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Flowers of *Impatiens glandulifera* as hubs for both pollinators and pathogens

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Abstract

Flower infestation by pathogens may influence pollination effectiveness. At the same time, by sharing infested flowers, pollinators increase transmission of pathogens. In the presented study we identified fungi that colonised flowers of the invasive alien Himalayan balsam *Impatiens glandulifera*, one of the most nectar rewarding plants in Europe, as well as its pollinators. We determined factors (e.g., plant size, length of flower lower sepal and the width of its entry, air temperature and sun illuminance) that affect pathogen species presence and pollinators numbers. The study was conducted in three regions in Poland differing in time from the *I. glandulifera* invasion onset. It allowed embedding our results in the context of the evolution of increased competitive ability (EICA) hypothesis. With reference to this hypothesis we tested whether *I. glandulifera* from the two younger populations are more frequently pollinated than individuals from the old one, which may be a result of the higher infection prevalence in the flowers of individuals from the latter population. Harmful primary pathogens of *I. glandulifera* (e.g., *Botrytis cinerea* and *Fusarium graminearum*) were identified from its flowers. Although the knowledge of the impact of the recorded pathogen species on the pollinators that transmit them is still limited, these pathogens are known to cause devastating diseases of native plant species and to incur significant economic losses in crops. Therefore, the facilitation of their transmission by *I. glandulifera* in the invaded communities may pose a serious threat both to native biodiversity and nearby crop production. We did not find support for the EICA hypothesis that flower release from pathogens may increase the pollinator's activity. *Bombus hortorum* was the most frequent visitor in the youngest surveyed population, while *B. pascuorum* was most frequent in the two others. So far the dominance of *B. hortorum* as a pollinator of *I. glandulifera* has not been recorded. A possible explanation is that flowers in the youngest population, with significantly wider entries than in the two older ones, were more accessible for this large bumblebee. We suggest that the shifts in flower dimensions may result from the evolutionary processes and/or phenotypic plasticity;

however, this suggestion needs to be confirmed in further studies. At the same time, it can be expected that exceptionally frequent visits of *B. hortorum* in flowers of *I. glandulifera* in the youngest population may contribute to increasing transmission rate of pathogen species to the new native host plants that are particularly associated with this pollinator.

Keywords
Bumblebee activity, EICA hypothesis, floral traits, Himalayan balsam, intraspecific and interspecific transmission, pathogen hotspot, post-introduction shifts, pathogen spillback

Introduction

Himalayan balsam *Impatiens glandulifera* is native to the western Himalayas, while as an alien species it is known from Europe (35 countries), North America (Canada, United States, Mexico), Oceania (New Zealand) and Asia (Japan) (CABI 2022). The negative impact of *I. glandulifera* on native biodiversity and human economy has been widely demonstrated. This invasive species was reported to compete successfully with co-occurring plants, reducing their diversity by 25% (Hulme and Bremner 2005); moreover, its negative impact on animals occurring in invaded habitats was also documented (Ruckli et al. 2013). *I. glandulifera* may decrease pollination of wild growing plants (Chittka and Schürkens 2001) as well as crops (Najberek et al. 2021), whose yield often depends on the availability of pollinators (Klein et al. 2007). At the same time, high costs of the species eradication, reaching £10/m^2 (Tanner et al. 2008), make its control complicated or even impossible to achieve in areas where *I. glandulifera* is widespread.

I. glandulifera is characterized by traits that facilitate its invasion success. It was shown that it has a high photosynthetic capacity and growth rate (Ugoletti et al. 2011). Impressive antimicrobial and antioxidant properties of the species were also discovered (Szewczyk et al. 2016) as well as its high dispersal abilities along watercourses: seeds of this balsam can float and persist viable for over 2 months in still water (Najberek et al. 2020a). *I. glandulifera* is an annual species which spreads only via seeds and its autonomous selfing abilities are relatively low (Vervoort et al. 2011; Najberek et al. 2022a), therefore, high attractiveness for pollinators is crucial. The species attracts pollinating insects by producing the extraordinary volumes of nectar, amounting to 0.3 mg hourly per flower (Chittka and Schürkens 2001). Therefore, in this study we investigated pollinators of *I. glandulifera* and factors that may influence their activity, with the main focus on pathogens that occur in flowers of the balsam species.

Flowers are part of the phyllosphere which contains all aerial organs of plants. In general, in contrast to the rhizosphere and endo-rhizosphere, the phyllosphere microbiome was poorly studied to date (Timmusk et al. 2020). In the presented study, we isolated fungal endophytes that colonised *I. glandulifera* flowers, identified them molecularly and assessed their harmfulness and economic impact for plants and crops. It is known that the shared use of flowers leads to pathogen transmission between plants and their pollinators and that floral traits may influence disease transmission (McArt et al. 2014). Although the first study on this phenomenon was published already in 1994 (Durrer and Schmid-Hempel 1994), only 11 experimental studies concerning flowers as hotspots aiding plant and/or pollinator pathogens dispersal have been reported since then (Shykoff and Kaltz 1997; Goodell 2003; Ruiz-González and Brown 2006; Ruiz-González et al. 2012; Fouks and Lattorff 2013; Cisarovsky and Schmid-Hempel 2014; Graystock et al. 2015; Adler et al. 2018; Bodden et al. 2019; Figueroa et al. 2019; Yousefi and Fouks 2019). In the presented study we focused on the two floral traits: the length of the flower's lower sepal and the width of its entry. These traits determine both the accessibility of the host plant for particular groups of pollinators (Inouye 1980) and likelihood of pathogen transmission (Bodden et al. 2019). Flower shape is associated with the length of pollinator visit, which in turn influences the likelihood of the defecation inside the flower and consequently – the risk of pathogen transmission via faeces (Bodden et al. 2019). Notably, longer visits increase also likelihood of transmission of pathogens attached to the surface of the pollinator's body (Cisarovsky and Schmid-Hempel 2014). It is also important that it takes longer for infected pollinators to learn floral cues than it does for healthy ones; also perception of these cues during a visit is longer for infected pollinators, which extends visiting time and consequently – may also increase the likelihood of pathogen transmission (Figueroa et al. 2019). The flower structures play also a role in protecting or exposing pathogens to environmental hazards, which infuences the likelihood of pathogen survival (Cisarovsky and Schmid-Hempel 2014). Longer nectar tubes, for example, protect pathogens better than shorter ones (Durrer and Schmid-Hempel 1994). In turn, secreting of unique chemical compounds (secondary metabolites), as is the case with *I. glandulifera* flowers (Vanderplanck et al. 2019), may decrease pathogen survival. On the other hand, the bell-shaped flowers in this species are more susceptible to infections than flat-formed flowers of other species (Graystock et al. 2015). It should also be noted that the flowering potential may be affected through infections that occur before the flowering phase. For example, Shykoff and Kaltz (1997) found that the initial flowering of diseased male plant is retarded, however, the infection results also in increased number of produced flowers. This in turn allows plants to conceal their infections from pollinators (Shykoff and Bucheli 1995).

In the presented study we selected three populations of *I. glandulifera* that significantly differed in age. The population age, determined on the basis of literature data documenting invasion of the species in Poland (Tokarska-Guzik 2005; Zając et al. 2011), was important in the context of the evolution of increased competitive ability (EICA) hypothesis (Blossey and Notzold 1995). The hypothesis assumes that the enemy pressure on newly introduced populations of alien species is low, hence individuals may reduce their investment into defence against enemies and re-allocate the saved resources into the improvement of competitive abilities. We assumed that *I. glandulifera* may re-allocate the saved resources into traits that determine its high attractiveness for pollinators. We tested whether *I. glandulifera* individuals from the young and middle aged population are more frequently pollinated than individuals from the old population.

Moreover, these populations were tested for fungal pathogens in the flowers visited by pollinators in order to indicate the population with the highest infections prevalence. Basing on the EICA assumptions, we hypothesised that young and middle aged populations of the species should be less infected than the old one. Importantly, such dependence may drive higher pollination success in the two former populations. The flower pathogens aspect has never been tested using invasive alien plants from populations varying in age.

In the presented study, pollinators and flower pathogens of the one of the most nectar rewarding plants in Europe, invasive alien *I. glandulifera* (Chittka and Schürkens 2001), were investigated. We tested also floral traits that determine pollinator visits and may influence pathogen transmission. In addition, the study was conducted using populations of different ages, which allowed to embed the collected dataset in the context of the EICA hypothesis assumptions. Moreover, based on our results as well as the reliable literture, we determined whether infected flowers of widely distributed *I. glandulifera* may pose a serious threat to the native plants and crops as well as to pollinators that also could become victims of pathogens that they are carrying between flowers.

Methods

Study area

The experiment was conducted in 2020 in southern Poland. Individuals of *I. glandulifera* were surveyed in three study regions – the Izerian Foothills, Kraków and Muszyna – differing in terms of the species introduction year (Table 1). The oldest Polish population of *I. glandulifera*, dating back to 1890s, occurs in the Izerian Foothills (Tokarska-Guzik 2005). From this source population the species dispersed to adjacent regions. In Kraków the species appeared in 1980s (Table 1), and it was probably the source of its further eastward expansion. In Muszyna *I. glandulifera* has been noted since 2010s (Zając et al. 2011). In each of the three regions the plants were surveyed along three rivers (Table 1). One survey per each locality was conducted, between 9 and 18 September (Table 1).

Plant measurements and weather conditions

At each locality 10 neighbouring individuals of *I. glandulifera* growing at the path margin were randomly selected and marked with a unique ID. Stem height and diameter of individuals were measured to include their size (Suppl. material 2: table S1) in the analyses; moreover, the number of flowers and seed capsules of individuals were also counted to assess their flowering power (see *Statistical analysis*). The hue of the flowers (pinkish or reddish) was also assessed.

The surveys were conducted on warm and rainless days. During each survey data loggers (i-Button DS1921G) were used to measure air temperature at 10-minute intervals, while hand-held environmental metre (Extech 45170CM) measured sun illuminance (lux) and wind speed (m/s) at 20-minute intervals.

Table 1. Characteristics of localities of the invasive alien *I. glandulifera* differing in population age in three study regions (Izerian Foothills, Kraków and Muszyna) in southern Poland; the surveyed plants always occurred at riversides.

Region	Year of introduction (references)	Locality with coordinates (latitude, longitude)	River name	Survey date
Izerian Foothills	1890s (Tokarska-Guzik 2005)	Zgorzelec (51.103805, 14.982722)	Nysa Łużycka	9/17/2020
		Leśna (51.021625, 15.269387)	Bruśnik	9/17/2020
		Mirsk (50.973962, 15.365690)	Czarny Potok	9/18/2020
Kraków and surrounding areas	1980s (Zając et al. 2011)	Kraków (50.038255, 19.897358)	Wisła	9/10/2020
		Szczyglice (50.086645, 19.814899)	Rudawa	9/10/2020
		Zabierzów (50.097423, 19.806040)	Rudawa	9/9/2020
Muszyna and surrounding areas	2010s (Zając et al. 2011)	Andrzejówka (49.342193, 20.819197)	Poprad	9/14/2020
		Żegiestów (49.374850, 20.785117)	Poprad	9/14/2020
		Milik (49.347806, 20.851281)	Milik	9/15/2020

Pollinators counting

Over a 60-minute period during each survey the number of pollinators visiting flowers of the selected 10 plants was counted. The counting was always conducted in the morning (at 9:30 h) or in the afternoon (at 13:30 h). Pollinators were defined as all insects visiting flowers to collect pollen or nectar that may carry pollen from male (anther) to female (stigma) flower organs. Following the methodology adapted for *I. glandulifera* and *I. parviflora* in our previous study (Najberek et al. 2021), flights of the pollinators were tracked, and IDs of subsequently visited plants were noted; the pollinators were not captured but identified at flowers and their size was roughly assessed by assigning to one of three categories (small, medium, large).

Flower measurements

At the end of each survey 10 flowers (or less, depending on availability) of each plant individual were cut off and their profiles were photographed against a millimetre paper background (Canon EOS 60D, Canon EF 100 mm f/2.8 Macro USM lens and ring flashlight). The images were used to assess: the length of lower sepal and the width of flower entry. Subsequently, digital images were analysed with ImageJ software (ver. 1.51 k), and the area of one flower side was evaluated; this corresponds to 1/2 of the total flower area (Najberek et al. 2021). The data on length, width and area of flowers are included in Suppl. material 2: table S1.

Detection and molecular identification of fungi

The cut off flowers were inserted in paper bags (one bag per one plant) and transported in a portable freezer. Molecular identification of fungal pathogen species was carried out in the laboratory of Department of Systematic and Environmental Botany of Adam Mickiewicz University in Poznań (Poland).

The flowers were surface-sterilized in 75% ethanol (30 s), 4.5% sodium hypochlorite (3 min 30 s), 75% ethanol (15 s), and then rinsed in sterile water. After sterilization, the flowers were cut into fragments and placed on Petri dishes with PDA medium and chloramphenicol (one flower per dish). To confirm the efficiency of the sterilisation process, 50 µl of rinse water was spread onto potato dextrose agar (PDA) and incubated at room temperature for 14 days. The addition of an antibiotic to the medium was used to exclude bacteria from the analysis, thus only pathogenic fungi were obtained from the plant tissues. In total, 585 flowers of 90 plant individuals were checked for the fungal presence. The dishes were placed in an incubator at 25 °C. They were observed every day, and emerging fungi were successively transplanted to new plates. For the identification of fungi, the fungal isolates were grouped into morphotypes based on macroscopic characteristics, such as the appearance and colour of the mycelium. Then, isolates representative of each morphotype were analysed using molecular methods.

The DNA was isolated using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, USA) according to the manufacturer's protocol and was stored at –20 °C. A pair of primers, ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990), was used to amplify the ribosomal cassette, which consisted of partial SSU, ITS1, 5.8S, ITS2 and partial LSU rDNA. The PCR reaction was conducted in a 25-µl volume containing 2.5 µl of 10X buffer, 2.5 µl of 2.5 mM dNTP mix, 0.5 µl of each primer at 10 µM, 0.5 µl of DNA Taq polymerase, 13.5 µl of nuclease-free water, and 5 µl of DNA template. Amplification was conducted in a thermocycler using a programme with the following parameters: 2 min at 95 °C; 37 cycles of 30 s at 95 °C, 30 s at 55 °C, and 60 s at 72 °C; and finally 5 min at 72 °C. The PCR products were purified using alkaline phosphatase and exonuclease I and directly cycle-sequenced with ABI BigDye Terminator ver. 3.1 (Applied Biosystems, USA). The obtained sequences were edited with Chromas (www.technelysium.com.au) software and were compared to those published in the European Molecular Biology Laboratory (EMBL) nucleotide databases and in the NCBI (www.ncbi.nlm.nih.gov) databases using BLAST (Altschul et al. 1990). A positive identification of a species was confirmed if ≥98% of the ITS region sequence identity was shared with the reference sequence from the databases. The obtained sequences were submitted to the GenBank.

Statistical analysis

The data were analysed with generalised linear models (GLMs) using SPSS ver. 26.0 (IBM Corp. 2016); all used data is presented as Suppl. material 1. The models with the lowest Akaike information criterion (AIC) were chosen (Burnham and Anderson 2002). Three GLM models were created: (1) for all recorded pollinators, (2) for bumblebees solely and (3) for pathogens identified from the flowers (Suppl. material 2: table S2). All models assumed the Poisson distribution for the numerical data. Pairwise contrasts were applied for between-group comparisons with adjustment for multiple tests using least significant difference (significance level 0.05).

In the first model the number of all recorded pollinators, calculated per plant individual per survey ('N pollinator records'), was a target variable (sample size = 216).

In the base model (Suppl. material 2: table S2), the following fixed effects were included: pollinator identified to a species or a higher taxonomic group ('Pollinator'; single records of *Bombus lapidarius* and *Psithyrus vestalis* were excluded from the analysis), study region representing population age of the surveyed plant individuals ('Region'), reproduction potential of the plant ('Flowering power'), area of flower profile ('Flower area'), hue of flower ('Flower hue'), height of the plant ('Stem height'), weather variables ('Temperature', 'Sun radiation' and 'Wind speed'), area of *I. glandulifera* patches at particular localities ('Cover area') and morning / afternoon survey time ('Day time'). Two interactions were also included: 'Region * Flowering power' and 'Region * Stem height' (Suppl. material 2: table S2). The 'Flowering power' was calculated from principal component analysis (PCA), based on two variables: (1) number of flowers and (2) number of seed capsules. In PCA, the Kaiser-Meyer-Olkin measure of sampling adequacy was 0.50, with a p-value in Bartlett's test < 0.001. The percentages of variance accounting for the two obtained components were 80.3% and 19.7%; the first component with an eigenvalue of 1.606 explained most of the variance.

The second GLM model was created only for bumblebee records as a target variable; as in the previous model, single records of *B. lapidarius* and *P. vestalis* were excluded from the analysis. This group of pollinators was most numerous. Data on bumblebees were also more precise than on other pollinators, thanks to the assessment of their size. Size assessment is particularly important in analyses of bumblebee effects, because meaningful size polymorphism occurs among workers (Mares et al. 2005). The following fixed effects were taken into consideration: bumblebee species ('Bumblebee") and its size ('Bumblebee size'), 'Flowering power', 'Flower area' and three weather variables ('Temperature', 'Sun radiation' and 'Wind speed').

In the last model the number of recorded pathogen species per each surveyed plant was used as a target variable. Moreover, the following variables were included in the model as fixed effects: the number of recorded pollinators ('N pollinator records'; single records of *B. lapidarius* and *P. vestalis* were excluded from the analysis), 'Region' differing in population age, two weather variables ('Temperature', 'Sun radiation'), 'Flowering power', three variables associated with flower dimensions ('Flower area', 'Length of lower sepal' and 'Width of flower entry') and a single variable representing size of the surveyed plants ('Plant size'). In addition, the interaction between flowering power and study region differing in age was included in the model ('Flowering power * Region'). 'Plant size' was obtained from principal component analysis (PCA), based on two variables: (1) stem height and (2) stem diameter. In PCA, the Kaiser-Meyer-Olkin measure of sampling adequacy was 0.50, with a p-value in Bartlett's test < 0.001. The percentages of variance accounting for the two obtained components were 82% and 18%; the first component with an eigenvalue of 1.640 explained most of the variance.

Data resources

Raw data used to perform statistical analyses are provided as a file with supporting information.

Results

In total, we recorded 1828 pollinator visits (Table 2) and 97.26% of them were identified at the species level, 1.04% at the family/superfamily level, while 1.7% belonged to the *Bombus lucorum* complex. Bumblebee species dominated regardless of the studied region differing in the age of surveyed plants; including a single record of *Psithyrus vestalis* we recorded 1625 of bumblebee visits (88.9% of all visits; Table 2). Most of them were recorded at plants surveyed in Muszyna and surrounding areas (N = 1014; 55.5%), where *B. hortorum* was the most numerous species (N = 988, 97.4%; Table 2). In turn, the Izerian foothills and Kraków, even if combined, had fewer records than Muszyna (N = 814; Table 2), with *B. pascuorum* as the most frequently noted species (N = 216 and 212, respectively; 52.7% and 52.5%, respectively; Table 2). Moreover, in the Izerian foothills the honeybee *Apis mellifera* was the second most abundant species (Table 2).

In the statistical analysis conducted for all recorded pollinators, the model with eight fixed effects and one interaction was selected (Suppl. material 2: table S2). During the model selection procedure, the following variables were excluded from the best-fit model: flower hue, wind speed, day time; also interaction between regions differing in age and flowering power was excluded (Suppl. material 2: table S2).

In the best-fit model, the three variables (pollinator species, flowering power and cover area) and the interaction between the regions differing in age and stem height played a significant role (Table 3; Suppl. material 2: table S2). The most frequently recorded species visiting *I. glandulifera* flowers was *B. hortorum* (at $p < 0.001$ in all comparisons; Fig. 1; all statistics are included in Suppl. material 2: table S3). The second important species was *B. pascuorum* (at $p < 0.02$ in 5 of 8 comparisons; Fig. 1; Suppl. material 2: table S3). We also found that the number of pollinators increased with the flowering power (Table 3). Moreover, in patches with larger cover area the higher pollinator activity was noticeable (Table 3). In turn, the interaction between the regions

Table 2. The number of pollinators recorded at flowers of of *I. glandulifera* differing in population age in the three study regions (Izerian Foothills, Kraków and Muszyna) in southern Poland.

Pollinator species	N records			TOTAL:
	Izerian Foothills	Kraków	Muszyna	
Bombus hortorum	27	29	988	1044
Bombus pascuorum	216	212	9	437
Apis mellifera	134	33	11	178
Bombus terrestris	20	82	0	102
Bombus lucorum complex	0	31	0	31
Syrphidae	3	5	6	14
Bombus sylvarum	9	0	0	9
Vespula vulgaris	0	6	0	6
Apoidea	0	5	0	5
Bombus lapidarius	1	0	0	1
Psithyrus vestalis	0	1	0	1
TOTAL:	410	404	1014	1828

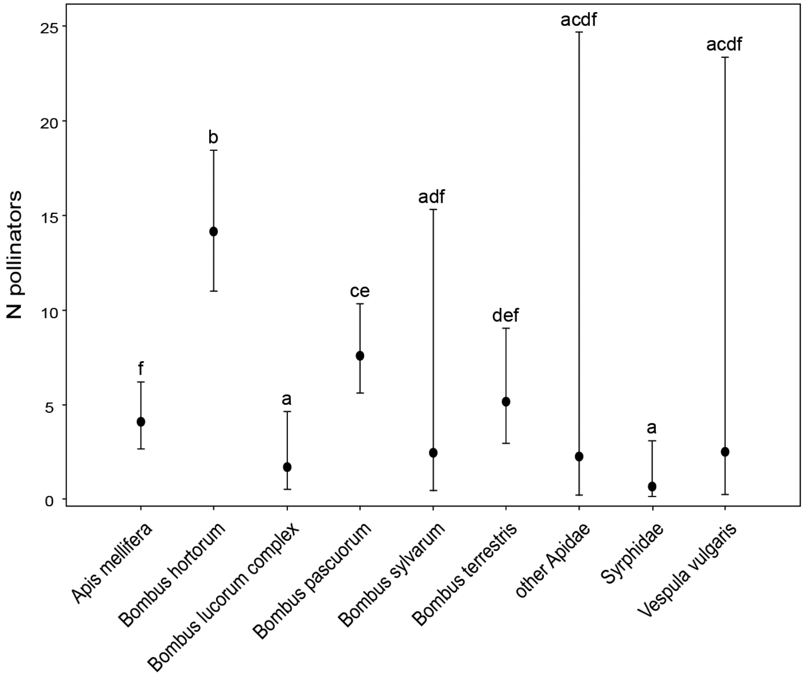

Figure 1. The estimated mean number of pollinators (± confidence intervals) recorded at flowers of *I. glandulifera* in the three study regions of different population age in southern Poland (Izerian Foothills, Kraków and Muszyna); groups with the same letter above the T-bars are not significantly different at p < 0.05; single records of *Bombus lapidarius* and *Psithyrus vestalis* were excluded from the analysis (see Methods).

Table 3. GLM best-fit model for the number of recorded pollinators. The following variables were included in the model: pollinator species (Table 2), three study regions differing in population age (Izerian Foothills, Kraków and Muszyna), flowering power (N of flowers and N of seed capsules computed into a single variable), flower area, stem height, weather conditions (air temperature, sun radiation), cover area as well as interaction between the study region differing in age and stem height. The estimated means ± SE for pollinator species and region are *Bombus hortorum*: 14.247 ± 1.876, *B. pascuorum*: 7.638 ± 1.170, *Apis mellifera*: 4.103 ± 0.851, *B. terrestris*: 5.200 ± 1.467, *B. lucorum* complex: 1.635 ± 0.861, Syrphidae: 0.734 ± 0.533, *B. sylvarum*: 2.502 ± 2.297, *Vespula vulgaris*: 2.530 ± 2.850, Apoidea: 2.250 ± 2.733, Izerian Foothills: 2.460 ± 0.731, Kraków: 2.904 ± 0.744, Muszyna: 4.774 ± 1.515.

Effect	F	df	p
Pollinator species	7.31	205	< 0.001
Region	0.45	199	0.6
Flowering power	8.30	198	0.004
Flower area	0.74	198	0.4
Stem height	0.29	198	0.6
Temperature	1.60	198	0.2
Sun radiation	2.83	198	0.094
Cover area	3.44	198	0.065
Region * Stem height	3.18	199	0.044

differing in age and stem height demonstrated a different role of plant height in various habitat conditions (Fig. 2, Table 3). In Muszyna (the young population) taller plants were visited significantly more often than shorter ones (Fig. 2, Table 3), whereas, in the two remaining regions (middle age and old populations) longer stems did not have any effect on the number of recorded pollinators (Fig. 2, Table 3). However, the result of the interaction was not associated with the age of *I. glandulifera* individuals in particular regions. In Muszyna, where the relationship was significant, plants co-occurred with equally tall and very dense bushes, which may determine this result. It should be stressed as well that the result for region variable, that also represents population age of the surveyed plants, was non-significant, thus it can be assumed that the obtained result was not consistent with the EICA hypothesis assumptions.

In the analysis carried out solely for bumblebees, the model with all seven fixed effects was selected (see base model in Suppl. material 2: table S2). Except for flower area, all included effects played a significant role in shaping the activity of bumblebees at *I. glandulifera* flowers (Table 4). The results for particular bumblebee species (Suppl. material 2: fig. S1, table S4) showed the same pattern as in the analysis for all pollinators (Fig. 1); there was only one exception – the numbers of visits of *B. pascuorum* and *B. sylvarum* did not differ between each other, while there was a difference in the

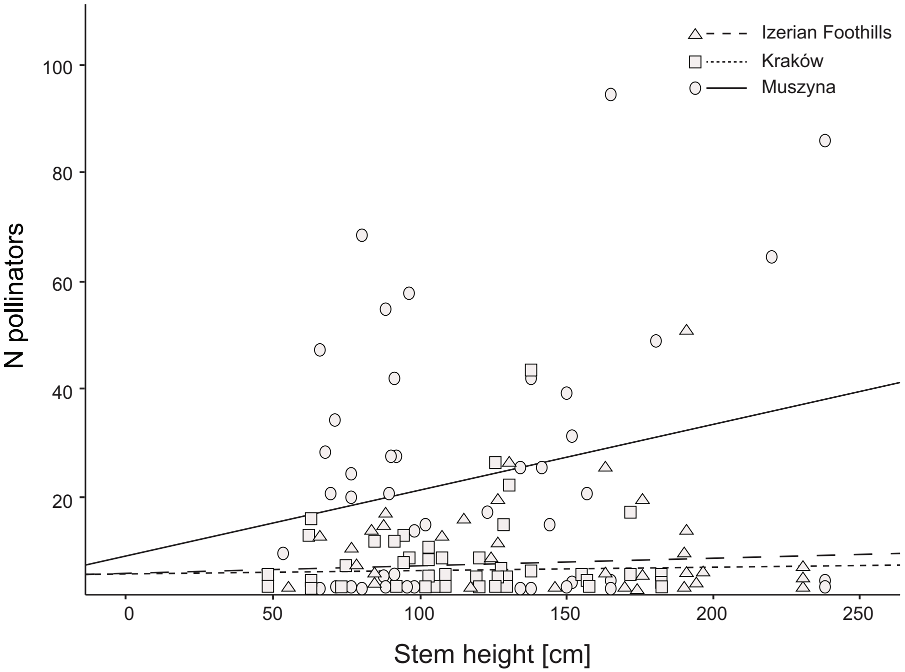

Figure 2. The scatter plot with fit lines for the relationship between the number of visiting pollinators and stem height of *I. glandulifera* individuals in the three study regions of different population age in southern Poland (Izerian Foothills, Kraków and Muszyna). The coefficients of determination are R^2 Linear: 0.011, Izerian Foothills: R^2 Linear 0.007, Kraków: R^2 Linear 0.002, Muszyna: R^2 Linear 0.065.

Table 4. GLM best-fit model for the number of recorded bumblebees. The bumblebee species (Suppl. material 2: fig. S1), their size, flowering power (combined N of flowers and N of seed capsules), flower area and weather conditions (air temperature, sun radiation and wind speed) were included in the model. The estimated means ± SE for bumblebee species and size are *Bombus hortorum*: 4.082 ± 0.522, *B. lucorum* complex: 0.525 ± 0.215, *B. pascuorum*: 2.678 ± 0.364, *B. sylvarum*: 1.096 ± 0.813, *B. terrestris*: 1.805 ± 0.433, small: 1.166 ± 0.273, medium: 4.664 ± 0.813, large: 0.790 ± 0.216.

Effect	F	df	p
Bumblebee species	8.82	233	< 0.001
Pollinator size	58.79	231	<0.001
Flowering power	18.45	230	<0.001
Flower area	1.60	230	0.2
Temperature	12.90	230	<0.001
Sun radiation	64.38	230	<0.001
Wind speed	7.38	230	0.007

model for all pollinators. We also found that medium-sized bumblebees were recorded more frequently than small and large ones (contrasts respectively: Estimate = 3.50, SE = 0.66, t = 5.33, p = 2.290E-7; Estimate = 3.87, SE = 0.71, t = 5.48, p = 1.091E-7; Suppl. material 2: fig. S2); at the same time, there were no differences between the numbers of visiting small and large bumblebees in this respect (contrast: Estimate = 0.38, SE = 0.71, t = -5.48, p = 1.091E-7; Suppl. material 2: fig. S2). Flowering power also influenced the obtained results (Table 4) in that the number of bumblebees visiting flowers increased with the estimate of the number of produced flowers. In turn, the area of flowers did not play any significant role (Table 4). Weather conditions had an impact on the obtained results (Table 4; Suppl. material 2: fig. S3) as shown by the number of recorded bumblebees increasing with air temperature (AVG = 19. 4 °C, MIN = 14 °C, MAX = 27.1 °C) and decreasing with both sun radiation (AVG = 11765 lux, MIN = 2000 lux, MAX >= 20000 lux) and wind speed (AVG = 0.6 m/s, MIN = 0 m/s, MAX = 2.5 m/s).

There were 16 species of fungal pathogens (Table 5) in the sampled flowers; GenBank IDs of the recorded species are included in Suppl. material 2: table S5. Some of them, including *Botrytis cinerea* and *Fusarium graminearum*, are very harmful pathogens of native plants and crops (Dean et al. 2012). The flowers from the Izerian Foothills were colonised by four times less pathogen species than the flowers from the two other regions (Table 5). Species belonging to the *Fusarium* genus were most frequently recorded (N = 27; 34.2% of all records in the studied regions). *F. sporotrichioides* dominated in the Izerian Foothills, *F. boothii* was the most frequent species in Kraków, and *Alternaria alternata* – in Muszyna (Table 5). It should be also noted that plant flowers were colonised by 1, 2 or 3 pathogen species (N = 36, 40.0%; N = 15, 16.7%; N = 4, 4.4%; respectively). Moreover, flowers of 35 of all plant individuals (38.9%) were not colonised by any pathogen species.

In the analysis of pathogen species records, the model with two fixed effects – study region and flowering power – and interaction between these two variables was selected (Table 6; Suppl. material 2: table S2). In turn, the following explanatory variables were

Table 5. The number of fungal species records revealed in three study regions differing in population age (Izerian Foothills, Kraków and Muszyna).

Species	N of records			TOTAL:
	Izerian Foothills	Kraków	Muszyna	
Alternaria alternata	1	6	8	15
Epicoccum nigrum	1	7	5	13
Fusarium boothii	0	8	0	8
Fusarium sporotrichioides	3	5	0	8
Alternaria tenuissima	0	1	3	4
Boeremia exigua	0	0	4	4
Epicoccum tritici	1	2	1	4
Fusarium acuminatum	1	2	1	4
Botrytis cinerea	0	0	3	3
Cladosporium cladosporioides	0	1	2	3
Fusarium avenaceum	1	2	0	3
Fusarium equiseti	1	2	0	3
Mucor hiemalis	1	0	2	3
Nigrospora oryzae	0	1	1	2
Fusarium graminearum	0	1	0	1
Stagonosporopsis ligulicola	0	0	1	1
TOTAL:	10	38	31	79

Table 6. GLM best-fit model for the number of recorded pathogen species. The pathogen species (Table 5), three study regions (Izerian Foothills, Kraków and Muszyna), flowering power (combined N of flowers and N of seed capsules) and interaction between region and flower power were included in the model. The estimated means ± SE for region are Izerian Foothills: 0.283 ± 0.096, Kraków: 1.098 ± 0.160, Muszyna: 1.120 ± 0.177.

Effect	F	df	p
Region	3.03	86	0.054
Flowering power	6.80	85	0.01
Flowering power * Region	4.92	86	0.01

excluded during model selection procedure: the number of recorded pollinators, temperature, sun radiation, flower area, length of lower sepal, width of flower entry and plant size (Suppl. material 2: table S2).

The number of pathogen species in flowers of *I. glandulifera* from the Izerian Foothills (the old population) was lower than that in flowers from the two younger populations from Kraków or Muszyna (contrasts respectively: Estimate = -0.81, SE = 0.187, t = -4.358, p = 3.692E-5; Estimate = -0.84, SE = 0.20, t = -4.157, p = 7.737E-5; Fig. 3); at the same time, Kraków and Muszyna did not differ in this respect (contrast: Estimate = -0.02, SE = 0.239, t = -0.091, p = 0.9; Fig. 3); the result was contrary to our assumption constructed based on the EICA hypothesis expectations (we assumed that the level of infestation should be lower in the young and middle age populations than in the old one). Moreover, we revealed that in Kraków and in the Izerian Foothills the number of pathogen species decreased with plants flowering power (Fig. 4, Table 6); interestingly, the result for Muszyna was opposite (Fig. 4).

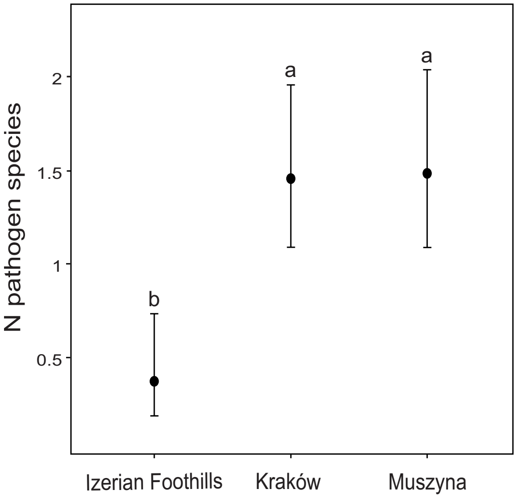

Figure 3. The estimated mean number of pathogen species (± confidence intervals) recorded from flowers of *I. glandulifera* in the three study regions of different population age in southern Poland (Izerian Foothills, Kraków and Muszyna); groups with the same letter above the T-bars are not significantly different at p < 0.05.

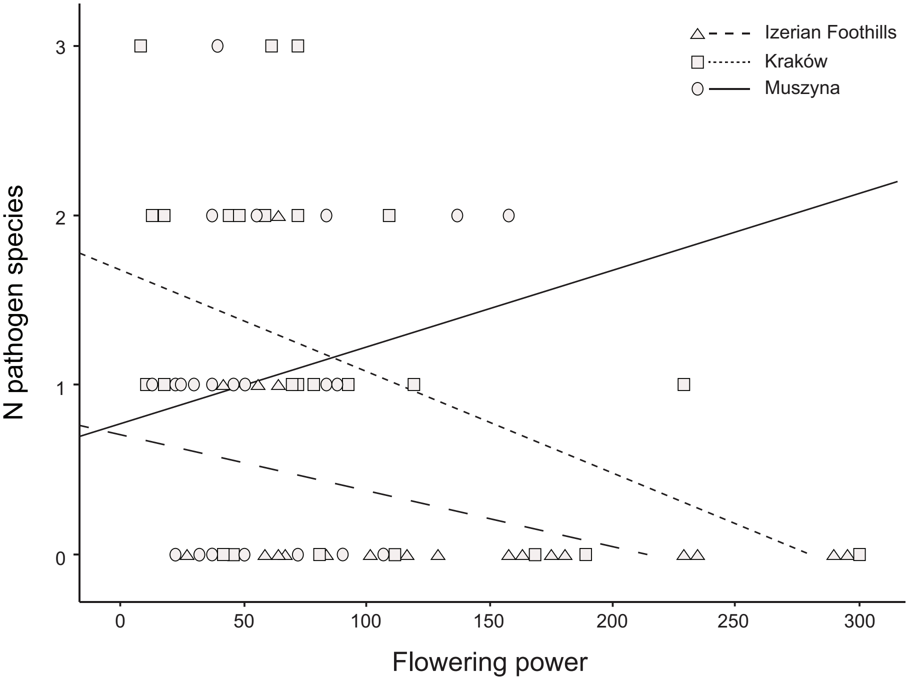

Figure 4. The relationship between the number of pathogen species and flowering power for different regions. Flowering power was calculated by PCA (combined N of flowers and N of seed capsules); the measurements were carried out in the three study regions of different population age in southern Poland (Izerian Foothills, Kraków and Muszyna).

Discussion

Infected plants may be less attractive to pollinators because pollinators can usually recognise contaminated flowers and thus they are able to avoid getting infected (Fouks and Lattorff 2011). For example, it was shown that bumblebees are able to detect the odour of a parasite *Crithidia bombi* (Fouks and Lattorff 2013). It was also demonstrated that larval honey bee parasite, the fungus *Ascosphaera apis*, decreases pollinator visits of several plant species (Yousefi and Fouks 2019). At the same time, *Drosophila melanogaster* may distinguish safe food source from food with toxic microbes (Stensmyr et al. 2012). However, plants try to compensate for pathogens presence, for instance by producing more flowers and/or more nectar (Shykoff and Bucheli 1995; McArt et al. 2014). In flowers of *I. glandulifera*, characterised by very high nectar content (Chittka and Schürkens 2001) and extended flowering, compensation may play an important role. If this is the case, pathogens occurring in frequently pollinated flowers of *I. glandulifera* may have a higher chance to be transmitted to other plants that share pollinators with this alien plant. It should be stressed that although it was not directly tested in this study, there is ample evidence that the shared use of flowers leads to horizontal pathogen transmission between plant individuals of the same generation. In the first experimental study on the transmission Durrer and Schmid-Hempel (1994) demonstrated that parasite *C. bombi* was horizontally transmitted through bumblebees between the inflorescences of *Echium vulgare*. The transmission of the same parasite between the flowers was confirmed also in other experiments (Ruiz-González and Brown 2006; Ruiz-González et al. 2012; Cisarovsky and Schmid-Hempel 2014). The mechanism was confirmed also for other pathogens and plants. For example, bumblebee parasites – *Apicystis bombi*, *C. bombi* and *Nosema bombi*, and honeybee parasites – *N. apis* and *N. ceranae*, can be rapidly transmitted between infected and healthy flowers of purple *Campanula cochleariifolia* (Graystock et al. 2015). In turn, Bodden et al. (2019) in their study of floral cues of eight plant species and bumblebee *Bombus impatiens* as a vector, provided evidence on pathogen transmission between the visited flowers.

The interspecific transmission of pathogens in *I. glandulifera* may determine the lower pollination rate of co-occurring native plants. The transmission probably occurs because native species have lower nectar content than *I. glandulifera* and it is probable that they are not able to mask the presence of pathogens so efficiently as this alien species. Its domination in terms of nectar production may also be manifested in neighbouring crops, whose yield success usually depends on the availability of pollinators (Klein et al. 2007). For example, it is likely that dangerous pathogens are interspecifically transmitted from flowers of *I. glandulifera* to flowers of tomato *Solanum lycopersicum* during alternating bumblebee visits to flowers of the two species (Najberek et al. in review). Such alternating visits were also noted between another alien balsam, *I. parviflora*, and co-occurring strawberries *Fragaria ×ananassa* (Najberek et al. 2021). Interestingly, in the presented study fungal pathogen *Alternaria tenuissima* (formerly *Alternaria tomato*) was identified in flowers of *I. glandulifera* (in Kraków and in Muszyna). This primary pathogen causes spot diseases of many crops, including nailhead spot of tomato and leaf spots of strawberries (Horst 2013a, Bagherabadi 2015). Thus, it cannot be excluded that *I. glandulifera* could facilitate spreading of this harmful crop pathogen and consequently – decrease yields.

In Muszyna, the cosmopolitan pathogen *Botrytis cinerea* was also recorded from the sampled flowers of *I. glandulifera*. This pathogen causes grey mould disease in more than 200 crop species and could be transmitted by insects (Woodford et al. 2002; Williamson et al. 2007). Moreover, *B. cinerea* is included as the second most important species in the list of top 10 fungal pathogens in molecular plant pathology (Dean et al. 2012); the list was constructed based on the scientific and economic pathogen importance. Interestingly, it was revealed that in greenhouse cultivations the pathogen spread was successfully reduced through inoculation with other fungal species, e.g., *Trichoderma harzianum*, used as biocontrol agents, distributed among flowers by bumblebees and bees. For example, they were successfully applied in strawberry cultivations using *B. terrestris* (Mommaerts et al. 2011). Moreover, it was also revealed that spores of *B. cinerea* may have some nutritional value for pollinators: their consumption with food increases longevity of workers of honey bees *A. mellifera* (Parish et al. 2020). A similar effect was obtained for diets supplemented with four species of *Cladosporium* genus, including *C. cladosporioides* (Parish et al. 2020). Notably, *C. cladosporioides* and *A. alternate* (both recorded from *I. glandulifera* flowers) may also play a positive role in plant-pathogen interactions. These two fungi are considered as less harmful secondary plant pathogens that may deter occurrence of primary pathogens with an invariably negative impact on plants and their propagules. In some circumstances the presence of secondary pathogens may even benefit a plant by limiting its infestation by primary pathogens (Liggitt et al. 1997; Najberek et al. 2018). In the sampled flowers we also found six *Fusarium* species (*F. acuminatum*, *F. avenaceum*, *F. boothii*, *F. equiseti*, *F. graminearum*, *F. sporotrichioides*). *Fusarium* species, classified as most harmful plant pathogens worldwide (Timmusk et al. 2020), are primary pathogens that cause a devastating fungal disease – Fusarium head blight (FHB). Like *B. cinerea*, *F. graminearum*, a highly destructive pathogen of all cereal species, is included in the list of top 10 fungal pathogens in molecular plant pathology (Dean et al. 2012); in our study, this pathogen was recorded in Kraków. Thus, it is highly possible that *I. glandulifera* invasion promotes transmission of at least two extremely dangerous plant pathogens.

Another fungal species detected during the study was a secondary pathogen *Mucor hiemalis* (recorded in the Izerian Foothils and Muszyna). It is a harmless saprophytic species producing secondary metabolites that are toxic to *Thielaviopsis paradoxa* – a pathogen that causes inflorescence brown rot disease; however, the same metabolites could be harmful to insects that may transmit them (Ziedan et al. 2013). Therefore, transmission of *M. hiemalis* may play both a positive and negative role in the pollinator-plant interactions. In Muszyna we also recorded *Boeremia exigua* which causes leaf spots of ornamental plants (e.g., hollyhock and rose-mallow; described as *Phoma exigua* by Horst 2013a) and likewise attacks crops (e.g., sweet potato and oregano; Zimowska 2015; Colmán et al. 2020). In turn, in Kraków and Muszyna we found *Nigrospora oryzae*. This is another pathogen that causes numerous crop diseases, e.g., Ripe Fruit Rot of tomato and Nigrospora Cob Rot of corn (Horst 2013b). This pathogen was found also in guts of bees (Moubasher et al. 2017); therefore, its transmission between flowers seems evident. Notably, *C. cladosporioides* was also identified from the same bee samples, as well as from samples of beetles and weevils (Moubasher et al. 2017). In Muszyna *Stagonosporopsis ligulicola* was also found. However, the impact of this pathogen has been poorly investigated to date. It is known that the species may be associated with ray blight disease of Asteraceae (Vaghefi et al. 2012); the major host of *Stagonosporopsis ligulicola* var. *ligulicola* is an ornamental hybrid *Dendranthema xgrandiflorum* (EPPO 2021).

In the presented study the aspect on floral traits that may influence transmission of pathogens between plant flowers was also taken into consideration. Because flower shape could play a significant role in pathogens transmission (Bodden et al. 2019), we tested the effect of width of flower entry and length of lower sepal of *I. glandulifera* flowers. They have a deep and tight corolla, and we assumed that the probability of pathogen deposition should increase with the dimensions of flower entry and its sepal. However, this expectation was not supported by our results, which is in line with the results obtained by Adler and co-authors (2018). The number of records of pathogen species was not associated with the number of visiting pollinators either. However, it should be stressed that we did not count the number of colonies per each recorded pathogen species. Thus, the quantitative data on infestation with particular pathogens was not taken into consideration in the statistical analyses, while such data may also yield additional insights into the studied phenomena. In turn, flowering power had an important role in the assessment. We found that the number of pathogen species may either decrease or increase with the number of flowers and seed capsules, depending on the study region invaded by populations of different age. In general, the probability of pathogen detection should increase with flowering power, because plants should tend to conceal their real infection level from pollinators by increased production of flowers (Shykoff and Bucheli 1995). This trend was noted in the youngest of the studied populations, in Muszyna, whereas in the middle age and old populations, in Kraków and in the Izerian Foothills, the opposite results were obtained. A possible explanation could be that the lower level of attack from the enemies in Muszyna (including all sources of damage) allowed the plants to relocate the resources into masking fungal infestation by producing more flowers. However, the result was not in line with this explanation, because the plants in Muszyna were not particularly effective in enemy release abilities (plants from the Izerian Foothills were most effective in this respect). Therefore, it is more probable that these differences are associated with secondary metabolites (i.e., polyphenol rich substances) secreted in *I. glandulifera* flowers (Vanderplanck et al. 2019). These metabolites might reduce prevalence of some of the pathogen species. The plants in Kraków and in the Izerian Foothills might have evolved to secrete more metabolites in flowers than the plants in Muszyna. However, this is only a supposition which needs to be tested in the future. Further tests should also include broad assessments of the impact of those metabolites on particular pathogen species. This aspect has been poorly explored to date. Vanderplanck et al. (2019) indicated only a single pathogen susceptible to *I. glandulifera* metabolites (*Apicystis bombi*) and three other species that were resistant to those metabolites (*C. bombi*, *Nosema bombi*, *N. ceranae*). In our tests, none of these pathogen species was detected. Notably, flowers of almost 40% of the surveyed plant individuals were not colonised by any pathogen species, while in the remaining 60% we always found between 1 and 3 species. We did not sample plants/crops co-occurring with

I. glandulifera, therefore, it cannot be assessed if the detected levels of floral pathogens were high, medium or low. However, we did it in a parallel experiment testing the impact of *I. glandulifera* on pollination of cultivated tomatoes *S. lycopersicum* (Najberek et al. in review). We identified significantly more pathogen species and their colonies (including *B. cinerea*) from the flowers of the invasive alien species than from the crop. Therefore, it can be assumed that although flowers of *I. glandulifera* may decrease prevalence of some primary pathogens (e.g., *A. bombi*), at the same time they provide a shelter for many other primary pathogens that are not prone to its floral secondary metabolites. Notably, the risk of primary pathogens transmission from flowers of this invasive alien species should be taken seriously even if the non-infected flowers of the species dominate in a given area. The extraordinary attractiveness of *I. glandulifera* for pollinators results in a large number of visits to its flowers (Chittka and Schürkens 2001; Najberek et al. 2021), which may in turn increase the overall pathogen transmission irrespective of its local pathogen loads.

In our experiment, bumblebees were the most frequently noted pollinator group. Two species dominated – *Bombus pascuorum* (in Kraków and Izerian Foothills) and *B. hortorum* (in Muszyna). The former species was recorded as the dominant pollinator of *I. glandulifera* also in other European studies (Chittka and Schürkens 2001; Nienhuis et al. 2009; Najberek et al. 2021), whereas domination of *B. hortorum* has not been recorded to date. Because the length of its tongue is larger than in *B. pascuorum* and it prefers deeper flower corollas, we assumed that its dominance in Muszyna could be associated with shifts in dimensions of *I. glandulifera* flowers in this region. However, the flowers from Muszyna were smallest and the length of their lower sepal did not differ in comparison with the two other populations. Moreover, we found no evidence that flower size had a significant influence on the results. Therefore, the dominance of *B. hortorum* cannot be explained by the occurrence of deeper flower corollas in the individuals in Muszyna. We found, however, that the width of flowers entry in this locality was larger than both in Kraków and in the Izerian Foothills, which could account for the frequent records of *B. hortorum*. This bumblebee species is meaningfully larger than *B. pascuorum*, therefore the narrower flower entry might be a barrier for this common bumblebee in two other surveyed regions. Such shifts in flower dimensions may appear with time after alien species introduction in new areas and may result from the evolutionary processes and/or phenotypic plasticity (Agrawal 2001; Colautti and Barrett 2013). We also found that in Muszyna *I. glandulifera* individuals co-occurred with equally tall and very dense bushes, and only in this population stem height positively influenced the frequency of pollination. Therefore, future monitoring of evolutionary processes of the young population of *I. glandulifera* in Muszyna, where habitat conditions are more competitive and where *B. hortorum* is, surprisingly, the dominant pollinator, is particularly recommended. It may yield insights into the mechanisms determining post-introduction shifts that alien species are manifesting in populations at the invasion forefront (Blossey and Notzold 1995; Gruntman et al. 2017). It should be also noted that the two bumblebee species, *B. pascuorum* and *B. hortorum*, pollinate different host plants. In Southern Poland, the former one prefers (in the descending order) *Trifolium pratense*, *Salvia glutinosa*, *Galeopsis speciose*, *Galeopsis pubescens* and *Lamium album*, while the latter one – *T. pratense*, *Centaurea jacea*, *Vicia cracca*, *Cirsium oleraceum* and *Cirsium vulgare* (A. Kosior, unpublished data). Therefore, it should be expected that frequent visits of *B. hortorum* to *I. glandulifera* with wider flowers entry in Muszyna may contribute to increased transmission of their pathogens to new native host plants that are pollinated by this species and commonly occur in this region (Flora Polski 2023). As pathogens of these native species have never been studied in Muszyna, it cannot be unequivocally assessed whether *I. glandulifera* was indeed the primary source of the infection, or if it had occurred before its invasion started. However, even if the native plants had already been infected from other source(s), frequent visits of *B. hortorum* to *I. glandulifera* are likely to enhance pathogen spread (spillback mechanism; see Kelly et al. 2009; Roy et al. 2017; Najberek et al. 2022b).

Brain size of bumblebees increases with their body size and it was revealed that individuals with larger brains have superior cognitive abilities to smaller ones (Mares et al. 2005). Larger brains allow, for instance, a better visual resolution (Spaethe and Chittka 2003) and faster learning (Worden et al. 2005). On the other hand, the colony costs of production of larger workers are relatively high, moreover, they have lower survivorship than medium and smaller ones (Kerr et al. 2019). In our experiment we found that bumblebee individuals of medium size were dominant visitors of flowers of *I. glandulifera*, while the numbers of large and small bumblebees were threefold lower. A similar trend was noted in the study on *B. vosnesenskii* (Kerr et al. 2019), where such intermediate-sized workers were assessed as the most beneficial for the colony. We also found that the number of the recorded bumblebees increased with air temperature and decreased with sun radiation and wind speed. The average air temperatures that we recorded were optimal for bumblebee flights (15–25 °C; Pawlikowski et al. 2020), while minimal and maximal temperatures only marginally exceeded this threshold. Under higher air temperatures (> 30°C), the number of visits would probably start to decrease. Notably, it was shown that the number of workers' revisits to probed flowers also increases with air temperature (Najberek et al. 2021); repeated visits of flowers decrease feeding efficiency and may have consequences for the colony economy, as well as for the pollination rate of host plants (Leonard et al. 2011; Najberek et al. 2021). In another study (Xu et al. 2021) it was shown that sun radiation increases the body temperature of insects, which may cause overheating. Therefore, decrease in bumblebee activity with sun radiation, found in our experiment, is well understood. Increasing wind speed that disturbs the bumblebee flight could also be a reason for lower pollinator activity. Thus, it should be expected that *I. glandulifera* individuals growing in shaded and sheltered localities under optimal temperatures (15–25 °C during flowering phase) are most frequently visited by bumblebees. In turn, increased pollination rate determines faster spread and higher invasiveness of the species. Interestingly, we also revealed that the number of pollinator records increased with the area of *I. glandulifera* patches as well as with the numbers of flowers and seed capsules (analysed as flowering power). The results showed that larger patches of this invasive alien plant receive more attention from pollinators than smaller ones, because of superior flowering power and greater nectar reward.

In the presented study the associations between the EICA hypothesis (Blossey and Notzold 1995), pollinator activity and level of fungal pathogens that colonised flowers in populations of different age were investigated. However, we found no confirmation that population age plays a role. Individuals from the young population were not more frequently pollinated than individuals from the two older ones. Therefore, it cannot be concluded that individuals from the young population could reduce their investment into defence against enemies and subsequently re-allocate the saved resources into the improvement of floral traits that facilitate their pollination success; e.g., the investment in larger flower size (non-significant in the presented study). The assumptions of the EICA hypothesis did not hold true in the results obtained for pathogens either. In this case, the lower number of pathogen species was recorded in the oldest population (the Izerian Foothills), while the most harmful plant pathogen species – *B. cinerea* and *F. graminearum* – were detected in Kraków (the middle age population) and in Muszyna (the youngest population). Interestingly, the association between the EICA hypothesis and pollinator activity was found in a non-invasive alien *I. balfourii* population in Zagreb, Croatia (Najberek et al. 2020b). It was demonstrated that the leaf damage by enemies in this population was significantly lower than in four older populations (Croatian Istria, Italian Insubria and Torino, and French Le Rozier), and at the same time pollination rate was highest. In addition, fungal pathogen *Aureobasidium pullulans* was frequently recorded from leaves and seeds in this population (Najberek et al. 2020b). This pathogen produces antibacterial and antifungal substances, which could increase the resistance against primary pathogens that permanently decrease the condition of the host plant (Bozoudi and Tsaltas 2018). Thus, although the invasive *I. glandulifera* and non-invasive *I. balfourii* are closely related (Janssens et al. 2009) changes that they undergo after the introduction in Europe are far from being parallel. It should be also noted that the EICA hypothesis was previously tested using *I. glandulifera* (Gruntman et al. 2017), however, different assumptions were verified. The authors assumed that the saved resources could be reallocated into allelopathic abilities of the species. They demonstrated that the leaf damage recorded in younger populations was significantly lower than that in the older ones, however, the damage was not associated with the level of allelopathic compounds secreted by the surveyed plants. Thus, neither in the previous studies on *I. glandulifera* (Gruntman et al. 2017), nor in the presented ones, the assumptions associated with the EICA hypothesis were confirmed.

Conclusions

Pollinators facilitate the spread of primary pathogens with invariably negative impact on plants, as well as less harmful secondary plant pathogens (their presence may even benefit a plant) and saprotrophs. In the presented study we demonstrated that the invasive alien *Impatiens glandulifera* may play a significant role in this process. The nectar-rich flowers of this species are very attractive for common pollinators and should be considered as a hot spot in intraspecific and interspecific pathogens transmission in the invaded communities. We identified each of three types of pathogens from the species flowers: primary pathogens, secondary pathogens and also saprotrophs. The most dangerous plant pathogens were *Botrytis cinerea* and *Fusarium graminearum*. These two fungal pathogens cause devastating diseases of native plant species and hundreds of crop species worldwide and are included in the list of top 10 fungal pathogens in molecular plant pathology (Dean et al. 2012). Therefore, facilitation of their transmission by *I. glandulifera* invasion decreases crop production and increases economic losses. Unfortunately, this aspect of harmful impact of alien plant species, as *I. glandulifera*, is neglected. There is only a single experiment on negative influence of invasive alien plant species on crops (Najberek et al. 2021), whereas biological invasions may have critical consequences for human economy. Although the European Union has tightened the law on the possibility of spread and new introductions of the worst alien species (*I. glandulifera* is included in the regulation; European Commission 2017), the level of control of the most invasive alien species is still insufficient.

The surveyed *I. glandulifera* flowers were pollinated mainly by two bumblebee species, *Bombus pascuorum* and *B. hortorum*. The former one is known from its association with this plant species, while the latter has never been recorded as its dominant pollinator. Its domination was recorded in the youngest surveyed population of *I. glandulifera*, in which the flowers had wider flower entries than in two other populations. *B. hortorum* is a large bumblebee and narrower flower entry may be a barrier for this species. We suppose that the revealed shifts in flower shape of *I. glandulifera* may have occurred after its introduction in Europe. Nevertheless, this supposition needs to be confirmed in further experiments testing a post-introduction shift of floral traits of *I. glandulifera*. It should also be noted that exceptionally frequent visits of *B. hortorum* to *I. glandulifera* in the population where plants have probably undergone a post-introduction shift may contribute to a significant increase in the transmission rate from *I. glandulifera* flowers to new native host plants that it also frequently pollinates.

It is noteworthy that the results of our study could not be explained based on the assumptions of the EICA hypothesis. We found that population age of plants surveyed in different regions was not associated with the numbers of recorded pollinators; moreover, the results of flowers' infestation by primary pathogens were even contrary to these assumptions. In addition, in the presented study the evidence of negative impact of recorded pathogen species on host plants (including crops) is provided, whereas still little is known on the impact of those pathogens on the pollinators that transmit them. Single studies demonstrated that the interactions between plant pathogens and pollinators could be beneficial (e.g., *B. cinerea* may have nutritional value for *Apis mellifera*; Parish et al. 2020). However, it cannot be excluded that for some other pollinators, such as hoverflies, the same pathogens could be detrimental. It is clear that flower visiting may increase the risk of acquiring parasitic infection by pollinators (Durrer and Schmid-Hempel 1994). Therefore, further studies on the impact of pathogens on pollinator that are carrying them are strongly recommended.

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Supplementary material 1

Raw data used to perform statistical analyses

Authors: Kamil Najberek, Wojciech Solarz, Wojciech Wysoczański, Ewa Węgrzyn, Paweł Olejniczak

Data type: Tables

Explanation note: Raw data contains datasets on recorded pollinators and floral pathogens used in statistical analyses.

Link: https://doi.org/10.3897/neobiota.87.102576.suppl1

Supplementary material 2

Data on *I. glandulifera* individuals, bumblebees pollinating them, and additional information on statistical analyses

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Data type: Tables, plots

Explanation note: Supporting tables contain data on the size of the surveyed *I. glandulifera* individuals as well as on the statistical models selection. Supporting figures demonstrate data on bumblebee species recorded from *Impatiens glandulifera* as well as the influence of weather conditions on the obtained results.

Link: https://doi.org/10.3897/neobiota.87.102576.suppl2