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EFFECTIVENESS OF NATIVE BUMBLEBEES AS POLLINATORS OF THE ALIEN INVASIVE PLANT *IMPATIENS GLANDULIFERA* (BALSAMINACEAE) IN IRELAND

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Abstract. Flowers of alien invasive plants can be pollen limited due to a lack of effective pollinators. The alien *Impatiens glandulifera* is predominantly visited by bumblebees in its invaded range. There bumblebees pollinate *I. glandulifera,* but it remains unclear whether foraging behaviour or bumblebee or flower morphology affects effectiveness. We investigated the effectiveness of native bumblebee (*Bombus pascuorum*) pollinators in Ireland by quantifying pollen deposition and removal, and seed production after a single bumblebee visit. Morphological characteristics of flowers and bumblebee body parts were measured to determine their influence on pollen deposition and removal. *B. pascuorum* is a highly effective pollinator of the alien due to its high visitation frequency, the morphological fit with flowers and individuals removing large pollen quantities and inducing maximum seed set after a single visit. The impact of native bumblebees on *I. glandulifera* pollination and the implications of the pollination mechanism of the alien for its successful spread are discussed.

Keywords: Alien plant, Bombus pascuorum, pollen deposition, pollen removal, morphological matching

INTRODUCTION

Although many alien plant species have been accidentally and deliberately introduced into ecosystems worldwide (Levine et al. 2003; Richardson 2004), only a small proportion of introduced species actually establish and persist in the newly invaded habitats (Mack et al. 2000; Manchester & Bullock 2000). For an alien plant to become a successful invader it has to be able to tolerate a range of biotic and physical characteristics (Milbau & Stout 2008; Richardson et al. 2000). In addition, many alien plants have to rely on mutualisms with symbiotic micro-organisms, seed dispersers and pollinators to not only facilitate their invasion and naturalisation process, but also aid their rapid spread (Richardson et al. 2000; Simberloff & Von Holle 1999). Interactions with mutualistic pollinators are often essential for the establishment and persistence of self-incompatible, out-crossing alien plants that depend on animal pollination (Morales & Aizen 2002; Parker 1997). For example, the seed production and perhaps the spread of the alien shrub *Lupinus arboreus* (Fabaceae) in Tasmania, is facilitated by pollination by exotic bees (*Apis mellifera* and *Bombus terrestris* (Hymenoptera, Apidae)) (Stout et al. 2002). If mutualistic pollinators are not introduced and are absent from the invaded ecosystem, the newly introduced alien plants either fail to establish (e.g. Faegri & Van der Pijl 1966) or have to interact with native pollinators already present to reproduce successfully (Parker & Haubensak 2002; Richardson et al. 2000; Valentine 1978). This is particularly the case if the introduction occurs between continents, as the alien plant is less likely to be served by its natural pollinators (Valentine 1978). The alien *Fuchsia magellanica* (Onagraceae) for instance, is pollinated primarily by hummingbirds (e.g. *Sephanoides galeritus*) in its native range (Traveset et al. 1998), whereas in Ireland its native pollinators have been replaced by generalist bumblebee species (e.g. *B. pascuorum*) (C. M. Nienhuis pers. obs.). Although many invasive alien plants are visited by a range of native generalist insects (Baker 1965; Dietzsch and Stout in press; Memmott & Waser 2002; Richardson et al. 2000; Stout et al. 2006), populations can undergo pollinator limitation due to a limited number or a complete lack of legitimate pollinators, which may constrain their ability to invade (Parker 1997; Parker & Haubensak 2002; Stout 2007a). Illegitimate pollinators either fail to transport pollen from the anthers or do not deposit pollen onto receptive stigmas effectively (Stout 2007a). This can be caused by a morphological miss-match between the plant and the insect visitor (Stang et al. 2006). For example, long-tongued *B. hortorum* individuals are too large to access nectar from the alien plant *Impatiens balfouri* (Balsaminaceae) legitimately, and have been observed robbing nectar via holes pierced through the corolla (C.M. Nienhuis pers. obs.).

The effectiveness of pollinators is related to floral structures and pollinator body shapes and sizes (e.g. Fukuda

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*Corresponding author: E-mail: nienhuic@tcd.ie et al. 2001; Herrera 1987; Hiei & Suzuki 2001; Hurlbert et al. 1996; Schemske & Horvitz 1984; Suzuki et al. 2002) and the mechanical fit of flowers around pollinators (Wilson 1995). Successful pollination is further impacted by insect foraging behaviour in terms of the method of pollen collection (from anthers) and deposition (on stigmas) (Thomson 1986), and visitation quantity (abundance of visitors and flower visitation rates) and quality (proportion of visitors carrying conspecific pollen and proportion of visits leading to positive out-crossing) (Fenster et al. 2004; Fumero-Cabán & Meléndez-Ackerman 2007; Stout 2007a; Young et al. 2007). Hence, foraging behaviour and morphology of native pollinators can have profound effects on the reproductive success and consequently the persistence of alien invasive plant populations.

Impatiens glandulifera Royle (*I. roylei* Walp.) (Balsaminaceae) originates from the Himalayas and East India and is a widely naturalised, prolific alien plant rapidly invading waterways, woodlands and waste grounds across continental Europe, Britain and Ireland (Beerling & Perrins 1993; Perrins et al. 1993; Pyšek & Prach 1995). In its native habitats the alien is visited by a variety of bumblebee species (e.g. *B. asiaticus*, *B. keriensis* and *B. rufofasciatus*) (Saini & Ghattor 2007). In its invasive range, *I. glandulifera* is regarded as an important nectar and pollen source (Showler 1989), especially in late summer (Starý & Tkalců 1998), and is visited by a range of generalist insects in Britain (Barrow & Pickard 1984; Fussell & Corbet 1992; Lopezaraiza-Mikel et al. 2007; Prowse & Goodridge 2000; Valentine 1978) and continental Europe (Sowig 1989; Starý & Tkalců 1998; Titze 2000). The medium- and long-tongued bumblebees, *B. pascuorum* and *B. hortorum* respectively, represent its main visitors in Ireland (Nienhuis et al. in press).

It has been proposed that the distribution of *I. glandulifera* is not pollinator limited (Willis & Hulme 2002) and native pollinators are thought to have aided the establishment and spread of *I. glandulifera* in Europe (Lopezaraiza-Mikel 2006). In Germany, bumblebees are known to be efficient pollinators of *I. glandulifera*, which is thought to be related to the morphology and the suspension mechanism of the flowers (Titze 2000). It remains unclear however, how effective native bumblebee visitors are as pollinators of the alien in Ireland and whether factors such as bumblebee and flower morphology are influencing this effectiveness.

The aim of this study was to investigate the role that native bumblebees play in the pollination of *I. glandulifera* in Ireland. We further considered how bumblebee and flower morphology influence pollen deposition on stigmas and pollen removal from androecia. In particular, we posed the following questions:

1) Is *I. glandulifera* pollen limited in Ireland? To deal with this question we examined the breeding system of *I. glandulifera* and its seed production in three treatments: open pollination, supplemental hand pollination and pollinator exclusion (bagged).

2) Are bumblebees (*B. pascuorum*) effective at pollinating and inducing seed set in *I. glandulifera*? To clarify this we explored pollination effectiveness after a single bumblebee visit (single visit experiments), by estimating firstly pollen deposition on stigmas and pollen removal from androecia, and secondly seed production.

3) Which bumblebee body parts and which floral parts are involved in the effective pollination of *I. glandulifera*? Here, we took morphological measurements of *I. glandulifera* flowers and their individual bumblebee visitors and conducted multiple linear regression analysis to investigate which bumblebee body parts and which floral parts influenced effective pollination.

MATERIALS AND METHODS

I. glandulifera and its pollinators

Impatiens glandulifera is an invasive annual plant and blooms from mid June until late October in Ireland (Nienhuis et al. in press). The alien produces strongly zygomorphic flowers, with the dorsal petals forming a hood above the androecium and the lower petals of the lateral united petals serving as a platform for landing insects (Beerling & Perrins 1993; Titze 2000) (Fig. 1a and b). Both the androecium and the stigma are suspended downward from the roof of the vestibular sepal (Wilson 1995). The flowers are protandrous (Bell et al. 1984) and flowering takes place over two to three days (Titze 2000), with the male phase lasting slightly longer than the female phase (Schemske 1978). The stigma is completely covered by the androecium during the male phase, and only becomes visible and receptive when it swells and pushes the completely dehisced androecium off, marking the beginning of the female phase (Wilson & Thomson 1991; Wilson & Thomson 1996). Insects passing through the lower sepal to obtain nectar in the posterior spur, contact the male and female parts with their dorsal body parts (Valentine 1978), and usually exit flowers with their dorsal parts covered in the characteristically white pollen of *I. glandulifera* (C.M. Nienhuis pers. obs.). Despite being self-compatible (via geitonogamy), the alien relies on insect pollination as flowers are not capable of automatic self-pollination due to protandry (Valentine 1978).

Study sites and species

From June to September 2007 and 2008, data were collected at two locations in Co. Dublin, Ireland: a naturally established *I. glandulifera* population along the River Liffey and a population of approximately 60 potted *I. glandulifera* plants at Trinity College Botanic Gardens, Dartry, Dublin 6. During the experimental period, we observed mostly *B. pascuorum* and hardly any other insect species visiting the alien and therefore the experiment was conducted on *B. pascuorum* exclusively.

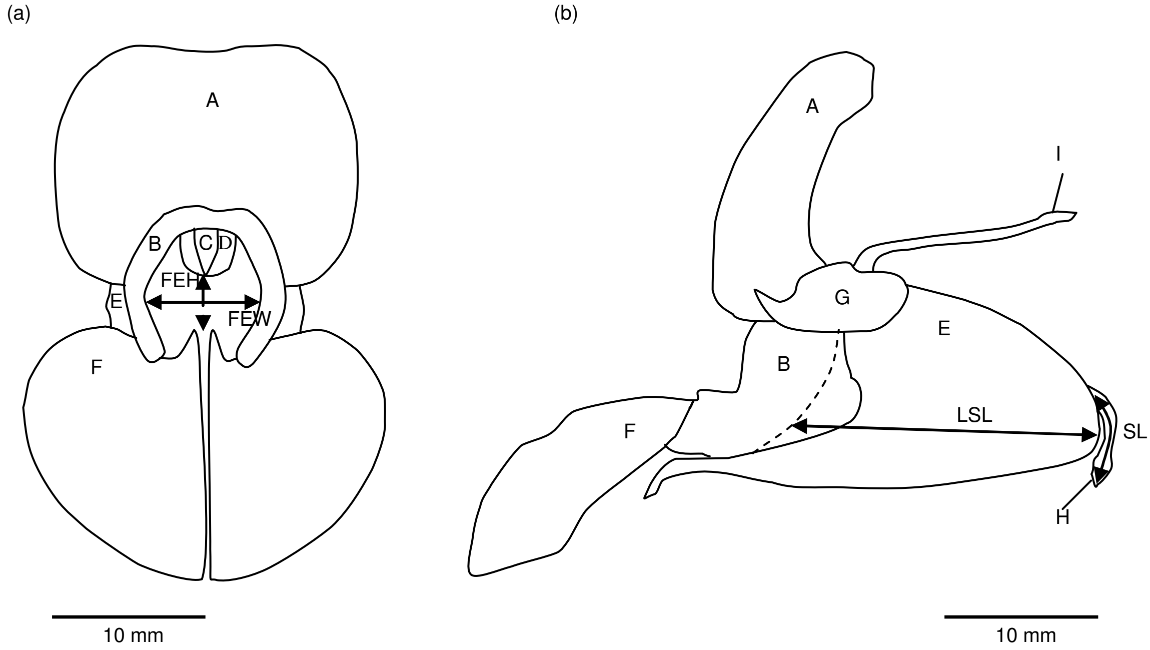

FIG. 1. Frontal (a) and lateral (b) view of *I. glandulifera* flower: A. dorsal petal, B. upper petal of the lateral united petals, C. stigma, D. androecium, E. lower sepal, F. lower petal of the lateral united petals, G. lateral sepal, H. spur, and I. peduncle (after Titze 2000). Morphological characteristics measured in *I. glandulifera* flowers: floral entrance height (FEH = height between the tip of androecium in male flowers / tip of stigma in female flowers to the lower petal of the lateral united petals), floral entrance width (FEW = width between the two upper petals of the lateral united petals), lower sepal length (LSL) and spur length (SL).

Breeding system of I. glandulifera

To determine whether *I. glandulifera* is pollen limited and to test the potential for self-pollination and self-fertilisation (autogamy) in the absence of bumblebees, we selected a total of 60 flower buds, each from different plants, and randomly assigned 20 flowers to each of three treatments: open pollination (OP (control)), supplemental hand pollination (SHP) and pollinator exclusion (PE (bagged)) between July and September 2007 and 2008. The experiment was carried out on the naturally established *I. glandulifera* population in 2007 and on the potted plants at Trinity College Botanic Gardens in 2008 (because the River Liffey plants were destroyed by flooding in 2008). For the open pollination treatment, flowers were left to be visited by insects. For the supplemental hand pollination treatment, pollen from a different plant (out-cross and at least 2 m away from test flowers) was added to receptive stigmas by removing whole anthers and applying pollen directly to stigmas of test flowers. Flowers were covered with bridal veil to exclude pollinators for the bagged treatment. Once matured, fruits were collected and the number of seeds per fruit (seed set) was counted.

Effectiveness of bumblebee pollination (single visit experiments)

Pollen deposition and removal – In June and August 2008, we randomly selected 40 open flowers that were at the end of the male phase, each from a different plant. These flowers were emasculated (emasculation is known to have no effect on insect floral preference or on nectar levels in *Impatiens* flowers (Bell et al. 1984)), by removing completely dehisced androecia, to expose stigmas, thus ensuring we had virgin female flowers which were then exposed to bumblebee visitation immediately. During the same period, an additional 40 flowers in bud, each from a different plant, were randomly chosen and bagged with bridal veil (for approximately four to five days, until flowers had completely opened) to exclude insects. After anthesis and when androecia were clearly visible and flowers were in the male phase but no pollen had been removed, flowers were uncovered to allow bumblebee visitation. When each of the 80 flowers had been visited once by *B. pascuorum* individuals, all stigmas (N = 40) and androecia (N = 40) were collected and stored in separate Eppendorf tubes containing 1.5 ml ethanol. In addition, 15 undehisced androecia were collected and stored as above. Immediately after each flower had been visited, four morphological characteristics were measured per flower (Fig. 1a and b) using digital callipers (150 mm ± 0.3 mm accuracy, Moore & Wright, Sheffield, UK). In addition, bumblebee behaviour (nectar and/or pollen collection) and the body parts which contacted the floral reproductive parts (stigma or androecium) were recorded.

In the laboratory, stigmas were positioned on a slide, dyed (with 0.5% safranin in 50% alcohol) and gently squashed with a cover slip to facilitate pollen grain counting. The number of intra- and inter-specific pollen grains deposited on each stigma were counted under a light microscope (x20), using a reference pollen collection of known pollen types (C.M. Nienhuis) and other accessible resources (Hodges 1964; Moore et al. 1999; Sawyer & Pickard 1981). Next, we quantified the number of pollen grains in undehisced flowers and remaining in androecia following a single bee visit (after Traynor 1981). Androecia were cut open in the Eppendorf tubes and vigorously shaken for five minutes to ensure that pollen grains were suspended in the solution (1.5 ml). For each androecium, the number of pollen grains was counted in three 0.5 µl samples, drawn from the 1.5 ml solution, by means of a Fuchs-Rosenthal haemocytometer (0.0625 mm^2, 0.200 mm depth). We calculated the total number of pollen grains in the 1.5 ml solution by multiplying the mean number of pollen grains in the three 0.5 µl samples by 3000. The number of pollen grains removed from an androecium by a single bumblebee visit was estimated by subtracting the number of remaining pollen grains from the mean total number of pollen grains in virgin androecia.

Seed production – In August 2008, a total of 30 flowers were selected at random, emasculated (as above) and each flower was permitted to be visited once by a *B. pascuorum* individual. Each individual bee was only observed once to prevent pseudoreplication. After flowers had been visited, they were bagged with bridal veil to prevent further visitation. Once fruits had matured (approximately three weeks later) they were collected and the number of seeds per fruit (seed set) was counted.

Morphological characteristics of bumblebees - The individual bumblebees visiting the corresponding *I. glandulifera* flowers (see above) were caught and frozen in labelled vials. Subsequently, we measured eight morphological characteristics for each of the collected bumblebees (Fig. 2a and b). For all morphological characteristics we took two measurements and calculated the mean. In addition, morphological characteristics were measured (as above) for three additional bumblebee species (*B. hortorum*, *B. pratorum* and *B. terrestris*) (N = 40 for each species) that sometimes visit *I. glandulifera* in Ireland (Nienhuis et al. in press), in order to establish morphological variations among the potential pollinator species.

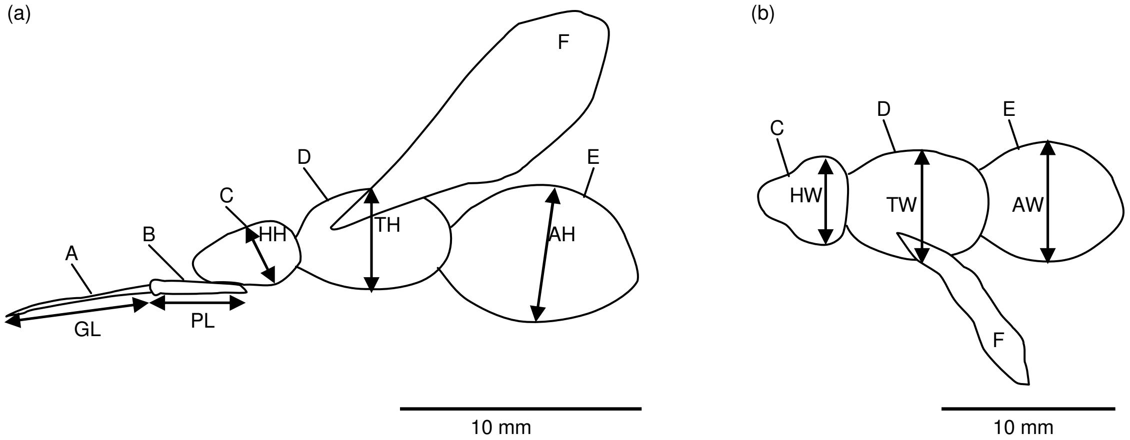

FIG. 2. Lateral (a) and dorsal (b) view of *B. pascuorum* individual: A. glossa, B. prementum, C. head, D. thorax, E. abdomen, F. wing. Morphological characteristics measured in *B. pascuorum* individuals: glossa length (GL), prementum length (PL), head height (HH), head width (HW), thorax height (TH), thorax width (TW), abdomen height (AH) and abdomen width (AW) (after Suzuki et al. 2002).

Statistical analysis

Breeding system of I. glandulifera – Seed set was compared among the three treatments (OP, SHP and PE) and between years (2007 and 2008) using a log-linear generalised linear model (Poisson distribution and log-link function) because our data consisted of small integer counts (N = 20, for each treatment), and lacked normality and constant variance (Crawley 1993). 'Treatments', 'years' and the interaction 'treatment x year', were used as fixed orthogonal factors within the model. Pearson chi-square statistics were employed to assess the goodness of fit of the model. *Post-hoc* comparisons between treatments were performed using Sequential Bonferroni tests (Crawley 1993).

Seed production – To analyse whether seed set varied between flowers subjected to the single visit experiment (N = 30) and flowers that had been openly pollinated by insects (N = 20), we compared the two using a non-parametric Fligner-Policello test (Hollander & Wolfe 1999).

Morphological match between B. pascuorum *and* I. glandulifera *flowers* – Due to heterogeneity of variances (determined using a Levene's test), we tested whether *B. pascuorum* proboscis length (PBL = PL (prementum length) + GL (glossa length)) differed from *I. glandulifera* spur length by means of a non-parametric Mood's Median test. The coefficient of variation (CV) for each morphological characteristic of all flowers visited and *B. pascuorum* individuals collected, was calculated to establish variability. To investigate which morphological characteristics of *I. glandulifera* flowers and *B. pascuorum* individuals influenced pollination effectiveness (pollen deposition and removal), we used multiple linear regression analysis (MLRA). The number of pollen grains deposited on stigmas and removed from androecia were selected as dependent variables and analysed in two separate models. Due to lack of normality the former was $\log_{10}$ transformed.

To reduce multi-collinearity among floral and bumblebee morphological characteristics ($r > 0.700$; Appendix 1) and consequently the number of independent variables in models, we used interaction terms of the height and width of floral entrance (FEH * FEW) and the height and width of bumblebee head (HH * HW), thorax (TH * TW) and abdomen (AH * AW). For each model, we used seven independent variables: lower sepal length (LSL), spur length (SL), proboscis length (PBL), and the four interaction terms (as above). All independent variables conformed to normality. For both models, we inspected the residuals, examined the variance inflation factor for each independent variable, and used Cook's distance (D_i) to determine outliers (Quinn & Keough 2002).

Morphological variations between bumblebee species – We investigated whether morphological characteristics varied between *B. pascuorum* and *B. hortorum*, *B. pratorum* and *B. terrestris* using one-factor ANOVAs and Dunnett's *post-hoc* tests. To test for heterogeneity of variances we employed Levene's test and where necessary data were $\log_{10}$ (x) transformed (HH, TH and AH). Non-parametric Mood's Median test was used when transformations were unable to reduce heterogeneity of variances (PBL).

All analyses were performed using MINITAB 13 (Minitab 2000) and SPSS 15 (SPSS 2006).

RESULTS

Breeding system of I. glandulifera

Bagged *I. glandulifera* fruits contained negligible numbers of seeds (mean ± S.E. = 0.05 ± 0.10 and 0.10 ± 0.10 for 2007 and 2008, respectively) and *post-hoc* tests confirmed that seed set of openly pollinated flowers (mean ± S.E. = 6.70 ± 0.47 and 8.15 ± 0.51 for 2007 and 2008, respectively) and supplementally hand pollinated flowers (mean ± S.E. = 6.85 ± 0.56 and 7.30 ± 0.49 for 2007 and 2008, respectively) was significantly larger than seed set of bagged (PE) flowers in both 2007 and 2008 ($X^2_2 = 71.31$, $p < 0.001$; Fig. 3). However, *I. glandulifera* did not produce more seeds when supplementally hand pollinated, suggesting no pollen limitation. In addition, this was consistent over the two years (Year: $X^2_1 = 0.74$, $p > 0.05$, Treatment x Year: $X^2_2 = 1.05$, $p > 0.05$; Fig. 3).

Effectiveness of bumblebee pollination

Pollen deposition and removal – Bumblebees visited *I. glandulifera* flowers primarily for nectar and no individuals were observed actively collecting pollen. However, bumblebees did deposit and remove pollen on entering and exiting flowers, as the androecium or stigma was always contacted (100%) with the dorsal parts of either the head, thorax or abdomen. After a single bumblebee visit, a mean (± S.E.) of 110.1 ± 19.3 pollen grains were deposited on *I. glandulifera* stigmas, although there was a large range in the number of grains deposited (range: 8 to 418 pollen grains). Heterospecific pollen was observed in minute quantities (1 to 18 heterospecific pollen grains) on only 4/40 stigmas collected. A mean (± S.E.) of 1.29 ± 0.09 million pollen grains (range: 0.19 to 2.29 million) remained in visited flowers from a mean (± S.E.) of 2.50 ± 0.1 million pollen grains (range: 0.18 to 2.99 million) in *I. glandulifera* flowers in bud, i.e. on average 48.4% of pollen grains were removed from *I. glandulifera* androecia after a single visit (mean number of pollen grains (± S.E.) removed = 1.21 ± 0.09 million, range: 0.20 to 2.30 million).

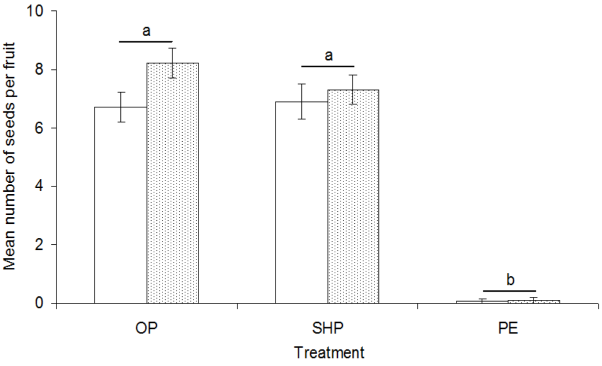

FIG. 3. Mean (± S.E.) number of seeds produced in *I. glandulifera* fruits (N = 20) after three treatments: open pollination (OP), supplemental hand pollination (SHP) and pollinator exclusion (PE) in 2007 (open bars) and 2008 (dotted bars).

Seed production after a single bumblebee visit – *I. glandulifera* fruits collected from the single visit experiment contained a mean (± S.E.) seed set of 7.1 ± 0.7, which did not differ significantly from the seed set of the open pollination treatment in 2008 (mean ± S.E. seed set = 8.15 ± 0.51, $\hat{U}_{20,30} = 1.191$, $p = 0.117$).

Morphological match between B. pascuorum *and* I. glandulifera *flowers* – *B. pascuorum* probosces (mean ± S.E. = 8.39 ± 0.05) were significantly longer than *I. glandulifera* spurs (mean ± S.E. = 7.90 ± 0.13) ($X^2_1 = 13.23$, $p < 0.001$; Fig. 4). Only two floral characteristics (FEW and LSL: mean ± S.E. = 10.34 ± 0.11 and 13.94 ± 0.36, respectively) were bigger than bumblebee body characteristics (Fig. 4). Overall, morphological characteristics showed low variability, with bumblebees being less variable than floral characteristics (Appendix 2). Regression models explained 3.6% and 25.1% of the variances in pollen deposition and removal, respectively. None of the seven morphological characteristics affected pollen deposition on stigmas ($F_{7,39} = 0.170$, $p = 0.989$, $r^2 = 0.036$; Tab. Ia). However, both lower sepal length (LSL) and thorax height * thorax width (TH * TW) influenced pollen removal negatively (Tab. Ib), indicating that with decreasing length of the lower sepal and decreasing height and width of the thorax more pollen was removed from androecia. None of the other characteristics had an effect on pollen removal ($F_{7,39} = 1.530$, $p = 0.192$, $r^2 = 0.251$; Tab. Ib).

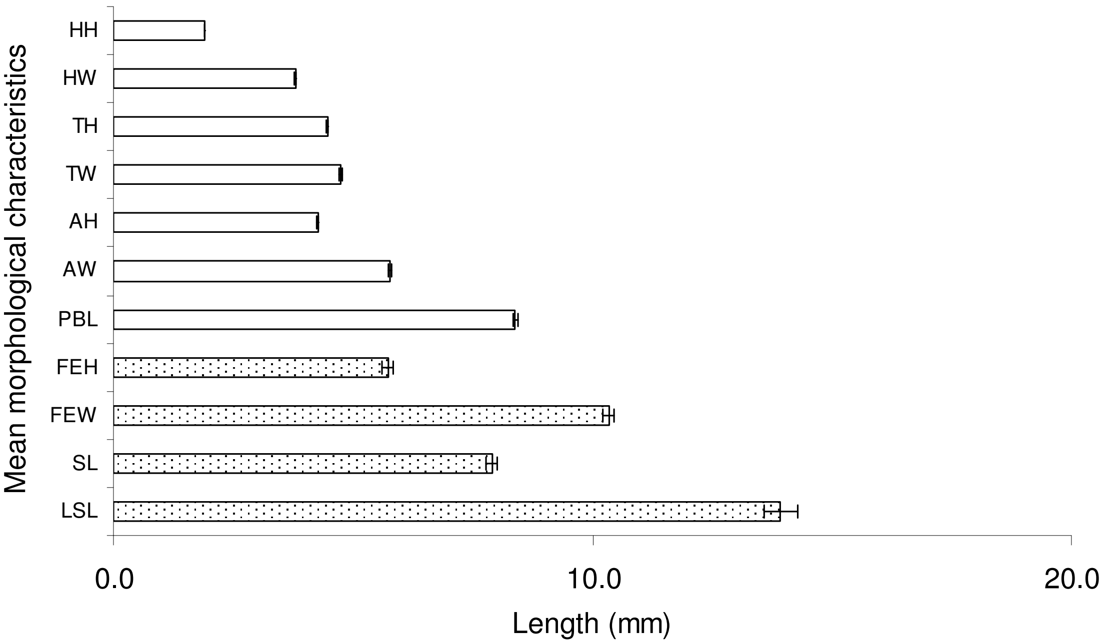

FIG. 4. Mean morphological characteristics of *B. pascuorum* individuals visiting female and male phase *I. glandulifera* flowers. *B. pascuorum* characteristics (open bars): head height (HH), head width (HW), thorax height (TH), thorax width (TW), abdomen height (AH), abdomen width (AW), proboscis length (PBL = prementum length + glossa length). Floral characteristics (dotted bars): floral entrance height (FEH), floral entrance width (FEW), lower sepal length (LSL) and spur length (SL). N = 80.

Morphological variations between bumblebee species – In general, morphological characteristics varied between *B. pascuorum* and *B. hortorum*, *B. pratorum* and *B. terrestris* (Appendix 3). In particular, *B. hortorum* and *B. terrestris* were bigger than *B. pascuorum* in most cases (except HH, TH and AW in *B. hortorum* and TBL in *B. terrestris,* which were not significantly different to *B. pascuorum*, and HW in *B. hortorum* and PBL in *B. terrestris*, which were smaller than *B. pascuorum*; Appendix 3). Conversely, *B. pratorum* was smaller than *B. pascuorum* in all variables measured (Appendix 3).

TABLE I. Multiple linear regression analysis (MLRA) relating pollen deposition on stigmas (a) and pollen removal from androecia (b) to floral and bumblebee morphological characteristics (for abbreviations refer to Fig. 4). b = regression coefficient.

(a) Dependent variable: pollen deposition (log10 transformed)

Independent variables	b ± S.E.	t	p
HH*HW	0.100 ± 0.325	0.31	0.759
TH*TW	0.025 ± 0.112	0.22	0.828
AH*AW	-0.072 ± 0.096	-0.75	0.458
PBL	0.290 ± 0.427	0.68	0.502
FEH*FEW	0.001 ± 0.008	0.16	0.875
SL	-0.019 ± 0.076	-0.25	0.805
LSL	-0.003 ± 0.037	-0.09	0.931

(b) Dependent variable: pollen removal

Independent variables	b ± S.E.	t	p
HH*HW	265 195 ± 369 307	0.72	0.478
TH*TW	-196 564 ± 87 752	-2.24	0.032
AH*AW	89 668 ± 91 617	0.98	0.335
PBL	126 353 ± 456 865	0.28	0.784
FEH*FEW	2 371 ± 9 006	0.26	0.794
SL	56 498 ± 104 182	0.54	0.591
LSL	-101 486 ± 40 946	-2.48	0.019

DISCUSSION

Native bumblebees are highly effective pollinators of invasive alien *I. glandulifera* in Ireland, as they deposit adequate amounts of pollen on stigmas to facilitate 100% seed set after a single visit. This is a result of bumblebee foraging behaviour, the morphological match between bumblebees and alien flowers and the pollination mechanism of *I. glandulifera*.

Impatiens glandulifera, although self-compatible (Valentine 1978), relies on native bumblebee pollination in Ireland, and is not capable of self-pollination nor self-fertilisation. Overall, *I. glandulifera* produced approximately seven seeds per fruit, which corresponds to previous studies (Könies & Glavač 1979; Titze 2000). Supplemental hand pollination of flowers did not improve seed set, suggesting that maximum pollination is achieved by bumblebee visitation and that *I. glandulifera* is not pollen limited in its invasive range. Studies on other invasive alien plant species with out-crossing breeding systems (e.g. *Cytisus scoparius* (Fabaceae), *Genista monspessulana* (Fabaceae), *Lonicera japonica* (Caprifoliaceae) and *L. arboreus*) suggest that populations can undergo pollen limitation due to low numbers or a complete lack of suitable pollinators (Larson et al. 2002; Parker 1997; Parker & Haubensak 2002; Stout et al. 2002; but see Stout 2007b). This difference may be explained by variations in floral rewards and morphological specificity. *C. scoparius*, *G. monspessulana* and *L. arboreus* are nectarless whilst *I. glandulifera* has abundant nectar production, and *L. japonica* has highly specialised flowers from which most generalist foragers cannot access nectar. As a highly rewarding plant, whose nectar is relatively easily accessed by generalist bumblebees, *I. glandulifera* does not suffer from pollen limitation. The same is true of other alien invasive plants that share these characteristics (e.g. *Rhododendron ponticum*; Stout 2007b).

Pollen deposition and removal

Bombus pascuorum has the potential to transfer large quantities of *I. glandulifera* pollen, as nectar-feeding individuals always contacted reproductive parts passively. Nevertheless, despite almost half of pollen grains being removed from *I. glandulifera* androecia (48.4%) after a single *B. pascuorum* visit, relatively low pollen quantities were found on stigmas. This suggests that *B. pascuorum* is a "high removal-low deposition" pollinator of the alien (cf. Young et al. 2007). Yet, several factors may be causing the observed discrepancy between pollen deposition and removal. Firstly, *I. glandulifera* androecia can accommodate larger pollen quantities (up to about 2 500 000 pollen grains) compared to the number that the relatively small stigmas can take up (up to 1 200 pollen grains (Titze 2000)). Secondly, pollen removal varied considerably and may have been overestimated by collecting androecia at different developmental stages that released varying amounts of pollen (this was not tested, but we attempted to collect androecia at similar stages), or by androecia releasing additional pollen due to wind movement and/or handling flowers. Thirdly, bumblebee foraging behaviour could also have caused variation in pollen removal. For example, bumblebees may have removed extra pollen by contacting androecia with their abdomen while foraging protractedly on nectar accumulated in the spur after bagging. It was unfeasible however, to extract the accumulated nectar before exposing flowers to bumblebee visitation, as androecia would have been disturbed. Fourthly, reduced pollen deposition may be further explained by individual bumblebees grooming pollen while flying between flowers (Thomson 1986), as the amount of pollen groomed off bodies can be proportional to the amount removed from anthers (Harder & Thomson 1989). Indeed, pollen sacs collected from bumblebees foraging on the alien contained small amounts of *I. glandulifera* pollen (Nienhuis et al. in prep.).

Seed production

We suggest that *I. glandulifera* has a highly effective pollination mechanism since maximum seed set was obtained after a single bumblebee visit. This confirms findings of studies conducted on *I. glandulifera* and other *Impatiens* species (*I. capensis* and *I. pallida*) elsewhere, where one bumblebee (e.g. *B. terrestris* and *B. vagans*) visit was sufficient to transfer the majority of pollen and fertilise all ovules (Bell et al. 1984; Titze 2000; Wilson 1995). This effectiveness is not surprising given that *I. glandulifera* requires only small pollen quantities to fertilise ovules and obtain maximum seed set (on average seven seeds per flower). In comparison, other alien plant species relying on insect pollination, such as *F. magellanica* and *R. ponticum*, produce far more seeds per flower (about 220 and 190 seeds per flower for *F. magellanica* and *R. ponticum*, respectively (unpublished data; Stout et al. 2006)), and they may therefore require more visits to attain full seed set. Hence, despite low pollen deposition, seed set in *I. glandulifera* seems to be secured by an over saturation in pollen production, high bumblebee effectiveness of pollen dispersal and removal, and an elevated pollen to ovule ratio (144 000 (Titze 2000)).

Morphological match between bumblebees and I. glandulifera *flowers*

Regression analysis revealed that little of the variance in both pollen deposition and removal is accounted for by bumblebee and flower morphological characteristics. However, firstly, more pollen was removed from male phase flowers with decreasing lower sepal length. This may be caused by androecia being in closer contact with the posterior abdomen of nectar-feeding bumblebees when sepal length is shorter, as androecia are suspended directly above the entrance to the sepal. Secondly, pollen removal increased with decreasing thorax height and width. This implies that the size of the thorax is important for bumblebees contacting androecia, and that smaller individuals are likely to remove more pollen. Hence smaller species, such as *B. pratorum*, may in fact remove more pollen. This may occur as smaller bees move around more in the entrance to the flower, thereby dislodging more pollen from the androecia. In addition, the fairly short spur of *I. glandulifera* enables *B. pascuorum* and other bumblebee species with shorter probosces (e.g. *B. pratorum* and *B. terrestris*) to access nectar in the spur easily.

Overall, bumblebee body parts were larger than *I. glandulifera* floral parts, and nectar-feeding *B. pascuorum* individuals were forced to contact reproductive parts of *I. glandulifera* flowers due to the relatively snug fit between the bee and flower (Wilson & Thomson 1996). Although other bumblebee species (*B. hortorum*, *B. pratorum* and *B. terrestris*) were not measured in direct relation to their ability to remove and deposit pollen, it is likely that, despite interspecific morphological variations, their similarity to *B. pascuorum* in their morphological proportions, enables them to effectively pollinate *I. glandulifera* flowers (Titze 2000). Bumblebees are relatively large bees, suited to the floral size and structure of *I. glandulifera* (Sowig 1989), and it is unsurprising that several species are capable of pollinating it in its invasive range since it is pollinated by multiple species of bumblebee in its native habitats (Saini & Ghattor 2007). The morphological match between bumblebees in general and *I. glandulifera* flowers may therefore explain why pollen deposition and removal are unlikely to be influenced by bumblebee or flower morphology. This morphological match does not necessarily occur in other alien plants, for example, the large flowers of *R. ponticum* are much bigger than most visitors' body size. Thus, some visiting insects can access nectar without contacting reproductive parts, resulting in only one third of insect visitors contacting stigmas (Stout 2007a).

Pollination effectiveness is further influenced by insect visitation frequency to flowers (Suzuki et al. 2002). Due to the high visitation rates by *B. pascuorum* (Nienhuis et al. in press) this species can be considered vital in contributing towards the pollination success of *I. glandulifera*. Ideally, we would have investigated the effectiveness of other less frequent bumblebee visitors (e.g. *B. hortorum* and *B. terrestris*), but their visitation was so rare when we conducted this study that it was not possible. Furthermore, it remains unclear whether smaller visitors that are known to visit and carry *I. glandulifera* pollen (e.g. *A. mellifera* and *Vespula vulgaris*) (Lopezaraiza-Mikel 2006) are effective pollinators. Due to their low visitation frequency in Ireland (Nienhuis et al. in press) however, they are unlikely to be of great importance.

CONCLUSIONS

Our study is the first to examine the mechanisms influencing the effectiveness of bumblebee pollination of the alien *I. glandulifera* in Ireland. *B. pascuorum* can be regarded as a highly effective pollinator of *I. glandulifera* due to transporting and removing large quantities of pollen, inducing maximum seed set after a single visit, and its high visitation frequency. Other bumblebee species (e.g. *B. hortorum*) are also likely to be effective pollinators of the alien because of the morphological match between bumblebees and *I. glandulifera* flowers. The effective pollination mechanism of *I. glandulifera* and a lack in pollinator limitation in its invaded range, which in combination with a high seed production (Beerling & Perrins 1993), extensive period of seed release (Willis & Hulme 2004), and a broad tolerance towards varying climates, soil types and light conditions (Chittka & Schürkens 2001), are likely to be contributing towards the spread and successful invasion of the alien in riparian ecosystems across Europe.

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Appendix

APPENDIX 1. Correlations among morphological characteristics of *I. glandulifera* flowers in the female (rf) and male (rm) phase and of *B. pascuorum* individuals visiting female (rf) and male (rm) flowers (for abbreviations refer to Fig. 4). rf and rm = correlation coefficients.

	HH	HW	TH	TW	AH	AW	PBL	FEH	FEW	SL
HW	$r_f = 0.738$ $r_m = 0.827$									
TH	$r_f = 0.692$ $r_m = 0.813$	$r_f = 0.767$ $r_m = 0.817$								
TW	$r_f = 0.728$ $r_m = 0.778$	$r_f = 0.856$ $r_m = 0.852$	$r_f = 0.716$ $r_m = 0.813$							
AH	$r_f = 0.696$ $r_m = 0.787$	$r_f = 0.802$ $r_m = 0.843$	$r_f = 0.927$ $r_m = 0.865$	$r_f = 0.766$ $r_m = 0.796$						
AW	$r_f = 0.724$ $r_m = 0.767$	$r_f = 0.804$ $r_m = 0.882$	$r_f = 0.776$ $r_m = 0.831$	$r_f = 0.749$ $r_m = 0.797$	$r_f = 0.795$ $r_m = 0.822$					
PBL	$r_f = 0.753$ $r_m = 0.804$	$r_f = 0.725$ $r_m = 0.795$	$r_f = 0.630$ $r_m = 0.811$	$r_f = 0.629$ $r_m = 0.758$	$r_f = 0.654$ $r_m = 0.778$	$r_f = 0.822$ $r_m = 0.886$				
FEH	$r_f = -0.178$ $r_m = 0.050$	$r_f = 0.022$ $r_m = 0.019$	$r_f = -0.123$ $r_m = 0.025$	$r_f = -0.024$ $r_m = -0.019$	$r_f = -0.062$ $r_m = -0.078$	$r_f = -0.036$ $r_m = 0.014$	$r_f = -0.083$ $r_m = -0.054$			
FEW	$r_f = -0.342$ $r_m = -0.298$	$r_f = -0.320$ $r_m = -0.423$	$r_f = -0.285$ $r_m = -0.370$	$r_f = -0.339$ $r_m = -0.522$	$r_f = -0.229$ $r_m = -0.431$	$r_f = -0.271$ $r_m = -0.335$	$r_f = -0.250$ $r_m = -0.320$	$r_f = 0.190$ $r_m = 0.178$		
SL	$r_f = -0.109$ $r_m = 0.291$	$r_f = -0.224$ $r_m = 0.248$	$r_f = -0.134$ $r_m = 0.306$	$r_f = -0.205$ $r_m = 0.261$	$r_f = -0.122$ $r_m = 0.190$	$r_f = -0.233$ $r_m = 0.213$	$r_f = -0.108$ $r_m = 0.137$	$r_f = 0.008$ $r_m = 0.306$	$r_f = 0.483$ $r_m = -0.088$	
LSL	$r_f = -0.336$ $r_m = 0.078$	$r_f = -0.219$ $r_m = 0.073$	$r_f = -0.357$ $r_m = 0.102$	$r_f = -0.265$ $r_m = -0.049$	$r_f = -0.413$ $r_m = 0.102$	$r_f = -0.295$ $r_m = 0.026$	$r_f = -0.186$ $r_m = 0.094$	$r_f = 0.061$ $r_m = -0.075$	$r_f = 0.113$ $r_m = 0.209$	$r_f = 0.031$ $r_m = -0.027$

APPENDIX 2. Coefficient of variation (CV = standard deviation / mean) for each morphological characteristic of *I. glandulifera* flowers in the female and male phase and of *B. pascuorum* individuals visiting female and male flowers. Proboscis length (PBL = PL (prementum length) + GL (glossa length)).

Morphological characteristics	Female flowers	Male flowers
Bumblebee	CV (%)	CV (%)
Head height (HH)	6.10	5.00
Head width (HW)	4.20	4.40
Thorax height (TH)	5.70	6.00
Thorax width (TW)	6.70	7.80
Abdomen height (AH)	6.10	6.30
Abdomen width (AW)	6.00	5.70
Proboscis length (PBL)	4.90	4.90
Flower		
Floral entrance height (FEH)	14.50	15.50
Floral entrance width (FEW)	8.80	9.60
Spur length (SL)	15.80	12.50
Lower sepal length (LSL)	22.80	13.80

APPENDIX 3 Morphological characteristics (for abbreviations refer to Fig. 4) compared among *B. pascuorum* and three bumblebee species (*B. hortorum, B. pratorum* and *B. terrestris*) using parametric tests (ANOVA F and Dunnett's *post-hoc* test) and non-parametric tests (Mood's Median X^2). ns = non-significant results, $*p < 0.05$, $**p < 0.001$. Arrows indicate when measurements are bigger (↑) or smaller (↓) than *B. pascuorum.*

	HH	HW	TH	TW	AH	AW	PBL
Post-hoc comparisons	$F_{3,199} = 26.21$ **	$F_{3,199} = 43.98$ **	$F_{3,199} = 35.08$ **	$F_{3,199} = 24.13$ **	$F_{3,199} = 23.78$ **	$F_{3,199} = 28.16$ **	$X^2_3 = 140$ **
B. hortorum	ns	* ↓	ns	* ↑	* ↑	ns	- ↑
B. pratorum	* ↓	* ↓	* ↓	* ↓	* ↓	* ↓	- ↓
B. terrestris	* ↑	* ↑	* ↑	* ↑	* ↑	* ↑	- ↓