Research article

Juvenile biological traits of *Impatiens* species are more strongly associated with naturalization in temperate climate than their adult traits

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**A B S T R A C T**

Potentially invasive species can be identified before they start to spread by comparing their traits with those of successful invaders. A powerful tool for delimiting the traits associated with invasiveness are analyses of a number of species of the same genus, where it is more likely to detect relevant differences because of elimination of biases that constrain the search for such traits in whole floras. Since the influence of traits on invasion success may differ with respect to the stage of the plant’s life cycle, comparative studies should address the whole life cycle, including early stages. Here we studied which biological traits are associated with the ability to naturalize within the genus *Impatiens*, how frequency of planting affects naturalization success, and whether naturalized species with biological traits similar to the native representative of this genus are more successful. The genus *Impatiens* includes a number of cultivated species popular in horticulture, among them several widespread invaders. We used one native and 10 alien annual taxa. This data set involved all commonly cultivated species, and representatives of different invasion status in Europe. In garden experiments and climatic chambers we measured seed mass, time to germination, percentage of seeds germinated, seedling growth rate, total seedling biomass, seedling root/total biomass ratio, adult biomass and fecundity. These traits and planting frequency were used to explain the invasion success of the species, expressed as (i) invasion status in Europe and (ii) the number of global temperate regions in which the species has been reported as naturalized. The frequency of planting was used as a proxy of propague pressure to separate this potentially biasing factor known to affect plant invasiveness from the effect of plant traits. We found that both species traits and frequency of planting were correlated with naturalization. Species naturalized in many temperate regions of the world had heavier seeds, high seedling growth rate and allocated low proportion of seedling biomass to roots. Importantly, common planting was more strongly correlated with naturalization success than with biological traits. *Impatiens* species naturalized in Europe exhibited better seed germination in the common garden, and it took a longer time for the seeds to germinate. Species escaped from cultivation but occurring only as casuals in Europe had heavy seeds and invested more resources into shoots than roots, whereas species not escaping from cultivation were characterized by fast seed germination and light seed. In general, traits linked to early stages of the life cycle were more strongly associated with invasion success than those of the adults. Frequently planted species tend to naturalize more easily than those planted scarcely. The successful invaders share traits similar to the one native *Impatiens* species in Europe and those with traits distinct from it do not invade. Our results indicate that many *Impatiens* species represent potential invaders should their planting become more widespread; this prediction is supported by the fact that *Impatiens* species included in the experiment completed their life cycles in an experimental garden in central Europe.

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1. Introduction

Effort to identify biological traits associated with plant species invasiveness has been central to plant invasion ecology (Roy, 1990; Pyšek and Richardson, 2007; van Kleunen et al., 2010a). As the traditional methodical approach towards identifying the role of species traits, comparisons of complete regional floras, is constrained by great variability within the species pools which makes it difficult to detect the respective traits (Moravcová et al., 2010), discovering which species traits promote invasiveness is thus more realistic at the generic or familial level (Rejmánek and Richardson, 1996; Burns, 2004; Cadotte et al., 2006; Pyšek and Richardson, 2007; van Kleunen et al., 2010b). Searching for traits linked to invasiveness in related species is also justified by the fact that most variation is observed among species within genera (Moravcová et al., 2010). This indicates that the predictions of species invasiveness should be done at the species level (Pyšek et al., 2009, 2014a; Moravcová et al., 2010).

Up to now, comparisons of closely related species have been used to reveal traits associated with invasiveness in several genera or families, including Pinus (Rejmánek and Richardson, 1996; Grotkopp et al., 2002; Matzek, 2011), Senecio (Sans et al., 2004), Rubus (McDowell, 2002), Oenothera (Mihulka et al., 2006), Eucalyptus (Radho-Toly et al., 2001), Lespedeza (Woods et al., 2009), Crepis and Centaurea (Muth and Pignlucchi, 2006; Muth and Pignlucchi, 2007), Impatiens (Perrins et al., 1993; Perglová et al., 2009; Skálová et al., 2011, 2012, 2013), bracheaeae (van Kleunen and Johnson, 2007). For details and other studies see review by Pyšek and Richardson (2007). To date, however, few studies have included more than four species within the genus, which somewhat limits the possibility to detect important traits and generalize beyond the specific circumstances of a given study (but see Rejmánek and Richardson, 1996; Grotkopp et al., 2002; Muth and Pignlucchi, 2006, 2007; van Kleunen and Johnson, 2007; Matzek, 2011).

Traits of invasive species have been found to differ from those of the native and non-invasive alien species (van Kleunen et al., 2010b), but the relevance of such traits for invasiveness is not the same throughout the whole life cycle. For example, the relative importance of two key concepts related to invasion from the perspective of community ecology, limiting similarity (Abrams, 1983) and competition displacement (Brown and Wilson, 1956), is thought to change during the invasion process, with trait similarity being more important for establishment (Williamson, 2006; Funk and Vitousek, 2007) and dissimilarity for naturalization and invasion (Blackburn et al., 2011; Richardson and Pyšek, 2012). However, studies assessing traits along the complete species’ life-cycle are rather rare (but see Radford and Cousins, 2000), despite the well known importance of early life stages, such as seed germination (Moravcová et al., 2010; Chrobok et al., 2011; Skálová et al., 2011) and seedling growth (Grotkopp and Rejmánek, 2007; Skálová et al., 2012) for population establishment and the beginning of invasion (van Kleunen and Johnson, 2007). The importance of seed and juvenile traits for invasion success was demonstrated e.g. for pines (Richardson, 2006).

Finally, it has been well established that whether a species becomes naturalized or invasive in a new region depends on the propogule pressure, a factor that acts in concert with species traits (Richardson and Pyšek, 2006; Pyšek et al., 2015). Propagule pressure is a function of reproduction and dispersal. Since the majority of naturalized and invasive species recruit from deliberately introduced plants (Mack, 2003; Hulme, 2011; Pyšek et al., 2011), horticulture represents the most important pathway of introduction (Groves et al., 2005; Dehnen-Schmutz et al., 2007a; Pyšek et al., 2012), and the frequency of planting can directly affect propogule pressure. Moreover, horticulture selects plants with fast growth and easy reproduction, namely high seed production, easy and massive germination or vigorous clonal reproduction, minimum gardening care, resistance to pathogens and other enemies and wide environmental tolerance (Mack, 2000). Studies searching for traits related to invasiveness revealed just the same traits (Pyšek and Richardson, 2007; van Kleunen et al., 2010b). In addition, plants introduced intentionally could have been preadapted to the local conditions by horticultural selection before they were launched on the market. Thus the probability of naturalization of such plants is greater (Milbau and Stout, 2008; Pyšek et al., 2011) and the process, including subsequent invasion, can be accelerated by horticulture (Pyšek et al., 2002; Hulme, 2011). Repeated introductions, which are typical of deliberately introduced plants, also increase the probability of naturalization and invasion (Richardson, 2006).

In this paper, we address the importance of traits for invasion success by using one native and 10 alien taxa of the genus Impatiens, all but one of which are annuals. We included all species within the genus that are commonly cultivated in the temperate climate. The genus includes ~1000 species (Grey-Wilson, 1980; Fisher, 2004), many of which are being introduced as popular ornamentals, and some have become invasive in various parts of the world (Adamowski and Tokarska-Guzik, 2008). The most prominent example is I. glandulifera, a highly invasive annual in temperate regions (Beerling and Perrins, 1993; Hejda and Pyšek, 2006; Clements et al., 2008). Impatiens parviflora has also invaded the temperate zone (Trepl, 1982; Hejda, 2012) while I. walleriana, the only perennial among the tested species, invades in the tropics (CABI, 2014; Pacific Islands Ecosystems at Risk, 2013). The other invasive species are I. balfouri, rapidly increasing its range in southern Europe (Adamowski, 2009; Schmitz and Dericks, 2010), I. capensis, with an invaded range in western and central Europe and eastern Asia (Perrins et al., 1993; Adamowski and Tokarska-Guzik, 2008), and I. balsamina, which has become widely naturalized in many areas of the warm temperate zones and the tropics. Impatiens balsamina has been known for ~4000 years in India (Grey-Wilson, 1983), but other Impatiens species have been grown for only the last ~150 years. The high number of planted Impatiens species (~90 species worldwide, according to their presence in horticultural databases; Plant Finder of the Royal Horticultural Society; PlantFiles of Dave’s Garden; HortiPlex Plant Database of GardenWeb), together with the fact that many of them have become invaders, some of them widespread, makes the genus a suitable model group to ask what drives the performance of those that succeeded. As the genus includes both successful invaders and species that do not invade despite being planted provides an opportunity to compare species traits of closely related species unbiased by phylogenetic effects. Moreover, the presence of both native and invasive Impatiens species in our study area of central Europe makes it possible to assess the role of invaders’ biological similarity to the native species, and whether or not it is beneficial for naturalization in the new range. To obtain insights into the mechanisms of invasion within the genus, we thus ask the following questions: (i) Which biological traits are associated with the ability to naturalize in the genus Impatiens? (ii) What is the role of the frequency of planting in the probability of becoming naturalized? (iii) Are the traits of the native species, that is successful in a given setting, close to those of the successfully naturalized species?

2. Material and methods

2.1. Study species

We selected nine alien species of Impatiens, and one cultivar (further termed ‘species’ for simplicity), differing in their invasion
Table 1
Overview of the Impatiens species with their characteristics. Invasion status (naturalized=established in DAISIE terminology) is taken from DAISIE (2015), number of global temperate regions where naturalized from the GloNAB database (van Kleunen et al., 2015 see text for details). Planting frequency was based on the knowledge of cultivation of the given species in the past, seed availability on Internet and availability on botanical gardens seed lists (Index semina 2009 and 2010). Information on the first report of cultivation, region of native distribution and invaded range is according to Adamowski and Tokarska-Guzik (2008), the Global Biodiversity Information Facility (www.gbf.org), Tropicos (www.tropicos.org), Baade and Gatte (2008), Baader (2014), http://waarneming.nl/waarneming/view/51036938. Seed sources: Antwerp Botanic Garden, Botanical Garden Graz, Botanical Garden in Krefeld, Botanical Garden of the Masaryk University, Botanical Garden of the Regensburg University, Botanical Garden of the University of Agronomic Science and Veterinary Medicine in Bucharest, Botanical Garden of the University of Hohenheim, Botanical Garden and Botanical Museum Berlin-Dahlem, Ecological-Botanical Garden in Bayreuth, Siauliai University Botanic Garden, Station of Nature Research and Environmental Education – Marijampole, W. J. Beal Botanical Garden of the Michigan State University; seeds were obtained via seed lists from years 2009 (in the table marked by *) and 2010.

<table>
<thead>
<tr>
<th>Invasion status</th>
<th>Number of naturalized temperate regions globally</th>
<th>Planting frequency</th>
<th>Cultivation</th>
<th>Region of native distribution</th>
<th>Invaded range</th>
<th>Seed sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. balsamina L. (incl. I. balsamina ‘alba’)</td>
<td>Casual</td>
<td>34</td>
<td>Common</td>
<td>2000 BC, India, 1542 Europe</td>
<td>India, Myanmar</td>
<td>W Himalaya</td>
</tr>
<tr>
<td>I. capensis Meerb.</td>
<td>Naturalized</td>
<td>10</td>
<td>Rare</td>
<td>&lt;1822 Great Britain</td>
<td>N America</td>
<td>W Himalaya</td>
</tr>
<tr>
<td>I. edgeworthii Hook. f.</td>
<td>Casual</td>
<td>1</td>
<td>None</td>
<td>17983, Germany</td>
<td>No data</td>
<td>W Himalaya</td>
</tr>
<tr>
<td>I. fluminensis Hook. f.</td>
<td>Not escaped</td>
<td>0</td>
<td>None</td>
<td>1839, Great Britain</td>
<td>No data</td>
<td>Europe, Asia, N America, New Zealand</td>
</tr>
<tr>
<td>I. glandulifera Royle</td>
<td>Naturalized</td>
<td>45</td>
<td>Common</td>
<td>1839, Great Britain</td>
<td>No data</td>
<td>W Himalaya</td>
</tr>
<tr>
<td>I. noli-tangere L.</td>
<td>Native</td>
<td>0</td>
<td>–</td>
<td>Europe, N &amp; E Asia, W N America</td>
<td>–</td>
<td>N America</td>
</tr>
<tr>
<td>I. parviflora DC.</td>
<td>Naturalized</td>
<td>30</td>
<td>Common</td>
<td>1830, Switzerland</td>
<td>C Asia</td>
<td>N America, E Asia</td>
</tr>
<tr>
<td>I. scabra DC.</td>
<td>Casual</td>
<td>0</td>
<td>Rare</td>
<td>1836</td>
<td>Himalaya</td>
<td>Europe, N America, E Asia</td>
</tr>
<tr>
<td>I. walleriana Hook. f.</td>
<td>Not escaped</td>
<td>9</td>
<td>Common</td>
<td>1883, Europe</td>
<td>E Africa</td>
<td>Tropical and warm temperate regions</td>
</tr>
</tbody>
</table>

status (sensu Richardson et al., 2000) in Europe: four are naturalized (i.e., create self-sustaining populations), three casual aliens (depend on repeated introductions by humans for their occurrence in the wild) and two are only planted and not escaping from cultivation, and the native species I. noli-tangere (Table 1). The cultivar I. balsamina ‘alba’ was included in the study because it exhibited the most vigorous growth and highest fecundity in comparison with all of other cultivars of I. balsamina (I. b. ‘flore’, and I. b. ‘violacea’) and the botanical species of I. balsamina in a pilot experiment. All species were annuals, except for I. walleriana, which is perennial, but it is commonly planted from seed as an annual plant in the temperate zone. The majority of species included in the study have been cultivated since ~1850, but only some of them to great extent such as I. balsamina and I. walleriana recently, and I. glandulifera in the past (Table 1).

2.2. Invasion success

We used two variables to describe the invasion success of the Impatiens species studied: First, (i) invasion status in Europe (from DAISIE, 2015), taken as the prevailing stage of the invasion process reached in countries where the species was recorded. According to DAISIE we distinguished whether a species is naturalized (‘established’ in DAISIE terminology), casual or did not escape from cultivation; this reflects the realized invasion potential in the target region of Europe. The second was (ii) the number of global temperate regions in which the species is known to occur as naturalized. Here, we restricted data to naturalization in temperate regions only, because this corresponds to experimental conditions under which Impatiens species were grown in our experimental garden. The data on naturalization was taken from the GloNAB database, the most comprehensive resource containing information on naturalized alien floras in 843 regions of the world (van Kleunen et al., 2015).

2.3. Frequency of planting

We included for each species the estimate of the frequency of planting in Europe, a proxy for propagule pressure, which is known to affect the invasion success (e.g. Dehnen-Schmutz et al., 2007a; Pyšek et al., 2015) and therefore could bias the effect of plant traits. The frequency of planting was scored on a rough scale (none; rare; common) based on the knowledge of cultivation of the given species in the past, seed availability on internet and availability on botanical gardens seed lists (Index Semina 2009 and 2010).

2.4. Species traits

For the study species, we measured traits that are supposed to be beneficial to plant fitness and potentially related to ability to naturalize or to invasiveness. Our aim was to cover plant performance over the whole life cycle, by including not only traits of adult plants that are most commonly addressed in studies on invasiveness, but also traits related to seeds, and seedling stage. We measured the following traits in the three experiments: (i) experimental garden – mostly adult traits (adult aboveground biomass – further referred as ‘adult biomass’; fecundity and seed mass), (ii) climatic chambers – seedling traits (seedling growth rate; total seedling biomass; and seedling root/total biomass ratio), (iii) experimental garden – seed germination (time since seed sowing to germination and seed germination rate). We measured also adult plant height, but we decided not to include it in analyses, because we consider the adult plant’s biomass a better proxy of competitive strength, and both measures were closely correlated (r = 0.73; also see Fig. 3F).
2.5. Seed sources

Seeds of Impatiens species occurring in the Czech Republic (I. noli-tangere, I. parviflora and I. glandulifera) were collected in summer 2011 in eastern and central Bohemia in the field; seeds of the other species were obtained from botanical gardens making seeds available via seed lists (Index Seminum) in 2009 and 2010 (Table 1). Seeds from the botanical gardens were sown in 2011 and plants grown in a glasshouse at the Institute of Botany CAS, Průhonice, Czech Republic, to obtain sufficient number of seeds for the experiments, to check species identity and avoid possible maternal effects (Roach and Wulff, 1987). After collection, seeds of I. noli-tangere and I. capensis were kept in a refrigerator at 3 °C on heat-sterilized wet river sand in Petri-dishes, as dry storage decreases the seed germination considerably (Perglová et al., 2009). Seeds of other species were stored in paper bags at room temperature.

2.6. Seed germination in the experimental garden

Twenty seeds were placed in a 10 cm × 10 cm × 15 cm pot filled with heat-sterilized common garden soil and covered by a thin layer (0.5 cm) of soil, in 10 replicates per species. For I. flemingii we were able to establish only seven replicates, because of the low number of available seeds. This germination experiment was established on 24 October 2012 in the experimental garden of the Institute of Botany CAS in Průhonice (49°59′38.972″N, 14°33′57.637″E; mean annual temperature 8.6 °C; mean annual precipitation 610 mm). The germination of seeds was recorded by counting the emerged seedlings once a week from 31 January 2012 to 4 June 2013; the recording ceased when the number of seeds germinated between the two weeks was negligible. These data were used to compute mean time to germination (MTG), further referred to as ‘time to seed germination’, which was computed as: $MTG = \sum (n \times d)/N$, where $n$ is the number of seeds germinated between scoring intervals, $d$ is the incubation period in days since the beginning of the experiment, and $N$ is the total number of seeds germinated in the treatment.

2.7. Seed mass and seedling growth

Seeds were weighed on a micro-balance with a precision of 10⁻⁴ g in groups of 10 in 15 replicates (I. flemingii only in 13 replicates, due to the low number of available seeds). The seeds were placed on heat-sterilized wet river sand into Petri-dishes and kept at 5 °C. The germinating seeds (with about 2 mm radicles) were transplanted into the plastic trays with individual 39 ml wells filled with sterilized river sand. Ten seeds per species were used, each planted in one well. Trays were put on the plastic plates and supplied with 50% Knop solution, which was maintained at the level of 1–2 cm during the experiment to provide plants with optimal conditions.

To achieve stable nutrient supply, conductivity of the solution was measured three times a week and nutrient solution or demineralized water was added to keep the conductivity at 1770 µS/cm. The nutrient solution was changed about every 10 days to prevent the growth of algae. The seedlings were cultivated in climatic chambers (Vötsch 1014) under a 16/8 h light/darkness regime, with mean humidity of 70% during the light and 80% during the dark period. The temperatures used simulated the gradually changing daily temperatures recorded in the field in spring from a minimum of 5 °C to a maximum of 19 °C (for details see Skálová et al., 2012). Seedling height was measured since the release of the cotyledons from the testa two times a week. After 10 measurements (~1 month in total) the seedlings were harvested and separated into roots and shoots. The biomass was dried at 70 °C for about 24 h and weighed.

2.8. Adult traits

Individuals were grown separately, 10 replicates per species, in 20 cm × 20 cm × 23 cm pots with ~5L of heat-sterilized common garden soil. Plants were watered daily by a micro-drip system (Hunter Industries, San Marcos, USA) to provide full water supply. To avoid full sunshine, shading net transmitting 50% of incident radiation was used, to meet the Impatiens species’ general requirement of partial shade (Morgan, 2007; Čuda et al., 2014). Plants were harvested individually, when the first symptoms of senescence appeared, and their height was measured. Peduncles were separated from the shoot biomass, counted, and considered as a substitute for the number of capsules; they were dried separately from the remaining shoot biomass at 70 °C for about 24 h and weighed. Fecundity was expressed as the number of seeds, which was calculated as the number of capsules × average number of seeds in the capsule, derived from seed counts in ~60 capsules (from 45 to 96) per species. The capsule sampling for seed counts was done continuously throughout the whole fruiting season, as the number of seeds in capsule varies over time (J. Čuda, personal observation). This experiment was carried out in the experimental garden of the Institute of Botany CAS, in 2012.

2.9. Data analysis

We analyzed the data obtained from experiments both by univariate and multivariate methods. First, we conducted separate analyses of variance in seed mass, time to seed germination, total seedling biomass, seedling root/total biomass ratio, adult biomass and fecundity, using species identity as the only predictor. These were followed by post hoc Tukey HSD tests. Meeting the assumptions of linear models was checked by means of regression diagnostic plots. This resulted in logarithmic transformation of some response variables (seed mass, adult biomass and fecundity) in order to improve the homogeneity of variance. Seed germination from the common garden was analyzed due to its binomial nature by means of Generalized linear models (GLM) of quasi-binomial family, since also considerable overdispersion was detected (dispersion parameter $\Phi = 3.35$). The seedling growth rate was computed as the species-specific regression slope derived from the linear mixed effect models fit to square-root transformed seedling height. Linear mixed effect models (LME) were used due to repeated measures of individual plants with time since planting and its interaction with species identity as fixed effects and random intercepts and slopes of time since planting for each measured individual. The post hoc tests cannot be conducted both in LME and quasi-binomial GLM analyses and therefore we specified the species identity predictors by use of treatment contrasts with I. noli-tangere set as a reference species and tested the differences of coefficient estimates of alien species from those of the native I. noli-tangere by means of Wald tests (Crawley, 2007). The residual degrees of freedom necessary for the Wald tests were calculated for LME-analysis by Kenward–Roger approximation (Halekoh and Højsgaard, 2012).

We used the obtained species traits, i.e., model estimates of species-specific coefficients (with exception of I. walleriana, which was missing in some of the experiments due to zero seed germination) as the main dataset for multivariate analyses (estimates of fixed effects were used in the case of seedling growth and back-transformed estimates were used in the case of seed germination rates in the common garden). The variation in the species traits was summarized by means of principal component analysis (PCA).

The main aim of the multivariate analysis was to relate the traits of nine (I. walleriana was excluded because it did not complete the life cycle, thus some traits were missing) alien species and their frequency of planting to their naturalization, expressed
as the naturalization status in Europe and the number of geographic temperate regions worldwide where naturalized. Since there is no clear causality between the species traits, and the measures of naturalization and frequency of planting (i.e., no response and predictor dataset), we decided to use co-inertia analysis to test for association between these two datasets (Dray et al., 2003). We utilized RV-coefficient, a multivariate extension of $R^2$, as a measure of agreement among the two datasets (Robert and Escoufier, 1976). The significance of its value was assessed by permutation tests with 9999 replications.

All analyses were conducted in R 3.1.1 statistical environment (available at www.r-project.org). Besides the base installation packages, we used packages: lm4 1.1-7 for fitting LME model; pbkrtest 0.4-1 for calculating the Kenward–Roger approximation of residual degrees of freedom of LME model; and ade4 1.6-2 for conducting the Co-inertia analysis and testing the significance of RV-coefficient by permutation test (Dray and Dufour, 2007). The ordination diagrams were drawn CANOCO v. 5.0 (ter Braak and Šmilauer, 2012).

### Table 2

<table>
<thead>
<tr>
<th>Seed mass</th>
<th>Naturalized regions</th>
<th>Naturalized</th>
<th>Casual</th>
<th>Not escaped</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.79</td>
<td>0.19</td>
<td>0.52</td>
<td>–0.75</td>
<td></td>
</tr>
<tr>
<td>Time to seed germination</td>
<td>0.51</td>
<td>0.54</td>
<td>0.37</td>
<td>–0.99</td>
</tr>
<tr>
<td>Seed germination</td>
<td>0.38</td>
<td>0.77</td>
<td>–0.2</td>
<td>–0.68</td>
</tr>
<tr>
<td>Seedling growth rate</td>
<td>0.75</td>
<td>0.4</td>
<td>–0.17</td>
<td>–0.29</td>
</tr>
<tr>
<td>Total seedling biomass</td>
<td>0.45</td>
<td>–0.03</td>
<td>0.25</td>
<td>–0.21</td>
</tr>
<tr>
<td>Seedling belowground/total biomass ratio</td>
<td>–0.55</td>
<td>0.23</td>
<td>–0.49</td>
<td>0.23</td>
</tr>
<tr>
<td>Adult biomass</td>
<td>–0.25</td>
<td>–0.12</td>
<td>0.24</td>
<td>–0.1</td>
</tr>
<tr>
<td>Fecundity</td>
<td>–0.6</td>
<td>–0.53</td>
<td>0.29</td>
<td>0.32</td>
</tr>
<tr>
<td>Planted not</td>
<td>–0.53</td>
<td>–0.58</td>
<td>0.33</td>
<td>0.33</td>
</tr>
<tr>
<td>Planted rare</td>
<td>–0.48</td>
<td>0.26</td>
<td>–0.45</td>
<td>0.15</td>
</tr>
<tr>
<td>Planted common</td>
<td>0.95</td>
<td>0.26</td>
<td>0.15</td>
<td>–0.45</td>
</tr>
</tbody>
</table>

3. Results

3.1. The relationship of biological traits and frequency of planting with the species’ ability to naturalize

The species traits and the frequency of species’ planting were closely correlated with the measures of naturalization success; the multidimensional correlation coefficient was 0.697, $P = 0.019$. Different biological traits were important in affecting the species’ naturalization in Europe, and the number of temperate regions where it has become naturalized globally (Fig. 1, see also Table 2). In general, seedling traits were more strongly correlated with main gradients of the ability to naturalize than were the adult traits (biomass and fecundity). High frequency of planting was strongly positively correlated with the number of naturalized temperate regions worldwide ($r = 0.95$, see Fig. 1 and Table 2) and negatively with not escaping from cultivation in Europe ($r = –0.45$).

Species naturalized in many temperate regions of the world were positioned close to each other within the trait space (I. glandulifera, I. parviflora, I. balsamina incl. I. balsamina ‘alba’, and I. capensis; see Fig. 2 and Table 2) and had heavy seed, high seedling growth rates and low proportion of total seedling biomass allocated to roots. On the contrary, species naturalized in few or no temperate regions showed much scatter in their traits. For instance, I. flemingii and I. scabrida, not reported as naturalized anywhere in the world, had shorter time to seed germination and low seed mass (see Table 2 for details). Common planting of Impatiens species was more strongly associated with naturalization success than were their biological traits ($r = 0.95$; see Table 2 and Fig. 1).

Plants naturalized in Europe exhibited high seed germination ($r = 0.77$, see Table 2), and long times to seeds to germinate; species known only to occur as casuals had heavy seeds and allocated more resources into shoots than roots (Fig. 1, see Table 2 details). Species not reported to escape from cultivation had short times to germination and light seeds. Commonly planted species are likely to escape from cultivation (see Table 2), the only exception being I. walleriana that does not escape although commonly planted.

3.2 Differences in traits between native and alien species

The native species, I. noli-tangere, did not substantially differ in biological traits from the alien species studied. Its position in the multidimensional space derived from biological traits lies among those of the other species (Fig. 2), and exhibited similar values of all traits (Fig. 3A–H). Traits of the two most invasive species in central Europe, I. glandulifera and I. parviflora, and I. balsamina, especially I. balsamina ‘alba’ were more similar to those of the native species than were traits of the other aliens, and the same was true for the
native species’ close relative I. capensis. On the contrary, traits of species which do not invade (I. flemingii, I. scabrida, see Figs. 2 and 3) were different from those of the native I. noli-tangere as well as from those that invade.

4. Discussion

4.1. Biological traits associated with naturalization within Impatiens

Impatiens species naturalized in many temperate regions had heavier seeds than less broadly distributed species, which contradicts the results of some previous studies that invasive species have on average lighter seeds (Rejmánek and Richardson, 1996; Moravcová et al., 2010; but see Crawley et al., 1996). Generally heavy seeds disperse worse than light seeds; this relationship was observed both among (Harper et al., 1970) and within species (Morse and Schmitt, 1985; Cappuccino et al., 2002). The trade-off between seed mass and dispersal capacity drives the balance between the probability of species’ establishment and spread. In the Impatiens species studied dispersal seems to be less important than establishment, or alternatively, seed mass may not be so closely related to seed dispersal. The explanation could be that invasive Impatiens species effectively disperse over long distances by soil movements, animal activity, forestry machines (Coome, 1956), and by water flow (Lhotská and Kopecký, 1966); these modes of dispersal are not much affected by seed mass. In a similar vein, species in cultivation are dispersed with garden waste (Adamowski and Tokarska-Guzik, 2008) where differences in seed mass are unlikely to play a role as well. The seemingly surprising negative relationship between naturalization success and fecundity is probably due to the general inverse relationship between seed mass and fecundity. One possible explanation is that the advantage gained from producing large seed outweighs possible constraints from a lower seed number, resulting from the trade off between the number and mass of the seed per plant (Westoby et al., 1992).

Heavy seeds are also advantageous for germination and seedling development, hence plant establishment (Baskin and Baskin, 1998). This is supported by the pattern found in our study where the seedlings of heavy-seeded species had higher germination rates and grew faster. The high performance of large-seeded Impatiens can be related to light requirements of the majority of species; i.e., their preference of shaded sites (Morgan, 2007). Shade-prefering plants in general tend to have larger seeds, ensuring enough stored reserves before the plant develops sufficient leaf area (Walters and Reich, 2000).

In our data, there was a strong relationship between Impatiens seed mass and time to seed germination; similarly to Tombach and Linhart (1990), who found that heavy seeds need a longer chilling period in order to overcome dormancy. This is one of the mechanisms involved in winter seed survival that is very important in temperate regions to ensure proper timing of germination (Baskin and Baskin, 1998). Proper seed germination is crucial for establishment of Impatiens species populations, as a mechanism to avoid winter frosts to which they are rather sensitive (Beerling, 1993; Skálová et al., 2011). The low hardness of Impatiens species probably causes their limited spread in colder regions, despite many of them being cultivated; the importance of hardness in general for plant invasion has been previously documented (Dehnen-Schmutz et al., 2007b; Hanspach et al., 2008).

The most successful invaders in Europe, I. glandulifera and I. parviflora, are both superior to their non-invasive congeners in at least one particular trait. Impatiens glandulifera is much taller and both its seedlings and adults produce much more biomass than all other species, making its individuals highly competitive. Impatiens parviflora had the highest germination rate of all species, indicating its perfect adaptation to local (climatic) conditions, which could be related to one of the earliest introduction dates to Europe of all species tested (Table 1). The invasion success of I. parviflora is also ascribed to its extreme plasticity (Skálová et al., 2012, 2013) and wide environmental tolerance, especially to shade and drought (Coome, 1956; Čuda et al., 2014), enabling growth both in spruce plantations and along river banks (Sádlo et al., 2007). We suggest that I. parviflora is an example of a species filling an empty niche not occupied by a native species (Hejda, 2012), which compensates for its low competitiveness (Skálová et al., 2013; Čuda et al., 2015).

The ability to naturalize of I. balfourii and I. capensis may be limited by poor seedling growth, which is not offset by the high germination of seeds. However, I. balfourii is highly invasive in western Europe and is currently spreading in southern Europe (Adamowski, 2009), which may be due to higher spring temperatures. Similarly I. capensis invades areas with a mild temperate climate in western Europe (Adamowski and Tokarska-Guzik, 2008; GBIF, 2012). Unclear is the future of invasion of I. edgeworthii, because this species has both traits of a successful invaders and species, which do not invade, but the growing number of records from Germany indicates its potential to spread (Baade and Gutte, 2008; Weiss, 2013; Kalveram, 2014).

The two proxies of naturalization were not associated with the same traits: for example the number of regions where the species is naturalized showed relatively weak relationship to seed germination, but the species naturalized in Europe were strongly positively linked with this trait. Invasive species in Europe (I. parviflora, I. capensis, I. glandulifera) had higher seed germination and germinated later than non-invasive species, similar to the pattern reported for Senecio species (Radford and Cousens, 2000). Conversely, I. flemingii, which is not known to escape from cultivation, had low seed germination and an extremely short time to germinate. Differences in predictive power between both measures of naturalization most likely reflect their nature: the
naturalization status in Europe is a qualitative trait related to the ability to become naturalized in a new region, whereas the number of temperate regions globally relates also to the species’ ability to become widespread. Therefore, the latter relates not only to ability to naturalize but also provides some indication of invasiveness at the global scale.

Both measures of naturalization were positively associated namely with delayed germination, followed by rapid growth of seedlings (see Fig. 1 and Table 2). This indicates that the seed and seedling traits were more closely associated with successful naturalization within Impatiens than the traits of adult plants, such as biomass and fecundity. The importance of early life stages for invasiveness (Grotkopp and Rejmánek, 2007; van Kleunen and Johnson, 2007; Dawson et al., 2011; Skálová et al., 2012) and fitness in general is crucial especially for annuals that have to succeed in early life stages to reach the only reproduction in their life, while perennial plants can persist in early life stages for many seasons before they reproduce. All but one species in our study were annuals that have very poor or non-existing seed banks (Perglová et al., 2009); this makes the early life stage even more critical because their persistence at a site is crucially dependent on successful reproduction and establishment in a given year.

4.2. Commonly planted species are more likely to become naturalized

Our results support the assumption that frequency of planting contributes to successful naturalization of plant species. This is
in accord with findings of previous studies that frequent planting increases propagule pressure and therefore promotes species invasiveness (Groves et al., 2005; Dehnen-Schmutz et al., 2007a; Pyšek et al., 2015). For Impatiens, planting was among the most important correlates in comparison with the biological traits of species, regardless of the measure of naturalization. Similarly, Hanspach et al. (2008) found that propagule pressure, expressed as the number of botanical gardens in Germany where an alien species was planted, was among the most important predictors of its naturalization success.

Interestingly, common planting was strongly correlated with naturalization in temperate regions worldwide (see Table 2), while in Europe the effects of common and rare planting were of lesser importance.

4.3. Successful aliens differ in traits from unsuccessful ones

In our model system the species that were more similar to the native I. noli-tangere tend to become naturalized in Europe and those that are most different do not. Nevertheless, the majority of naturalized species had traits similar to each other, and different from non-invasive species, while the native species possess traits intermediate between these two groups. However, since our data set includes only one native species, the aforementioned pattern needs to be taken with caution. Interestingly, the invasive aliens I. parviflora and I. glandulifera, long established in central Europe, and also I. capensis, were the most similar to the native I. noli-tangere when considering all traits together. The same time to seed germination for all four species, including the native, indicates that the right timing of germination is crucial for successful performance of invaders in the temperate zone (Hanspach et al., 2008). Impatiens capensis, closely relative to native I. noli-tangere (Yuan et al., 2004) seems to be limited in its spread to continental Europe by its poor seedling performance (Beerling and Perrins, 1993; Skálová et al., 2012). In Europe it is invasive predominantly in the western part of the continent (Adamowski and Tokarska-Guzik, 2008; GBIF, 2012), where seasonality and also diurnal fluctuations are less pronounced as the temperature is buffered by oceanic water masses. In the same vein, I. flemingii and I. scabrida, which markedly differ in their traits from the native species, do not represent a potential threat, because they are limited by climatic conditions; their seeds germinate too early.

In conclusion, it is important to stress that 9 of 10 alien Impatiens species included in the experiment completed their life cycles in an experimental garden in central Europe (the only exception being I. walleriana). This indicates that those species up to now not reported as naturalized are unlikely to be limited by the regional climate as to their escape from cultivation, and that the majority of Impatiens species potential invader should their planting become more widespread. For prediction, it is important to emphasize that invasiveness in Impatiens is associated namely with the traits of early life stages, such as high seed mass, delayed germination and fast seedling growth, while the traits of adult plants are of lesser importance.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jpees.2016.02.007.

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