

## Differences in germination and seedling establishment of alien and native *Impatiens* species

Rozdíly v klíčivosti semen a vzházení semenáčů nepůvodních a původních druhů rodu *Impatiens*

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Comparative studies of closely related species may provide useful insights into the effect of species traits on invasion success since some of the biases associated with multispecies studies, such as phylogenetic effects, are considerably reduced by virtue of the experimental design. In this study seed and seedling traits of three congeneric alien species in Europe, differing in their region of origin, invasion status and history (*Impatiens glandulifera*, *I. parviflora*, *I. capensis*), were compared with the native *I. noli-tangere* in laboratory and common garden experiments. Seeds of *I. glandulifera* required the shortest period of stratification, germinated well both under laboratory and experimental garden conditions and the seedlings produced more biomass than those of the other species. Seeds of *I. parviflora* required a longer period of stratification, had the highest percentage germination but seedling emergence in the experimental garden was poorer than in *I. glandulifera*. Neither of these two species invasive in the Czech Republic formed soil seed banks. The native *I. noli-tangere* had the lowest percentage germination and formed a short-term persistent seed bank. *Impatiens capensis* germinated well in the laboratory, had the highest seedling emergence in the garden and its seed remained viable in the soil for three years. This indicates that in terms of germination and emergence, this species is comparable with the two invasive alien congeners and there appear to be no constraints to its invasion in the Czech Republic where it does not occur yet. Its absence may be due to a low propagule pressure; in the national flora *I. capensis* is listed as a potential future invader without mentioning it being cultivated in this country. Our results indicate that differences in the invasiveness of three alien species of balsams in the temperate zone of Central Europe can be attributed, at least in part, to their differing performances in the early stages of their life cycle. The short period of time required for seed stratification and the high seedling biomass of *I. glandulifera* might have increased its invasion potential compared to other *Impatiens* species occurring in the Czech Republic.

**Key words:** alien species, congeneric comparison, dormancy, germination, *Impatiens*, invasion, RGR, seed bank

### Introduction

Biological invasions by alien species represent a major threat to native biodiversity (Vitousek et al. 1996, Mack et al. 2000, Millennium Ecosystem Assessment 2005, Heinze 2008, Lambdon et al. 2008, Mácová 2008, Chytrý et al. 2009). Only a small percentage of introduced species become invasive (Williamson & Fitter 1996) and the processes resulting in successful invasions are widely studied (Vitousek 1990, Richardson & Pyšek 2006, Pyšek et al. 2008). Furthermore, an understanding of the key traits of invasive species is necessary for informed management decisions. What makes a species invasive is one of

the most studied questions in invasion ecology (Rejmánek et al. 2005). Recent studies based on large species datasets have contributed to identifying traits associated with invasiveness (Hamilton et al. 2005, Pyšek & Richardson 2007, Küster et al. 2008, Pyšek et al. 2009a, b). However, comparisons of closely related native and alien species, and of aliens showing different degrees of invasiveness, are better for identifying the characteristics of successful invaders because this approach minimizes biases associated with phylogenetic distance and habitat affinities of the species compared (Caldwell et al. 1981, Rejmánek & Richardson 1996, Radford & Cousens 2000, Mihulka et al. 2003, Pyšek et al. 2003, Burns 2004, Gravuer et al. 2008; see Pyšek & Richardson 2007 for review).

Many studies exploring traits associated with invasiveness highlight the importance of reproductive characteristics (Rejmánek 1996, Rejmánek & Richardson 1996, Rejmánek et al. 2005, van Kleunen & Johnson 2007, van Kleunen et al. 2007; see references in Pyšek & Richardson 2007). Seed production and germination, two key processes associated with the initial phase of plant establishment, may determine the outcome of invasion in the new region (Moravcová et al. 2006), especially for annual species reproducing exclusively by seed (Crawley et al. 1996). In some genera, differences among congeners in germination characteristics separate successful and non-successful invaders (Forcella et al. 1986, Dreyer et al. 1987, Vilà & D'Antonio 1998, Mandák 2003) as does the persistence, size and dynamics of seed banks (Pyke 1990, Radford & Cousens 2000, van Clef & Stiles 2001, Krinke et al. 2005). Of the traits important during the early stages of the life cycle, high relative growth rate (RGR) of seedlings is identified as contributing to invasiveness (Rejmánek & Richardson 1996, Grotkopp et al. 2002, Burns 2004).

The present study addresses the role of seed and seedling traits in four species of the genus *Impatiens* (*Balsaminaceae*). Three species alien to the Czech Republic, differing in origin, invasive status and history (*I. glandulifera* Royle, *I. parviflora* DC., *I. capensis* Meerb.) are compared with the native *I. noli-tangere* L. These species have similar life-histories and reproductive characteristics. Although the seed ecology and reproductive biology of *Impatiens* species are well studied (Coombe 1956, Daumann 1967, Jouret 1976, Grime et al. 1981, Mumford 1988, Beerling & Perrins 1993, Thompson et al. 1997, Hatcher 2003) and these species have been used in studies on the evolution of cleistogamy (Waller 1979, 1984, Lu 2002), a comparative study under standard conditions has not been published. This is needed for determining the effect of reproductive characteristics on the invasion potential of the three alien species. The published results are difficult to compare due to the different methods used, especially variation in the period of dry storage prior to germination, which is known to affect germination (Jouret 1976). Moreover, it is known that the germination pattern within the genus *Impatiens* is not simple and that individual species are likely to differ in the dynamics of their seed banks.

The comparative study of four species of *Impatiens* presented here is aimed at determining the differences between these species in: (i) germination requirements; (ii) effect of temperature and dry seed storage on seed germination; (iii) dynamics of seedling emergence; (iv) soil seed bank dynamics (temporal pattern of depletion, the dormancy state of seeds, proportion of seeds persisting longer than one year); and (v) growth rate of seedlings. Finally, we interpret the differences in these characteristics in terms of the invasion success of the species studied, with the objective of understanding why *I. glandulifera* and *I. parviflora* are such successful invasive species and whether *I. capensis*, invasive in other countries, is likely to invade the Czech Republic.

## Study species

### *Distribution and history of invasion*

*Impatiens glandulifera* is a native of the Western Himalayas where it grows along river banks, in floodplain forests and ruderal sites at altitudes of 1600–4300 m a.s.l. (Adamowski 2008); it is one of the most invasive species in Europe (Beerling & Perrins 1993, Perrins et al. 1993). The species was introduced into Europe (UK) in 1839 as a garden ornamental and it was first recorded as escaped in 1855 (Beerling & Perrins 1993); now it is recorded from 35 European countries (Lambdon et al. 2008). In the Czech Republic, the first record of planting is 1846 and that of occurrence outside cultivation 1896 (Slavík 1997). At present it is reported from 428 grid cells ( $10' \times 6'$ , longitude  $\times$  latitude), i.e. 63% of the total number in the Czech Republic (P. Pyšek et al., unpubl. data). This massive invasion has resulted in a conservation problem in riparian habitats (Pyšek & Prach 1993, 1995). As one of the European tallest annual herbs (up to 250 cm), *I. glandulifera* is highly competitive, replacing native flora at invaded sites (Trewick & Wade 1986, Perrins et al. 1990, 1993, Hulme & Bremner 2005; but see Hejda & Pyšek 2006, Hejda et al. 2009).

*Impatiens parviflora* is a native of the mountains of Central Asia (1000–3000 m a.s.l.), where it grows along river banks and streams, on stony mountain slopes, in moist shady places and broadleaved forests (Coombe 1956, Adamowski 2008); this species is also a widely distributed invasive species in Europe. It was first recorded for this continent in 1831 from a botanical garden in Geneva (Coombe 1956); at present the species is reported from 34 European countries (Lambdon et al. 2008). In the Czech Republic it was first recorded in 1844 in a botanical garden in Prague, and in the wild around 1870 (Slavík 1997); currently it is reported from 588 grid cells (86%; P. Pyšek et al., unpublished data). *Impatiens parviflora* is of a similar stature to the native *I. noli-tangere* (usually 30–60, max. 130 cm; Coombe 1956), and both species occur in similar habitats, i.e. shaded and humid forest, and riparian shrub and woodland.

*Impatiens capensis* is native and widely distributed in North America where it grows in open forests, along river banks and in other half-open habitats (reported from 55 American states and Canadian provinces; Kartesz & Meacham 1999). The species is reported from eight European countries, in five of which it is naturalized (DAISIE European Alien Species Gateway 2009). It is invasive in western Europe, and the closest localities to the Czech Republic are in central Germany and northern Poland, which makes the species a potential future invader of the Czech Republic.

The European native species, *Impatiens noli-tangere*, is widely distributed from Europe to Russia, and Siberia to Japan and Kamchatka; whether it is native to North America is not clear (Hatcher 2003). In the Czech Republic it is known from 630 out of the total 679 grid cells (93%) (P. Pyšek et al., unpublished data). *Impatiens noli-tangere* is part of the herb layer of deciduous or mixed forests and its occurrence is supported by soil disturbances and nitrification. It grows on damp to wet, occasionally waterlogged soils in forests and at the edges of rivers, streams and lakes. Depending on local conditions it reaches a height of 20–180 cm.

### *Germination requirements and seed banks*

Most seeds of *Impatiens* species germinate simultaneously in the first spring following cold winter stratification (Coombe 1956, Beerling & Perrins 1993, Hatcher 2003). However, their stratification demands are different (Jouret 1976). Seeds of all the species studied have a physiological dormancy (Nikolaeva et al. 1985, Baskin & Baskin 1998), which is broken by a cold moist period (Mumford 1988). The reports on the length of stratification needed for individual species differ and the results of individual studies are difficult to compare because of the different conditions and length of dry storage used prior to cold-wet stratification. Storage in dry conditions at room temperature results in a shorter stratification period in *I. parviflora* (Jouret 1976) and *I. glandulifera* (Jouret 1976, Mumford 1988), but not *I. noli-tangere* (Jouret 1976). Seeds of *I. glandulifera* stored for more than 200 days germinated after 15 days of chilling at 4 °C but those stored for less than 50 days required more than 70 days of chilling at 4 °C (Mumford 1988). The length of dry storage affects also the percentage of seeds that germinate and this effect is most pronounced in *I. noli-tangere* (Jouret 1976).

*Impatiens* species are reported to have transient or short-term persistent soil seed banks (Thompson et al. 1997). However, good data is scarce and this part of the life cycle has not been thoroughly studied by means of burial experiments. Grime et al. (1988) state that *I. glandulifera* does not form a persistent seed bank. In contrast, Beerling and Perrins (1993) conclude, on the basis of an experimental removal of plants before flowering, that seeds can persist in the soil for at least 18 months.

## **Methods**

### *Seed collection*

Seeds of *Impatiens glandulifera*, *I. parviflora* and *I. noli-tangere* were collected in August 2005 from three sites in the Czech Republic located 60–130 km apart: Jihlava (coded as J; N 49°24', E 15°35'), Příbram (P; N 49°41', E 13°59') and Volyně (V; N 49°09', E 13°53'). The localities are located between 280–500 m a.s.l. and experience similar climates with precipitation ranging between 500–600 mm·yr<sup>-1</sup> and mean annual temperature between 6.5–7.5 °C (ČHMÚ, Climate atlas of Czechia, 1961–1990 average). Seeds of *I. capensis* were collected in September 2005 from three sites in central Germany (Frankfurt am Main region) located 20–90 km apart: Fronhausen (Site 1; N 50°42', E 8°41'), Großseelheim (Site 2; N 50°48', E 8°51') and Schloßborn (Site 3; N 50°12', E 8°22'). Annual precipitation (1961–1990) at these sites is 650–700 mm·yr<sup>-1</sup> and mean annual temperature 8.7–9.3 °C (Mitchell et al 2004).

### *Germination requirements and effect of dry storage*

A subset of seeds was dry-stored for 2 months and germinated, without cold stratification, in climate chambers with either 12-h light: 12-h dark, 15 °C: 5 °C or 12-h light: 12-h dark, 25 °C : 10 °C diurnal cycles (for simplicity called temperature regimes 15/5 °C and 25/10 °C) to simulate autumn and late summer temperatures in the field. Another subset was dry-stored at room temperature for 2 or 6 months. After each storage period, seeds were cold-stratified in the dark and wet at 5 °C. Since the length of the stratification period

needed for germination was not known and this temperature was reported as suitable for germination (Jouret 1976), the seeds were also germinated at 5 °C.

Seeds were stratified and germinated in plastic Petri dishes filled with heat-sterilized river sand. Each treatment consisted of three replicates of 20 seeds for each species and site. Seeds were kept continuously moist by adding tap water weekly. The number of germinated and decayed seeds was determined three times a week, and the experiments lasted for 8 months. At the end of the experiment, seed viability of all remaining seeds was tested by staining with tetrazolium. Viable seeds that did not germinate were recorded as dormant, decayed seeds as dead.

The response of individual species was measured in terms of percentage germination (% of seeds that had germinated at the end of experiment) and time to germination (time to when half of the germinable seeds had germinated). The day on which the seeds were transferred from dry storage to a particular temperature regime was taken as the starting day.

Preliminary analyses showed significant interactions between length of dry storage and sites nested within species that cannot be reasonably interpreted, therefore the percentage germination and time to germination was averaged within sites to avoid a nested design. This allowed the model to be simplified without losing any important information. A factorial ANOVA design, with species and length of storage as fixed factors was used to test for differences between species, and the effect of length of dry storage on percentage germination and time to germination.

The effect of temperature on germination was not determined for the 15/5 °C and 25/10 °C regimes (without cold-wet stratification), because germination under these conditions was extremely low for all species and sites. To normalize the error distribution, germination percentages were angular-transformed (Sokal & Rohlf 1995). Where appropriate, the differences between species were tested using Tukey HSD multiple comparisons.

### *Seedling emergence*

In October 2005, seeds were sown into 10 × 10 cm pots and covered with a thin layer of soil. The pots were placed in the Experimental Garden of the Institute of Botany in Půhonice (N 49°59', E 14°34'). Three replicates were used for each species and site; each pot contained 25 seeds; pots were randomly distributed within the bed. The number of seedlings was monitored twice a week from the emergence of the first seedling (7 April 2006) until new seedlings ceased to appear at the end of May 2006.

Seedling emergence was measured as percentage emergence (% of seedlings that emerged in the course of the experiment) and time to emergence (time to when half of the seedlings had emerged). The starting day was when the first seedling was observed. Data were analysed using nested ANOVA, with species as a fixed factor and sites nested within species. To normalize the error distribution, percentage emergence was angular-transformed (Sokal & Rohlf 1995). Where appropriate, the differences between species were tested using Tukey HSD multiple comparisons.

### *Relative growth rate and seedling biomass*

The seedlings that developed in the germination experiments were used to measure relative growth rate (RGR). After emergence of the radicle from the seed coat, seeds were placed in pots filled with heat-sterilized river sand, moved to a climate chamber (Fitotron,

Sanyo SGC970) with a standard diurnal regime (12-h light : 12-h dark; 22 °C : 15 °C; 80% relative humidity) and supplied with Rorison nutrient solution (Hendry & Grime 1993). Twenty seedlings of each species and site were harvested after 7 and 21 days. In the case of *I. noli-tangere*, germination of the seeds collected in 2005 was very poor. Therefore, the RGR of this species was measured using seeds collected at Volyně in 2006. Because of the low number of seedlings of *I. capensis* from Site 3, RGR was measured only for seedlings from two sites.

Relative growth rate and variation in RGR were calculated according to Hoffman & Poorter (2002) and Hunt et al. (2002). Sequential tests based on comparisons of confidence intervals, after Bonferroni correction, were used to test the differences between species and sites (Sokal & Rohlf 1995, Zar 1999). Dry weight of 21-day old seedlings was used as the measure of seedling biomass.

#### *Depletion of soil seed bank*

For each species, mixed samples from three sites were placed in nylon bags. Bags with *I. glandulifera*, *I. parviflora* and *I. noli-tangere* contained 40 seeds, those of *I. capensis* only 30 seeds. In November 2005, six replicates of 13 bags for each species were buried at a depth of 5 cm in the Experimental Garden of the Institute of Botany, Průhonice. The seeds were dug up at monthly intervals from March 2006 to November 2006, and then in March and May of 2007 and 2008.

The number of seeds that (i) germinated or (ii) decayed in the soil (this category consisted of dead non-germinated seeds and seeds that germinated but then decayed) were counted after excavation. The remaining excavated seeds were placed in plastic Petri dishes filled with heat-sterilized river sand for one month in a chamber at 10/5 °C (corresponding to the mean early spring temperature in the Czech Republic). Those seeds that subsequently germinated were considered as (iii) non-dormant, those that did not were stained with tetrazolium to differentiate between (iv) dormant and (v) dead seeds. The pattern in the early depletion of the seed bank during March and April 2006 was described in terms of the total numbers of living seeds (i+iii+iv) and total number of non-dormant seeds (i+iii). Later in the season, only the numbers of dormant (iv) and non-dormant (iii) seeds were distinguished, as no seeds germinated at that time.

To describe the pattern in depletion of the soil seed bank in early spring (March and April 2006) the number of seeds in each category from the total sample size was analysed using generalized linear models with binomial errors and logit link function. To avoid an undue influence of small sample size, the numbers of seeds in each category were weighted by the total number of seeds in the sample (Crawley 2002). The numbers of dormant and total non-dormant seeds among the total number of living seeds were taken as response variables, and time and species as explanatory variables. The minimal adequate model was obtained by simplification of the maximal model by using deletion tests (Crawley 2002). In case of over-dispersion, the scale parameter was estimated by dividing Pearson chi-square by the degrees of freedom, and model tested using F statistics. The same approach was used to test the percentage of dead seeds among all seeds. All calculations were made using statistical software S-Plus ® v. 6.2 (Insightful Corp.).

## Results

### *Germination requirements and the effect of dry storage*

Germination at 15/5 °C and 25/10 °C without cold-wet stratification was very low in all four *Impatiens* species (Table 1), which confirmed that stratification is needed for germination. At 15/5 °C, some seeds of *I. glandulifera* started to germinate after 3 months. However, germination was very slow, resulting in a final germination of 29%. Except for *I. capensis* (11% germination at 15/5 °C), germination in other treatment/species combinations was negligible (up to 1.1%) or zero. Most seeds of the native *I. noli-tangere* (89–94%) decayed, whereas the vast majority of those of alien species remained dormant (Table 1).

Germination at 5 °C after different periods of storage indicates that the four species differed in the time needed for seed stratification (Fig. 1), which was shortest for the highly invasive *I. glandulifera*; the germination of this species was also fastest. In contrast, the native *I. noli-tangere* germinated slowly and needed a considerably longer period of stratification.

Germination at 5 °C was affected by the length of the storage period and by species (Table 2). For all species studied, a higher percentage of seeds germinated after 2 than after 6 months of dry storage. Multiple comparisons showed that the percentage germination of the native *I. noli-tangere* (average across the storage regimes: 15%) was significantly lower than that of *I. capensis* (56%) and the two other invasive species (*I. glandulifera* and *I. parviflora*), whose average percentage germination was 95%.

Table 1. – Percentages of seeds of four *Impatiens* species that germinated, remained dormant and were dead (see text for definitions) after 8 months at 15/5 °C and 25/10 °C (seeds were not previously subjected to cold-wet stratification). Values are averages, minima and maxima across sites based on three replicates per species and site, and 20 seeds per Petri dish.

Temperature regime	Species	Germinated [%]	Dormant [%]	Dead [%]
15/5 °C	<i>I. glandulifera</i>	29.4 (0–70)	67.2 (25–100)	3.3 (0–15)
	<i>I. parviflora</i>	0.0 (0–0)	99.4 (95–100)	0.6 (0–5)
	<i>I. noli-tangere</i>	0.6 (0–5)	10.0 (0–25)	89.4 (75–100)
	<i>I. capensis</i>	11.1 (0–45)	64.4 (45–80)	24.4 (0–45)
25/10 °C	<i>I. glandulifera</i>	1.1 (0–10)	83.9 (60–100)	15 (0–40)
	<i>I. parviflora</i>	0.0 (0–0)	94.4 (85–100)	5.6 (0–15)
	<i>I. noli-tangere</i>	0.6 (0–5)	5.6 (0–20)	93.9 (80–100)
	<i>I. capensis</i>	0.0 (0–0)	65.0 (30–90)	35.0 (10–70)

Table 2. – Summary of ANOVAs showing the effect of the length of dry storage and species on percentage germination and time to germination in *Impatiens* species.

Factor	DF	Percentage germination			Time to germination		
		MS	F	P	MS	F	P
Storage	1	0.240	7.893	0.012	7788	60.553	< 0.001
Species	3	1.398	46.013	< 0.001	13682	106.382	< 0.001
Storage × Species	3	0.030	0.977	n.s.	1255	9.754	< 0.001
Residuals	16	0.030			129		

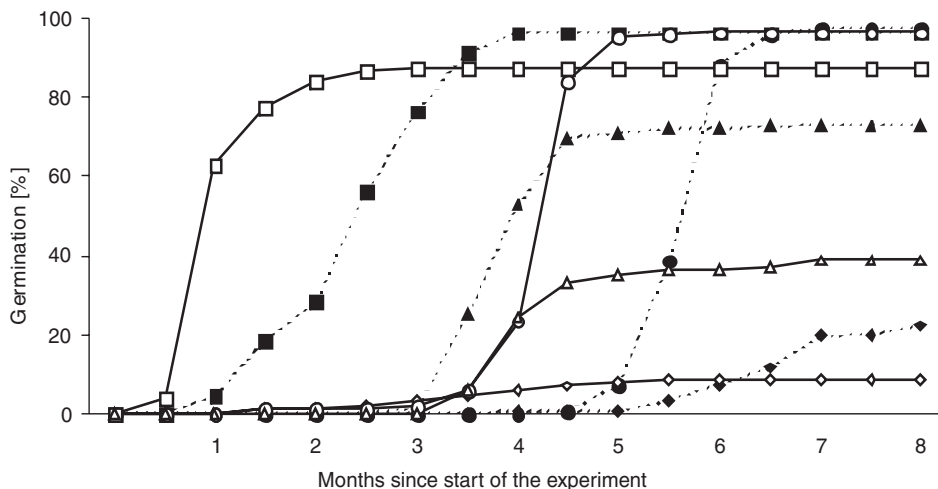


Fig. 1. – The time needed for seed stratification indicated by the pattern in seed germination of the four *Impatiens* species after 2 (dotted lines, solid symbols) and 6 (full lines, empty symbols) months of dry storage (■ *I. glandulifera*, ▲ *I. capensis*, ● *I. parviflora*, ◆ *I. noli-tangere*; averages across three sites).

However, the pattern in the time to germination was more complex as the interaction term between storage and species was significant (Table 2). Therefore the analysis was done separately for each species/storage combination. The differences in time to germination between storage regimes were not significant for *I. capensis* (ANOVA;  $F_{1,4} = 0.45$ , n.s.), whereas for *I. parviflora* (ANOVA;  $F_{1,4} = 191.78$ ,  $P < 0.001$ ), *I. glandulifera* (ANOVA;  $F_{1,4} = 12.26$ ,  $P < 0.05$ ) and *I. noli-tangere* (ANOVA;  $F_{1,4} = 24.67$ ,  $P < 0.01$ ) the time to germination was shortest for seeds stored for 6 months, indicating that those stored for a long period of time germinated earlier (Table 3). There were significant differences between species whose seeds were stored for 2 months (ANOVA;  $F_{3,8} = 89.30$ ,  $P < 0.001$ ); those of *I. glandulifera* germinated approximately two months earlier than those of *I. capensis* and almost 4 months earlier than those of the remaining two species, *I. parviflora* and *I. noli-tangere*, which did not differ significantly in their times to germination (Table 3). For seeds stored for 6 months, the only significant difference was that between *I. glandulifera*, which germinated first, and the other three species (ANOVA;  $F_{3,8} = 39.80$ ,  $P < 0.001$ ; Table 3).

#### Seedling emergence in experimental garden

Seedlings started to emerge in mid April, when for *I. glandulifera*, *I. parviflora* and *I. capensis* 15–20% of the total number had emerged and for *I. noli-tangere* 5%. Final values were reached at the end of April, when seedlings ceased to emerge. The final seedling emergence outdoors was overall lower than seed germination in the laboratory at 5 °C. The highest percentage of emerged seedlings was recorded for *I. capensis* (on average 64%). The species studied significantly differed in percentage emergence, but not in time of emergence (Table 4). Similarly no significant variation was explained by sites. Only 13% of the seeds of *I. noli-tangere* produced seedlings, which is the lowest value for all species studied (Table 5).



Table 3. – Percentage germination and time to germination for the four *Impatiens* species after 2 and 6 months of dry storage. Mean time to germination followed by the same letter are not significantly different at  $P < 0.05$  (Tukey HSD), lower case and capital letters are used for the two storage regimes. See text for details on statistical analysis and for analysis of differences in percentage germination between species and regimes.

Period	Species	Percentage germination [%]		Time to germination [days]	
		Mean	Range	Mean	SD
2 months	<i>I. glandulifera</i>	96.7	75–100	66.7 <sup>a</sup>	17.6
	<i>I. parviflora</i>	97.8	90–100	166.1 <sup>c</sup>	5.7
	<i>I. noli-tangere</i>	22.2	5–55	183.2 <sup>e</sup>	17.9
	<i>I. capensis</i>	73.3	40–100	111.4 <sup>b</sup>	5.1
6 months	<i>I. glandulifera</i>	87.8	55–100	26.6 <sup>a</sup>	11.5
	<i>I. parviflora</i>	96.7	90–100	125.1 <sup>B</sup>	3.7
	<i>I. noli-tangere</i>	8.3	0–20	116.9 <sup>B</sup>	55.4
	<i>I. capensis</i>	38.9	10–95	114.8 <sup>B</sup>	11.4

Table 4. – Summary of ANOVAs showing the effect of species and site on percentage emergence and time to emergence of seedlings of the four *Impatiens* species in the experimental garden.

Factor	DF	Percentage germination			Time to germination		
		MS	F	p	MS	F	p
Species	3	0.694	14.356	0.001	15.73	0.967	0.454
Site (Species)	8	0.048	1.490	0.213	16.27	0.946	0.499
Residuals	24	0.032			17.20		

Table 5. – Percentage emergence and time to emergence of seedlings of four *Impatiens* species recorded in the experimental garden. Means followed by the same letter in a column are not significantly different at  $P < 0.05$  (Tukey HSD).

Species	Percentage emergence [%]		Time to emergence [days]	
	Mean	Range	Mean	SD
<i>I. glandulifera</i>	44.0 <sup>bc</sup>	32.4–55.6	18.6 <sup>a</sup>	3.1
<i>I. parviflora</i>	27.1 <sup>b</sup>	19.8–34.5	17.6 <sup>a</sup>	5.6
<i>I. noli-tangere</i>	12.4 <sup>a</sup>	0.0–25.2	20.7 <sup>a</sup>	4.2
<i>I. capensis</i>	63.6 <sup>c</sup>	50.5–76.6	18.9 <sup>a</sup>	3.0

#### Relative growth rate and seedling biomass

The values of RGR varied between  $0.05 \text{ g}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$  for *I. capensis* and  $0.17 \text{ g}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$  for *I. glandulifera* (Table 6). Although the mean values of RGR for all sites for *I. capensis* ( $0.072 \text{ g}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$ ) was less than half of that of *I. parviflora* ( $0.146 \text{ g}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$ ) and *I. glandulifera* ( $0.156 \text{ g}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$ ), they were not statistically different, based on the comparison of confidence intervals for estimates of RGR between individual species and sites, due to a high variance in data.

The greatest seedling biomass was recorded for *I. glandulifera* (average across localities  $0.128 \text{ g}$ ), the lowest for *I. capensis* ( $0.024 \text{ g}$ ) and *I. noli-tangere* ( $0.029 \text{ g}$ ).

Table 6. – Relative growth rate (RGR) and seedling biomass (dry weight of 21-day old seedlings) of four *Impatiens* species. Codes in brackets refer to particular sites (see Methods).

Species	RGR [ $\text{g}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$ ]		Seedling biomass [g]	
	mean	SD	mean	SD
<i>I. parviflora</i> (J)	0.132	0.042	0.066	0.026
<i>I. parviflora</i> (P)	0.169	0.034	0.097	0.033
<i>I. parviflora</i> (V)	0.136	0.046	0.060	0.029
<i>I. glandulifera</i> (J)	0.159	0.038	0.126	0.067
<i>I. glandulifera</i> (P)	0.171	0.043	0.140	0.056
<i>I. glandulifera</i> (V)	0.138	0.027	0.119	0.030
<i>I. capensis</i> (1)	0.058	0.076	0.026	0.023
<i>I. capensis</i> (2)	0.085	0.066	0.021	0.014
<i>I. noli-tangere</i> (V)	0.101	0.042	0.029	0.013

### Early depletion of soil seed banks

The depletion of the seed bank during March and April 2006 depended on species, which significantly affected the percentage of dormant and total non-dormant seeds among living; the effect of time was non-significant (deletion test:  $\chi_{42,43} = 0.832$ ). *Impatiens glandulifera* and *I. parviflora* did not differ in the percentage of total non-dormant seeds among living (100%), differences between other species were significant (*I. capensis* 96.3%, *I. noli-tangere* 58.2%) (Fig. 2).

The analysis of the number of seeds that germinated in the soil, using GLM with binomial errors, revealed a significant interaction between time and species. Thus, the effect of these two variables was analysed separately. In both months, all species significantly differed from one another in the number of seeds that germinated. Germination in all species was significantly higher in April than March, with the exception of *I. glandulifera*, which reflects the earlier germination of this species.

The percentage of dead seeds in the seed bank in early spring was significantly affected by time ( $F_{43,44} = 4.70$ ,  $P = 0.036$ ) and species ( $F_{43,46} = 285.06$ ,  $P < 0.001$ ), with all differences being significant in multiple comparisons. The percentage of dead seeds recorded was highest for the native *I. noli-tangere* (92.3%) and lowest for *I. glandulifera* (7.1%) and *I. parviflora* (1.0%).

### Long-term dynamics of soil seed banks

No further seeds germinated in the soil after April in 2006, but there were dormant seeds of all species except *I. parviflora* present in the samples (Fig. 3A). The highest percentage of dormant seeds recorded in the second half of 2006 was for *I. noli-tangere* (up to 4.6%). A small percentage of seeds of *I. noli-tangere* and *I. capensis* were non-dormant in March 2007 (2.9% and 1.7%, respectively) and March 2008 (1.6% and 2.2%, respectively; Fig. 3B), i.e. in the second and third season after burial. Nevertheless, in contrast to May 2007, when dormant seeds of both species were found, there were no dormant seeds in May 2008, which indicates a complete depletion of their seed banks during the third spring following burial; seeds that were dormant in 2007 germinated in 2008. Only one dormant seed of *I. parviflora* was found in March 2007 and one germinated seed in March 2008, suggesting

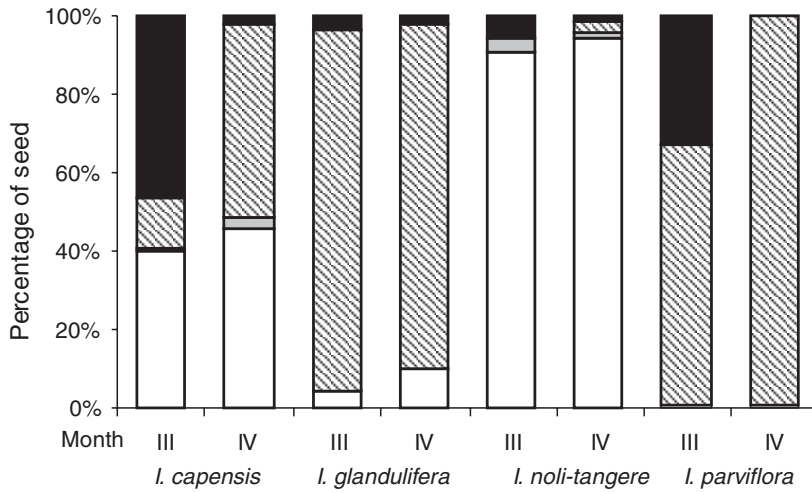


Fig. 2. – Average percentages of dead seeds (white), dormant seeds (grey) and seeds that germinated in the soil (hatched), and of seeds that germinated after excavation (black) in March and April 2006, for four *Impatiens* species.

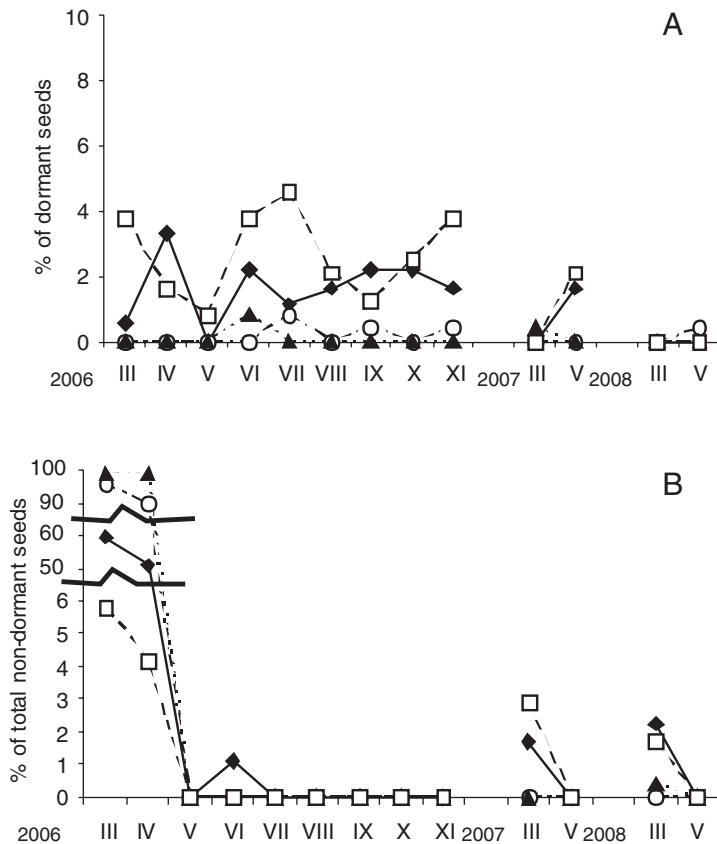


Fig. 3. – Average percentages of dormant (a) and total non-dormant (b) seeds. For details see Methods. ◆ *I. capensis*, ○ *I. glandulifera*, ▲ *I. parviflora*, □ *I. noli-tangere*.

that this species has little or no potential for developing a seed bank and that the presence of dormant seeds may be incidental. The same holds for *I. glandulifera*, where one dormant seed was found in May 2008, i.e. in the third season after the seeds were buried.

## Discussion

### *Stratification demands*

Comparative studies of closely related species can provide some understanding of why some species are invasive (Roy 1990, Rejmánek 1996, Pyšek & Richardson 2007, van Kleunen et al. 2007, Gravuer et al. 2008) and the wider the range of traits studied the more informative the results (Forcella et al. 1986, Perrins et al. 1993, Mihulka et al. 2006). This paper focused on the comparison of seed and seedling characteristics of closely related species of the genus *Impatiens*. The species in this genus are annuals. Thus, seed ecology and soil seed banks play an important role in their population and invasion dynamics. Several species of *Impatiens* are highly invasive in Europe (Lambdon et al. 2008, Hejda 2009), and a frequent subject of comparative studies. Our results show that the four *Impatiens* species studied differ markedly in their germination and seedling characteristics. All species require cold-wet stratification (Coombe 1956, Jouret 1976, Nikolaeva et al. 1985, Mumford 1988), for which a constant temperature of 5 °C is suitable. The experiments lasted long enough to conclude that the maximum germination of all the species was achieved at this temperature (this is supported by the fact that there was only one dormant seed in four of the Petri dishes after 8 months). Only in the highly invasive *I. glandulifera* were the stratification requirements partly met by the 15/5 °C regime, as almost a third of the seed completed their low temperature stratification requirements, even so germination started later and the percentage germination was much lower than at 5 °C.

Almost no seeds germinated when kept at 25/10 °C. In both the 15/5 and 25/10 °C regimes most seeds of *I. noli-tangere*, from all three sites, decayed, whereas the majority of seeds of the three alien species remained dormant until the end of the experiment, indicating that their stratification requirements were not met at these temperatures. This is consistent with Mumford (1988) who found that if the stratification requirements are not met, fully imbibed seeds of *I. glandulifera* could remain dormant for in excess of 3 years at 20 °C under laboratory conditions without any signs of deterioration.

### *Effect of dry storage on stratification time and germination*

We showed that the duration of dry storage influences the time needed for stratification as well as the final percentage germination of seeds. Unfortunately, details of seed storage are often not given in the literature on germination, which makes the published results difficult to compare.

In general, the percentage of seeds that germinated decreased with the length of the dry storage period, and in agreement with the remark of Jouret (1976), the effect is most pronounced in the native *I. noli-tangere*, in which we found that germination after 6 months storage was less than half that after 2 months storage. It is thus possible that germination of *I. noli-tangere* under natural conditions, where they are usually not exposed to such a long period of drought, is higher than that recorded in our experiments. The longer they are kept

in dry storage, the less time the seeds need for stratification; this also accords with the results of Jouret (1976) for *I. glandulifera* and *I. parviflora*, but not *I. noli-tangere*. In our study, it was true for all species studied, except *I. capensis*, where the effect was non-significant due to variation among sampled populations. The highly invasive *I. glandulifera* needed the shortest period of stratification of all the species studied; seeds kept in dry storage for 6 months started to germinate after two weeks at 5 °C (cf. Mumford 1988). The stratification time for *I. parviflora* also decreased with increasing period of dry storage, but the effect was less pronounced than that reported by Coombe (1956), who records that seed kept in dry storage for 6 months germinated after two weeks at 5 °C; the corresponding value obtained in this study was approximately 3.5 months.

#### *Seedling emergence and growth*

The early germination of the seed of *I. glandulifera* in the laboratory was not confirmed outdoors. In the experimental garden, seedlings of all species appeared during April, which corresponds to the results of a field study in UK, where *I. glandulifera*, *I. parviflora* and *I. capensis* emerged simultaneously in the second half of March and beginning of April (Perrins et al. 1993). It is likely that in the experimental garden, the early emergence of *I. glandulifera* seedlings was prevented by the extremely harsh winter, with snow and frost lasting until March in 2006. In the mild winter of 2007, seedlings of this species appeared in mid January (H. Skálová, unpublished data). The early emergence in years when seedlings are not constrained by harsh conditions is likely to give *I. glandulifera* a competitive advantage (Begon et al. 2006), but imposes a considerable risk of death due to late frosts. This risk, however, may be diminished because *I. glandulifera* is more frost resistant than *I. parviflora* (Beerling & Perrins 1993, Perrins et al. 1993).

*Impatiens capensis* was the only species for which we recorded a similarly high germination in the garden, measured in terms of the final percentage of seeds that produced seedlings, and in the laboratory. The three species occurring in the Czech Republic exhibited considerably lower percentages of germination in the garden than in the laboratory. Perrins et al. (1993) report values similar to ours for the establishment of seedlings of *I. glandulifera* and *I. parviflora* in a garden, but the values they recorded in the field were an order of magnitude lower; no seeds of *I. capensis* and *I. noli-tangere* germinated in their experiment.

#### *Soil seed bank dynamics*

The depletion of seed in the garden experiment mimicked the pattern observed in the laboratory. *Impatiens glandulifera* germinated first – all species except *I. glandulifera* exhibited a progressive increase in percentage of seeds that had germinated from March to April. However, the emergence of seedlings of all species occurred at the same time in mid April, probably due to cold weather, as discussed above.

The long-term pattern in the depletion of the seed banks indicates that of the four species only *I. noli-tangere* and *I. capensis* can form short-term persistent seed banks (sensu Thompson 1993), with seeds remaining viable in the soil for up to three years. These results are the first reliable evidence of the formation and dynamics of seed banks for *Impatiens* species based on a burial experiment. Reports in the literature are rather vague and refer to all balsams as having a transient seed bank, with only a single reference to

a short-term persistent seed bank in *I. capensis* (Thompson et al. 1997). Other reports of the short-term persistence of seed in the soil relate to *I. noli-tangere* and are inferred from the reappearance of plants in the year following mowing (Austad & Skogen 1990) or germination of seed in two-year old soil samples (Jankowska-Błaszczuk & Grubb 1997; but see Markov 1991). Similarly, Beerling & Perrins (1993) observed seedlings of *I. glandulifera* 18 months after the removal of fruiting plants and concluded that this species has a seed bank in nature. Our results contradict this; seeds of *I. glandulifera* germinated in the first spring after release and the persistence of dormant seeds in the soil is very unlikely (see also Clements et al. 2008). However, the results of common garden experiment might differ from seed performance in natural conditions as a considerable number of dormant seeds of *I. noli-tangere* and a limited number of dormant seeds of *I. glandulifera* were found in the wild after the first year of an ongoing field experiment (L. Moravcová & H. Skálová, unpublished data). Although the presence of a persistent seed bank was suggested to be associated with invasiveness (Pyke 1990, Radford & Cousens 2000, van Clef & Stiles 2001), its absence in both of the highly successful invaders, *I. glandulifera* and *I. parviflora*, has not constrained their invasion of the temperate zone of Europe.

#### *Can seed traits explain differences in invasiveness of Impatiens species?*

This study is based on a model system, which includes two invasive species, a native congener and a potential invader. Of the two invasive species that are now widely distributed in the Czech Republic, *I. glandulifera* spread faster than *I. parviflora* (Williamson et al. 2005) and its success seems to be associated with certain seed characteristics, such as a very high percentage germination and the short time needed for stratification. These features, together with others not examined here, e.g. competitive ability, prolific seed production, introduction history and increased propagule pressure (Perrins et al. 1993, Collauti & MacIsaac 2004, Puth & Post 2005, Muth & Pigliucci 2006) can be assumed to contribute to this species' invasiveness.

As the performance and competitive advantage of invasive species depends on various environmental factors and interspecific interactions (Sea & Chesson 2002, Goergen & Daehler 2002, Suding et al. 2004, Morrison & Mauck 2007), the results of microcosmos experiments must be interpreted with care. However, the results presented in this paper follow a similar pattern to that recorded in a field experiment (Skálová & Pyšek 2009) in which the invasive *Impatiens* species performed better than the native *I. noli-tangere* in terms of the number of emerged seedlings, although all the species performed worse than in our common garden experiment.

Our results also suggest that seedlings of *I. glandulifera* and *I. parviflora* grow faster than those of *I. capensis* and the native *I. noli-tangere*, even though the marked differences in the RGRs of these two species were not statistically significant. High seedling biomass of *I. glandulifera* may also be advantageous, because there is a close relationship between plant size and competitive ability (Cahill et al. 2008). Daumann (1967) placed *I. glandulifera* at the top of a competitive hierarchy for this genus on the basis of reproductive characteristics, such as production of floral nectar, fecundity, phenology and interaction with pollinators. In the UK, Perrins et al. (1993) studied the emergence of seedlings of three balsam species in two contrasting habitats and concluded that shade tolerance and fast germination of *I. glandulifera* accounted for its successful spread. The competitive ability

and plasticity of *I. glandulifera* is further indicated by the fact that plants affected by viral infections seem to have lower vigour, but their reproductive characteristics, such as seed production, remain unaffected (Kollmann et al. 2007).

Considering the traits shared by the two invasive species, it seems that an invasion of the Czech Republic by *I. capensis* is unlikely to be constrained by either percentage germination or seedling establishment. Its seeds can persist in the soil for 3 years, germinate well in the laboratory and it performs better in terms of seedling emergence than the other species. However, low RGR and seedling biomass indicate a weak competitive ability, similar to that of the native *I. noli-tangere*, which is often outcompeted by surrounding vegetation (Hatcher 2003). Since *I. capensis* has a similar ecology to *I. glandulifera*, it seems unlikely that the former will succeed when in direct competition with the latter species. So far, *I. capensis* has invaded only a few areas of Europe. It was suggested that the rather limited invaded range of this species could be, at least in part, attributed to a lower propagule pressure since *I. capensis* was never so extensively cultivated in Europe as its more successful congeners, *I. glandulifera* and *I. parviflora* (Perrins et al. 1993, Adamowski 2009). This also holds for the Czech Republic since *I. capensis* is reported in the national flora as a potential future invader without mentioning it being cultivated (Slavík 1997). Therefore, it seems likely that the absence of this species from the Czech Republic is mainly due to dispersal limitation resulting from an absence of propagule pressure.

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## Souhrn

Studie se zabývá srovnáním ekologických vlastností semen semenáčů tří nepůvodních druhů netýkavek, lišících se oblastí původního rozšíření, invazním statutem a historií invaze (*Impatiens glandulifera*, *I. parviflora*, *I. capensis*), s původním druhem *I. noli-tangere* ve standardních podmínkách. Semena *I. glandulifera* vyžadovala nejkratší dobu stratifikace, dobře klíčila jak v laboratoři, tak v experimentální zahradě a semenáče měly největší biomasu. Semena *I. parviflora* vyžadovala dlouhodobou stratifikaci; ačkoli dosahovala mezi studovanými druhy nejvyšší klíčivosti, počet semenáčů zaznamenaný na pokusné zahradě byl nižší než u *I. glandulifera*. Žádný z těchto druhů nevytvářel semennou banku. Původní druh *I. noli-tangere* vykazoval nejnižší klíčivost, ale vytvářel krátkodobou semennou banku. Semena *I. capensis* klíčila dobře v laboratoři; v experimentální zahradě byl u tohoto druhu zaznamenán nejvyšší počet semenáčů a v půdě přetrvala živá semena po tři roky. Výsledky pro *I. capensis* jsou srovnatelné s oběma dalšími nepůvodními druhy, což nasvědčuje tomu, že z hlediska studovaných charakteristik neexistují zásadní překážky invaze tohoto druhu na území České republiky; tento druh se u nás pěstuje jen zřídka a jeho nepřítomnost může být tedy spíše důsledkem nedostatečného přísunu diaspor. Naše výsledky nasvědčují tomu, že rozdíly v invazivnosti druhů mohou být alespoň částečně způsobeny rozdíly v raných vývojových stádiích jejich životního cyklu. Krátká doba stratifikace semen a velká biomasa semenáčů *I. glandulifera* pravděpodobně představují vlastnosti, které jsou z hlediska invaze výhodné a zajišťují tomuto druhu výhodu oproti ostatním studovaným příbuzným druhům.

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