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Original Article

Biological Traits of *Impatiens parviflora* DC. under Different Habitat Conditions

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Abstract

Small balsam *Impatiens parviflora* DC. (Balsaminaceae) is the invasive species that colonises natural European forest. The morphological and physiological traits of the species under different natural conditions were analysed. The studies were carried out in the forest communities in the National Park (Ojców National Park – Southern Poland) with the known history of the plot: P1 – bottom of the valley, *Alno-Ulmion* Br.-Bl. et R.Tx. 1943, P2 – terrace of the valley, *Tilio-Carpinetum* Tracz. 1962 *stachyetosum*, P3 – south slope of the valley, *Tilio-Carpinetum* Tracz. 1962 *typicum*. The plots differed with the soil parameters and microclimatic conditions as well as with species richness, species composition and cover of *I. parviflora*. The significant statistical differences in the length of the aboveground parts of collected small balsam specimens on the studied plots were showed. The longest shoots among specimens growing in plot P1, and the shortest in plot P3 were observed. In the underground part of plants, the highest values of water content among the specimens on plot P1 and the lowest on plot P3 were revealed. The highest percentage of electrolytes leakage among the specimens from the plot P2 and the lowest of the plot P1 were observed. The significant differences of the chlorophyll *a* fluorescence of *I. parviflora* leaves on the plot P2 were observed, compared to specimens from the two remaining plots. The results pointed that disturbance light availability in dense forest canopy could influence on abundance the local population of small balsam.

Keywords: Braun-Blanquet method, electrolyte leakage, Ellenberg's indices, environmental conditions, fluorescence, invasive species, protected areas

Abbreviations: Chl *a* – chlorophyll *a*; Chl *b* – chlorophyll *b*; Chl *a* + *b* – total chlorophyll; Chl *a*/*b* – ratio chlorophyll *a* to *b*; ChlF – fluorescence imaging of Chl *a*; FW – fresh weight; *I. parviflora* – *Impatiens parviflora* DC.; LHCII – light harvesting complex of photosystem II; ONP – Ojców National Park; P1 – bottom of the valley, *Alno-Ulmion*; P2 – terrace of the valley, *Tilio-Carpinetum stachyetosum*; P3 – south slope of the valley, *Tilio-Carpinetum typicum*; PSII – photosystem II

Introduction

Impatiens parviflora DC. (small balsam) is an invasive annual herb in Europe originated from mountain areas of central Asia. The species was first observed in the 30-ies of XIX-th century. The first specimens occurring in natural conditions were recorded in 1831 in the neighbourhood of the botanical garden in Geneva (Switzerland), where the species was cultivated (Trepl, 1984), however the exact date of its introduction to Europe is not known. The history of the spread of this species in Europe and individual countries was the subject of many studies (Coombe, 1956; Trepl, 1984; Zając *et al*., 1998; Tokarska-Guzik, 2005; Galera and Sudnik-Wójcikowska, 2010). From the end of the 19th century began its expansion, mainly in forest communities (Trepl, 1984). It grows in diverse plant communities – both disturbed and natural ones (Obidziński and Symonides, 2000; Chmura and Sierka, 2007; Towpasz and Stachurska-Swakoń, 2011; Chmura, 2014), also in the National Parks areas (Łysik, 2008; Sołtys-Lelek and Barabasz-Krasny, 2010). Most easily it invades disturbed forest with depauperate herbal layer. Its habitat requirements have a wide ecological amplitude and seems to be variable in different parts of Europe (Godefroid and Koedam, 2010).

The expansion of *I. parviflora* is connected with its ability to produce huge number of seeds; one plant can yield

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up to 2000, and even up to 10000. The time from germination to flowering is 8-9 weeks while the ripe seeds appear already in the third or fourth week. The seeds are actively thrown under pressure 12 atmospheres up to 3.4 m. They can be also transported by the water flows, the fur of mammals and the ground in the roots of garden plants (Coombe, 1956; Trepl, 1984). The influence of *I. parviflora* on native flora is referred to as significant in terms of reduction other herbaceous forest species in good lighting conditions (Kujawa-Pawlaczyk, 1991). Population densities of the species were found to negatively correlate with herbal layer diversity of forest communities (Obidziński and Symonides, 2000; Chmura, 2014). Probably it has the ability of growth inhibition of other plants, through the secretion of allopathic compounds (Hierro and Callaway, 2003).

I. parviflora was the model species in experimental studies of the impact of light and selected environmental factors (e.g. Evans and Hughes, 1961; Elias and Causton, 1975; Peace and Grubb, 1982; Ugoletti *et al*., 2011; Skalova *et al*., 2013). The studies indicate the significant increase of dry plant weight while good light conditions and if the proper nitrogen and phosphorus fertilisation was supplied, however nitrogen is more important. The quantum efficiency for apparent photosynthesis was almost identical in blue and red light and it appeared to be optimum at 15 °C. Blue light and low temperature resulted in a restriction in the rate of leaves growth. A high nutrient status increased leaves weight ratio (Hughes, 1965). The species showed a marked and rapid response to changes in the photochrome photoequlibrium with low-fluence-rate sources as an answer to low photon influence (Whitelam and Johson, 1982).

The studies on certain physiological processes in response to stress factors give opportunities for a new look at the adaptations of invasive species to natural habitat conditions. The environmental stress is the harmful factor that might destruct the structures of the plant cells membranes, responsible for maintaining integrity and stability of the cells. The degree of their damage stands for evidence of disorganisation of the cells by the action of a stressor and can be easily measured as the percentage of electrolytes leakage from cell membranes (Sutinen *et al*., 1992; Bajji *et al*., 2001). Chlorophyll fluorescence (ChlF) imaging has become one of the most powerful and known tools to track changes in the photosynthetic capacities of plants in response to abiotic and biotic factors in experimental conditions. Pulse-amplitude modulated ChlF techniques provide a non-invasive assessment of the photosystem II (PSII) efficiency to supply electrons to the photosynthetic system (Krause and Weis, 1991; Bresson *et al*., 2015). Light energy absorbed by chlorophyll molecules can undergo one of three competing fates: driving photosynthesis, being dissipated as heat, or being re-emitted as ChlF. These three processes take place in a competitive way and under stress conditions; the photochemistry declines whereas heat dissipation and ChlF emission increase. Chlorophyll content and the ChlF imaging allow for a better understanding of both: the photochemical and non-photochemical processes that take place in the thylakoid membranes. Fluorescence induction kinetics of chlorophyll provides an excellent insight into the amount of energy used by the PSII, and indirectly also by other complexes in the thylakoid membranes, that reflect changes in the efficiency of the photosynthesis (Roháček, 2002; Murchie and Lawson, 2013).

The aim of the study was to investigate the influence of environmental conditions to biological traits of invasive *Impatiens parviflora*. The study was carried in the National Park territory, that allowed for the selection of suitable areas, with a known forest history. The natural lighting and soil conditions were considered as a stress factors and the studied morphological and physiological parameters were: the growth and weight of specimens, electrolyte leakage, chlorophyll content and fluorescence. The co-occurrence of other species on individual plots was also analysed.

Materials and Methods

Study area

The study was carried out in the Ojców National Park (Southern Poland, 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). The three plots 10 m×10 m were selected along the Sąspowska Valley transect (S-N direction, the middle part of the valley) (Fig. 1). The plots differed with abiotic and biotic

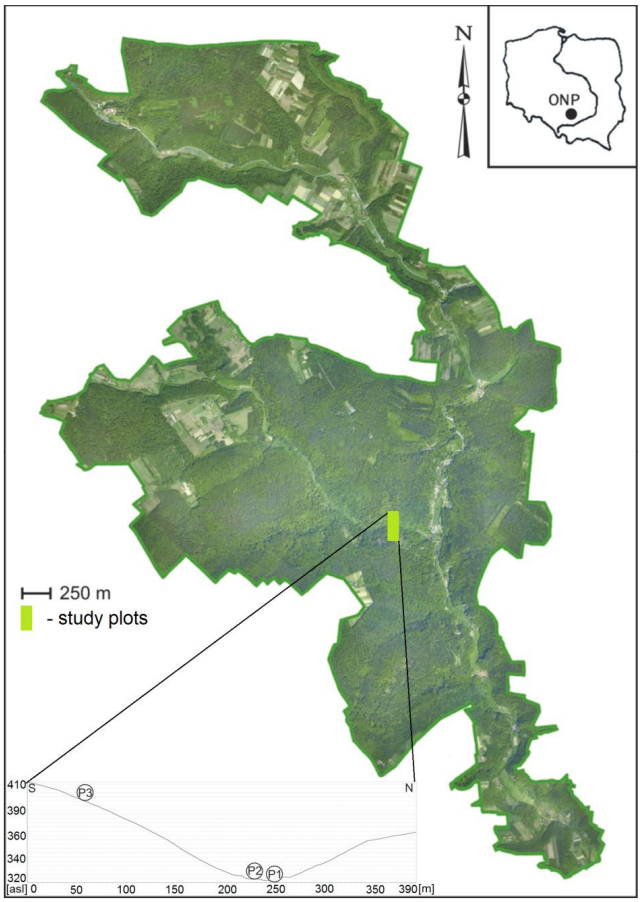

Fig. 1. Localisation of study areas in Ojców National Park; distribution of study plots (P1-P3) in Sąspowska Valley. P1 – bottom of the valley, *Alno-Ulmion*, P2 – terrace of the valley, *Tilio-Carpinetum stachyetosum*, P3 – south slope of the valley, *Tilio-Carpinetum typicum*

conditions: soil type and soil properties, topography, altitude, lighting, air temperature and humidity, plant species composition. The P1 plot was located at the bottom of the valley at the 320 m a.s.l., in the riverside carr of *Alno-Ulmion*. The P2 plot was situated at the foot of the southern slope of the valley, at 330 m a.s.l., in the fertile hornbeam forest of *Tilio-Carpinetum stachyetosum* association. This plot was situated close to the tourist trail. The P3 plot was located at the southern slope of the valley, at 390 m a.s.l., the dry variant of hornbeam forest *Tilio-Carpinetum typicum* association. The study was carried out in the growing seasons 2014 and 2015. The type of forest associations were recognised using the detailed vegetation map of the Ojców National Park (Medwecka-Kornaś and Kornaś, 1963).

Habitat analysis

To estimate the habitat conditions soil analyses were completed for each of the plots at the beginning of the study. The soil type was determined using the standard method of the soil profile description. In order to examine soil properties five random soil samples were collected from every plot. The soil reaction (measured in KCl and water) was determined using Elmetron measuring device (Elmetron, Poland). The soil grain size was determined in the range 80 nm –2 mm with Fritsch laser meter. Percentage of the granular fraction of the soil samples was measured with a Bouyoucosa-Casagrande method with Prószyński modification.

Light intensity (using *LI-189* light meter, Lincoln, USA), relative air humidity and air temperature (*PWT-221* thermo/hygrometer, Elmetron, Poland) were measured in the phenological optimum for *I. parviflora* growth (July in the Ojców National Park). The measurements were performed on a cloudless day before noon, to provide the similar conditions for all plots. All parameters were measured in 10 repetitions on 40 cm above the ground level (average height of herbaceous plants).

Phytosociological method (Braun-Blanquet, 1964) was used to assess the biotic conditions of *I. parviflora* growth. Phytosociological relevés were made three times in every plot: the optimum of the growing season (July 2014), the end of the growing season (September 2014) and the beginning of the growing season (April 2015). The 100 m square plots were characterised by species composition, species richness, and the cover of species, including tree canopy cover. Plant species nomenclature follows Mirek *et al*. (2002).

Ellenberg indicator values were used to estimate species habitat requirements: soil nitrogen availability (N), soil reaction (R), soil moisture (F), and light (L) (Ellenberg *et al*., 1992). Weighted averages of each index were calculated for every plot during seasons.

Plant material

Biometric analysis, dry weight, water content, and electrolyte leakage

Impatiens parviflora individuals were collected from each plot to examine the influence of habitat conditions for its growth and biomass allocation in July of 2014 (the optimum growing season for small balsam in ONP). The biometric analyses were done separately for roots and aboveground shoots. The separated plant parts were weighed as fresh (fresh weight) and after drying at 105 °C (Wamed SUP 100, Poland) for 48 h (dry weight). The percentage of water content was calculated.

The analysis of cell membranes disorganisation was made separately for roots, stems and leaves on independently collected *I. parviflora* individuals. The conductivity measurements were done on both – fresh (L_Z) and macerated (L_M) cellular tissues. Plant material was transferred to polypropylene falcon containing 30 ml of deionised water with a conductivity of 0.05 μS cm^{-1}. Next the tubes were placed on a shaker (Labnet Rocker International, New York, USA) for 3 h and for 5 min on Vortex (Biomix BVX-10, Blizne Jasiński, Poland). After this time, the measurement of electrolytes leakage from cell membranes was made, with the help of MFP (CX-701 Elmetron, Zabrze, Poland). After measuring, the plant material was frozen at -80 °C in order to kill the cells. Subsequently, the material was defrosted and subjected to the same shaking procedure as before, and conductivity of the total electrolytes content of the tissue was measured. The total degree of cell membranes disorganisation was calculated according to the formula $EL = (L_Z/L_M)\times100\%$ (Sutinen *et al*., 1992).

Chlorophyll content and fluorescence

In order to check the activity of photosynthesis system of *I. parviflora*, the content of chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) was measured using the method of Barnes *et al*. (1992). Measurements of chlorophyll fluorescence (ChlF) were performed using a closed fluorometer FluorCam (*Photon Systems Instruments*, Brno, Czech Republic), according to the method of Lichtenthaler *et al*. (2004). In order to quench the reaction of light phase of photosynthesis, the leaves were cut and placed in the measuring chamber on filter paper lightly dampened with water into the darkness for 20 min. Then the parameters: maximum photochemical efficiency of PSII (F_v/F_m), non-photochemical quenching (NPQ), an indicator of the activity of PSII (Rfd), photochemical quenching (qP) were analysed using the FluorCam7-v.1.5.0.46 software.

Data analysis

All measurements of physiological activity of *I. parviflora* were done in 10 replicates. The variances between groups of habitat traits as well as biological traits were tested with Kruskal-Wallis rank test (Statistica 10.0 for Windows software). The significance of the influence of investigated habitat conditions and biological traits was tested with Spearman correlation test. The 0.05 level of probability was accepted as significant.

Species composition and their variability between plots and seasons were analysed with Detrended Correspondence Analysis (DCA, CANOCO software, ter Braak 1991). DCA was made twice: using presence/absence species data and species abundance data. In the last case, Braun-Blanquet cover-abundance scale was transformed by the corresponding cover percentage values (median of each scale interval: 87.5 for 5 in the Braun-Blanquet scale, 62.5 for 4, 37.5 for 3, 17.5 for 2, 5 for 1 and 0.5 for +). Correlations between DCA axes and Ellenberg indexes and species

richness measured for the individual plot were calculated using Kendal's rank correlation coefficient (Statistica 10.0 for Windows software). Correlations were calculated twice: for presence/absence data and for abundance data.

Results

Habitat and biotic analysis

The statistical analysis of the air parameters measured on the cloudless day of July 2014 indicated significant differences between plots (Table 1). The light intensity was the highest at the P2 plot with the value of 83.93 μmol $m^{-2} \cdot s^{-1}$, at the same time, the value of air temperature was the highest here: + 16.02 °C and relative air humidity was the lowest: 80.15%. The lowest value of the light intensity described the P3 plot: 5.60 μmol $m^{-2} \cdot s^{-1}$. The P1 plot was characterised by the lowest air temperature and the highest relative air humidity: + 14.18 °C and 87.84% respectively.

The pH of the soil showed significant differentiation between the plots. The P3 soil reaction was the most acidic (pH_{H2O} = 6.01 and pH_{KCl} = 4.57). The P2 soil reaction was close to neutral (pH_{H2O} = 7.10 and pH_{KCl} = 6.60), while in the case of the P1 plot was clearly alkaline (pH_{H2O} = 8.47 and pH_{KCl} = 7.93) (Table 1).

The soils profiles in the plots represent brown rendzinas derived from Jurassic limestones with different thickness of surface horizon. Silt predominate in the granulometric fraction with marked sand content (Table 1). There are differences among studied plots concerning physical properties of soil. The silt content was between 49.83% (P1) and 61.55% (P3), the sand 31.41% (P3) to 46.88% (P1) and the clay 3.31% (P1) to 7.01% (P3). Those fractions increase sorption capacity of the soil and create favourable conditions for plant growing, especially in the P1 plot.

Detrended Correspondence Analysis pointed at a distinct grouping of plots, however, the plots ordination were more related with their location in the Sąspowska valley relief than to the time of taking the phytosociological relevés (Fig. 2). The light (L), soil humidity (F) and the soil nitrogen (N) were the factors with the biggest influence upon the distribution of the plant species along DCA axes (Fig. 3, Table 2). The species were ordinate from shade-tolerant, nitrophilous, moisture-loving forest plant species (the left side of the diagram) to their opposite (the right side of the diagram). The highest number of shade-tolerant and moisture-loving forest herbaceous plant were in the P1 plot. The species of less demand to light, nitrogen and humidity

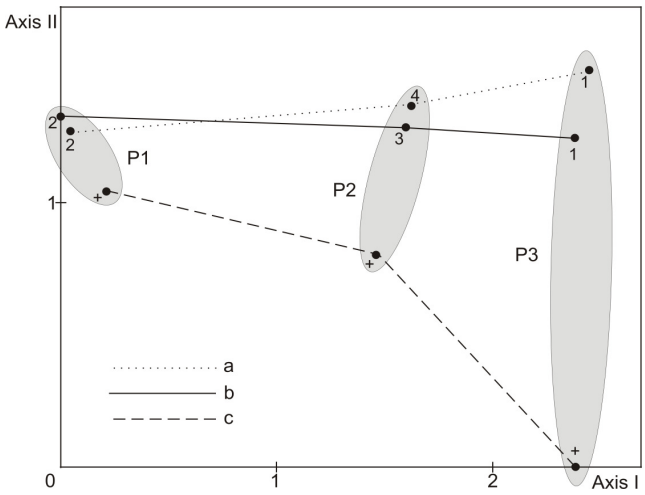

Fig. 2. Plot ordination along the first two axes of DCA based on the species composition. P1 – bottom of the valley, *Alno-Ulmion*, P2 – terrace of the valley, *Tilio-Carpinetum stachyetosum*, P3 – south slope of the valley, *Tilio-Carpinetum typicum*; a – beginning of the growing season (April), b – optimum of the growing season for *Impatiens parviflora* (July), c – the end of the growing season (September); for the each plot are given of cover by *I. parviflora* in the Braun-Blanquet scale

occurred on the P3 plot. The P2 plot is located with the intermediate position in the DCA diagram, at the same time the highest cover of *I. parviflora* was noticed in that plot (Fig. 2).

The location of the plots in different forest associations was clearly reflected in the species composition and cover of small balsam. The highest cover during the optimum of the growing season (July 2014) of *I. parviflora* was recorded on the P2 plot (Fig. 2). The species composition of the P1 plot was dominated by *Urtica dioica* and *Aegopodium podagraria* with an overall cover of herbaceous plants at 95% (total of 31 species in July – Table 1). The cover of plant species on the P2 plot was 90% and *Galium odoratum* was the dominant species besides *I. parviflora* (total of 45 species). The lowest cover of the herbaceous plant with 70% was at the P3 plot in July 2014 and the main role here played *Galeobdolon luteum*, *Mercurialis perennis* and *Galium odoratum* (total of 41 species).

Table 1. Habitat conditions on the plots with *Impatiens parviflora* in the Ojców National Park (southern Poland) during optimum of vegetation season (July 2014)

Parameters		Plot P1	Plot P2	Plot P3
Species richness (100 m^2)		31	45	41
Intensity of light (μmol $m^{-2} \cdot s^{-1}$)		$15.66^{b} \pm 8.65$	$83.93^{a} \pm 9.09$	$5.60^{c} \pm 1.14$
Temperature (°C)		$14.18^{b} \pm 0.12$	$16.02^{a} \pm 0.07$	$14.20^{b} \pm 0.09$
RH – relative air humidity (%)		$87.84^{a} \pm 0.80$	$80.15^{b} \pm 2.41$	$82.66^{b} \pm 2.34$
pH soil	H_2O	$8.47^{a} \pm 0.32$	$7.10^{ab} \pm 0.22$	$6.01^{b} \pm 0.68$
	KCl	$7.93^{a} \pm 0.18$	$6.60^{ab} \pm 0.21$	$4.57^{b} \pm 0.21$
The content of the grainy fractions (%)	2-0.05 mm sand	$46.88^{a} \pm 2.39$	$36.60^{ab} \pm 4.72$	$31.41^{b} \pm 11.17$
	0.05-0.002 mm silt	$49.83^{b} \pm 2.11$	$58.03^{ab} \pm 3.36$	$61.55^{a} \pm 10.46$
	<0.002 mm clay	$3.31^{b} \pm 0.35$	$5.39^{ab} \pm 1.44$	$7.01^{a} \pm 2.10$
Granulometric fractions		LS	SiL	SiL

P1 – bottom of the valley, *Alno-Ulmion*; P2 – terrace of the valley, *Tilio-Carpinetum stachyetosum*; P3 – south slope of the valley, *Tilio-Carpinetum typicum*; LS – loamy sand; SiL – silt loam; average ± standard deviation; a, b, c – statistical significance with Kruskal-Wallis test, P≤0.05

In September 2014, the cover of *I. parviflora* significantly lowered and it occupied only 1% (+ in Braun-Blanquet scale) of the plot area (Fig. 2) while the cover of all herbaceous plant species was still high and amounted 90% on P1, 80% on P2 and 40% on P3 respectively. Among species forming the herbaceous layer in the P1 plot were *Urtica dioica*, *Lamium maculatum*, *Aegopodium podagraria*, *Galeobdolon luteum*. *Galium odoratum* dominated on the P2 and P3 plots.

The highest cover in the spring of next year (April 2015) of *I. parviflora* was recorded on the P2 plot (Fig. 2). At that time, the cover the remaining plants of herbal layer was: 90% on P1, 85% on P2, 85% on P3. The dominant species in the P1 plot were: *Urtica dioica*, *Lamium maculatum*, *Aegopodium podagraria*, *Ficaria verna*, in the P2 plot: *Impatiens parviflora* and *Galium odoratum*, in the P3 plot *Galeobdolon luteum*, *Galium odoratum*, *Mercurialis perennis*, *Asarum europaeum* and *Hepatica nobilis*.

Biometric analysis, dry weight, water content, and electrolyte leakage

Biometric analyses of *I. parviflora* roots showed statistically significant differences in their lengths only between the specimens growing on the P1 and P3 plots. In the case of the length of shoots, the longest ones were from the P1 plot (*Alno-Ulmion*) while the shortest grew on P3 (*Tilio-Carpinetum typicum*) (Table 3). There were significant differences of roots and shoots dry weight. Considering the roots, the highest dry weight was recorded for specimens from the P1 plot – 0.14 g while only 0.04 g from the P2 plot and 0.06 g from the P3 plot. The values of shoot dry weight had a similar pattern: the lowest – 0.15 g from P2 plot, the highest – 0.68 g from P1 (Table 3).

The difference in water content of the roots was statistically significant between the specimens of P3 and P1. The largest amount of water content (92.36%) was recorded in the roots from P1 plot and the smallest amount (85.95%) in roots from P3 plot. The smallest water content for aboveground parts was recorded in individuals from the P2 plot – 92.90% (Table 3).

The analysis of the electrolyte leakage (EL) revealed statistically significant differences in disorganisation of roots and leaves membranes. In the case of the roots: the highest EL was found among plants from P2 plot – 21.69%. The lowest degree was observed among the plant roots from P3 plot – 9.22%. No difference was recorded for the stalks EL. The percentage EL of leaves was found the largest among the plants from P2 plot – 23.91% and the lowest from P1 – 10.49% (Table 3).

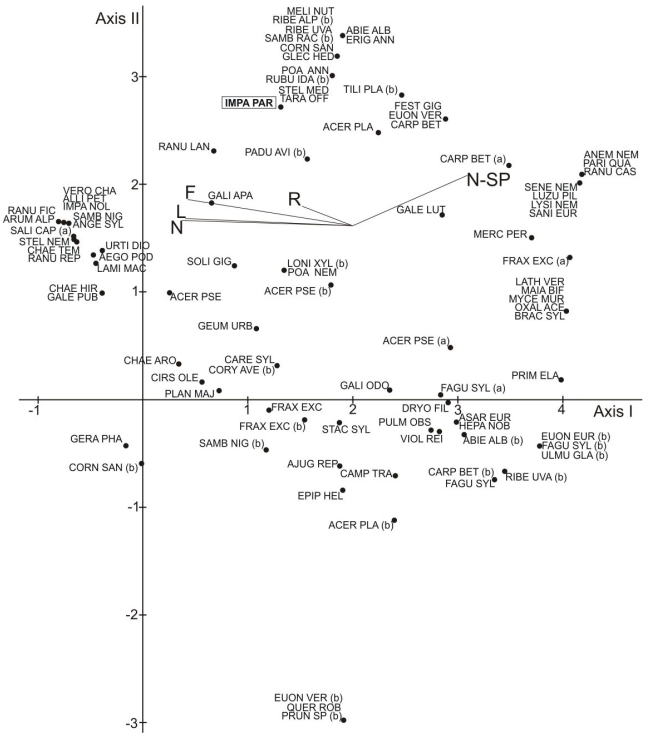

Fig. 3. Species ordination along the first two axes of DCA based on the species composition of plots with *Impatiens parviflora* based on their abundance. Species names are given as acronym of first four letter of genus and of species. Letter a in the end of acronym is for tree layer, b – for shrub layer. Additionally relationship with Ellenberg habitat indices is presented: L – light, F – soil humidity, R – soil reaction, N – soil nitrogen availability; N-SP – species richness

Content of chlorophyll and fluorescence

The spectrophotometric measurement of chlorophyll content in *I. parviflora* leaves revealed the significant statistic differences between plots (Table 4). The lowest content of Chl *a*, Chl *b* and the sum of Chl *a* + *b* was found in the leaves from the P2 plot with the value of 1.25 mg/g FW, 0.39 mg/g FW and 1.64 mg/g FW, respectively. The lowest value of Chl *a/b* was in the P3 plot. The highest values of Chl *a*, Chl *a* + *b* and Chl *a/b* was noticed in the leaves from the P1 plot.

The fluorescence imaging of Chl *a* (ChlF) distinguished the parts of *I. parviflora* leaves susceptible to changes in the PSII activity (Fig. 4). The reduced content of minimum

Table 2. Rank correlation (value of Kendall's τ) between 1st and 2nd DCA axes and ecological indicators of plots with *Impatiens parviflora*

Variables	Quantity		Quality	
	Axis I	Axis II	Axis I	Axis II
Ellenberg indicator values:				
Light (L)	-0.83**	0.06	-0.83**	0.11
Soil moisture (F)	-0.89***	0.11	-0.78**	0.06
Soil reaction (R)	-0.17	0.17	-0.17	0.00
Soil nitrogen (N)	-0.78**	0.00	-0.67*	0.05
Species richness	0.29	0.40	0.51	0.40

Quantity – DCA with species presence/absence; Quality – DCA with cover abundance scale of species. *0.01 < P < 0.05, **0.001 < P< 0.01, ***P < 0.001

Table 3. Biomass, length and electrolyte leakage of *Impatiens parviflora* organs in different habitat conditions in the Ojców National Park

Organ	Parameters		Plot P1	Plot P2	Plot P3
Roots	Length (cm)		$4.60^{b} \pm 0.20$	$5.01^{ab} \pm 0.29$	$5.07^{a} \pm 0.50$
	DM (g)		$0.14^{a} \pm 0.03$	$0.04^{b} \pm 0.01$	$0.06^{ab} \pm 0.02$
	WC (%)		$92.36^{a} \pm 1.46$	$90.11^{ab} \pm 3.51$	$85.95^{b} \pm 2.16$
	EL (%)		$18.83^{ab} \pm 2.37$	$21.69^{a} \pm 2.76$	$9.22^{b} \pm 1.79$
Stems	Length (cm)		$67.28^{a} \pm 8.83$	$30.60^{ab} \pm 7.44$	$22.14^{b} \pm 4.59$
	DM (g)		$0.68^{a} \pm 0.11$	$0.15^{b} \pm 0.03$	$0.17^{b} \pm 0.07$
	WC (%)		$93.48^{ab} \pm 0.88$	$92.90^{b} \pm 0.54$	$93.84^{a} \pm 0.46$
	EL (%)	stalks	$11.48^{a} \pm 1.51$	$9.14^{a} \pm 2.15$	$10.37^{a} \pm 2.40$
		leaves	$10.49^{b} \pm 1.20$	$23.91^{a} \pm 1.32$	$16.03^{ab} \pm 2.73$

DM – dry weight; WC – water content; EL – electrolyte leakage; P1 – bottom of the valley, *Alno-Ulmion*; P2 – terrace of the valley, *Tilio-Carpinetum stachyetosum*; P3 – S slope of the valley, *Tilio-Carpinetum typicum*; average ± standard deviation; a, b, c - statistical significance with Kruskal-Wallis test, $P \leq 0.05$

Table 4. Chlorophyll content [mg / g FW] in leaves of *Impatiens parviflora* in different habitat conditions in the Ojców National Park

Content of chlorophyll	Plot P1	Plot P2	Plot P3
Chl *a* (mg g^{-1} FW)	$1.77^{a} \pm 0.06$	$1.25^{b} \pm 0.02$	$1.55^{ab} \pm 0.14$
Chl *b* (mg g^{-1} FW)	$0.48^{ab} \pm 0.04$	$0.39^{b} \pm 0.02$	$0.60^{a} \pm 0.04$
Chl *a*+*b* (mg g^{-1} FW)	$2.25^{a} \pm 0.01$	$1.64^{b} \pm 0.04$	$2.14^{ab} \pm 0.19$
Chl *a*/*b*	$3.67^{a} \pm 0.30$	$3.25^{ab} \pm 0.17$	$2.58^{b} \pm 0.05$

P1 – bottom of the valley, *Alno-Ulmion*; P2 – terrace of the valley, *Tilio-Carpinetum stachyetosum*; P3 – S slope of the valley, *Tilio-Carpinetum typicum*; average ± standard deviation; a, b, c – statistical significance with Kruskal-Wallis test, $P \leq 0.05$

fluorescence (F_0) was observed in the leaves from the P1 and P2 plots. The largest surface with the reduced F_0 was visible in the leaves: upper part of the leaf blade (P1), upper part of the leaf blade, leaf edges and in the vicinity of leaf venation (P2). The largest changes of maximum fluorescence (F_m) in the leaves of the small balsam with plots P2 and P3 and the smallest with P1 plot were observed. The maximum photochemical efficiency of PSII (F_v/F_m) was high on the whole surface of leaves (except the edge of the leaf blade) from the P1 plot. In the case of specimens of the plot P2, significant growth of F_v/F_m along venation leaves and significant reduction of activity in the vicinity of the margin and the lateral venation leaves were observed – the lack of fluorescence was especially visible in the upper part of leaves (black spot). Non-photochemical quenching (NPQ) achieved the lowest values in the leaves from the P1 plot, especially in the oldest parts of the leaves (the upper part) and along venation. In case of the other two plots (P2 and P3), there was an increase in the value of this parameter in both the marginal zone and the central of leaves. Photochemical quenching (qP) reached the highest values in the leaves from the P1 plot and the lowest values from P2. The similar values of indicator of the PSII vitality (Rfd) in specimens from plots P1 and P3 were recorded. Analysed parameter of small balsam leaves on the plot P2 showed higher activity.

Discussion

In the natural or close to natural conditions, the light is a potent regulator of growth and development of vegetable organisms. The light intensity and irradiance spectrum change during the growing season. It diminishes gradually when going through the forest layers from tree canopy to the bottom of the forest floor (Thery, 2001). In the investigated area of the Ojców National Park (ONP), the highest light intensity was observed in the plot of *Tilio-Carpinetum stachyetosum* (P2) located at the bottom of the

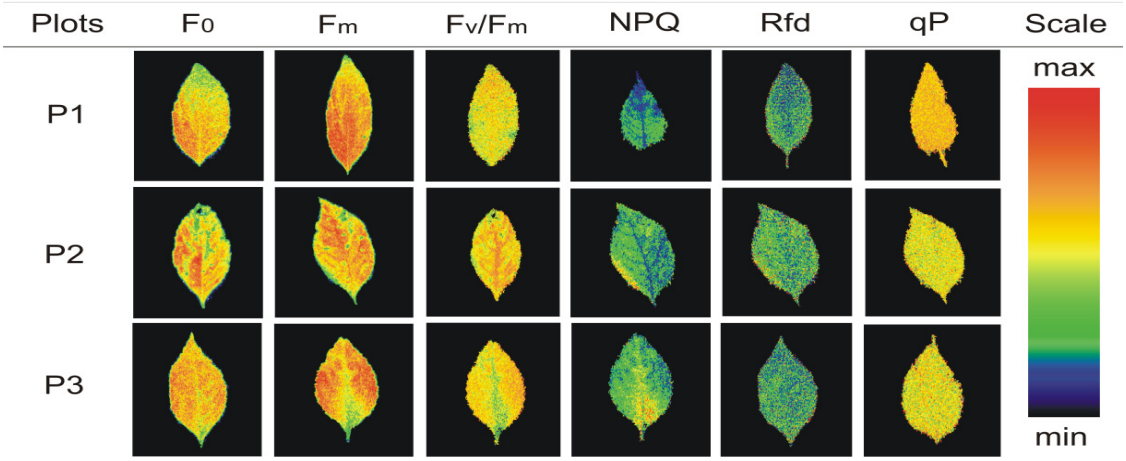

Fig. 4. Imaging chlorophyll fluorescence in the *Impatiens parviflora* leaves collected from three studied areas (1 – *Alno-Ulmion*, 2 – *Tilio-Carpinetum stachyetosum*, 3 – *Tilio-Carpinetum typicum*); F_0 – zero fluorescence, F_m – maximum fluorescence, F_v/F_m – maximum photochemical efficiency of PSII, NPQ – non-photochemical quenching, qP – photochemical quenching, Rfd – an indicator of the activity of PSII

slope where the cover of *I. parviflora* was the highest. This relatively bigger amount of daylight was a consequence of the tourist trail vicinity creating clearances in the tree crowns. It also causes higher temperature on the plot, an average of 2 °C compared to the other plots. The lowest light intensity was recorded on P3 plot, caused by the maximum stand density, that took place during this part of vegetation season (Table 1). However, considering the whole period of vegetation season, the situation seems not to be so clear. The DCA showed that the largest number of heliophilous forest species occurs on the P3 plot which is situated in the highest part of the valley, while the least amount of them grows on P1 plot situated in the bottom of the Sąspowska valley, where numerous shade-loving species appear (Fig. 1-3, Table 2). Many of the heliophilous species from the P3 plot belong to early spring plants, which are in their optimum before the maximum of leafage density, which results in abundance of ground vegetation cover occurring in spring. In this case, the mean Ellenberg values seem to better describe light condition than the measurements of light intensity on the given day of the vegetation season.

The diversity of forest habitat conditions could cause the significant differences in morphological traits of *Impatiens parviflora* (Coombe, 1956; Trepl, 1984). Elemans (2004) pointed that the main factor affecting the biomass distribution and production was light availability. In low light intensity, the increase is usually small, which is associated with low demand for nutrients. The longest stems (statistically significant) of *I. parviflora* was recorded for the specimens growing on the P1 plot in the *Alno-Ulmion* community, where the light intensity was intermediate compared to other plots and relatively air humidity was the highest. Their roots were the shortest ones, but with the highest dry weight (Table 3).

Shallow root system is considered to help avoid root competition and allows developing in the shallow soils (Godefroit and Koedam, 2010). In the P1 plot the plants grew on the place situated at the bottom of the valley, so the factors like: soil properties and high air humidity might have caused the good condition for the growth (Tables 1-3). The species composition of the P1 plot indicated a good amount of the soil nitrogen as the group of nitrophilous species occurred here e.g.: *Urtica dioica*, *Alliaria petiolata*, *Chaerophyllum hirsutum*, *Ch. temulum*, *Sambucus nigra* and other (Fig. 3). Soil fertility definitely has an effect on the value of length, dry weight of roots and stems of *I. parviflora* growing on this plot (Table 3).

The exposure of plot P1 in compare to the P2 and P3 surfaces may also have relevance. Chmura (2014) noticed statistically significant differences in biometry of *I. parviflora* between the specimens growing in southern and northern slopes. The longest specimens grew in northern slope while the shorter ones in southern slope. In the ONP, the P2 and P3 plots are situated in the southern slope, the P1 plot was in the bottom of the valley (the longest stems occurrence). Additionally, comparing to the physiological parameters from all studied plots, the best results were observed for *I. parviflora* specimens from the P1 plot. The values for Chl *a*, Chl *a* + *b* content were the highest and also Chl *a*/*b* (Tables 3-4), as well as the chlorophyll fluorescence parameters (Fig. 4). For example, the ChlF imagine showed that F_m from P1 was characterised by the same activity all over the whole leaf surface, that confirmed either the lack or presence of weak environmental stress factors.

The *I. parviflora* populations growing close to the forest edges are usually larger in size and produce the bigger amount of seeds which stays in contrary to the natural forest habitats, where the plants are smaller and their seeds productivity is lower (Chmura, 2014). The studies carried out in ONP partly confirm this conclusion. The stems of *I. parviflora* from P2 plot in *Tilio-Carpinetum stachyetosum* near the path were longer that specimens from the shaded in July the P3 plot in *Tilio-Carpinetum typicum*. However, comparing to P1 plot of *Alno-Ulmion* the stems were shorter (Table 3). It is interesting that the cover of *I. parviflora* was the highest in the P2 plot and here it was the dominant even that the habitat condition for plant growth was worse than in the P1 plot. At the beginning and optimum of vegetation season small balsam had the largest quantity in the herbal layer (Fig. 2). It is noteworthy, at the same time specimens from P2 had the low chlorophyll content and the largest electrolytes leakage from the leaves and stems cells that points towards the disorganisation of their cells membranes (Table 3-4). Both of the phenomena could be explained by the high daylight intensity observed on this plot in July (Table 1). The negative light influence for the chlorophyll content of shadow-tolerant plants is indicated by the studies e.g. on *Oxalis acetosella*, where the chlorophyll content was 15% lower after the 30 minutes period of its exposition for intensive irradiance (Hoel and Solhaug, 1998). Comparing to plants growing in the shadow, the amount of Chl *a* decreased in leaves of plants growing in sunny areas, due to the dispersion of chloroplasts and not an efficient use of supplied solar energy (Agusti *et al*., 1994). In the ONP, the amount of chlorophyll content was higher in the P1 and P3 plots than the P2 plot where probably the light was the stress factor. The electrolytes leakage from the cells due to damages in their membranes is the plants answer for the environmental stress (Liu *et al*., 2006). Demidchik *et al*. (2014) confirmed that it is mainly connected with the outflow of potassium ions (K^+). The process is often accompanied by accumulation of reactive forms of oxygen which leads to the apoptosis of the cell. In moderate stress conditions, the outflow of potassium ions may essentially influence the initiation of catabolic processes, and to record the energetic changes for the plants adaptation needs. The additional stress factor for the *I. parviflora* in the investigated P2 plot could be air temperature (+ 16 °C July 2014), connected with the light intensity. Elias and Causton (1975) suggest, that + 13 °C is the optimum temperature for the growth and development of *I. parviflora*.

ChlF imaging seems to be an important tool for the early detection of stress on the whole surface of the leaves, which provides fast and precise information about the induced stress. The zero fluorescence (F_0) was similar on leaves from the P1 and P2 plots, and little higher on the whole surfaces of leaves of specimens from P3. According to Murkowski (2005) and Havaux (1993), the high value of F_0 confirms either the lower ability of transmitting energy between the molecules in PSII or the smaller absorption of

energy because of the LHCII defects. In the case of maximum fluorescence (F_m) all the changes confirm the lack of reduction in all electron acceptors in PSII. The changes were visible on the leaves from the P2 and P3 plots.

The maximum photochemical efficiency of PSII (F_v/F_m) for most plants in non-stressed conditions is about 0.83. The low values are connected with the difficulties in electron transport chain and the dysfunction of the potential effectiveness of photochemical reaction in PSII. Comparing plants from all plots the biggest changes F_v/F_m for *I. parviflora* were observed in plants growing on P2. The indicator of the activity of PSII (Rfd) factor informs about the disturbances in the photochemical reaction process in thylakoids and the enzymatic reaction in the chloroplast stroma (Croxdale and Omasa, 1990). The high values of Rfd confirm the high photosynthetic activity, the low ones present the disturbances during the CO_2 assimilation processes. The non-photochemical quenching (NPQ) varies with the changes of light intensity (Robakowski *et al*., 2013). Kovar *et al*. (2001) reported increase the NPQ of barley with simultaneous lowering of photochemical quenching (qP). The similar results were observed for *I. parviflora* in the investigated area.

The essential factor limiting the expansion of *I. parviflora* on the P3 plot in *Tilio-Carpinetum typicum* association seems to be the low light intensity caused by a dense cover of the tree canopy. The specimens of *I. parviflora* collected here contained a significantly smaller percentage of water content in the roots, the shorter stems, and the high electrolytes leakage from the leaves cells (Table 3). They also contained the biggest amount of Chl *b* in leaves (Table 4). The plants cope with light stress by various adaptation and acclimatisation mechanisms. Liu *et al*. (2006) observed the higher chlorophyll content for *Heptacodium miconioides* growing in the conditions of low light intensity compared to plants growing in the highest sun. The similar results were obtained for *I. parviflora* in ONP. The individuals from P1 contained the higher amount of chlorophyll comparing to the plants from P2 plot (Table 4).

Conclusions

On the basis of above considerations the following question could be put: *Does the environmental stress associated with high intensity light stimulate the production of a higher number of seeds, and thus a more frequency of I. parviflora population*? Probably it is true, because on the P2 plot the highest cover of small balsam was observed where the high light intensity as the stress factor caused negative changes in the physiological processes of individuals. Basing on former studies, it has been assumed that the light supported the *I. parviflora* expansion (Kujawa-Pawlaczyk, 1991; Chmura and Sierka, 2006, 2007; Chmura, 2014) but the light was not considered as the stress factor. *I. parviflora* possesses the ability to develop in a variable environment, so it can grow despite the various stress conditions (Peace and Grubb, 1982; Golivets, 2014). The problem of seeds productivity, considering the physiological changes which are the response for stress, requires the further detailed studies, especially in strictly controlled conditions.

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