL – electrolyte leakage

L_Z – electrolyte leakage from live seedlings

L_M – electrolyte leakage from dead seedlings

Chlorophyll *a* and *b* content was determined according to the method of Barnes et al. (1992), at wavelengths: λ=665 and 648 nm, using an Aquarius 9500 spectrophotometer (*Cecil Instruments*, Cambridge, Great Britain). For each of the studied objects, measurements were performed in 5 repetitions. The amount of chlorophyll was converted to concentration in fresh mass, with an accuracy of ±0.01 g.

Chlorophyll *a* fluorescence imaging was performed using FluorCam (*FC 800C, Photon Systems Instruments*, Czechia). In order to extinguish the light phase reaction of photosynthesis, each time *B. ×oycoviensis* leaves were placed on filter paper moistened with distilled water, in the measuring chamber of the fluorimeter for 20 min (Lichtenthaler et al., 2004). We used the method of quenching analysis which consists of three phases: measurement of dark adapted levels F_0, and F_m, measurement of the Kautsky effect and non-photochemical quenching in the light (with actinic and super light exposure). Non-photochemical quenching (NPQ), photochemical quenching (qP) and PSII vitality index (Rfd) were analysed. FluorCam 7 and Microsoft Paint were used to process the results. Due to the destructive nature of the method, measurements were performed on a different leaf of *B. ×oycoviensis* each time.

Statistical analysis

The significance of differences between the objects was tested with a parametric statistical test – one-factor (simple) ANOVA using the Tukey test (HSD) for homogeneous groups ($n = 5$ or $n = 10$) at the level of $p \leq 0.05$. The calculations were performed using Statistica 10.0 for Windows.

Results

Data from the measurements of electrolyte leakage from leaf cells of *Betula ×oycoviensis* growing in the area of the Park Zamkowy and Góra Zamkowa in the ONP showed no statistically significant difference in the values of the tested parameter. The percentage of electrolyte leakage from leaf cell membranes ranged between 7 and 9% with the disorganisation of Ojców birch membrane structures (Tab. 1).

Tab. 1. Electrolyte leakage from leaf cells of specimens of *Betula ×oycoviensis* Besser, growing in the Park Zamkowy and Góra Zamkowa in the Ojców National Park (Southern Poland); different letters represent significant differences ($p \leq 0.05$) according to Tukey's test

Percentage of electrolyte leakage from leaf cells	Stands	
	Góra Zamkowa	**Park Zamkowy**
	7.82^{a} ±0.32	8.39^{a} ±0.29

The determinations of chlorophyll content in the leaves of *B.* ×*oycoviensis* specimens showed statistically significant differences in the values of the tested parameter. The individuals growing in the area of the Góra Zamkowa contained 2.28 mg/g FW of chlorophyll *a*, and from the Park Zamkowy 1.29 mg/g FW. The chlorophyll *b* content in the leaves of *B.* ×*oycoviensis* plants occurring on the Góra Zamkowa in relation to this pigment content in the leaves of plants from the Park Zamkowy area, no statistical differences were found. In general, Ojców birch plants growing in the Góra Zamkowa area in the ONP had a significant higher chlorophyll content (Tab. 2).

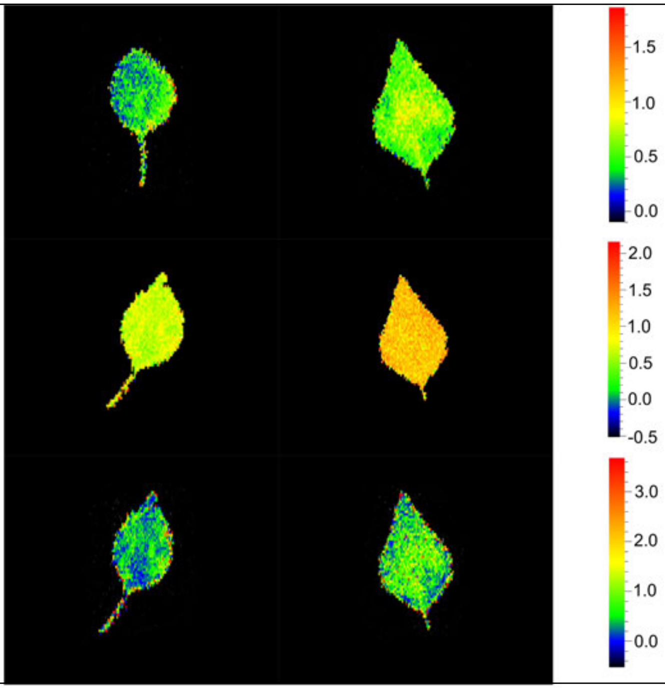
Tab. 2. Chlorophyll content [mg/g FW] in leaves of specimens of *Betula* ×*oycoviensis* Besser, occurring in the area of the Góra Zamkowa and Park Zamkowy in Ojców National Park (Southern Poland); different letters represent significant differences ($p \leq 0.05$) according to Tukey's test

Chlorophyll	**Stands**	
	Góra Zamkowa	**Park Zamkowy**
Chl *a*	$2.28^{a} \pm 0.06$	$1.29^{b} \pm 0.29$
Chl *b*	$0.35^{a} \pm 0.01$	$0.29^{a} \pm 0.03$
Chl *a*+*b*	$2.63^{a} \pm 0.07$	$1.60^{b} \pm 0.28$
Chl *a*/*b*	$6.53^{a} \pm 0.18$	$4.45^{b} \pm 0.10$

Areas of Ojców birch leaves with different fluorescent activity depending on the stands in ONP were identified as a result of chlorophyll *a* fluorescence imaging measurements (Tab. 3–4; Fig. 4 – Appendix 1).

In the case of trees growing in the Góra Zamkowa area with higher light intensity, lower values of all tested parameters were shown in comparison to leaves of *B.* ×*oycoviensis* from the Park Zamkowy (Tab. 4). Non-photochemical quenching (NPQ) reached values from 0 to 0.5 in case of the trees growing in the Zamkowa Góra (shades of blue and green) whereas in case of the trees from the Park Zamkowy the values range was respectively from 0.5 to 1 (shades of green and yellow). The photochemical quenching parameter (qP) had values above 1 in case of the leaves of Ojców birch from the Park Zamkowy area (shades of yellow and orange), and below 0.8 for the plants occurring in the Góra Zamkowa area (shades of yellow and light green). The PSII vitality index (Rfd) values among *B.* ×*oycoviensis* plants occurring in the area of Góra Zamkowa were below 1 (blue) in the majority of the leaf area, compared to the leaves of Ojców birch growing in the area of the Park Zamkowy, for which values of about 1 (green) were recorded (Tab. 3; Fig. 4 – Appendix 1).

Tab. 3. Imaging of chlorophyll *a* fluorescence in leaves of specimens of *Betula ×oycoviensis* Besser, growing in the area of the Góra Zamkowa and Park Zamkowy in Ojców National Park (Southern Poland); non-photochemical quenching (NPQ), photochemical quenching (qP) and PSII vitality index (Rfd)

Parametr	Stands		
	Góra Zamkowa	Park Zamkowy	Scale
NPQ			
qP			
Rfd			

Tab. 4. The comparison of mean values of chlorophyll *a* fluorescence parameters in leaves of *Betula ×oycoviensis* Besser specimens growing in the area of Góra Zamkowa and Park Zamkowy in Ojców National Park (Southern Poland); different letters represent significant differences ($p \leq 0.05$) according to Tukey's test

Parameter	Stands	
	Góra Zamkowa	**Park Zamkowy**
non-photochemical quenching NPQ	0.45^{b} ±0.27	0.59^{a} ±0.16
non-photochemical quenching qP	0.70^{b} ±0.19	1.13^{a} ±0.26
PSII vitality index Rfd	0.71^{b} ±0.56	0.88^{a} ±0.54

Discussion

Changes in the activity of abiotic factors, including light intensity significantly deviating from optimal values, cause "plastic deformations" of plant organisms and changes in the intensity of metabolic processes, disturbing their course, resulting from, for example, damage to cell membranes, denaturation of macromolecules, or inactivation of enzymes. Plastic damage is usually irreversible and in extreme cases leads to the death of the cell or the whole organism (Starck, 2005). Measurements of electrolyte leakage from cell membranes of Ojców birch leaves from the areas of the Góra Zamkowa and Park Zamkowy in the ONP did not show any statistically significant effect of light intensity on changes in protein-lipid membranes. It means that both stands are suitable for the growth and development of this species, and the differences in their sunlight do not cause significant environmental stress. The percentage of disorganization of membranous structures ranged from approximately 7 to 9% of the total electrolyte leakage (Tab. 1). Plants protect plasma membranes from degradation by synthesizing substances that protect protein macromolecules and cell membrane lipids. For this purpose, they induce and inhibit the expression of appropriate genes, protecting cells against secondary damage (Seki et al., 2002; 2003).

The analysis of chlorophyll content in leaves is key information about the physiological responses of plants to environmental light factors. According to Pilarski et al. (2012), the main factor determining the absorption of radiation by leaves is their anatomical structure, degree of hydration, and pigment content. Pettersen et al. 2010 and Gajc-Wolska et al. 2013 found that additional light sources affected the chlorophyll content, e.g. in cucumber (*Cucumis sativus* L.) and tomato (*Lycopersicon esculentum* Mill.) leaves. Mänd et al. (2012) showed differences in the amount of chlorophyll in the leaves of *Populus tremula* L. and *Tilia cordata* Mill., growing at different altitudes in the forests of Estonia. Leaves of *Fagus sylvatica* L. from areas exposed to intense sunlight have a smaller surface area, lower water content, and higher chlorophyll *a*/*b* ratios compared to leaves of plants growing in shaded places (Lichtenthaler et al., 1981). In the case of Ojców birch (*Betula* ×*oycoviensis*), a significantly higher content of chlorophyll *a* was found in the leaves of trees from the Góra Zamkowa area with higher light intensity, compared to the leaves of birches from the Park Zamkowy area with lower light intensity (Tab. 2). The lower amount of chlorophyll *a*/*b* in plants from the Góra Zamkowa area is probably a sign of leaf aging due to the breakdown of chlorophyll molecules and thus the disorganization of PSII functioning (Mittal et al., 2011). In addition, changes in Chl *a*/*b* values are associated with the light-capturing capacity of the LHC proteins (Saldaña et al., 2010).

Light as a heterogeneous factor in correlation with other abiotic environmental factors contributes to the disruption of the balance of physiological processes, including the destabilisation of the photosynthetic apparatus of plants (Valladares, Niinemets, 2008; Bercea et al., 2012; Możdżeń et al., 2014). Plants growing in excessive light intensity have smaller antennae, which partially protect them from photoinhibition. Under light stress conditions, their chlorophyll *a* content, cyclic electron transport activity, and the ability to release oxygen and assimilate CO_2 increase. A large amount of absorbed energy is dissipated as heat. Partial degradation of D1 protein occurs and the pool of plastoquinones decreases. In addition, the impact of toxic oxygen species contributes to the damage of photosystem II (PSII) (Kalaji, Łoboda, 2010; Kalaji et al., 2016). In the case of Ojców birch, among the trees growing in a stand with higher light intensity (Góra Zamkowa), lower values of the tested fluorescence parameters were found (Tab. 3–4). Non-photochemical quenching, illustrating heat losses of absorbed energy, had lower values from 0 to 0.5 in case of the leaves of *B. ×oycoviensis* from the Góra Zamkowa area, and from 0.5 to 1 for leaves of birches growing in the Park Zamkowy area (Tab. 3–4, Fig. 4 – Appendix 1). Kovar et al. (2001) obtained similar results for barley plants *Hordeum* sp., adapted to high and low light intensities. The number of open PSII reaction centres (Maxwell, Johnson, 2000), inferred from the value of the photochemical quenching parameter (qP), was significantly lower among plants from the Góra Zamkowa area with higher light intensity, compared to the birches of the Park Zamkowy. According to Murkowski (2003), increased PAR levels at low temperatures caused a significant reduction in the values of all fluorescence parameters, especially the vitality index (Rfd) of spring rape (*Brassica napus* L.). In the case of Ojców birch, lower values were observed in the plants growing in the Góra Zamkowa in the form of a blue colouration practically on the entire leaf surface (values close to 0), compared to the leaves from the Park Zamkowy, in which higher values were observed with a light green colouration from 0 to 1 (Tab. 3).

In the natural environment, there are no habitats that completely protect plants from stress. The phenomenon of stress can determine the fate of organisms at every stage of their development. The intensity and spectral composition of light-reaching to plants have a fundamental influence on their growth and development. These factors determine the efficiency and possibility of photochemical processes, contain information about the surrounding environment and allow for adaptation to existing conditions (Thery, 2001; Pilarski, 2005; Pilarski et al., 2012; Kalaji et al., 2016). According to Davies et al. (1986), plant acclimatisation is a dynamic process in which plants respond rapidly to changes in their surrounding environment. The physiological measurements of *B. ×oycoviensis* from the Góra Zamkowa and the Park Zamkowy in the ONP showed a significant effect of light intensity on the chlorophyll content and PSII activity. However, both sites are characterised by fairly good lighting conditions. They are thus favourable for the development and growth of plants of this rare species, as evidenced by the statistically insignificant result of electrolyte leakage from cell membranes (Tab. 1) as an indicator of environmental stress. This is very important because the natural regeneration of the studied birch population is very slow.

To strengthen the population of *B. ×oycoviensis* in the Kraków-Częstochowa Upland (Southern Poland), six specimens of Ojców birch were introduced to the ONP in 2012. It was planted in places often visited by tourists, among others, at the viewing point on the Skała Jonaszówka and on the educational rockery behind the "Hotel pod

Łokietkiem" building in Ojców. In Czechia, research was recently conducted into the potential of *B. ×oycoviensis* for micropropagation, grafting, and propagation through cuttings. The results of this experiment indicated that *in vitro* propagation and grafting protocols may be used to successfully mass-reproduce this birch, although these processes are genotype-dependent responses (Vítámvás et al., 2020). All such treatments and experiments are aimed at one thing – maintaining and protecting *B. ×oycoviensis* as an important component of biodiversity at all levels.

Conflict of interest

The authors declare no conflict of interest related to this article.

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Appendix 1

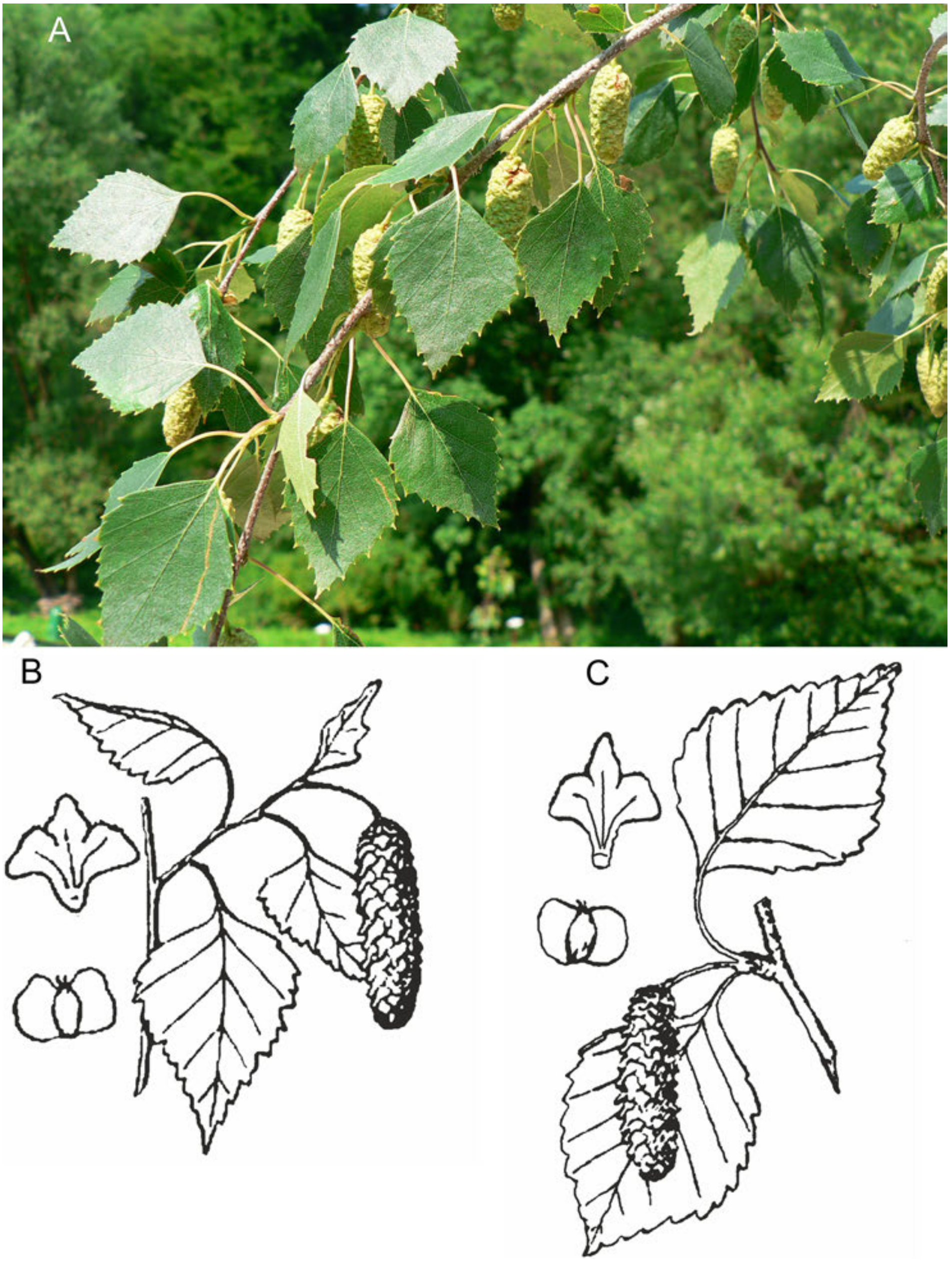

Fig. 1. *Betula ×oycoviensis* Besser: fragment of a leafy twig with catkins A (Photo. A. Sołtys-Lelek); leafy short shoot, scale, nutlet B; *Betula pendula* Roth: leafy short shoot, scale, nutlet C (after J. Szaferowa 1933 – modified)

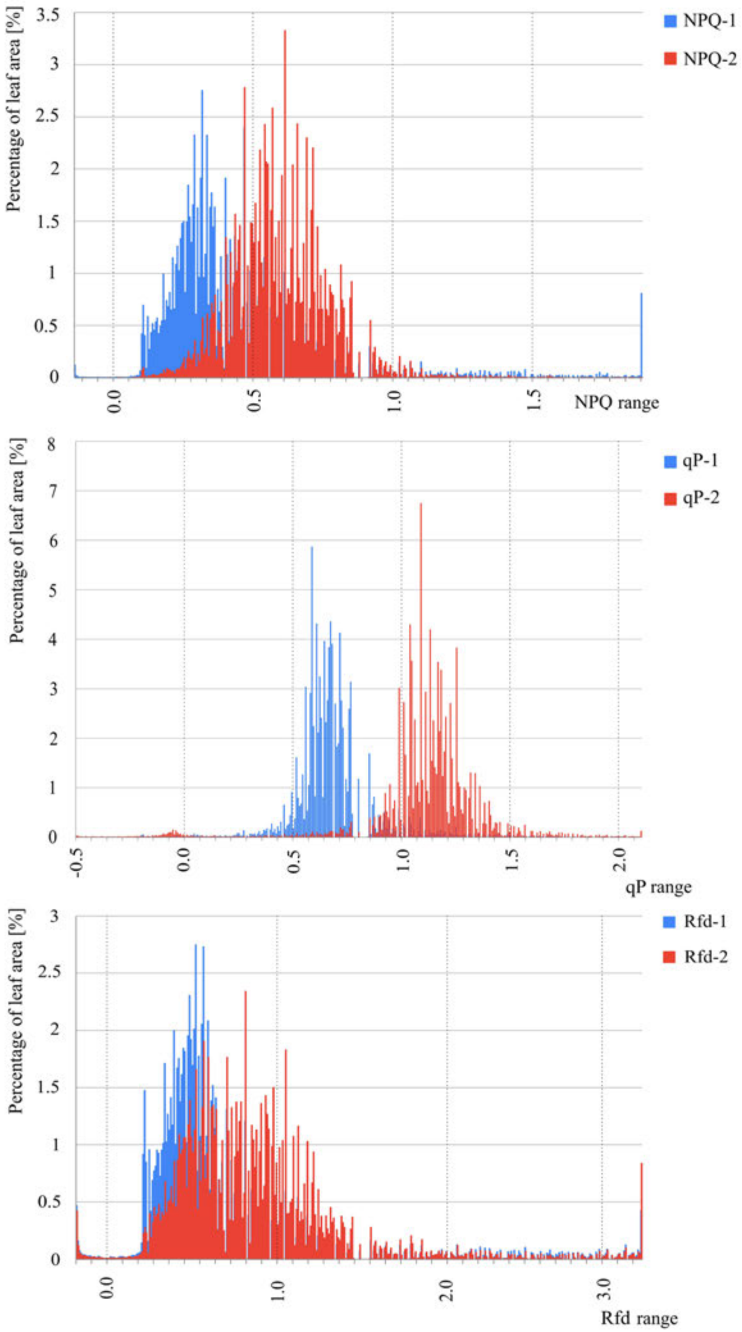

Fig. 4. Percentage comparison of chlorophyll *a* fluorescence parameter values (non-photochemical quenching NPQ, photochemical quenching qP and PSII vitality index Rfd), recorded on the leaf surface of *Betula ×oycoviensis* Besser collected from the analysed stands: Góra Zamkowa (NPQ-1, qP-1, Rfd-1), Park Zamkowy (NPQ-2, qP-2, Rfd-2)

Wpływ zróżnicowanych warunków siedliskowych w Ojcowskim Parku Narodowym (Południowa Polska) na wybrane procesy fizjologiczne *Betula ×oycoviensis* Besser

Streszczenie

W Polsce brzoza ojcowska *Betula ×oycoviensis* Besser jest taksonem bardzo rzadkim. Rośnie najlepiej w warunkach pełnego nasłonecznienia. Celem eksperymentu była analiza wybranych parametrów fizjologicznych związanych z funkcjonowaniem aparatu fotosyntetycznego *Betula ×oycoviensis* Besser i ogólną kondycją osobników tego gatunku, występujących w zróżnicowanych warunkach siedliskowych na dwóch stanowiskach w Ojcowskim Parku Narodowym (Południowa Polska). Wśród okazów brzozy ojcowskiej rosnących na stanowisku o większym natężeniu światła (Góra Zamkowa) stwierdzono istotnie niższe wartości badanych parametrów fluorescencji (NPQ, qP i Rfd) chlorofilu *a*. Generalnie przeprowadzone pomiary fizjologiczne wykonane dla tego taksonu z terenu Góry i Parku Zamkowego wykazały istotny wpływ natężenia światła na zawartość chlorofilu i aktywność PSII. Jednak obydwa stanowiska stwarzają dość dobre warunki oświetlenia, a tym samym rozwoju i wzrostu tego rzadkiego gatunku.

Słowa kluczowe: brzoza, chlorofil, FluorCam, fluorescencja, rzadki gatunek

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