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Pollen Morphology and Pollen Production of Invasive and Native Impatiens Species Growing in Bulgaria

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Abstract. The purpose of this study was to establish baseline data concerning certain aspects of pollen biology of the invasive alien species *Impatiens balfourii* and *I. glandulifera,* and the native *I. noli-tangere* to the flora of Lozenska Mt. The aims of this study were: 1. To evaluate variation in pollen morphology, pollen production and fertility/sterility in the populations of *I. glandulifera* in order to obtain data for its invasive success and compare with the other two species; 2. To analyze differences in pollen biology in two flower color forms (pink and violet). The pollen morphology was investigated with light microscope and the pollen grains are 4(5)-zonocolpate oblate or peroblate in equatorial view, and rectangular-obtuse or suborbicular in most 4-zonocolpate grains in polar view, with a reticulate ornamentation. The mean pollen production per flower and stamen was very high in all three species comparable with anemophilous plants. The sterile pollen was between 8.9% and 17.54%. The variations in pollen biology can be related to different evolutionary adaptations of the flowers to be more attractive for pollinators and successful in reproduction and distribution. The results are of importance for taking measures to reduce the invasiveness of these alien species.

Key words: Bulgaria, invasive plant, Impatiens, pollen, reproduction.

Introduction

The genus *Impatiens* L. (Balsaminaceae) is the largest in the family, with c. 1000 species, and occurs mainly in the montane regions in the Old World tropics and subtropics (Grey-Wilson, 1980; Christenhusz et al., 2017). In Europe, most *Impatiens* species are neophytes from Asia and North America except *I. noli-tangere* L. (Maslo & Šarić, 2019) and some of them are worst invasive alien species threatening biodiversity in Europe (Petrova et al., 2013). The genus *Impatiens* contains several entomophilous species which vary in their invasive capacity (Perrins et al., 1993; Ugoletti et al., 2013) and represent a significant threat on the reproductive success of native plants. Among them are *Impatiens glandulifera* Royle, commonly known as Himalayan balsam, spread in almost all European countries, including Bulgaria (Petrova et al., 2013), and *I. balfourii* Hook.f.

The invasive species compete with native plants for nutrients, water, light, space and may also exert indirect pressure by competing for pollinators (Chittka & Schürkens, 2001). *Impatiens glandulifera* has been reported to effectively outcompete native species for pollination services in riparian sites and to represent a significant

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threat on the reproductive success of natives (Chittka & Schürkens, 2001; Vervoort et al., 2011, Cawoy et al., 2012). This plant has numerous attractive large size flowers producing large amounts of pollen and high-sugar content nectar (Vervoort et al., 2011; Ugoletti et al., 2013). These characteristics in the biology of the species attract many insects, and as visitation increases, so does the probability of pollen deposition and seed production (Titze, 2000). Studying the biology of invasive plant species is an essential component for understanding plant invasions in introduced regions (Tanner et al., 2014) and for its sustainable utilization. There are several reports in the literature concerning the pollen biology of *Impatiens* species (Emer et al., 2015; Jacquemart et al., 2015; Vinogradova et al., 2020).

Variations in pollen biology (i.e. pollen morphology, pollen production, pollen viability) can be related to different evolutionary adaptations of the flowers to make them more attractive to pollinators. The earliest description of Balsaminaceae (*Impatiens*) pollen morphology was done on light microscopy (LM) observations in several regional pollen morphological studies and in general surveys of the family (Erdtman et al., 1963; Erdtman, 1966; Huynh, 1968; Moore & Webb, 1978; Moore et al., 1991; Faegri & Iversen, 1989). Later, based on scanning electron microscopy (SEM), more precise characteristic of Balsaminaceae pollen morphology was done (Grey-Wilson, 1980; Lu, 1991). The information for separate *Impatiens* species was presented by different authors who accepted pollen aperture, shape, ornamentation, and density of columella in the lumina as the most important characters for distinguishing species (Perveen & Quiser, 2001; Pudjoarianto & Utami, 2002; Beug, 2004; Janssens et al., 2019; Vinogradova et al., 2020; Wang et al., 2020). Such information was provided for the populations of the species from different part of their area, but no data so far are reported for Bulgaria. Pollen morphology was used for classification of the taxa and decisions related to the systematic position in the family Balsaminaceae. A general survey of palynological variation in Balsaminoid Ericales was presented by Janssens et al. (2005) where LM, SEM and TEM results were combined to present a complete pollen description. Palynological characteristics of Balsaminaceae such as small to medium-sized pollen grains with three to four apertures, which can be either colpate or porate, and a sexine sculpturing varying from coarsely reticulate to almost microreticulate is mainly based on the pollen characters of the larger genus *Impatiens*. Despite the young evolutionary age of *Impatiens* and its rapid radiation, a palynological variability could be observed, as the ancestral pollen type in *Impatiens* being a triangular, tri-aperturate pollen grain with reticulate sexine ornamentation has evolved into a 4-aperturate, rectangular pollen grain with the same ornamentation (Janssens et al., 2012).

The production and the dispersal of pollen have both biological and genetical implications for the quality and genetic value of the seed produced (Khanduri & Sharma, 2002). Studies on integration of alien species into native communities emphasize the role of pollinators in dispersal of alien pollen and a "magnet effect" of the bigger and more colorful flowers, high amounts of nectar and pollen production (Chittka & Schürkens, 2001, Vervoort et al. 2011; Emer et al., 2015). The viability of pollen is important for species dispersal, fitness, and survival (Impe et al., 2020). Pollen mass and the nutritional value of pollen per flower are positively related to pollen viability. Conditions that lead to reduced pollen viability, especially early in pollen development, may greatly reduce the rewards to plant pollinators (Yeamans et al., 2014). Katiyar (2012) demonstrated differences in pollen fertility in five different flower color forms varieties of *Impatiens balsamina* from India.

The purpose of this study was to establish baseline data concerning certain aspects of pollen biology of the invasive alien and native *Impatiens* to the flora of Lozenska and Plana mountains. Our sampling represents a part of the geographic and taxonomic diversity in the family on the territory of Bulgaria, mainly Lozenska Mt. and Plana Mt., where three *Impatiens* species are present: the alien species *Impatiens balfourii* and *I. glandulifera* and the native *I. noli-tangere* which are sympatric in some places with the invasive alien species *I. glandulifera*. The aims of this study were: 1. to evaluate variation in pollen morphology, pollen production and fertility/sterility in the populations of *I. glandulifera* in order to obtain useful data for its invasive success and compare with the other two species; 2. to analyze differences in pollen biology in two flower color forms (pink and violet) of *I. glandulifera*.

Material and Methods

Sampling of material

The pollen samples were collected in June 2020 from five different populations of *Impatiens glandulifera* in Lozenska and Plana mountains at a distance of 1-3 km between them (Table 1). Three of them (№ 1, № 3 and №5) are situated at shadow places in riparian communities at the banks of Iskar river, while the other two (№2 and №4) are situated along the roads far from the river on dry and sunny places. In all sampling places, except for № 2 and № 4, the species appear with two different flower color forms (pink and violet). The population of the native for the flora of Lozenska Mt. species *I. noli-tangere* (№ 7*)* is very close to population №5 of *I. glandulifera. Impatiens balfourii* population (№ 6) is isolated from *I. glandulifera* situated under the *Acer pseudoplatanus* L. trees in the village Passarel.

Flowers (20-0) from 10 individuals with same color were collected before opening, placed in plastic bags, transported to the laboratory and separated for analyses. The material was collected and stored as described by Shivanna & Rangaswamy (1992). The voucher specimens of our study are stored in the Herbarium at the University of Sofia (SO).

Pollen morphology

The collected samples for pollen morphological studies were totally ten: eight for *I. glandulifera*, one for *I. balfourii* and one for *I. noli-tangere.* For each population a mixed sample from a minimum of five flowers with the same flower color from different individuals were prepared for light microscopy (LM) analysis. The pollen samples were acetolysed according to Erdtman (1966) and Skvarla (1966). For LM analysis slides were prepared by mounting pollen in glycerol jelly and the observations were made with an Olympus BX-51 (Tokyo, Japan) microscope under E40, 0.65 and oil immersion (E100, 1.25) magnification, using 10× eyepiece. Pollen grains (30) were measured for six features: polar diameter (P), equatorial diameter (E), apocolpium (A), distance between colpi edges in polar view (M), long diameter in polar view (Ld), and short diameter in polar view (Sd). The P/E ratio and Ld/Sd ratio were calculated to determine the pollen shape. The microphotographs were prepared using Zeiss Axiocam ERc5s (Zeiss, Jena, Germany) camera to show the pollen morphology of the species. The pollen terminology in general follows Faegri & Iversen (1989) and Hesse et al. (2009).

Pollen production and fertility / sterility

The analysis of pollen production and pollen fertility/sterility was conducted on the same samples prepared by following Godini (1981). Eighteen anthers from unopened flowers from 6 different individuals (three flowers per individual) per population were placed in separate vials and treated with 1 mL of 0.1% aqueous solution of a detergent and Alexander stain (Alexander, 1969) in the ratio 9 : 1. The Alexander stain is used for differential staining of aborted and non-aborted pollen (grains stained in red are considered fertile). A Fuchs-Rosenthal haemocytometer was used to count pollen grains and calculate pollen production following procedure described by Pavlova & Bani (2019).

Statistical analysis

Univariate and multivariate statistical procedures were applied to examine variation among the *Impatiens* populations. Data for pollen production and fertility/sterility were analyzed using analysis of variance (ANOVA) and the means were statistically grouped by Tukey's (HSD, honestly significant difference) test ($P < 0.05$). Means and standard deviations were calculated for measured pollen characters, pollen production and fertility/sterility.

Pearson correlations between flower pollen production, fertility and pollen morphological characters P, E, A, M, Ld, and Sd as variables were performed. Correlations among pollen production and fertile/sterile pollen were statistically evaluated by calculating the correlation coefficients (r) at different P-values. The results were considered significant at $P < 0.05$.

Cluster analysis using Euclidean distances and unweighted pair group average (UPGMA) was also used to express the similarities between studied populations of *Impatiens* species based on mean values of all measured pollen characters, pollen production and pollen fertility/sterility. Statistical analyses were carried out using Statistica 7.0 program (Statsoft Inc., Tulsa, OK, USA).

Results

Pollen morphology

Pollen description is presented for each species based on light microscopy (LM) observations and measurements of six characters. A summary of the results are presented in Table 2. *Impatiens glandulifera* (Fig.1A-E) pollen grains are 4(5)-zonocolpate, oblate or peroblate (P/E 0.42-0.6) in equatorial view and rectangular-obtuse or suborbicular in most 4-colpate grains in polar view (Ld/Sd 1.93-2.8), mean dimensions P × E = 16.5–18.54 × 34.88–39.38μm and Ld × Sd = 35.14–41.2 × 26.04–30.4 μm. The apertures are simple and consist only of an ectocolpus. The ectocolpi are very narrow. The colpus margin is uneven, the colpus membrane is usually invisible covered by the margins of the colpus. The exine is 1-2 μm thick. The ornamentation is reticulate with difference in shape of lumina (0.5-2.5 μm). The number and size of the granules inside the lumina are variable. The granules are solitary or fused in clusters of two or more. The populations of *I. glandulifera* can be divided into two groups (small and medium) considering the dimensions of the pollen grains, their shape in equatorial and polar view. The smallest pollen grains of *I. glandulifera* were observed in populations №2 and №4, both having pink flowers and differentiated from all other studied populations.

Impatiens balfourii (Fig. 1F-J) pollen grains are 4-zonocolpate, peroblate (P/E 0.34–0.48) in equatorial view and rectangular-obtuse in polar view (Ld/Sd 1.96–3.1), mean dimensions P × E = 15–22.5 × 40.5–49.5 μm and Ld × Sd = 39.5–52.5 × 19.5–34.5 μm. Ectocolpi are very short and narrow, not clearly visible. The exine is 1.5–3 μm thick. The ornamentation is reticulate with larger lumina and sometimes coalescences between adjacent lumina are visible caused by an incomplete surrounding by the muri (Fig. 1J). *Impatiens balfourii* pollen grains are morphologically well distinguished from the other two species because of the longer equatorial diameter (E), shortest and very narrow colpi and larger lumina. The density of the lumina is smaller compared to the other two species. Moreover, 5-zonocolpate pollen grains were not found this species.

Impatiens noli-tangere (Fig. 1 K-O) pollen grains are also 4-zonocolpate oblate or peroblate (P/E 0.39–0.63) in equatorial view and rectangular-obtuse in polar view (Ld/Sd 1.68–3), mean dimensions P × E = 15–25.5 × 37.5–46.5 μm and Ld × Sd = 35.5–49.5 × 21–36 μm. The ectocolpi are shorter compared to *I. glandulifera* and slightly wider with uneven margin. In LM observation in polar view margo is clearly visible around the ectocolpi area (Fig. 1K). It is differentiated from the rest of the sexine most probably by the ornamentation thickness which appears as a main difference from the other two species. The exine is up to 2 µm thick. The ornamentation is reticulate with larger lumina and more granules inside compared to *I. glandulifera*. The pollen grains of the only native to the flora of Bulgaria species *I. noli-tangere* are closer to *I. glandulifera* than to *I. balfourii* based on their dimensions, shape in polar and equatorial views and ornamentation. Similarly to the other species crystals of calcium oxalate (raphide) were found in all analyzed samples.

Table 1. List of the localities of the studied populations of *Impatiens* species.

Population №	Species	Locality	Site code	Geographical coordinates	Altitude [m]
1	*I. glandulifera*	Sredna gora floristic Region, Lozenska Mt., near the "Devil's Bridge"; soil type: Fluvisols (WBR, 2014), part of hygrophyte grass community.	Lz 1	42°34'58.674''N 23°25'39.212''E	650
2	*I. glandulifera*	Vitosha Mt. floristic Region, Plana Mt., near village Dolni Okol, soil type: Fluvisols (WBR, 2014), part of hygrophyte grass community.	Pl 2	42°30'27.873''N 23°30'29.057''E.	880
3	*I. glandulifera*	Sredna gora floristic Region, Lozenska Mt., near the "Fallen tree bridge" on the left bank of the Iskar River, soil type: Fluvisols (WBR, 2014), part of hygrophyte grass community.	Lz 3	42°34'5.006''N 23°25'42.95''E	610
4	*I. glandulifera*	Sredna gora floristic Region, Lozenska Mt., along the road Sofia -Samokov, dry open terrain, soil type: Leptosols (WBR 2014), part of ruderal grass community.	Lz 4	42°34'06.8"N 23°25'50.1"E	680
5	*I. glandulifera*	Sredna gora floristic Region, Lozenska Mt., screes on the right bank of the Iskar River; initial stage of soil formation, community of *Fagus sylvatica* L. close to the screes.	Lz 5	42°34'2.076''N 23°25'48.594''E.	630
6	*I. balfourii*	Sredna gora floristic Region, Lozenska Mt., Passarel village soil type: Leptosols (WBR 2014), with partial shade under the *Acer pseudoplatanus* trees.	Lz 6	42°32'29.0"N 23°29'52.9"E	700
7	*I. noli-tangere*	Sredna gora floristic Region, Lozenska Mt., soil type: Fluvisols (WBR, 2014), part of community *Alnus glutinosa and Alnus incana* community on the right bank of the Iskar River.	Lz 7	42°34'02.1"N 23°25'48.6"E	615

Table 2. Pollen morphological data of the examined populations of *Impatiens* species with measurements (µm) for each flower form with mean (in brackets) ± standard deviation and ranges of the pollen characters: polar (P) and equatorial (E) diameter, apocolpium (A), long distance between colpi in polar view (M), long diameter in polar view (Ld), short diameter in polar view (Sd), index P/E, and index Ld/Sd. Site codes correspond to data in Table 1. Abb. pink flower (p); violet flower (v).

Species	Site code	P	E	A	M	P/E	Ld	Sd	Ld/Sd
I. glandulifera	Lz 1p	16.5-19.5 (18.59±0.7)	33-39.8 (37.2±1.36)	22.5-30 (26.46±1.6)	18-28 (22.71±1.49)	0.5-0.6 (0.5±0.03)	33-40.5 (37.1±1.36)	25.5-31.5 (28.77±1.23)	1.7-2.3 (1.94±0.09)
	Lz 1v	16.5-22.5 (18.54±0.59)	34.5-42.0 (38.2±1.17)	24-37.5 (37.5±1.96)	18-28.5 (24.54±1.66)	0.4-0.6 (0.6±0.04)	36-42.0 (38.7±1.03)	24-30 (28.61±1.09)	1.8-2.4 (2.03±0.09)
I. glandulifera	Pl 2p	13.5-19.5 (16.5±0.82)	31.5-37.5 (34.88±1.24)	19.5-28.5 (25.55±1.29)	15-24 (20.57±1.41)	0.39-0.59 (0.47±0.05)	30-39 (35.46±1.52)	24-42 (27.7±2,3)	1.28-2.2 (1.94±0.15)
I. glandulifera	Lz 3p	15-22.5 (17.36±1.17)	31.5-43.5 (39.38±1.46)	22.5-31.5 (27.96±1.39)	19.5-28.5 (24.27±1.7)	0.34-0.62 (0.44±0.03)	34.5-43 (39.21±1.41)	24-30 (27.16±1.17)	1.8-2.6 (2.18±0.14)
	Lz 3v	15-19.5 (17.41±0.88)	34.5-45 (38.2±1.5)	15-30 (26.46±1.91)	12-27 (21.75±1.88)	0.4-0.52 (0.46±0.03)	30-39 (37.07±1.33)	25.5-33 (28.93±1.08)	1.67-2.16 (1.93±0.09)
I. glandulifera	Lz 4p	15-19.5 (16.77±0.94)	30-40.5 (35.41±1.75)	22.5-30 (25.88±1.29)	16.5-25.5 (20.89±1.33)	0.4-0.59 (0.48±0.04)	30-40.5 (35.14±1.64)	22.5-28,5 (26.04±1,06)	1,66-2,4 (2.03-0.13)
I. glandulifera	Lz 5p	16.5-19.5 (17.7±0.59)	36-45.5 (38.9±0.86)	22.5-33 (28.8±1.41)	21-30 (24.4±1.26)	0.41-0.52 (0.45±0.02)	36-48 (41.2±1.73)	25.5-36 (30.4±2.12)	1.75-2.45 (2±0.1)
	Lz 5v	15-18 (16.7±0.7)	34.5-40.5 (37.39±0.94)	22.5-31.5 (26.89±1.36)	18-28.5 (22.88±1.6)	0.37-0.52 (0.45±0.03)	30-40.5 (37.23±1.36)	22.5-30 (26.03±1.06)	1.81-2.5 (2.15±0.11)
I. balfourii	Lz 6	15-22.5 (19.23±0.92)	40.5-49.5 (45.99±1.31	30-37.5 (34.13±1.53)	34.5-42 (38.04±1.47)	0.34-0.48 (0.42±0.03)	39-52.5 (45.71±2.01)	19.5-34.5 (28.29±1.95)	1.96-3.1 (2.44±0.17)
I. noli-tangere	Lz 7	15-25.5 (20.19±1.38)	37.5-46.5 (41.36±1.48)	18-36 (28.55±2.06)	18-30 (24.43±1.66)	0.39-0.63 (0.49±0.05)	34.5-49.5 (42.08±2.08)	21-36 (29.87±2.3)	1.68-3 (2.13±0.14)

Pollen production and fertility/sterility

The pollen production per flower and stamen, and fertility/sterility percentage for studied *Impatiens* species and populations was calculated and the mean values are presented in Table 3. The lowest flower pollen production-was calculated for *I. noli-tangere* and the highest was calculated for *I. glanfulifera* population №4. The mean flower pollen production of *I. balfourii* was also low (mean 197639) and not significantly different from FPP of *I. noli-tangere* (mean 194444). All studied populations of the invasive alien species *I. glandulifera* show higher FPP compared to the other two species and flower pollen production varied between 772613 and 1350000 pollen grains. The lowest flower pollen production was recorded in population №5 and the highest in population №4. The flower pollen production was higher in flowers with pink color compared to violet flowers of the same population and between populations. The analysis of variance (ANOVA) performed on the flower pollen production show violet flowers from population №5 was statistically different from flower pollen production for the pink flowers from populations №№2, 3 and 4 (F = 4.637, P = 0.00017). Variation of pollen production was found for the flowers of the same individual as well. Statistically differences were proved by the post-hoc test (Tukey's HSD) (Table 3).

The percentage of fertile pollen varies between 82.49% and 91.09% (Table 3). The sterile pollen is above 5%, a limit considered as a normal abortion (Mičieta & Murin 1996), and is between 8.91% and 17.54%. The analysis of variance (ANOVA) performed on the percentages of the fertile and sterile pollen (Table 3) and proved by the post-hoc test (Tukey's HSD) demonstrated they were not significantly different (P = 0.05).

Correlations between variables such as flower pollen production and pollen morphological characters P, E, A, M, Ld, and Sd ($^{**}P< 0.01$; $^{*}P<0.05$, n=10) were calculated (Table 4). Positive correlation coefficients were found between polar diameter (P) and equatorial diameter (E), long and short diameters (r=0.72, r=0.74, and r=0.63, respectively, P<0.05); equatorial diameter (E) and apocolpium (A), distance between colpi edges in polar view (M), and long diameter in polar view (Ld) (r=0.69, r=0.94, and r=0.96, respectively, P<0.05 and P<0.01); apocolpium and distance between colpi edges in polar view (M) from one hand and with distance between colpi edges in polar view (Ld) from the other (r=0.80, and r=0.68, respectively, *P*<0.01 and *P*<0.05). Correlation between M and Ld were statistically significant and positive as well. Significant negative correlations (*P*<0.05 and *P*<0.01) were found only between flower pollen production and all measured pollen characters except apocolpium (A) and pollen short diameter in polar view (Sd). Pollen fertility/sterility as a variable does not show significant correlations with all other variables and is excluded from Table 4.

The relations between species populations based on pollen morphological characters, flower pollen production and fertility/sterility were examined using cluster analysis (Fig. 2). The populations of *I. balfourii* and *I noli-tangere* demonstrate the shortest Euclidean distance and they were separated from *I. glandulifera* populations. Very close to each other were populations № 2, 3 and 4 of *I. glandulifera*, all with pink color of their flowers. The individuals with violet flowers from population №3 were separated from them because of significant differences in pollen fertility and Ld:Sd ratio. The populations № 2, 3 and 4 are clustered separately from populations №1 and 5 at linkage distance 100000. The last two populations are separated from each other based on the pollen characters, mainly by the polar diameter (P).

Table 3. Mean pollen production, fertile and sterile pollen ± standard deviation of three replicates for each flower form of the *Impatiens glandulifera* populations, *I. balfourii,* and *I. noli-tangere*. Significant differences (based on one-way ANOVA and Tukey HDS-tests) are indicated with different letters, *P* < 0.05. Site codes correspond to data in Table 1. Abb. pink flower (p); violet flower (v).

Site Code	**Pollen production**		**Fertile pollen (%)**	**Sterile pollen (%)**
	flower	**stamen**		
		Impatiens glandulifera		
Lz 1p	1022951 ± 82405abc	204590 ± 16481abc	89.3 ± 4.53a	10.7 ± 4.53a
Lz 1v	997413 ± 245688abc	199483 ± 49138abc	85.82± 10.39a	14.18± 10.39a
Pl 2p	1358602 ± 469551a	271720 ± 93910a	87.95± 9.57a	12.05 ± 9.57a
Lz 3p	1350000 ± 126997a	270000 ± 25399a	88.27 ± 5.53a	11.73 ± 5.53a
Lz 3v	1239392 ± 227536ac	247878 ± 45507ac	82.46 ± 3.87a	17.54 ± 3.87a
Lz 4p	1368637 ± 378471a	273727 ± 75694a	91.09± 1.58a	8.91± 1.58a
Lz 5p	885677 ± 79850bc	177175 ± 15970bc	88.6 ± 4.1a	11.4 ± 4.1a
Lz 5v	772613 ± 181566b	154523± 36313b	82.49 ± 6.89a	17.47 ± 6.89a
		Impatiens balfourii		
Lz 6	197639 ± 53351d	39528 ± 10670d	87.59 ± 5.85a	12.41 ± 5.85a
		Impatiens noli-tangere		
Lz 7	194444 ± 62765d	38889 ± 12553d	88.45 ± 7.23a	11.55 ± 7.23a

Table 4. Pearson correlation coefficients between measured flower pollen production (FPP) and pollen morphological characters P, E, A, M, Ld, and Sd (***P* < 0.01;**P* < 0.05, n=10).

Variable	**FPP**	**P**	**E**	**A**	**M**	**Ld**	**Sd**
FPP	ns	-0,79**	-0,81**	ns	-0,72*	-0,83**	ns
P	-0.79**	ns	0,72*	ns	ns	0,74*	0,63*
E	-0,81**	0,72*	ns	0,69**	0,94**	0,96**	ns
A	ns	ns	0,69*	ns	0,80**	0,68*	ns
M	-0,72*	ns	0,94**	0,80**	ns	0,91**	ns
Ld	-0,83**	0,74*	0,96**	0,68*	0,91**	ns	ns
Sd	ns	0,63*	ns	ns	ns	ns	ns

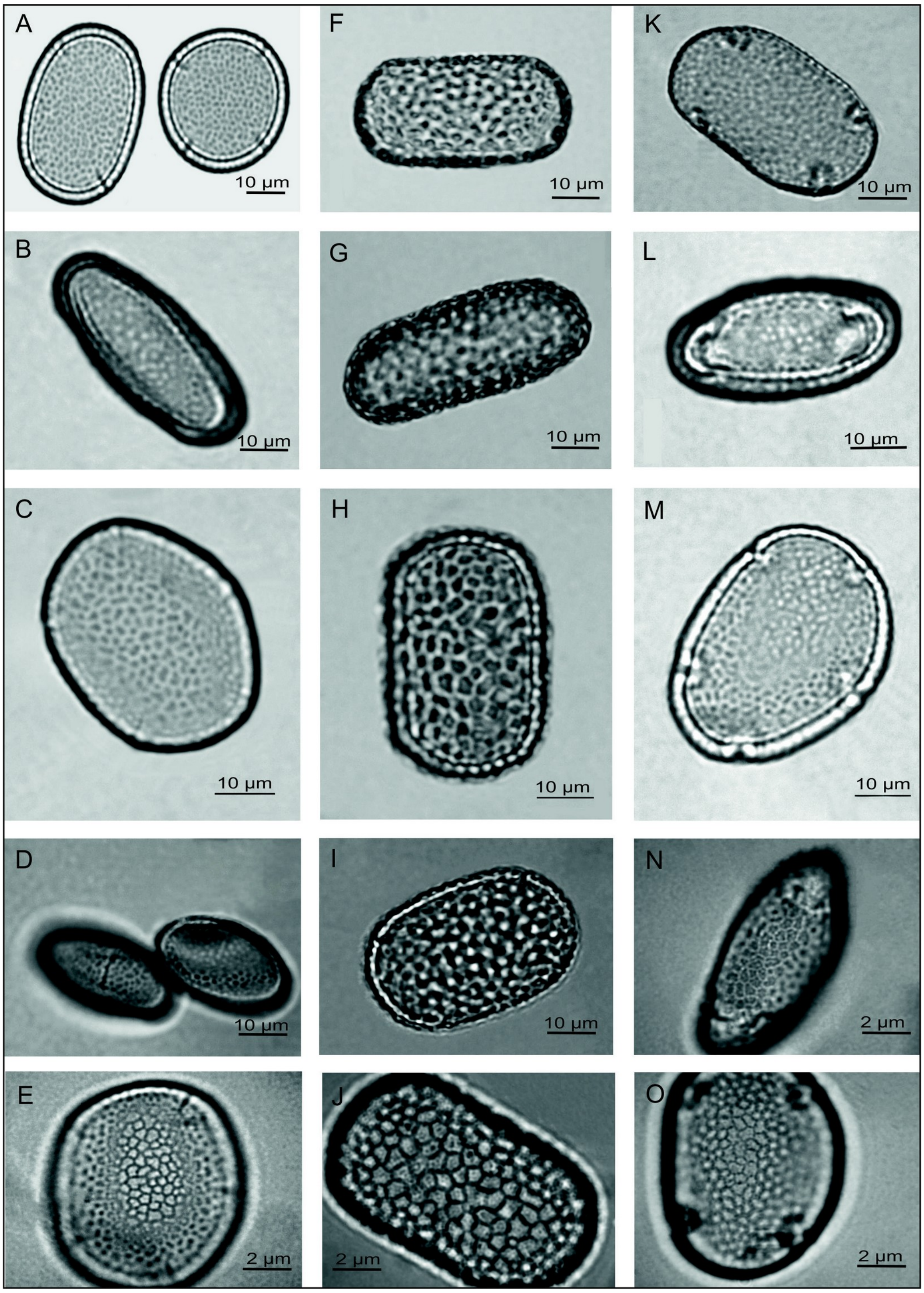

Fig. 1. LM micrographs of *Impatiens glandulidera* (A-E), *I. balfourii* (F-J), and *I. noli-tangere* (K-O) pollen grains: (A) rectangular-obtuse and subcircular pollen in polar view; (F, I, K) polar view; (B, G, L) peroblate pollen in equatorial view; (C, M) 5-colpate and (H) 4-colpate pollen; (D, N) colpus; (D, J, O) reticulate ornamentation. (Photo: D. Pavlova).

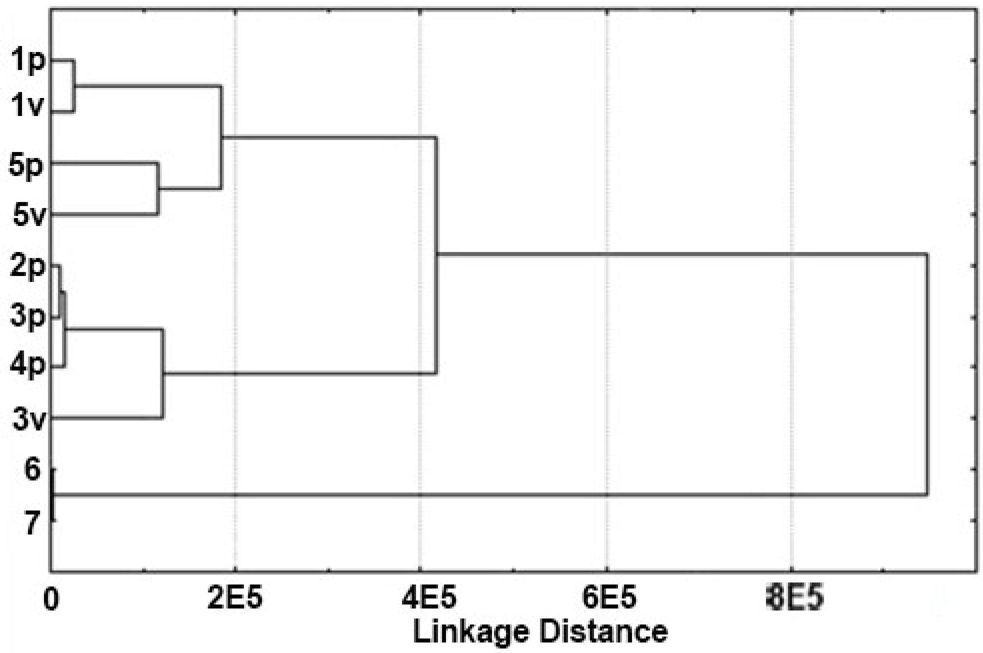

Fig. 2. Similarity dendrogram obtained by cluster analysis (Euclidean distances) applied to data of the measured pollen characters and calculated flower pollen production and fertility.

Discussion

The pollen morphological data for the studied *Impatiens* species confirm previous information about these species (Huynh, 1968; Moore et al., 1991; Beug, 2004; Janssens et al., 2005; Vinogradova et al., 2020). The three studied species are 4(5)-zonocolpate, except *I. balfourii* where no 5-zonocolpate pollen grains were found. According to Janssens et al. (2005) the main *Impatiens* pollen type is 4-aperturate, rectangular pollen grain with reticulate sexine ornamentation which originated from a triangular, 3-aperturate pollen grain with reticulate sexine ornamentation, considered as ancestral pollen type in *Impatiens*.

The apertures variation between 4 and 5-zonocolpate pollen grains confirms data presented by many authors and do not agree with Janssens et al. (2005). Studying pollen morphology of African *Impatiens* species Grey-Wilson (1980) mentions that the presence of 5-aperturate pollen in *Impatiens* is typical for hybrids. Bearing in mind that there are no described native hybrids for *I. glandulifera, I. balfouri* and *I. noli-tangere* till now, we can conclude that this phenomenon is more widely distributed between species in the genus and it is probably related to the pollination success of these plants. Similarly, high pollination success has been observed for other generalist and attractive alien species, such as *Rhododendron ponticum* and *Senecio inaequidens* (Vervoort et al., 2011). Pollen aperture number heteromorphism is considered as an evolutionary advantage if there is a trade-off between germination speed and life expectancy of the pollen grain, and if pollination conditions vary unpredictably (Nadot et al., 2000). The apertures provide exits for recognition proteins (Moore et al., 1991) as well and this is related to the distribution success of the species. As pollen of *Impatiens* species is rich in proteins, it is one of the most abundant pollen in bee breads (Donkersley et al., 2017).

The abundance of pollen and nectar in the invasive species *I. glandulifera* attracts potential bee pollinators from other native plants resulting in reduced seed set and fitness among other plants (Chittka & Schürkens, 2001).

Differences in pollen size and shape are found not only between species but also at the population level. The size of the pollen grains decreases in the order *I. balfourii* > *I. noli-tangere* > *I. glandulifera*. Our results confirm data presented for *I. noli-tangere* and *I. glandulifera* by Vinogradova et al. (2020), for *I. noli-tangere* by Beug (2004), and differ from Janssens et al. (2005) where pollen of *I. glandulifera* was bigger from the pollen of *I. noli-tangere*. The relation between studied species is proved by the performed cluster analysis (Fig. 2), where *I. balfourii* and *I. noli-tangere* are separated from *I. glandulifera* populations. The pollen size in *I. glandulifera* is quite variable between populations and two groups are formed. One of the factors influencing pollen size proved in our study, is the flower pollen production which negatively statistically correlates with polar and equatorial diameters, apocolpium, and distance between colpi edges in polar view. The second factor can be soil moisture and soil nutrients. The populations of *I. glandulifera* that are at distance from the rivers demonstrate smaller pollen size. Infraspecific pollen variation is frequently linked to the size of the species area and then in turn, to the ecological diversity (Guinet & Ferguson, 1989). The growth under unusual ecological conditions and changes in soil mineral nutritive elements also increase the amplitude in the variation of pollen characters (Guinet & Ferguson, 1989; Katiyar et al., 2012). However, such a relationship should be proved in future studies under experimental conditions.

Variation in pollen shape and outlines in polar and equatorial view found in *Impatiens* pollen confirm Janssens et al. (2005). The rounded pollen for *I. noli-tangere* reported by Vinogradova et al. (2020) is not confirmed. The most variable in shape, oblate or peroblate (P/E 0.42-0.6) in equatorial view and rectangular-obtuse or suborbicular in most 4-zonocolpate grains in polar view, were the pollen grains of *I. glandulifera*. Because of the variation found this pollen character should be used carefully in the identification of the species. The pollen grains are quite variable in shape and size even in the same individual and in the same anther. The smallest pollen grains in the anthers were sterile. Our results demonstrated a higher proportion of sterile pollen in *I. glandulifera* with violet flowers compared to pink flowers (Table 3) and could be a result of genetic and environmental factors. Katiyar et al. (2012) suggest that differences in *I. balsamina* pollen are due to meiotic abnormalities as a result of hybridization and polyploidy. A genetic reason for variation in pollen size and shape can be the diverse reproductive systems in studied *Impatiens* species. Their reproductive systems are prepositions for distribution success as well. According to Vervoort et al. (2011) some alien species such as *I. glandulifera* are successful because of their autogamous breeding system or because of their ability to attract pollinators successfully. The self-compatible species *I. glandulifera* does not suffer from inbreeding depression while this is not true for the native species *I. noli-tangere* (Vervoort et al. 2011). Studies on genetic diversity in *Impatiens* species confirm a low rate of DNA polymorphism in plants from natural populations of *I. noli-tangere* (7.3%) and higher DNA polymorphism in invasive populations of *I. glandulifera* (45.6%) (Kupcinskiene et al., 2015).

Pollen ornamentation in studied species is reticulate but differences in size and number of lumina, granules inside the lumina, and muri shape and height are enough informative to differentiate each of them. The differences in ornamentation between species should be related to their adaptation to specific pollinators as it was previously pointed out for different genera (Guinet and Ferguson, 1989; Pavlova et al., 2016). The reticulate ornamentation is considered ancestral for the *Impatiens* species but variation in ornamentation along with other pollen characters outlines phylogenetical lineage in the genus and Balsaminaceae as well (Janssens et al., 2005, 2012).

High flower pollen production, comparable to anemophilous plants, was found in the studied species and confirmed previous data (Vervoort et al., 2011). The highest amount of pollen produced per flower and stamen was found in the invasive *I. glandulifera*, followed by *I. balfourii* and the native *I. noli-tangere*. As pollen production is genetically and physiologically controlled and species-specific (Ferrara et al., 2007) this result and the high percentage of fertile pollen in invasive species directly can be referred to its pollination and distribution success, but future investigations are needed to elucidate the specifics in this aspect.

Conclusions

Although the pollen morphology of *I. glandulifera* is similar to the native *I. noli-tangere* some adaptations in pollen biology can favor the capacity of invasion. Pollen heteromorphism is more widely distributed in *I. glandulifera* related to the pollination success and distribution of the species. The abundance of pollen and nectar in the invasive species *I. glandulifera* attracts much more pollinators from other native plants, including *I. noli-tangere* in their populations, resulting in reduced seed set and fitness among other plants. Variation in pollen size and shape is found both at species and population level, being the highest in the invasive species *I. glandulifera*. This variation is directly correlated with flower pollen production, soil moisture, and soil nutrients. Variation in pollen size and shape is found between the pink and violet flowering forms of *I. glandulirera* altogether with the variation of the percentage of fertile/sterile pollen and could be a result of genetic and environmental factors. The high percentage of fertile pollen in invasive species is referred as its pollination and distribution success.

As a result of the study, we propose a careful use of pollen character in the identification of the species because of the variation established. Further studies related to the pollination biology of *Impatiens* species are needed to evaluate the invasive success of the alien species.

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