

DO ABUNDANCE AND PROXIMITY OF THE ALIEN *IMPATIENS GLANDULIFERA* AFFECT POLLINATION AND REPRODUCTIVE SUCCESS OF TWO SYMPATRIC CO-FLOWERING NATIVE SPECIES?

Valérie Cawoy¹, Mathieu Jonard², Carolin Mayer¹ and Anne-Laure Jacquemart^{1,*}

¹Research group « Genetics, reproduction, populations » - Earth and Life Institute - Université catholique de Louvain, 2 (box L7.05.14) Croix du Sud, 1348 Louvain-la-Neuve, Belgium

²Research group « Forest sciences » - Earth and Life Institute - Université catholique de Louvain, 2 (box L7.05.09) Croix du Sud, 1348 Louvain-la-Neuve, Belgium

Abstract— In invasion ecology, potential impacts of aliens on native flora are still under debate. Our aim was to determine the pollinator mediated effects of both proximity and abundance of an alien species on the reproductive success of natives. We chose the highly invasive *Impatiens glandulifera* and two native species: *Epilobium angustifolium* and *Aconitum napellus* ssp. *lusitanicum*. These species share characteristics allowing for pollination interactions: similar biotopes, overlapping flowering periods and same main pollinators. The effects of abundance (5, 25 and 100 individuals) and proximity (0 and 15 m) of the alien on visitation rate, insect behaviour, pollen deposition and reproductive success of both natives were investigated during 2 flowering seasons. We used centred visitation rates as they can be directly interpreted as a positive or negative effect of the invasive.

Both abundance and proximity of the alien increased bumblebee visitation rates to both natives. On the other hand, abundance of the exotic species had a slight negative effect on honeybee visits to natives while its proximity had no effect. The behaviour of bumblebees changed as visitors left significantly more often the native plants for *I. glandulifera* when its abundance increased. As a consequence of this “inconstancy”, bees deposited considerable quantities of alien pollen on native stigmas. Nevertheless, this interspecific pollen transfer did not decrease seed set in natives. Self-compatibility and high attractiveness of both native species probably alleviate the risk of altered pollinator services and reproductive success due to the invader in natural populations.

Keywords: flower constancy, facilitation, pollen deposition, seed set, visitation rate

INTRODUCTION

Entomophilous invasive plant species usually present floral traits that are highly attractive to pollinators like showy and numerous flowers, an extended flowering period, a high nectar production and large amounts of pollen (Ghazoul 2002; Traveset & Richardson 2006). Examples include species introduced for ornamental purposes such as *Heracleum mantegazzianum* (Nielsen et al. 2008), *Impatiens glandulifera* (Chittka & Schürkens 2001; Lopezaraiza-Mikel et al. 2007) or *Lythrum salicaria* (Brown et al. 2002). These ‘mass flowering’ species are supergeneralists as they attract polylectic generalist insects present in their neighbourhood. Their potential impacts on pollen transfer and subsequent seed set of sympatric co-flowering natives they share pollinators with have been increasingly studied. Insect visitors of native plants are often generalists and readily include aliens in their diets (Memmott & Waser 2002; Lopezaraiza-Mikel et al. 2007; Aizen et al. 2008; Vilà et al. 2009). Therefore, extended resources offered by mass-flowering invaders and the prevalence of generalist

pollinators in natural communities may evoke changes in pollinator services to native plants.

The floral composition of the local neighbourhood influences plant-pollinator interactions (Rathcke 1983). An alien plant species might alter these interactions (Bjerknes et al. 2007) but it remains difficult to draw general conclusions. So far, three types of interactions between alien and native plant species have been described: first, a competitive interaction, where an alien reduces pollinator visits to native plants (e.g. Brown et al. 2002; Totland et al. 2006; Kandori et al. 2009); second, a facilitative interaction where a ‘magnet-invader’ attracts pollinators which would visit natives less in the absence of the invader (e.g. Lopezaraiza-Mikel et al. 2007; Nielsen et al. 2008; Bartomeus, Vilà, et al. 2008); and third, a neutral interaction where an alien does not influence pollinator visits to natives (e.g. Bjerknes et al. 2007; Kaiser-Bunbury & Müller 2009; Vanparys et al. 2011).

The presence of an alien may affect both quantity and compatibility of pollen grains deposited on native stigmas. Less conspecific pollen is deposited in case of competition (Larson et al. 2006; Matsumoto et al. 2010) or due to pollen loss during their flights between species (Flanagan et al. 2009). On the other hand, in case of facilitation, more

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*Corresponding author: Anne-Laure.Jacquemart@uclouvain.be

conspicuous pollen is deposited. Still, if pollinators also forage on the invader and show inconstant foraging behaviour, loads of alien pollen have been observed on native stigmas (Moragues & Traveset 2005; Cariveau & Norton 2009).

A recent quantitative synthesis compiling data from 40 studies revealed an overall negative impact of aliens on visitation rates and reproductive success of native species (Morales & Traveset 2009). Thus, identifying invasion-sensitive native plant species is crucial to improve conservation strategies.

With numerous showy flowers producing large amounts of nectar and pollen, *Impatiens glandulifera* has been reported to effectively outcompete native species for pollination services in riparian sites and to represent a significant threat on the reproductive success of natives (Chittka & Schürkens 2001; DAISIE 2009; Nienhuis et al. 2009; Vervoort et al. 2011). However, also facilitation effects of *I. glandulifera* on an entire community of co-flowering natives have been observed in field experiments by Lopezaraiza-Mikel et al. (2007), while Bartomeus et al. (2010) did not detect any effect.

Experimental studies usually investigated the effects on visitation rates on a selection of native species or on the entire community of co-flowering natives but have rarely examined seed set (e.g. Chittka & Schürkens 2001; Lopezaraiza-Mikel et al. 2007; Vilà et al. 2009; but see Bartomeus et al. 2010). Diverging results between visitation rates and reproductive success highlight the importance to study both components simultaneously, and to consider changes in conspecific and heterospecific pollen deposits (Moragues & Traveset 2005; Morales & Traveset 2009). Moreover, the effect of the presence of the alien may differ according to its abundance (number of individuals or flowers) and to proximity of alien individuals to natives (Muñoz & Cavieres 2008; Cariveau & Norton 2009; Morales & Traveset 2009). It is therefore necessary to disentangle these two aspects when looking at pollination and reproductive success of natives. To our knowledge, no study has assessed the effect of an alien species at all reproduction levels, from visitation rates to seed set focusing on native species sharing biotopes and presenting similar flowering traits.

We investigated the impact of *Impatiens glandulifera* on pollinator visits, pollen transfer and reproductive success of two sympatric co-flowering native species, the rare *Aconitum napellus* ssp. *lusitanicum* and the common *Epilobium angustifolium*. We chose these species because (1) they share biotopes with *I. glandulifera* like wet meadows, edges of marshes and river banks (Lambinon et al. 2004), (2) their flowering periods overlap (July-August) and (3) their main pollinators are bumblebees and honeybees (Myerscough 1980; Le Cadre 2005; Vervoort et al. 2011). We might thus expect competition for pollinator services and that the alien might affect the reproductive success of the natives, especially threatening the rare native *A. napellus*. All three species are considered valuable sources of nectar and, despite being protandrous, all are self-compatible (Myerscough 1980; Le Cadre 2005; Vervoort et al. 2011).

Using an experimental design, we examined whether the effects of interaction may increase with the abundance and the proximity of the alien. Four main questions were addressed: Does the abundance and/or proximity of the alien *I. glandulifera* (1) modify visitation rates of the main pollinators to the native individuals? (2) change the behaviour of their main pollinators? (3) lead to deposition of alien pollen on native stigmas? and (4) affect the seed set of the natives?

MATERIALS AND METHODS

Studied species

Impatiens glandulifera Royle (Balsaminaceae) is an herbaceous annual introduced from the Himalayas in Europe for horticulture in the 1830s (Beerling & Perrins 1993; Titze 2000). Due to its numerous large flowers with copious nectar (up to 8µl per flower.day) attracting large numbers of insects, the species has been favoured by beekeepers and nursery gardeners (Beerling & Perrins 1993; Nienhuis et al. 2009). *Impatiens glandulifera* has spread in the majority of temperate communities in Europe, growing in riparian biotopes and in other disturbed sites with good water and nutrient supply. It is now considered as one of the 100 worst invasive species in Europe (DAISIE 2009). Its inflorescences consist of 3-12 white to pink or purple flowers. Flowers are 3-4 cm long, zygomorphic, helmet-like and spurred (Beerling & Perrins 1993; Titze 2000; Fig. 1A). Anthesis lasts two days.

Aconitum napellus L. ssp. *lusitanicum* Rouy (Ranunculaceae) is a herbaceous perennial native to Europe. This rare taxon grows in wet biotopes, primarily riparian zones, along rivers and marshes, or in wet forest edges (Lambinon et al. 2004; Le Cadre et al. 2008). Its recent decline has been attributed to destruction and fragmentation of suitable biotopes (Le Cadre 2005; Le Cadre et al. 2008). The main raceme bears up to 40 zygomorphic dark blue or violet helmet shaped flowers (Lambinon et al. 2004; Le Cadre 2005; Fig. 1B). Anthesis lasts 1-2 weeks and flowers produce large amounts of nectar (up to 8µl per flower.day; Heinrich 1979; Marden 1984).

Epilobium angustifolium L. (Onagraceae) is a common herbaceous perennial native to Europe, growing in open and disturbed biotopes like clear-cuttings, wood edges and wet meadows (Myerscough 1980; Lambinon et al. 2004). The main raceme bears up to 400 actinomorphic pink flowers (Sargent & Roitberg 2000; Fig. 1C). Anthesis lasts 2-7 days. *Epilobium angustifolium* is also considered an attractive source of nectar for bees (up to 12µl per flower.day; Schmid-Hempel & Speiser 1988; Sargent & Roitberg 2000).

Cultivation

Native species were cultivated from pieces of rhizomes collected in April 2008 in three large populations from South Belgium (50 fragments per population). The annual *I. glandulifera* was cultivated from seeds collected in 2007 in two river bank populations in Central Belgium. Seeds were sown each year and germinated in a climate chamber as described by Vervoort et al. (2011).

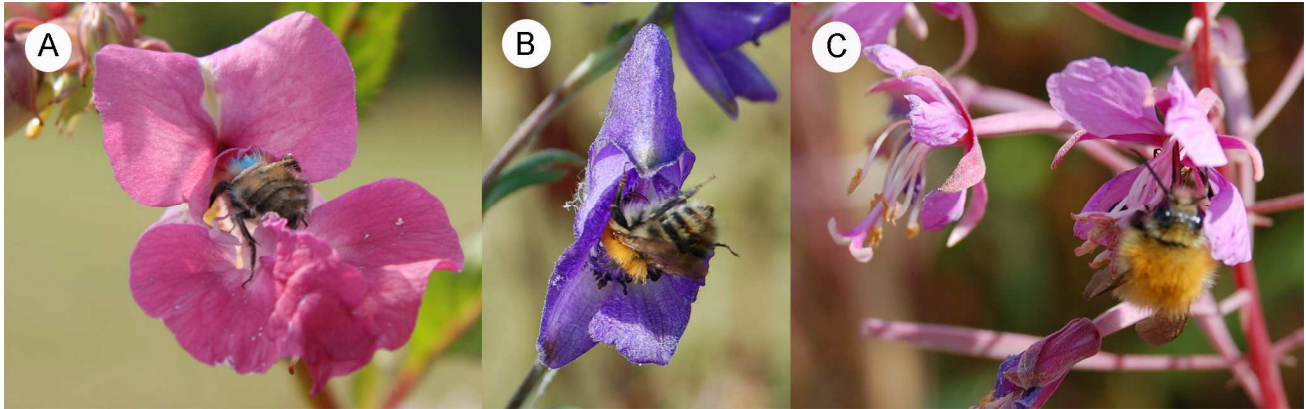


FIG. 1. Morphology of the three plant species and visiting behaviour of bumblebees (*Bombus pascuorum*). (A) When a bumblebee saps nectar from the spur of *I. glandulifera*, it touches anthers with dorsal parts (head, thorax and abdomen). Prior to insect visits, blue fluorescent dye had been deposited on open anthers of *I. glandulifera* and a dye load is visible on the back of the insect. (B) Bumblebee legitimately visiting *A. napellus* in search for nectar. When it enters the flower, dorsal parts touch anthers and stigmas. (C) Bumblebee drinking nectar on *E. angustifolium*. The ventral parts of the insect body are in contact with stigmatic lobes and anthers.

All plants were grown outdoors in 5 L pots filled with peat compost at the Université catholique de Louvain (UCL, Louvain-la-Neuve). During summer 2008 and 2009, flowering individuals were placed in two experimental sites (see below). The same native individuals were used during the two years.

Site description and experimental design

Independent experiments with both alien - native pairs (*I. glandulifera* - *E. angustifolium* and *I. glandulifera* - *A. napellus*) were carried out in two experimental sites separated by 5 km, in Central Belgium, Louvain-la-Neuve (50°40' N, 4°37' E). Both sites were mown grasslands of approximately 170 x 55 m. They were homogeneous and poor in entomophilous flowering plants. The vegetation was dominated by grasses, 10-25 cm high. *Trifolium repens* was the only insect pollinated species flowering during experiments, at maximum covering 10% of the surface.

The experimental design combined different degrees of *I. glandulifera* abundance and proximity. In 2008, we tested two degrees of *I. glandulifera* abundance (5 and 25

individuals) and two degrees of proximity (0 and 15 m). In 2009, we tested three degrees of *I. glandulifera* abundance (5, 25 and 100 individuals) at close proximity (0 m). Both years, we added plots of natives without *I. glandulifera* (see below centred visitation rate calculation). Native species were grouped in 1 m² clumps of seven individuals (Fig. 2). In both years, experiments started with the pair *I. glandulifera* - *E. angustifolium* in mid July. Observations on the second pair *I. glandulifera* - *A. napellus* began immediately afterwards until mid August.

Insect observations

Insect visits and foraging behaviour were recorded during 10-min periods on 23 days for the pair *I. glandulifera* - *E. angustifolium* and on 28 days for *I. glandulifera* - *A. napellus*. Observations were carried out at both sites simultaneously on sunny days between 10h00 and 17h00. Between five and 42 observations (24 on average) were conducted per site per day, resulting in total a observation time of 181 hrs. Open flowers per individual were counted daily. Number of visitors and visitor category were

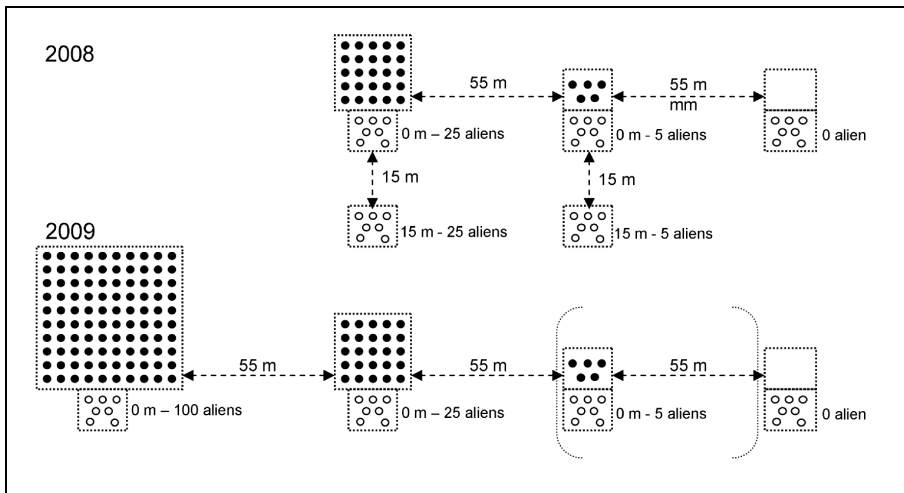


FIG. 2. Per year (2008 and 2009), experimental design for both alien-native pairs (*I. glandulifera* - *A. napellus*; *I. glandulifera* - *E. angustifolium*). Each dot represents one individual of an alien (black dots) or a native plant (white dots). In 2009, the treatment “0 m -5 aliens” was only present in the site A.

noted. Insect categories were limited to the four main previously determined visitor groups: honeybees, bumblebees, small-sized syrphids and other insects. During observations, a total of 14 349 insects were recorded. Insect category proportions varied among sites and years but social bees and syrphids were the most frequent for all three studied species. Observations confirmed that *A. napellus*, *E. angustifolium* and *I. glandulifera* shared the main visitors: bumblebees (65.5% of visits), small-sized syrphids (17.2%), and honeybees (15.3%). An insect was considered a pollinator when it touched the reproductive organs. Social bees were effective pollinators due to high abundances (74.4% on *A. napellus*, 55.7% on *E. angustifolium* and 93.5% on *I. glandulifera*) and frequent contacts with anthers and stigmas (Fig. IB-C). Honeybees (*Apis mellifera*) and two bumblebee species were identified in the field: *Bombus pascuorum* and *B. terrestris* s.l. On the contrary, small-sized syrphids, mainly *Episyrphus balteatus*, despite their large numbers (21.3% on *A. napellus*, 38.5% on *E. angustifolium* and 5.8% on *I. glandulifera*), did not act as pollinators and were not included in analyses.

Total visitation rate was calculated as the total number of visits per 10-min period divided by the number of open flowers observed. We also calculated visitation rates for honeybees and for bumblebees separately. For both natives, all flowers of the seven individuals from each patch were observed simultaneously. For *I. glandulifera*, due to the numerous flowers and the high visitation rates, observations were made on a group of 20-30 flowers.

To detect a possible change in insect behaviour in 2009, the direction of insect transitions was recorded when they visited native plants (i.e. if they came from or left a native for an alien).

Pollen load on native stigmas

In order to test if alien pollen contaminated native stigmas as a result of interspecific insect movements, we collected native flowers in female phase (12 per treatment and per site) in 2008. Flowers were fixed in FAA before staining stigmas in acid fuchsin solution (Kearns & Inouye 1993). The red-coloured pollen grains were immediately observed under a light microscope. Pollen grains of the three species were easily distinguished according to size and shape, and counted.

Reproductive success: seed set of natives

On each inflorescence of the native individual plants, all flowers open during observations were marked. Of these, five ripe fruits per plant were collected in August for *E. angustifolium*, and eight ripe fruits per plant were collected in September for *A. napellus*. Seeds of *A. napellus* were counted by naked eye. Wind-dispersed plumed seeds of *E. angustifolium* were scattered on a sticky transparent sheet and scanned at 800 dpi. Seeds were automatically counted using the MacBiophotonics ImageJ software (Plugins: Particle Analysis, Cell counter; Abramoff et al. 2004).

For both species, ripe viable seeds, aborted seeds (flattened and smaller size) and unfertilized ovules were

distinguished. Viability was confirmed via germination tests after stratification during three months at 4°C. The reproductive success (seed set) was estimated as number of viable seeds divided per number of total ovules per fruit.

Statistical analyses

Visitation rates were transformed into centred visitation rates (VR_C) by subtracting each visitation rate (VR) by the visitation rate of the plot without *I. glandulifera* within a radius of 55m (VR_o) on the same date and time of day.

$$VR_C = VR - VR_o \quad [\text{eq. 1}]$$

The advantage of the centred visitation rate is that it can be directly interpreted as a positive or negative effect of the alien and that its absolute value provides the magnitude of the effect. This transformation removes part of the site effects independent from *I. glandulifera* abundance (a comparable method was used by Price et al. 2005). Centred visitation rates were analyzed using linear mixed models with the MIXED procedure in SAS. Mixed models allow for correct analysis of such intercorrelated data sets (here: repeated measurements in time) and effectively deal with pseudoreplication (van de Pol & Wright 2009), which has been defined as the misanalyses or misinterpretation of replicates that are not statistically independent (Hurlbert 1984).

The effect of *I. glandulifera* abundance on the centred visitation rate was analyzed using only the data from 2009 (three levels of abundance). A linear mixed model describing the variation of the centred visitation rate with the natural logarithm of *I. glandulifera* abundance was adjusted for each native plant species and pollinator category. We log-transformed abundance to take into account the fact that an increase of 10 individuals has a much stronger effect when it occurs between 0 and 10 than between 100 and 110 or 1000 and 1010. The formula of the model is:

$$VR_C = a \times \ln(n_{imp}) + \delta(0, \sigma^2_\delta) + \varepsilon(0, \sigma^2) \quad [\text{eq. 2}]$$

where:

- VR_C is the centered visitation rate,
- n_{imp} is the abundance of *I. glandulifera* (number of plants in the patch),
- a is a model parameter,
- δ is a random effect accounting for the census date effect,
- ε is the residual term whose normality was checked.

The relevance of introducing a random effect in the model was evaluated on the basis of the AIC (Brown & Prescott 1999).

The impact of the proximity of *I. glandulifera* on the effect of its abundance on the centred visitation rate was analyzed based on the data collected in 2008. A linear model of the same form was used except that the parameter a varied according to the proximity level (0 or 15 m). Contrasts were used to test the *I. glandulifera* proximity effect on the parameter a of the model (Brown & Prescott 1999).

To test whether changes of visitation rates to the native species would simply mirror an increase in patch size, we compared visitation rates of bumble and honeybees to *I. glandulifera* for the different abundances with a generalized linear mixed model with the different abundance classes and years as fixed effects and included date and site as random factors (Procedure GLIMMIX). To test the effects of *I. glandulifera* abundance on the proportions of bumblebee transitions from and towards *I. glandulifera* we used a Chi-square test using data collected in 2009 (not enough data for honeybee movements). Effects of *I. glandulifera* abundance on the number of pollen grains deposited on native stigmas in 2008 were tested for pollen origins, alien (*I. glandulifera*), conspecific (*A. napellus* or *E. angustifolium*), or other (other species) with one-way ANOVA. Finally, to test the effects of *I. glandulifera* abundance and proximity on seed set we used one-way ANOVA. Data for seed sets were arcsin (*A. napellus*) or log (*E. angustifolium*) transformed to achieve normality. All statistical analyses were performed using SAS (version 9.1; SAS Institute Inc., Cary, N.C.). Means are given with their standard errors.

RESULTS

Effects of *I. glandulifera* on the visitation rates to natives

The total visitation rate to the alien (0.34 ± 0.1 visits 10 min^{-1} flower $^{-1}$; years and sites pooled) was 10.4 times higher than that to *A. napellus* and 6.1 times higher than that to *E. angustifolium*. Visitation rates to *I. glandulifera* did not change with increasing abundance ($F_{1,741} = 0.60$, $P = 0.549$). However, the abundance of the alien modified visitation rates to natives.

Bumblebee and honeybee visitation rates responded differently to the abundance and proximity of the alien (Fig. 3). Higher abundance of *I. glandulifera* increased the centered visitation rates of bumblebees to native plants, especially for *E. angustifolium* ($P = 0.137$ for *A. napellus* and $P < 0.001$ for *E. angustifolium*). On the contrary, higher abundance of *I. glandulifera* decreased the visitation rate of honeybees to both natives (Fig. 3; $P = 0.022$ for *A. napellus* and $P = 0.003$ for *E. angustifolium*).

Proximity of *I. glandulifera* significantly increased the effect of its abundance on bumblebee visitation rate mainly for *E. angustifolium* (Fig. 4; $P < 0.001$) but also for *A. napellus* ($P = 0.007$). Not surprisingly, the abundance effect was much larger when *I. glandulifera* plants were adjacent to the natives. For *A. napellus*, the abundance effect disappeared at 15 m distance between the alien and the native but for *E. angustifolium*, a small facilitation effect could still be shown at 15 m (Fig. 4; $P = 0.035$). Regarding honeybees, proximity had no significant impact on the effect of alien abundance on the visitation rate ($P = 0.102$ for *A. napellus* and $P = 0.980$ for *E. angustifolium*).

Behavioural changes for pollinators

Bumblebees shifted frequently between *I. glandulifera* and the native species as indicated by the proportions of

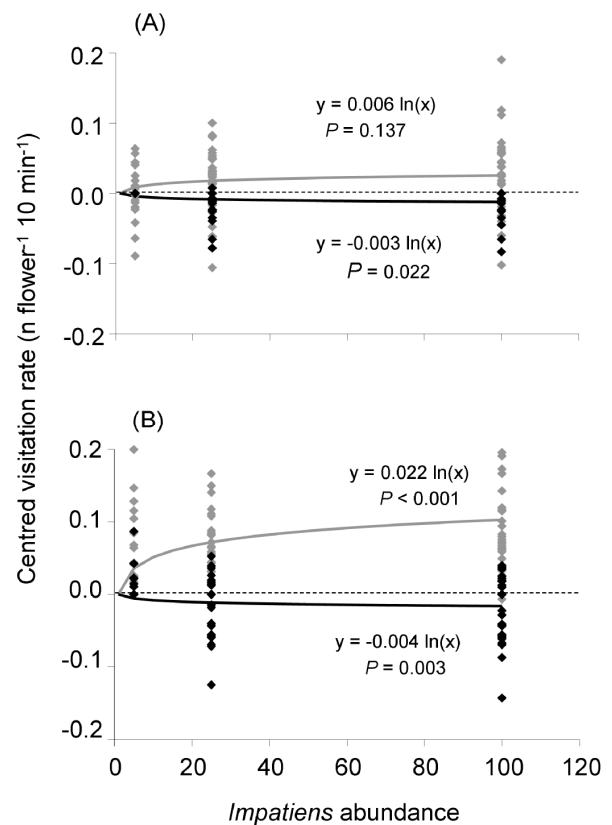


FIG. 3. Centred visitation rate in 2009 as a function of *I. glandulifera* abundance (5, 25 and 100 individuals) for both native plant species, (A) *A. napellus* and (B) *E. angustifolium* and for the two main pollinator categories (grey diamonds: bumblebees; black diamonds: honeybees). For both pollinator categories, model predictions are represented by a solid line and described by the equation with the corresponding P -value indicating significance.

individuals coming from or leaving for an alien flower (Table 1). When alien abundance increased, a significantly higher proportion of bumblebees left both natives for the alien but proportions of bumblebees coming from the alien did not change significantly (Table 1). In all cases, the majority of the observed movements were interspecific.

Pollen loads on native stigmas

Insects deposited considerable quantities of pollen from *I. glandulifera* on native stigmas (Fig. 5). The abundance of the alien affected heterospecific pollen deposition since the quantities of alien pollen on stigmas of both natives increased with higher *I. glandulifera* numbers of individuals (Fig. 5; $F_{1,84} = 15.42$; $P < 0.001$ for *A. napellus* and $F_{1,85} = 48.67$; $P < 0.001$ for *E. angustifolium*). This increase of heterospecific pollen was much lower for *A. napellus* than for *E. angustifolium*. At the same time, quantities of conspecific pollen on stigmas of both natives remained more or less equal and unaffected by alien abundance ($F_{1,84} = 0.86$; $P = 0.427$ for *A. napellus* and $F_{1,85} = 0.25$; $P = 0.783$ for *E. angustifolium* respectively). As a consequence, total pollen loads on stigmas of *A. napellus* remained mainly conspecific, even at high abundances of *I. glandulifera* (Fig. 5A). On the

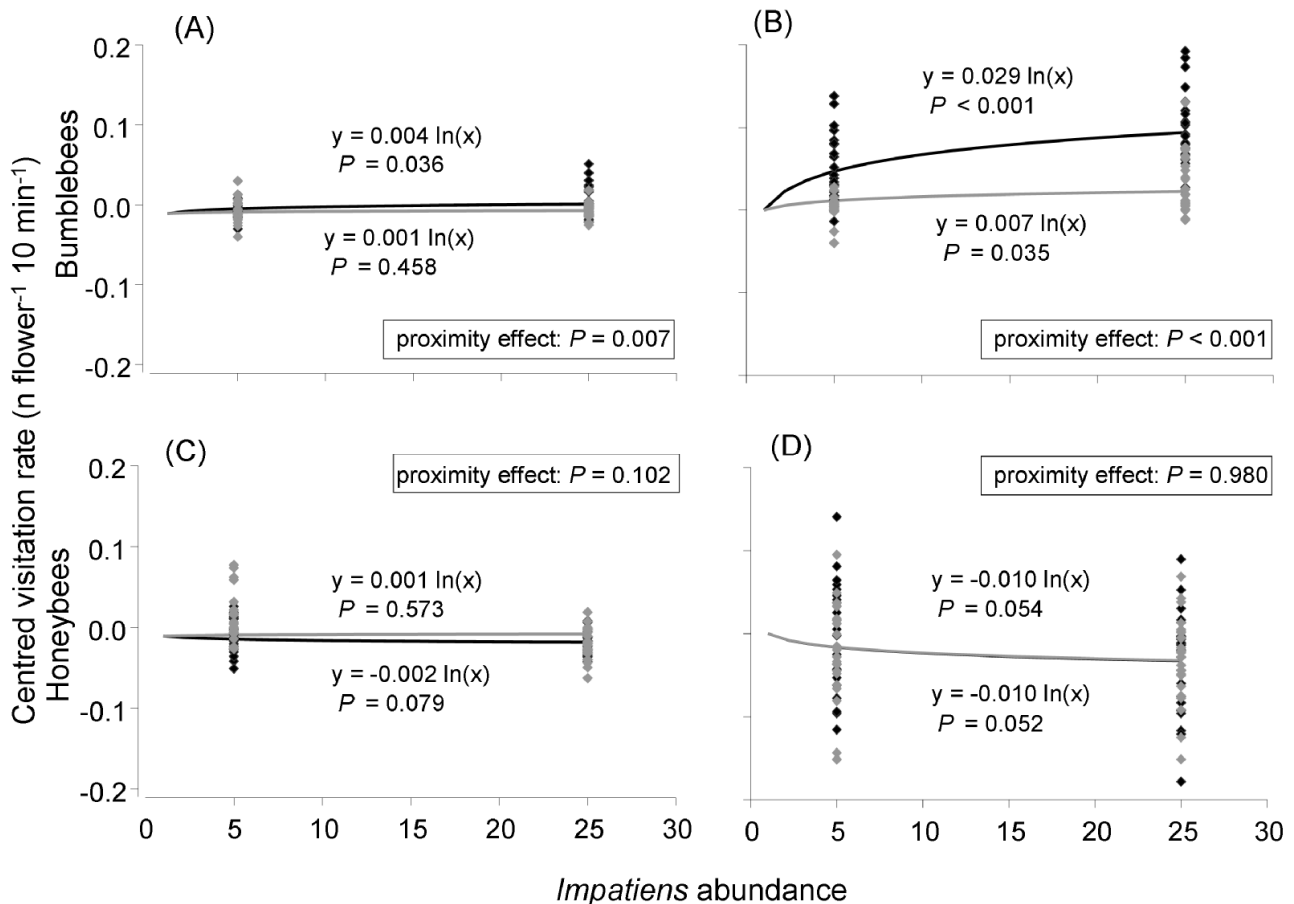


FIG. 4. Impact of the proximity (black diamonds: 0 m distance, grey diamonds: 15 m distance) of *I. glandulifera* on the effect of its abundance on the centred visitation rate in 2008 for both native plant species, *A. napellus* (A) & (C) and *E. angustifolium* (B) & (D), and for the two main pollinator categories (bumblebees and honeybees). For each combination of plant species, pollinator category and proximity level, model predictions are represented by a solid line and described by the equation with the corresponding *P*-value indicating significance. Another *P*-value indicates the significance of the proximity effect.

contrary, total pollen loads on stigmas of *E. angustifolium* increased with growing alien abundance and were finally dominated by alien pollen (Fig. 5B).

Impacts on seed set of natives

Seed set ranged between 71 and 85% for *A. napellus* and between 53 and 64% for *E. angustifolium* (Fig. 6). The alien abundance did not affect the seed set of *A. napellus* ($F_{1,191} = 0.46$; $P = 0.781$) and *E. angustifolium* ($F_{1,119} = 0.42$; $P = 0.762$). Neither proximity nor abundance of the alien influenced the seed sets of both natives in both sites (Fig. 6).

DISCUSSION

To determine a possible influence of the alien species on the reproductive success of natives, all steps during the pollination process, from insect visitation rates to pollen deposition and seed set, will be discussed sequentially.

Do abundance and/or proximity of the alien modify the visitation rates to natives?

Also other studies observed high visitation rates to *I. glandulifera*, probably due to its valuable sources of both

	<i>I. glandulifera</i> abundance			χ^2	<i>P</i>
	5	25	100		
<i>A. napellus</i>					
Coming from alien	93.6	92.5	90.9	0.79	NS
Leaving for alien	69.2	83.3	87.8	15.41	<0.0001***
<i>E. angustifolium</i>					
Coming from alien	86.2	89.8	87.8	0.72	NS
Leaving for alien	73.1	90.4	90.6	22.00	<0.0001***

TABLE I. Total percentage of bumblebee transitions from (“coming from”) or towards (“leaving for”) *I. glandulifera* observed on *A. napellus* (total number of transitions observed: n = 1 045) and *E. angustifolium* (n = 898) for the three abundance levels of the alien (5, 25 and 100 individuals) in 2009.

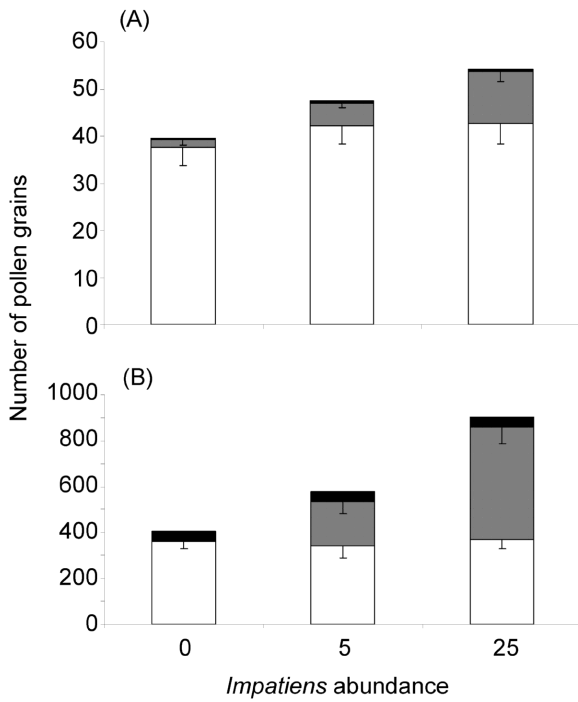


FIG. 5. Origin (white: conspecific, grey: alien, black: other) and quantity of pollen grains deposited on stigmas of native species according to *I. glandulifera* abundance (0, 5 and 25 individuals). (A) *A. napellus* (one stigma per flower). (B) *E. angustifolium* (one stigmatic lobe per flower). Data were collected in 2008 in plots adjacent to *I. glandulifera* and are presented as mean \pm s.e. (n = 24–30 flowers).

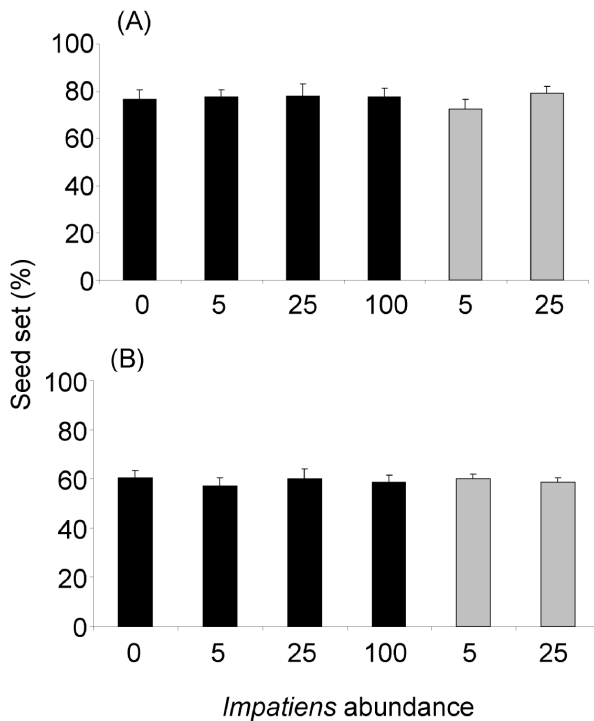


FIG. 6. Seed set of (A) *A. napellus* and (B) *E. angustifolium* for both years and sites pooled, according to the abundance (0, 5, 25 and 100 individuals) and proximity (black bars: 0 m distance, grey bars: 15 m distance) of the alien.

nectar and pollen (Lopezaraiza-Mikel et al. 2007; Nienhuis et al. 2009; Bartomeus et al. 2010; Vervoort et al. 2011). As already reported elsewhere, *Bombus pascuorum* was the main pollinator we observed (Chittka & Schürkens 2001; Nienhuis et al. 2009; Vervoort et al. 2011). Our results confirmed that the alien *I. glandulifera* received higher total visitation rates than both natives studied, comparable to other comparisons, e.g. with the native *I. noli-tangere* (Vervoort et al. 2011). Therefore, *I. glandulifera* can be considered as a potential competitor for pollinators of natives (Chittka & Schürkens 2001). In case of competition, the reproductive success of natives could be reduced. This would imply a greater threat for *A. napellus* ssp. *lusitanicum*, a rare species of patrimonial interest mainly pollinated by long-tongued bumblebees (Le Cadre 2005).

However, our results demonstrated a facilitative effect for *A. napellus* with an increase of visitation rates at least for bumblebee visitors (mainly *B. pascuorum*, a long-tongued bumblebee). On the contrary, for honeybees, a small competitive effect with lower visitation rates was detected. Similarly, for *E. angustifolium*, an increase of visitation rates was observed for bumblebees (*Bombus pascuorum* and *B. terrestris* s.l.) whereas lower visitation rates were registered for honeybees. The alien was more attractive than both natives, even for honeybees. We can posit that the alien provided such large quantities of nectar and pollen that hive recruitment increased honeybee worker visits. Honeybees might further have difficulties to exploit the native *A. napellus*, and they were often observed to rob nectar. For bumblebees, the facilitation effect obviously outweighed possible competition which might be due to the otherwise poor flower resources at the experimental sites and could be different in natural populations.

Five *I. glandulifera* individuals were sufficient to facilitate visitation of bumblebees to the investigated native species, an effect that strongly increased with higher alien abundance. This facilitation effect was not simply due to a rise in patch size, since at the same time, the visitation rate to *I. glandulifera* did not increase with higher abundances. We did, however, not control for increased abundance of native floral resources. Our results show effects at the local scale but data are still needed for non invaded large natural populations. Even though our patches were spatially not independent (bees can forage over larger ranges than 50 m), the potential of *I. glandulifera* to influence pollination of natives increased at short distances. Similar trends have been reported in previous studies using experimental design with potted plants (Nielsen et al. 2008; Cariveau & Norton 2009). Also the meta-analytical approach of Morales and Traveset (2009) confirmed the importance of alien abundance for interactions for insect visits. Of course, facilitation and competition for pollinator services between invasive and native species are extremes on a continuum that is a function of population density or relative abundance of floral resources (Bernhardt et al. 2008). And they definitely depend upon the insect species that visit. It is therefore not surprising that we obtained different results for honeybees and bumblebees.

Does pollinator behaviour change according to abundance and/or proximity of the alien?

The majority of bumblebees showed inconstant foraging behaviour and switched between the alien and the adjacent native. Moreover, interspecific movements increased with the abundance of the alien, and higher proportions of bumblebees left natives for the alien. Bumblebee constancy is often higher when pollinators forage among flowers that differ in size and odours than when flowers differ in colour only (Goulson 2003; Gegear & Thomson 2004; Gegear 2005; Raine & Chittka 2007). Flower constancy in pollinators is related to their inability to perceive process or recall multicomponent floral signals (Gegear 2005). In our case, bumblebee constancy was low at close vicinity of the alien (contrary to honeybees). This infidelity could be due to flower similarities among the three species. However, colours are more similar between the alien and *E. angustifolium* than with the violet *A. napellus*. This fact is compensated by flower morphology which is more similar between the alien and *A. napellus* (helmet-shaped and zygomorphic). More detailed analyses about flower odour or size could disentangle attractiveness components among our studied species.

Do visitors deposit alien pollen on native stigmas?

The amount of alien pollen deposited by pollinators on native stigmas is usually considered rather low, suggesting that differences in pollen placement on insect bodies might avoid interspecific pollen deposits (Lopezaraiza-Mikel et al. 2007; Jakobsson et al. 2008). In our study, we observed that honeybees crawled into the flower of *I. glandulifera* in order to reach the nectar without touching the anthers (also reported by Sowig 1989) and they did not visit this species for pollen. It was therefore unlikely that they contributed significantly to alien pollen transfer to natives. On the contrary, dorsal parts (head, thorax and abdomen) of visiting bumblebees were covered with alien pollen (see also Nienhuis & Stout 2009). When they visited natives, they turned inside the flowers thereby touching stigmas with their dorsal parts. As they moved from invasive to native flowers, they deposited pollen of *I. glandulifera* on native stigmas. As a consequence, when both natives were placed close to *I. glandulifera*, alien pollen was deposited on their stigmas. Therefore, conspecific native pollen could experience competition with alien pollen that can impede its placement, its germination or the growth of pollen tubes by stylar inhibition or stigma clogging (Brown & Mitchell 2001; Morales & Traveset 2008; Nielsen et al. 2008).

Variations in the visitation rates of bumblebees and honeybees can induce fluctuations in the amount of conspecific pollen deposited on native stigmas (Ghazoul 2002; Larson et al. 2006; Bartomeus, Bosch, et al. 2008; Flanagan et al. 2009). However, as reported for other alien-native pairs, even if the amount of alien pollen increased with alien abundance, the amount of conspecific pollen on *A. napellus* and *E. angustifolium* stigmas was globally not affected (Grabas & Laverty 1999; Larson et al. 2006).

What are the consequences on the seed set of natives?

Conspecific pollen deposition allowed for sufficient native fertilization as seed set of both natives was not affected by the abundance and/or the proximity of the alien. Thus, even if the quantity of heterospecific pollen increased due to the increase of alien pollen at high alien abundances, this did not reduce subsequent seed set. It has already been reported that in some cases, even when a large amount of heterospecific pollen was deposited, no germination interference and no reduction of reproductive success were observed (Morales & Traveset 2008). Closer investigation of pollen germination and pollen tube growth could give further insights into possible lack of competition.

Conclusions

Abundance and proximity of the alien increased bumblebee visitation rates to natives. Even if bumblebees moved frequently between species allowing for alien pollen deposits on stigmas of the natives *A. napellus* and *E. angustifolium*, no negative impacts of *I. glandulifera* on native reproductive success were found. Probably owed to self-compatibility and high attractiveness of both native species, the risk that the invader impedes their reproductive success by altering pollinator services may assumed to be low in natural populations. Still, effects of *I. glandulifera* on bumblebee population dynamics need further evaluation. Social bees, like bumblebees and honeybees, have broad foraging ranges and benefit from mass-flowering resources at large spatial scales (Westphal et al. 2003). Therefore, the alien *I. glandulifera* could even have a positive effect on colony fitness of *Bombus* species (Westphal et al. 2009; Bartomeus et al. 2010).

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