

DO NATIVE BEES HAVE THE POTENTIAL TO PROMOTE INTERSPECIFIC POLLINATION IN INTRODUCED *IMPATIENS* SPECIES?

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Abstract— Artificial introductions of non-native species by humans can remove geographical barriers between species. In the absence of reproductive barriers, closely related introduced taxa may be able to hybridize, resulting in the formation of novel genotypes. These may be more suited to the new environment than either of their parent taxa, and have the potential to become more invasive. We investigated potential reproductive barriers between the non-native invasive *Impatiens glandulifera* and its less aggressive non-native congener *I. balfourii*. We examined behaviour of pollinators, including their foraging preferences and whether they switched from one species to the other. Moreover, conspecific and interspecific artificial crosses were performed between these species and seed production and the germination success of hybrid seeds were assessed.

Both *I. balfourii* and *I. glandulifera* had relatively long flowering periods which mostly overlapped. Insect visitors were observed to switch from flowers of one species to the other during a single foraging bout, confirming that natural pollen transfer between species is possible. Artificial interspecific pollination resulted in the production of seeds, suggesting the presence of incomplete reproductive barriers between the two species. However, hybrid seeds mostly failed to germinate making the production of invasive hybrids unlikely. Nevertheless, because of the prolific reproductive output of these species we would not exclude the possibility that some viable hybrid seeds could be formed and become established. Further investigation of the fitness of hybrid seeds is required as hybridisation may allow *Impatiens* to exploit different ecological niches.

Keywords: *Bombus*, *heterospecific pollen transfer*, *invasive alien species*, *non-native species*, *reproductive barriers*

INTRODUCTION

One important indirect consequence of the increasing number of introduced plant species is the possible hybridisation between closely related taxa (Abbott 1992; Bleeker 2007). While natural hybridisation can be responsible for a greater genetic diversity, hybridisation between anthropogenically introduced species may lead to increased invasiveness for some introduced taxa, since it may result in genotypes more adapted to or fitter in novel habitats (Anderson and Stebbins 1954; Ellstrand and Schierenbeck 2000). Although many interspecific hybrids are sterile, more than half of all hybrid plants between non-native species in the UK were found to be fertile (Abbott 1992). Fertile hybrids may become invasive because they are more versatile than parents and/or able to tolerate a wider range of environments, including habitats which may not have been colonised by the species originally introduced. Alternatively, fertile hybrids may backcross with parental taxa with subsequent introgression, which could result in increased invasiveness in the parental species (e.g. Milne and Abbott 2000). For example, fertile allotetraploid hybrids resulting from interbreeding between non-native

Tragopogon species have become invasive in the USA (Novak et al. 1991). Also Tiébré et al. (2007) concluded that sexual reproduction among non-native *Fallopia* species can increase the invasive capacity of the genus. Furthermore, Ellenstrand and Schierenbeck (2000) reported 28 examples across 12 families of hybrid plants that had become invasive, mostly in Europe and North America.

In order for hybridisation to occur between non-native plant species, they must overlap in their flowering phenology, and their pollination mechanisms and breeding systems must allow interspecific pollen transfer. In addition, animal-pollinated species require appropriate flower visitors who include both taxa in their diet and who switch between taxa during a single foraging bout, thus mediating interspecific pollen transfer. Most non-native plants are well served by generalist native pollinators (Valentine 1978). Some generalists such as bumblebees (*Bombus* spp.) visit a range of taxa during a single foraging bout (i.e. are not flower constant; Heinrich 1976); particularly if those taxa are morphologically similar (Goulson 2000) as this increases their foraging efficiency. Hence, co-flowering, closely-related, non-native plant species may well have pollen transferred between them as a result of pollinator foraging strategies. However, the transfer of pollen is not sufficient for hybridisation in itself. Interspecific pollen must be able to germinate on the stigma and grow a pollen tube through the style, fertilize the ovule and form a functional seed, and the

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seed must be able to germinate and grow into a new plant (Wolf et al. 2001).

The genus *Impatiens* contains several biotically pollinated species which vary in their invasive capacity throughout their introduced range (Perrins et al. 1993). A great number of hybrids have been bred for ornamental purposes. Besides the many ornamental *Impatiens*, natural hybridisations are reported to occur in wild populations of *Impatiens* species, both in tropical (Grey-Wilson 1980; Tsukaya 2004) and temperate regions (Zika 2006). Although frequently co-occurring native species in north-east USA do not appear to hybridize (*I. capensis* and *I. pallida*; Randall and Hilu 1990; Tabak and von Wettberg 2008), hybridisation between *I. capensis* and the closely related introduced species, *I. noli-tangere* (native to Europe) is possible (Tabak and von Wettberg 2008). Ornduff (1967) reported that *I. capensis* can hybridize with native *I. ecalcarata* in the north-west USA. However, little information is available on boundaries between other temperate *Impatiens* species (Tabak and von Wettberg 2008), particularly regarding those which are problematic invaders.

The most common *Impatiens* species in Ireland, Britain and continental Europe is the aggressive invader *I. glandulifera* (DAISIE 2009; NOBANIS 2010), which was first introduced from the Himalayas to Europe (to Kew Gardens) in 1839 (Britten 1900, Beerling and Perrins 1993). Originating from the same native range, *I. balfourii* was introduced to the south of France about 50 years later (Beerling and Perrins 1993; Adamowski 2009) and since then has spread to southern and central Europe (Schmitz and Dericks 2010). *Impatiens glandulifera* is currently much more invasive than *I. balfourii*. Even though it appears that *I. glandulifera* requires higher soil moisture (Beerling and Perrins 1993; Schmitz and Dericks 2010), both species have similar ecological requirements and have been observed to grow together in the same habitat (Fig. 1). It is possible that *I. balfourii* is only in an early stage of invasion and may become more established and widespread in the future (Adamowski 2009; Schmitz and Dericks 2010). This might result in the frequent occurrence of this species in habitats already invaded by *I. glandulifera*. In its introduced range, *I. glandulifera* has been reported to flower from July to October (Beerling and Perrins 1993) and although there is little information available on the phenology of *I. balfourii* in its introduced range, this species flowers from the end of July to August in its native range (Adamowski 2009).

Unlike the perennial *Impatiens* characteristic of the tropical regions which reproduce vegetatively, the annual *Impatiens* species, including *I. glandulifera* and *I. balfourii*, completely rely on sexual reproduction (Grey-Wilson 1980; Beerling and Perrins 1993). The understanding of the hybridisation potential of these species is therefore important to evaluate their possible invasion dynamics (Ellstrand and Schierenbeck, 2000, Richardson and Pysek, 2006).

Impatiens glandulifera produces both flowers and inflorescences that are slightly larger in dimension than *I. balfourii* (Beerling and Perrins 1993; Adamowski 2009)



FIG. 1. *Impatiens glandulifera* (the larger species) and *I. balfourii* growing and flowering together in Saint Christophe en Oisans (Rhône-Alpes, Isère, France), August 2010. Photograph by P. Ugoletti.

(Fig. 1). However, the two species present a similar flower morphology and reproductive system. The male phase precedes the female phase with the stigma becoming visible when the androecium has completely dehisced and fallen off (Wilson and Thomson 1991; 1996). *Impatiens glandulifera*'s flowers have traditionally been described as strongly protandrous (Valentine 1978; Bell et al. 1984; Titze 2000). However, Vervoort et al. (2011) reported that *I. glandulifera*'s stigma is already receptive at bud stage but flower morphology prevents self pollination and pollinator activity is needed for this species to maximise fruit set. *Impatiens glandulifera* has been reported to attract great numbers of insect visitors in its introduced range, especially generalist *Bombus* species, attracted by its plentiful nectar production (Lopezaraiza-Mikel et al. 2007; Nienhuis and Stout 2009). As far as we are aware, little is known about the insect visitors or the floral rewards of *I. balfourii* (but see Elias and Gelband 1977; and Vervoort et al. 2011).

Impatiens glandulifera is not reported to hybridize with other species (Beerling and Perrins 1993). In "Flora of Pakistan" the possibility of hybridisation between *I. balfourii* and *I. bicolor* Royle is hypothesized, but the potential for crosses between *I. balfourii* and *I. glandulifera* is not considered (Nasir 1980).

By focussing on these two species in Ireland, we examined the potential for hybridisation and for production of hybrid offspring. We hypothesised that: 1) insect visitor guilds overlap between *I. glandulifera* and *I. balfourii* allowing natural pollen transfer between the two species to

occur; 2) pollen from *I. balfourii* and *I. glandulifera* are capable of germinating on and penetrating through stigmas of the other species, and producing hybrid seeds; and 3) that hybrid seeds are viable and can germinate.

MATERIALS AND METHODS

Insect behaviour

Seeds of *I. glandulifera* and *I. balfourii* were obtained from several Botanic Gardens across Europe (Appendix 1). Approximately equal numbers of seeds from each source were mixed within species and stratified at 4°C for about 50 days to break dormancy (Mumford 1988). Seeds were then germinated on moist filter paper in 90 mm diameter Petri dishes in a growth chamber (Ugoletti et al. 2011). Seedlings were kept inside in a greenhouse at Trinity College Botanic Garden, Dublin, for an initial period to avoid any frost damage. Sixty plants each of *I. glandulifera* and *I. balfourii* were randomly chosen and potted in 20-litre pots (one seedling per pot). In early June 2008, the plants from the two species were randomly interspersed and positioned approximately 1 m apart from each other in an open, unshaded area in Trinity College Botanic Garden. During July, 56 individual bees of three species (*Apis mellifera*, *Bombus hortorum* and *Bombus pascuorum*) were observed for approximately 7 minutes each. The number of visits made by each individual bee to flowers of each *Impatiens* species and the number of switches between *Impatiens* species were recorded during the 7-minute period. Observations were made opportunistically, and so the number of bees of each species observed depended on the abundance of those bees at the site on days with appropriate weather conditions for insect foraging (i.e. no or light wind, temperatures >15°C).

Experimental crosses

In 2009, 15 plants of *I. glandulifera* and 15 plants of *I. balfourii* were grown as described above and again placed outside in the Trinity College Botanic Gardens. On each plant, 30 flower buds were bagged with bridal veil material (with a mesh < 1 mm) to exclude pollinators. If more than 30 flowers were produced per plant, excess flowers were removed. Each plant was randomly allocated to one of three treatments (5 plants per treatment): interspecific cross pollination (treatments B×G or G×B); conspecific cross pollination (treatments B×B or G×G); and control flowers receiving no outcross pollen (treatments B0 and G0; included to confirm absence of autogamous selfing and the effectiveness of the pollen exclusion bags). When, at the end of the male phase of flowering, the androecium fell off exposing the gynoecium (Wilson and Thomson 1991), flowers were hand pollinated by removing the whole anthers from flowers of pollen donors and applying the pollen directly to receptive stigmas of test flowers (Nienhuis and Stout 2009). Hand pollinated flowers were marked by applying a strip of black adhesive tape around the flower stem and re-bagged. Once mature, fruits were collected and the number of seeds per fruit was counted. Seeds were stored dry in paper bags at 20°C for a period of 8 months, after which they received a stratification of 20 days at 4°C to

break dormancy and they were subsequently placed to germinate at 20°C (Ugoletti et al. 2011). Germination was compared among four treatments (B×G, G×B, B×B and G×G) since the unpollinated flowers (B0 and G0) produced only very low numbers of seeds. For each treatment, different numbers of seeds were germinated depending on seed availability. Seed production was higher when flowers had been pollinated with conspecific pollen. For the conspecific treatments B×B and G×G, 300 and 273 seeds were germinated, while for the heterospecific G×B and B×G treatments, only 91 and 84 seeds were available for germination tests. Seed germination was followed for eight weeks. However, no seeds germinated after the fifth week.

Data analysis

For all bee species, the numbers of visits by each individual bee to flowers of *I. glandulifera* or *I. balfourii* were compared between plants using a non-parametric Wilcoxon test (Z) for two related samples (since the same individual bees were visiting both plants). For the most abundant bee species (*B. pascuorum*), numbers of switches of each bee from *I. glandulifera* to *I. balfourii* and from *I. balfourii* to *I. glandulifera*, were compared with Wilcoxon test (Z) for two related samples. For *B. pascuorum*, also the number of consecutive visits to *I. glandulifera* and the number of consecutive visits to *I. balfourii* were compared with Mann-Whitney test (U) for two unrelated samples.

Data on the number of seeds per fruit consisted of integer counts; therefore differences between treatments were tested with a generalized linear model (GLM) with Poisson distribution and log-link function (Crawley 1993). Since data showed overdispersion an overdispersed Poisson model was fitted, including the inverse of Pearson chi-square/df as scale weight, which increased the standard error and made the test more conservative. Treatment (B×B, B×G, B0, G×G, G×B, and G0) was included in the model as categorical predictor (factor). To investigate differences in the fruit set (proportion of fruits containing seeds) according to the different types of cross, a GLM with binomial distribution and logit-link function was used. The type of cross (B×B, B×G, B0, G×G, G×B, and G0) was included in the model as categorical predictor (factor).

Germination rates after 8 weeks were calculated for each treatment and compared with a binomial logistic GLM with logit-link function with the type of cross (B×B, B×G, G×G and G×B) as categorical predictor.

Pair-wise comparisons of number of seeds per fruit, fruit set and germination percentage between each pair of crosses were performed using sequential Bonferroni *post-hoc* test. All the data analyses were run using SPSS 16 (SPSS, Inc. Chicago IL).

RESULTS

Bee behaviour

Visitation patterns were recorded for eight *Apis mellifera*, six *Bombus hortorum* and 42 *Bombus pascuorum* workers, the number of bees of each species reflecting its

	Visits to flowers			Switches between plants			Consecutive visits				
	Total	To IG	To IB	Wilcoxon test	Total	From IG to IB	From IB to IG	Wilcoxon test	To IG	To IB	MWU test
<i>A. mellifera</i>	11.88±1.90	11.25±2.04	0.63±0.63	Z=2.37, P=0.018	0.13±0.13	0.13±0.13	0		11.25±2.04	2	
<i>B. hortorum</i>	22.00±7.03	17.50±4.91	4.50±4.31	Z=1.78, P=0.075	0.83±0.54	0.50±0.34	0.33±0.21		13.13±4.25	9.00±7.50	
<i>B. pascuorum</i>	15.83±1.31	10.02±1.03	5.81±1.14	Z=2.31, P=0.021	1.76±0.31	0.79±0.16	0.98±0.16	Z=34, P=0.0455	6.34±0.66	4.75±0.50	U=1861.5, P=0.194

TABLE 1. Number of visits to flowers of *I. glandulifera* (IG) and *I. balfourii* (IB) average number of switches between different plant species, and average number of consecutive visits to *I. glandulifera* and to *I. balfourii* recorded for the three bee species during single foraging bouts. Data are presented as mean ± SE.

relative abundance at the site. During a 7-minute foraging bout, all three bee species visited more *I. glandulifera* flowers than *I. balfourii*, but this difference was only significant for *A. mellifera* and *B. pascuorum* (Tab. 1)

Individuals of all bee species switched between the two *Impatiens* species. The honeybees (*A. mellifera*) were observed foraging mainly on *I. glandulifera* flowers and were seen switching only from *I. glandulifera* to *I. balfourii*. The other two bumblebee species switched either from *I. glandulifera* to *I. balfourii* or from *I. balfourii* to *I. glandulifera*. The most abundant bee species, *B. pascuorum*, showed a preference regarding the direction of the switch; it was observed to switch more often from *I. balfourii* to *I. glandulifera* than vice versa (Tab. 1). This bumble bee species further showed the lowest (though not significantly) average number of consecutive visits to the same plant species than the other two bee species (Tab. 1).

Fruit set and seed production

Most fruits from flowers which did not receive any pollen did not develop (Fig. 2A), and those that did reach maturity contained very low numbers of seeds per fruit for both species (Fig. 2B). This confirms a low autogamous self-pollination for both species and the effectiveness of bags at excluding pollen from other sources. No differences between *I. glandulifera* and *I. balfourii* were found in fruit set in

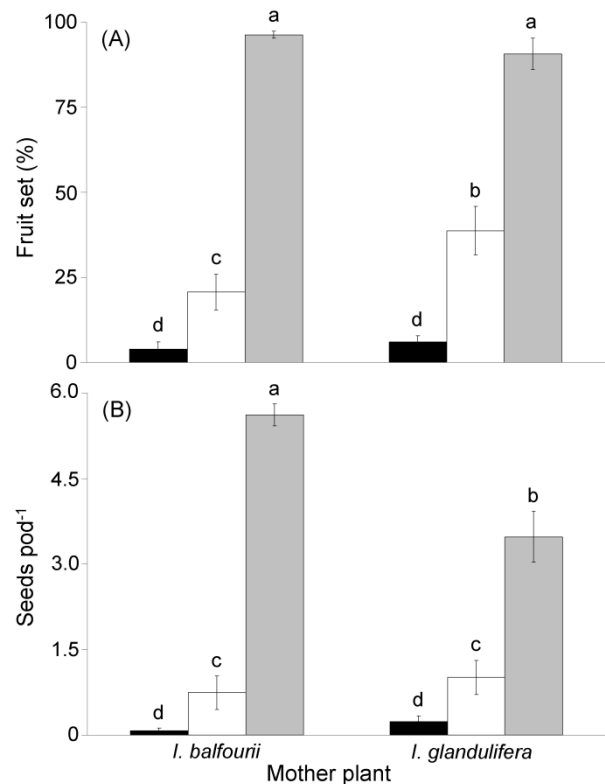


FIG. 2. Mean (\pm SE) fruit (A) and seed (B) production following pollen exclusion (black bars, treatments B0 and G0), interspecific crosses (white bars, treatments B×G and G×B) and conspecific crosses (grey bars, treatments B×B and G×G). Letters above columns in the same graph indicate significant differences among types of cross (Sequential Bonferroni test, $P < 0.05$).

flowers pollinated with conspecific pollen (mean difference = -0.06 ± 0.03 , $P = 0.063$), and conspecific crosses produced higher fruit set than interspecific crosses (-0.58 ± 0.04 to 0.76 ± 0.04 , $P < 0.001$, Fig. 2A). Differences in fruit set were significant between the two interspecific cross types: *Impatiens glandulifera* flowers pollinated with *I. balfourii* pollen had higher fruit set than *I. balfourii* flowers pollinated with *I. glandulifera* pollen (0.18 ± 0.05 , $P = 0.001$, Fig. 2A).

Fruits resulting from conspecific crosses contained significantly more seeds than those from interspecific crosses (2.46 ± 0.30 to 4.87 ± 0.36 , $P < 0.001$). Conspecific crosses resulted in higher seed numbers for *I. balfourii* than *I. glandulifera* (2.15 ± 0.43 , $P < 0.001$). There was no difference in seed numbers between the two interspecific cross types (B×G and G×B) (-0.27 ± 0.19 , $P = 0.155$, Fig. 2B).

Seed germination

None of the seeds produced from *I. balfourii* flowers pollinated with *I. glandulifera* pollen (B×G) germinated. Hybrid seeds with *I. glandulifera* as mother and *I. balfourii* as the pollen donor showed negligible germination ($1.1\% \pm 1.1$, $N = 91$, Fig. 3). In the group of control seeds, produced by pollinating flowers with conspecific pollen, germination reached $28.3\% \pm 7.5$ ($N = 273$) for B×B and $14.1\% \pm 6.2$ ($N = 300$) for G×G (Fig. 3).

DISCUSSION

The reproductive biology of *I. glandulifera* has been extensively studied because of its widespread invasive capacity (Stary and Tkalcu 1998; Titze 2000; Nienhuis and Stout 2009). Some attention has been given to *I. balfourii*, which has recently been suggested as a potential invader (Schmitz and Dericks 2010). However, so far no study has investigated the potential for sexual reproduction between these two species in their introduced range.

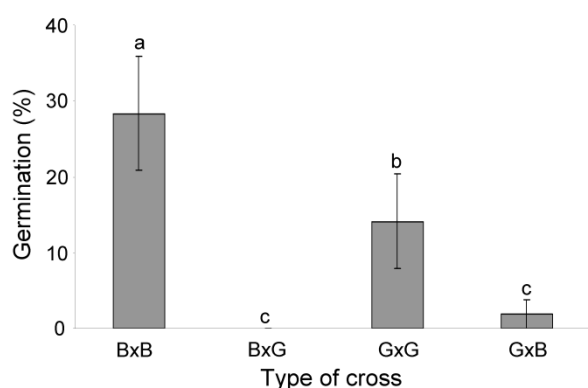


FIG. 3. Mean percentage of germination (\pm SE) for the conspecific (B×B and G×G) and interspecific (B×G and G×B) crosses. Different letters above columns indicate significant differences among species (Sequential Bonferroni test, $P < 0.05$).

Impatiens balfourii is reported as established in a few countries in the south of Europe but not in Ireland (DAISIE 2009). However, during our experiments this species was able to grow, flower and produce seeds under local climate, suggesting that it might also become established in Ireland. We observed a longer flowering period for *I. balfourii* (from mid June to October) than has been reported for this species in its native range (end of July to August, Adamowski 2009). Its flowering period overlapped with that of *I. glandulifera*, which began flowering only slightly later in our common garden experiment. Perrins et al. (1993) also reported *I. glandulifera* flowering from the end of June to October. For *I. balfourii* they found that, in England, plants of this species started flowering more than a month later and continued flowering until they were killed by frost (Perrins et al. 1993). Our experiment took place in 2008, when mean air temperature and sunshine totals for May were above normal. As a consequence, *I. balfourii* might have flowered earlier than in years with a cooler spring. There is no information available on *I. balfourii*'s flowering phenology in its introduced range in the south of Europe.

Bee visitation

Native honey- and bumblebees visited both species in our study. *Impatiens glandulifera* seemed to outcompete *I. balfourii* for pollinators, which could promote its reproductive success and invasion capacity. Two of the three bee species visited significantly more *I. glandulifera* flowers, perhaps because of the large nectar and pollen reward of this species (Nienhuis et al., 2009) and/or because of other flower characteristics (e.g. larger flower size, brighter colour and possibly stronger odour). No study has been done to assess *I. balfourii*'s floral rewards. *Apis mellifera* seemed to express fidelity to *I. glandulifera*, but due to the weather conditions during observations, and possibly because of the suburban location of the study site, not many honeybees were observed. However, especially the two bumblebee species were frequently observed to switch between plant species during a single foraging bout. Since pollen placement on the bees is identical for both *Impatiens*, there is a high probability that a switch between species by a foraging bee results in interspecific pollen transfer. *Bombus pascuorum*'s observed preference to switch from *I. balfourii* to *I. glandulifera* makes it more likely that hybrid seeds will be formed when *I. glandulifera* is the mother plant.

Fruit set and number of seeds per fruit

A previous comparison of reproductive output showed no significant difference in natural levels of fruit set and seed production per plant between *I. glandulifera* and *I. balfourii* (Ugoletti et al., 2011). Perrins et al. (1993) found that seed set was approximately double in *I. glandulifera* compared with *I. balfourii* (6 seeds pod^{-1} in *I. glandulifera* and less than 3 seeds pod^{-1} in *I. balfourii*). Conversely, in our current study, we found that conspecific crosses resulted in more seeds per pod in *I. balfourii* than in *I. glandulifera*. Knowing *I. balfourii*'s ovule number would give a better insight into this species' reproductive traits. Vervoort et al. (2011) reported an ovule number of 6.8 for *I. glandulifera*, but the authors did not include *I. balfourii* in their study. However, besides ovule number, other factors (e.g. environmental

stress) could affect the fertilization or post-fertilization abortion of an ovule. *Impatiens glandulifera* is a larger, more moisture-loving plant, which might have suffered more than *I. balfourii* from experimental conditions consequently reducing seed set.

Following heterospecific crosses, we found that fruit formation was higher when *I. glandulifera* was the maternal plant. This could be due to mechanical or chemical characteristics of the stigma affecting the degree of adhesion and germination of interspecific pollen. Seed production following interspecific pollen deposition was lower than after conspecific crosses, suggesting that interspecific pollen deposition would reduce the reproductive success/output of plants. This has been shown for other plant species (e.g. Levin et al. 1996). Nagamitsu et al (2006) hypothesised that hybrid seeds could be produced at the expense of conspecific ones. Consistently, Randall and Hilu (1990) found that when interspecific crosses were made between *I. capensis* and *I. pallida*, pollen mixtures of the two species reduced fruit set, which was greater in *I. capensis* than in *I. pallida*. Improper pollen transfer has been indicated as a primary competition mechanism also for other species that rely on generalist pollinators; for instance, *Claytonia virginica* and *Stellaria pubera* (Campbell and Motten 1985). The negative effects of improper pollen transfer can even lead to the exclusion of one of the two species, representing an important selective force (Waser 1978). Nevertheless, though large quantities of *I. glandulifera* pollen were detected on two co-flowering native species, their seed set was not reduced (Cawoy et al. 2012). We observed that conspecific flowers were frequently visited consecutively, resulting in conspecific pollination. So far, there is no evidence that the co-occurrence of *I. glandulifera* and *I. balfourii* would reduce (or even exclude) one or both species through improper pollen transfer. However, our hand pollination experiments showed that interspecific pollen can adhere and germinate on the other species' stigmas and progress through the style to reach the ovary to produce hybrid seeds.

Seeds produced from interspecific crosses mostly failed to germinate, suggesting the presence of post-fertilization reproductive barriers. Hybrid seeds were only able to germinate when *I. glandulifera* was the mother plant but the germination rate was very low (1.1%). Germination rates of seeds produced by conspecific crosses were 3-4 times lower than previously observed for these two species (Ugoletti et al. 2009; 2011). This could be due to collection of seeds before fruits were fully ripe, which was done to avoid seed loss when fruits explode. Consequently, we may have underestimated the potential for hybrid seed germination. Considering the total number of *I. glandulifera* flowers for each treatment (150) and the average number of ovules in this species (6.8, Vervoort et al., 2011), we can estimate that per treatment approximately 1020 ovules could have been fertilized. According to our germination rate, the probability of a seedling originating from a conspecific cross is approximately 0.035 while the probability of a new individual originating from the interspecific crosses is only approximately 0.001. Considering this ratio of conspecific to hybrid seedlings of 35:1, but given the vast number of *I.*

glandulifera plants in its introduced range, we cannot exclude the possibility that hybrids could establish.

CONCLUSION

Natural pollen transfer between *I. glandulifera* and *I. balfourii* is possible due to overlapping flowering periods and to the presence of generalist pollinators that include both species in their diet and switch between species during single foraging bouts.

Seeds developed from interspecific crosses. Thus, the formation and establishment of hybrids with *I. glandulifera* as mother plant is possible. However, hybrid seeds mostly failed to germinate making it unlikely that hybridisation between these two taxa will result in a more aggressive invasive hybrid taxon. The probability that seedlings originate from hybrid crosses is much lower than the chance that seedlings originate from conspecific crosses. It is more likely that interspecific pollination could reduce conspecific seed production in either species through improper pollen transfer. From a conservation point of view, it would be worth to further investigate the possibility that the coexistence of these two introduced species could limit each other's spread.

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APPENDIX

Additional supporting information may be found in the online version of this article:

APPENDIX I. Seeds providers of the *Impatiens* plants used for the experiment.

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