

LETTER

The impact of an alien plant on a native plant–pollinator network: an experimental approach

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Abstract

Studies of pairwise interactions have shown that an alien plant can affect the pollination of a native plant, this effect being mediated by shared pollinators. Here we use a manipulative field experiment, to investigate the impact of the alien plant *Impatiens glandulifera* on an entire community of coflowering native plants. Visitation and pollen transport networks were constructed to compare replicated *I. glandulifera* invaded and *I. glandulifera* removal plots. Invaded plots had significantly higher visitor species richness, visitor abundance and flower visitation. However, the pollen transport networks were dominated by alien pollen grains in the invaded plots and consequently higher visitation may not translate in facilitation for pollination. The more generalized insects were more likely to visit the alien plant, and Hymenoptera and Hemiptera were more likely to visit the alien than Coleoptera. Our data indicate that generalized native pollinators can provide a pathway of integration for alien plants into native visitation systems.

Keywords

Competition, ecological networks, facilitation, food webs, generalization, *Impatiens glandulifera*, invasive species, pollen transport webs, pollination, visitation webs.

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INTRODUCTION

The scope for alien plants to influence essential ecosystem services such as pollination has only recently been revealed. Chittka & Schurkens (2001), Brown *et al.* (2002), Ghazoul (2002) and Larson *et al.* (2006) all reported that alien plants compete with natives for flower visitors; furthermore, Moragues & Traveset (2005) reported a facilitative effect on natives. However, these studies consider the effect of one alien plant species on one or a few native plant species and little is known of the alien's impact at the community level. Most plants have several pollinators and most pollinators visit several plants (Waser *et al.* 1996), which results in generalized communities. Given that plant–pollinator communities are widely considered to be generalized (Waser *et al.* 1996; Blüthgen *et al.* 2006), there is a pressing need for more data at the community level. In generalized systems the impact of an alien species can potentially ripple through the whole plant–pollinator network, as most species will be directly or indirectly connected to the alien species.

Chittka & Schurkens (2001), looking at a single pairwise interaction between a native and a magnet alien species,

clearly demonstrated that an alien plant can appropriate the pollinators of a native plant, in their case leading to decreased visitation and thereby decreased seed set of the native species. Community level studies such as those of Olesen *et al.* (2002), Morales & Aizen (2006) and Memmott & Waser (2002) have begun to address the impact of alien plants on plant–pollinator networks. Although arguably compromising the precision of pairwise plant studies (Morales & Aizen 2006), their research has provided novel insights into the mechanisms by which alien species can integrate into a pollination system. In none of these three community level studies though, did the alien plants serve as a magnet for native visitors.

In order for native plants to compete with alien plants for native pollinators, native pollinators must change their foraging behaviour to include alien plants in their diets (Ghazoul 2002) and pollination must be a limiting service in the environment for plant reproduction. Both the quantity (e.g. visits to flowers) and quality (e.g. pollen deposition on stigmas) of pollinator visits to native plants can be affected through a switch in foraging preferences. These ultimately affecting fertilization through reduced conspecific pollen loads on stigmas (Larson *et al.* 2006), or through the

deposition of heterospecific pollen on stigmas (Brown & Mitchell 2001; Moragues & Traveset 2005). To better predict the impact of alien plant species, it is important to identify which native pollinators will visit alien plant species. This is because native pollinators can provide them with a direct route for integration into native pollination networks. Although evidence is limited, generalized pollinator species are believed to be more likely to include new plant species in their diet (Richardson *et al.* 2000; Memmott & Waser 2002).

We used a manipulative field experiment to quantify the impact of an alien plant, *Impatiens glandulifera* Royle, Balsaminaceae, the same species studied by Chittka & Schurkens (2001), on a native plant–pollinator community. Our objectives were threefold: (1) To use replicated visitation networks to determine how the presence of an alien plant species affects insect visitation to native plant species. We predict that native species will receive fewer visits in the presence of the alien species. (2) To quantify how the alien plant disrupts insect-mediated pollen transport. Given the copious amount of pollen produced by our chosen alien species, we predict that alien pollen will dominate the pollen transport network. (3) To determine whether the level of generalization of flower visitors influences whether they visit the alien plant species. We predict that the most generalized pollinator species are most likely to visit the alien plant and are thereby crucial to the integration of alien plants into a native network.

METHODS

The underlying rationale of our approach is to remove the floral resources of an alien plant species from replicated field plots, leaving the rest of the plant *in situ*, whilst maintaining unmanipulated plots as controls. The structure of the plant–pollinator communities at each site will be quantified by constructing quantitative visitation networks and pollen transport networks which will be compared between the flower removal and the control plots. Visitation networks provide the pollinators' perspective of the interaction by showing how insects respond to the diversity, quantity and regularity in nectar and pollen supplies (Memmott 1999). Constructing pollen transport networks is a recently developed approach (Forup & Memmott 2005; Gibson *et al.* 2006) that shows which insect species carry which pollen species. These 'pollen vectors' are considered the likely pollinators.

Pollinator-mediated interactions among plant species can potentially result in reduced reproductive success (competition for pollination, Waser 1983) or increased reproductive success (facilitation for pollination, Rathcke 1983) of one or more participants, via the interaction between the quantity (frequency of pollinator visits) and quality (amount of compatible pollen deposited on stigmas) components of

pollinator visits (Rathcke 1983; Kunin & Iwasa 1996). Here we refer to competition and facilitation for visits, relate visitation patterns to pollen transport patterns, and discuss the possible final outcome of pollination.

The alien plant species

I. glandulifera (Himalayan or Indian balsam), one of the top 20 invasive species in the UK (Crawley 1987), was chosen as the subject for the experiment. First introduced into Kew Gardens in 1839 (Beerling & Perrins 1993) it rapidly spread throughout the UK and invaded most European countries (Grime *et al.* 1988). *I. glandulifera* flowers have a high rate of sugar production, high nectar sugar concentration (Chittka & Schurkens 2001) and produce large amounts of pollen (Titze 2000). Working in central Germany, Chittka & Schurkens (2001) reported that the native species *Stachys palustris*, Lamiaceae (marsh woundwort) suffered reduced visitation rates and seed set when growing with *I. glandulifera*, compared to when growing with conspecifics.

Field sites and experimental design

Field sites were in natural or semi-natural vegetation in the city of Bristol, Great Britain. Two *I. glandulifera* patches were selected at each of four sites, each pair containing plots matched for a high density of this alien species, habitat type and common native plant species. *I. glandulifera* was the dominant plant in all eight plots. Plot size varied slightly due to variation in the extent of *I. glandulifera* and the nature of the landscape, but was always consistent within a pair (range = 360–450 m²).

As pollinators feed on floral resources, only the flowers of *I. glandulifera* were removed from the experimental plots (vs. the entire plant); in this way the effects of direct competition for other resources (e.g. water, light and nutrients) between *I. glandulifera* and other plant species were kept constant between control and experimental plots. The experimental plot in each pair was subjected to an *I. glandulifera* flower removal treatment, which was applied every 10–14 days throughout the experiment, this being done by hand using scissors. The control plots, henceforth referred as invaded plots, were left untreated. Permanent transects were placed every 5 m across each plot, the length of each transect was variable, depending on the shape and the accessibility of each plot; however, within each pair the sampling effort was consistent (range 65–90 m).

Visitation data

Each site was sampled every 7–16 days from 2 July 2003 to 23 September 2003; this sampling period encompassing

most of *I. glandulifera*'s flowering season (July–October, Rose 1981). The weather determined the timing of consecutive samples, as plots could only be sampled under warm, dry conditions. A total of eight samples were collected from each plot and by the last sample insect abundance had dropped to just a few individuals per plot. A site was sampled in a single day, each plot during either the morning or afternoon, alternating the order of sampling on each occasion. Before sampling plant–pollinator interactions, the identity of all plant species 1 m either side of each transect was recorded and floral abundance was measured in floral units. A floral unit was defined as a single flower head, or part of a multiple head, from which a medium-sized bee has to fly, rather than walk to reach to another floral unit of the same species (Dicks *et al.* 2002).

To collect the visitation data, we slowly walked each transect once, collecting insects observed visiting flowers up to 1 m either side of the transect line and 1 m ahead. Thus an interaction was recorded if a floral unit was visited while walking the transects. While the amount of time spent in each plot was allowed to vary depending on insect and floral abundance (and the time processing the insects), each flower was sampled by the same amount. This approach standardized the sampling effort per flower over a standardized unit area. All flower visiting insects were collected, i.e. no *a priori* decisions were made concerning a visitor's likely pollinator status. Insects were caught using a net or they were captured directly into a killing vial. Killing vials were lined with paper bags to prevent insects from contaminating the tubes with pollen and insects were kept in their individual paper bags until processed in the laboratory. Plants and insects were identified to species or morphospecies by taxonomists. Finally quantitative visitation webs were constructed, these showing the frequency with which each insect species visits each plant species (Memmott 1999).

Pollen transport data

Before mounting the collected insects, a small cube (*c.* 3 mm × 3 mm × 2 mm) of glycerine jelly containing basic fuchsin stain (Kearns & Inouye 1993) was systematically dabbed around the insect's body to sample its pollen. Corbicula of bees were avoided as this pollen is unlikely to be available for pollination. After sampling the pollen, the jelly was placed on a microscope slide, melted and covered with a cover slip for analysis. Due to time constraints, a stratified random sub-sample of the pollen slides (one-fifth of each insect order for each plot) was analysed. Using a light microscope the pollen grains from each insect were identified and counted using a pollen reference collection made from plants found in the plots and adjacent vegetation. In a few cases it was impossible to differentiate pollen grains of closely related species. If this occurred, the

pollen species were grouped together, thus *Epilobium* spp., *Cirsium* spp. and Lamiaceae were considered as pollen groups. However, *I. glandulifera* pollen is distinctive and was always identifiable to species. Pollen data were used to construct quantitative pollen transport webs showing the identity and quantity of each pollen species transported by each insect species (Forup & Memmott 2005; Gibson *et al.* 2006).

Data analyses

Except when otherwise stated, all statistical analyses were done using SPSS 12.0, and measures of dispersion are presented as standard errors throughout.

Visitation data

To look at differences in insect diversity between invaded and flower removal plots, we checked whether the total number of native floral units and the number of native plant species differ among the treatments using paired *t*-tests. Then, we compared the observed species richness of flower visitors, the total visitor abundance at plots (i.e. number of individuals), the observed species richness of flower visitors to native plant species, and the visitor abundance on native plant species (i.e. total number of individuals collected visiting all native plants) between invaded and flower removal plots using univariate general linear models. Two factors were used, treatment (invaded and flower removal) as fixed factor and site (1–4) as random factor. The interaction treatment × site was not included in the analyses because the experimental design did not replicate for treatment–site combinations. Interaction plots suggested that the interaction between treatment and site is minimal (treatment effects were consistent across sites, see Fig. S1 in Supplementary Material).

To determine if any effect of *I. glandulifera* on visitation to native plants was consistent throughout the flowering season, visitor abundance on native plant species was also compared with a repeated measures multivariate analysis of variance. Plots were used as subjects and treatment (invaded and flower removal) as between subject factor, with visitation to native plants being considered monthly using samples in July (three samples), August (three samples) and September (two samples); thus month was used as the within subject factor and the interaction treatment × month was included in this model. Although sampling intensity (and thus the number of visitors) was lower in the last month, in this analysis we are interested only in the interaction effect treatment × month. The natural logarithm of visitor abundance was used.

We also used rarefaction curves to compare the rarefied species richness between invaded and flower removal plots (Gotelli & Colwell 2001), looking at all flower visitors and at

visitors to native plants only. This approach looked at species richness for a standardized sample of individuals, and allowed us to determine whether differences in observed numbers of visitor species among treatments are explained by differences in insect abundance. Considering the eight samples from each plot we used the EstimateS software (Colwell 2005) to compute, for both responses, sample-based rarefaction curves (the analytical version) and their 95% confidence interval curves for each plot. Curves were rescaled by number of individuals to allow for species richness comparison (Gotelli & Colwell 2001).

Pollen transport data

Two tests were run, the first asking if the quantity of alien pollen was different between the invaded and flower removal plots and the second asking whether the quantity of native pollen on the insects was affected by the presence of the alien plant. The amount of pollen (alien and native separately) carried by insects in the flower removal and invaded plots was compared using univariate general linear models. Only insects belonging to the orders common to all plots were considered (Coleoptera, Diptera, Hemiptera and Hymenoptera), comprising 99% of all the visiting insects. To normalize data, and avoid pseudoreplication, the pollen per individual was averaged for each order at each plot, and the natural logarithm of alien pollen and logarithm of native pollen were used. Treatment and order were used as fixed factors and site as random factor. Interactions among factors were included when variances were homogeneous across cells.

Most visitors to *I. glandulifera* belonged to Hymenoptera and Diptera and we also tested whether the average alien and native pollen carried per visitor by these orders was affected by the treatment when just looking at the individuals visiting native flowers. This was done to determine the impact of the treatment from the perspective of just the native plants and paired *t*-tests were used (natural logarithm of alien pollen and logarithm of native pollen). Finally, we compared the total number of native pollen grains in the control and experimental plots with a paired *t*-test.

Insect generalization and visitation to alien flowers

Here, we define generalization as the number of interacting partners of a species, without considering the frequency of the interactions. A forward stepwise (likelihood ratio) logistic regression, which models the probability of an event with two outcomes (here whether a species visits or does not visit the alien *I. glandulifera*), was used to investigate whether three predicting variables, insect generalization (the number of plant species visited by an insect species), insect abundance (total number of individuals of each species) and insect order affect the likelihood of an insect species visiting

an alien species. This statistical analysis is suitable because our response variable is binary (a species found either visiting the alien or not visiting it). Data from the four invaded plots were pooled in order to maximize the number of insect species in the analysis and only species represented by three or more individuals (57 species, 39% of the species from invaded plots) were included.

The analysis was repeated for data from pollen transport webs, to determine whether insects that carry more species of pollen are more likely to carry alien pollen. Here the predicting variables were insect order, generalization (number of pollen types carried by insect species) and number of pollen slides analysed for each species, the last included because it could increase the chances of finding *I. glandulifera* pollen carried by a species. Note that this approach told us which species are capable of probing the alien and transporting alien pollen, but did not give information on the frequency of these interactions. Given that *I. glandulifera*'s alien status was potentially confounded with its dominance status, we compare the visitation data results with those of the same analysis for the most generalized native species at flower removal plots (*Heracleum sphondylium* L., Apiaceae commonly known as hogweed). Using the same criteria as above, 31 species, 37% of species from flower removal plots, were included in the analysis.

RESULTS

Visitation data

A total of 1741 flower visitors were collected in the eight plots. These comprised 173 species of insects which visited 27 of the 38 recorded flowering plant species in the plots. Seven orders of insects were found visiting flowers: Diptera (100 species, 541 individuals), Hymenoptera (31 species, 665 individuals), Coleoptera (25 species, 359 individuals), Hemiptera (10 morphotypes, 165 individuals), Lepidoptera (4 species, 6 individuals), Dermaptera (2 species, 4 individuals) and Mecoptera (1 species, 1 individual).

In each invaded plot *I. glandulifera* was embedded in a complex network of plant pollinator interactions. Space limitations preclude us from presenting all 16 networks from the study (eight visitation webs and eight pollen transport webs), so we present visitation networks (Fig. 1a) and pollen transport networks (Fig. 1b) from one representative site (networks for all sites are shown in Figs S2 and S3). While neither the number of native floral units nor the number of native plant species differed between control and flower removal plots (paired *t*-tests: $t = 0.180$, d.f. = 3, $P = 0.869$, 2308 ± 452 floral units per plot; $t = 0.649$, d.f. = 3, $P = 0.563$, 13.3 ± 1.9 native plant species per plot), the alien plant species provided a high proportion of the overall floral resources in the invaded plots (Fig. 1a).

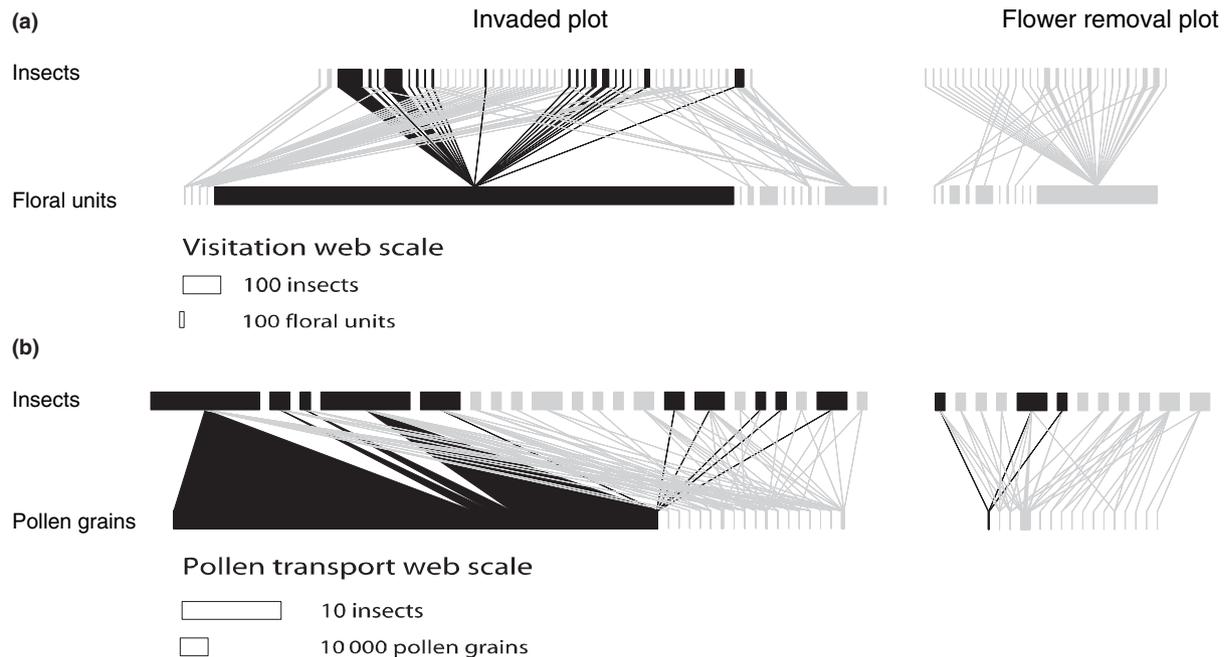


Figure 1 Visitation (a) and pollen transport (b) webs from invaded and flower removal plots for Site 1. In each web the rectangles represent insect species (top of each web) and plant species (bottom of each web), and the connecting triangles and lines represent links among species. In the visitation webs, the abundance of all species and the frequency of visitation are reflected by the width of the rectangles and basal width of the connecting triangles respectively. *I. glandulifera* and its links with visitors are shown in black and the two visitation webs are drawn to the same scale and thus are comparable. In the pollen transport webs pollen abundance is reflected by the rectangle width, and the pollen load carried by each insect species is reflected by the width of the connecting triangles. *I. glandulifera* and its links with visitors are shown in black. Note the pollen transport webs are based on a sub-sample of 20% of flower visiting insects whilst the visitation webs show all insects. The pollen transport webs are drawn to the same scale and are comparable.

Overall species richness of flower visitors and insect abundance were both significantly higher at invaded plots ($F_{1,3} = 69.610$, $P = 0.004$ and $F_{1,3} = 114.245$, $P = 0.002$, respectively, Fig. 2a,b). Species richness of flower visitors on native plants was negatively affected by *I. glandulifera* removal ($F_{1,3} = 44.611$, $P = 0.007$, Fig. 2c). A significantly higher number of insects were collected visiting native flowers at plots where *I. glandulifera* was present than at plots where *I. glandulifera* flowers were removed ($F_{1,3} = 16.363$, $P = 0.027$, Fig. 2d). Site had a significant effect on all four variables.

Given that differences in flower abundance are confounded with our treatment, we reanalysed these data adding the total flower abundance in the plots as a covariate. This does not change the results of our treatment effect on the variables; moreover, the effect of flower abundance is only significant for species richness and marginally significant for species richness on native plants. For the visitor abundance on natives, the assumption of equal slopes for all treatment groups is violated, as indicated by a significant interaction effect treatment \times covariate, rendering this test inappropriate for use with these data. Nevertheless, flower abundance is not significantly

related to the number of visitors to native plants ($R^2 = 0.337$, $F_{1,6} = 3.049$, $P = 0.131$), which suggests that the higher visitation is not, or is not exclusively, explained by flower abundance. With respect to the repeated measures analysis looking at the experiment during monthly samples, the interaction treatment \times month was not significant (Pillai's Trace = 0.282, $F_{2,5} = 0.984$, $P = 0.436$), thus the trend of higher visits at invaded plots was consistent throughout the 3-month flowering period.

To determine whether the increased visitation to native species can be attributed to a particular insect order, the difference in visitation to native flowers between invaded and flower removal plots at each site was compared with a general linear model, using order (Coleoptera, Diptera, Hemiptera and Hymenoptera) as the single factor. No differences among orders were found ($F_{3,12} = 2.327$, $P = 0.126$), suggesting that insects from all four orders respond to the presence of *I. glandulifera* in a similar manner.

Sample-based rarefaction curves reveal that when standardizing the number of individuals, species richness is not significantly different between invaded and flower removal plots, as confidence interval curves for each pair overlap (see Fig. S4). This indicates that it is through increased

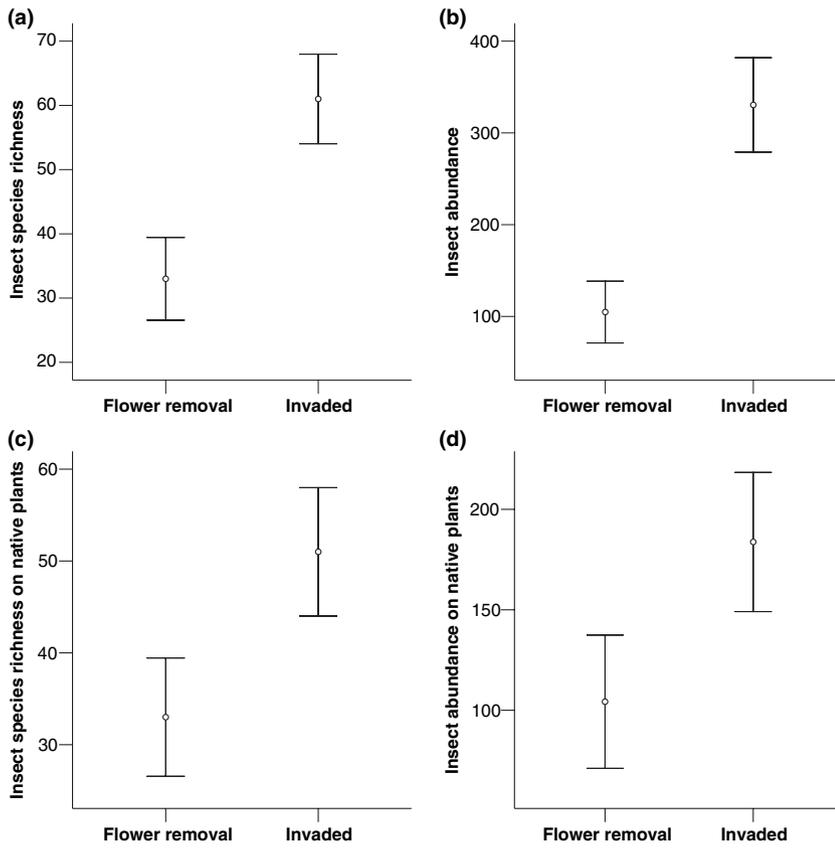


Figure 2 Flower visitor diversity and abundance at *I. glandulifera* invaded and *I. glandulifera* flower removal plots. (a) Overall species richness of flower visitors, (b) overall visitor abundance, (c) species richness of flower visitors to native plants, (d) visitor abundance on native plants. The error bars represent standard errors.

visitor abundance at invaded plots, that visitor diversity and diversity of visitors to native plants is higher at these plots.

Pollen transport data

Overall, 774 951 pollen grains were counted; 25 pollen groups and species from plants inside and around the plots were identified, plus a further 28 unknown pollen types. The quantitative pollen transport webs were completely dominated by *I. glandulifera* pollen (Fig. 1b). Overall, $95.5 \pm 2.5\%$ of pollen at invaded plots belonged to *I. glandulifera*, while only $36.6 \pm 17.4\%$ of pollen at flower removal plots was *I. glandulifera*'s. Thus, although the removal treatment reduced the amount of *I. glandulifera* pollen available, the visiting insects still foraged on *I. glandulifera* outside the plots. As predicted though, alien pollen swamped the networks, and insects carried significantly more *I. glandulifera* pollen at invaded plots (Table 1). However *I. glandulifera* pollen was not distributed evenly among the insect orders (Table 1, Fig. 3a). Hymenoptera carried significantly more alien pollen than any other order, and Diptera carried more alien pollen than Coleoptera. In fact, at invaded plots 10% of insects, mainly *Apis mellifera* Linnaeus and *Bombus* spp., carried 53% of the total alien pollen, and 98% of alien pollen was carried by Hymenoptera, this order comprising

Table 1 Results for the general linear model analyses for native and alien pollen carried per insect. Degrees of freedom (d.f.), *F*-value (*F*) and significance (Sig.) are shown for each variable.

Source	d.f.	<i>F</i>	Sig.
<i>I. glandulifera</i> pollen			
Treatment	1, 3	13.150	0.036*
Order	3, 18	39.550	<0.001*
Site	3, 3	0.828	0.560
Treatment × order	3, 18	0.839	0.490
Treatment × site	3, 18	0.980	0.424
Native pollen			
Treatment	1, 3	2.353	0.223
Order	3, 18	5.130	0.010*
Site	3, 3	2.780	0.212
Treatment × order	3, 18	3.567	0.035*
Treatment × site	3, 18	1.309	0.302

*Significant effects.

only 54% of insect individuals at the invaded plots. Nevertheless, all insect orders carried significantly more *I. glandulifera* pollen at invaded plots as indicated by the non-significant effect of the interaction treatment × order (Table 1, Fig. 3a).

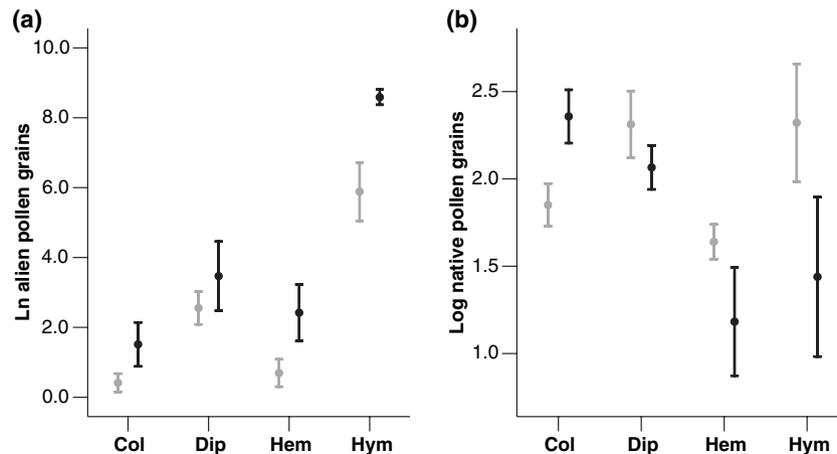


Figure 3 Pollen carried by the different insect orders at *I. glandulifera* invaded and *I. glandulifera* flower removal plots. (a) Mean alien pollen grains carried per individual and (b) mean native pollen grains carried per individual, for the four most abundant insect orders at the plots. Col, Coleoptera; Dip, Diptera; Hem, Hemiptera; Hym, Hymenoptera. Dark error bars represent the invaded plots, grey error bars represent the flower removal plots. The error bars represent standard errors.

Most insects carried native pollen, with $89.4 \pm 4.5\%$ of insects carrying native pollen at the invaded plots and $97.5 \pm 1.5\%$ carrying native pollen at the flower removal plots. There was no significant difference in the amount of native pollen carried by insects in the invaded and flower removal plots (Table 1). Nevertheless, the interaction treatment \times order was significant (Table 1) as the Coleoptera carried on average more native pollen at invaded plots, while the other orders showed the opposite trend (Fig. 3b). Contrasts for the different orders revealed that Coleoptera carries significantly more native pollen at invaded plots. When accounting for site variation, Hymenoptera carries significantly more native pollen at flower removal plots. In addition, for invaded plots we used contrasts to compare native pollen carried by Hymenoptera (the main alien pollen carriers) and the other orders, and found that Coleoptera carries more native pollen than Hymenoptera. Order as main effect was also a significant variable (Table 1); Coleoptera and Diptera were found to carry significantly more native pollen than the Hemiptera.

Interestingly, the amount of alien pollen carried by Diptera and Hymenoptera individuals visiting native flowers is not significantly different between invaded and flower removal plots (paired *t*-tests: $t = 0.787$, d.f. = 3, $P = 0.489$, and $t = 1.640$, d.f. = 3, $P = 0.200$ respectively). The amount of native pollen carried by these visitors does not differ significantly either (paired *t*-tests: $t = 0.523$, d.f. = 3, $P = 0.637$, and $t = 2.311$, d.f. = 3, $P = 0.104$ respectively). Despite the higher insect abundance at invaded plots, we found no significant difference in the total amount of native pollen grains in the system between invaded and flower removal plots (paired *t*-test: $t = 1.508$, d.f. = 3, $P = 0.229$), probably because there was considerable variation in the

amount of native pollen carried per insect, and because for three orders, the average number of native pollen grains was slightly higher at flower removal plots (Fig. 3b).

Generalization

Given that the solutions for both visitation and pollen transport analyses are qualitatively very similar, with the same significant variables, we report only the results for the visitation data here. At the invaded plots, 37% of the insect species, belonging to six orders, visited *I. glandulifera*. The main visitors to this species were Hymenoptera (81.6% visitors at invaded plots) and Diptera (15.5% of visitors). Notably, 95.7% of *Apis mellifera* and 88.1% of *Bombus pascuorum* (Scopoli) individuals were collected visiting *I. glandulifera*. These were the most abundant insect species at the invaded plots. In the logistic regression analysis, generalization was the first variable to be included in the model and thus an important parameter explaining the inclusion of the alien in a species diet (test of model coefficients for first step, $\chi^2 = 7.536$, d.f. = 1, $P = 0.006$); insect order was incorporated in the second step as a significant parameter (test of model coefficients for second step, $\chi^2 = 10.094$, d.f. = 3, $P = 0.018$). Abundance was not incorporated in the model by the stepwise regression method, nor was it significant if considered as single variable.

The model with generalization and insect order as meaningful predictor variables for visitation to *I. glandulifera*, adequately fits the data (Hosmer and Lemeshow goodness of fit test: $\chi^2 = 6.112$, d.f. = 7, $P = 0.527$), and the variation it explains is reasonable (Nagelkerke $r^2 = 0.364$). The odds ratio of a predictor variable (Table 2) is the predicted change in odds for a unit increase in the predictor, and its natural

Table 2 Variables in the logistic regression equation: coefficients (*B*) and their standard error (SE), the Wald statistic, degrees of freedom (d.f.), significance (Sig.), and the odds ratio for each variable at steps 1 and 2 are reported

Step	Variable	<i>B</i>	SE	Wald	d.f.	Sig.	Odds ratio [Exp(<i>B</i>)]
Step 1	Generalization	0.565	0.247	5.237	1	0.022	1.759
	Constant	-1.027	0.695	2.186	1	0.139	0.358
Step 2	Generalization*	0.659	0.295	4.985	1	0.026	1.933
	Order†‡			7.438	3	0.059	
	Coleoptera-Diptera	2.279	1.274	3.198	1	0.074	9.764
	Coleoptera-Hemiptera	3.290	1.680	3.835	1	0.050	26.848
	Coleoptera-Hymenoptera	3.583	1.401	6.544	1	0.011	35.995
	Constant	-3.731	1.567	5.668	1	0.017	0.024

*Change in $-2 \log$ likelihood if the variable removed from the model is significant at the level of $P = 0.006$.

†Change in $-2 \log$ likelihood if the variable removed from the model is significant at the level of $P = 0.018$.

‡The contrast method used to compare categories is Indicator, and Coleoptera was used as reference category.

logarithm corresponds to the coefficient of the predictor (Table 2) in the logistic regression equation. The odds ratio of 1.933 for generalization (from Table 2), for example, indicates that the odds of visiting *I. glandulifera* for an insect species that feeds on two plant species are 1.933 times the odds of visiting *I. glandulifera* for an insect that feeds on one plant species. An increase in odds with increasing generalization (odds ratio > 1) results in generalized insect species having a greater probability of visiting *I. glandulifera* than more specialized insect species. Overall, the results of the logistic regression show that, as we predicted, the probability of visiting the alien species increases with the generalization of insect species; and that the insect order to which a species belongs also influences the outcome, with Hemiptera and Hymenoptera being more likely to visit *I. glandulifera* than Coleoptera (Table 2, see odds ratios).

H. sphondylium was visited by 61% of the insect species at flower removal plots, and by 58% of the species at invaded plots (five insect orders). Interestingly, for *H. sphondylium* the only variable included in the model was order (test of model coefficients for first step, $\chi^2 = 8.212$, d.f. = 3, $P = 0.042$) where Diptera were significantly more likely to visit this native species than Hymenoptera (Wald statistic = 6.5, d.f. = 1, $P = 0.011$, Exp(*B*) = 14). The model adequately fits the data (Hosmer and Lemeshow goodness of fit test: $\chi^2 < 0.001$, d.f. = 2, $P = 1$), and explains a reasonable amount of variation (Nagelkerke $r^2 = 0.320$). Generalization and abundance were non-significant variables, even when entered separately.

DISCUSSION

We found that *I. glandulifera* did not compete with native plants for visits, but instead we found evidence of facilitation for visits to native plants (Fig. 2d). As predicted, the more generalized an insect species, the more likely it was to include *I. glandulifera* in its diet and in contrast,

generalization of species did not influence visitation to the most generalized native plant. Despite the facilitation for visits, the pollen transport networks were dominated by *I. glandulifera* pollen, potentially negating any increase in seed set due to increased visitation. In this section we discuss the limitations of this study, and consider our results in the light of our three predictions. We end by discussing the implications of our data to the study of ecological networks.

Limitations of the study

Ultimately, it is the effect of visitation and pollination on seed set and seedling recruitment that has the potential to impact plant populations and communities (Turnbull *et al.* 2000). While there was some overlap in native plant species within a pair of plots, these plants were not found at the same abundance thus confounding any comparison between treatment and control. To determine the impact of *I. glandulifera* on seed set, experimental plots planted with a constant array of native plants will be needed. And ideally the paired design would incorporate a third treatment, that of a plot which had never been invaded in the first place, in order to determine how the attractiveness of this alien species compares to the attractiveness of the native species from which it has usurped the dominant role. Adding a further four plots was unfeasible with the resources available to this project. Nevertheless, our treatment effect was significant even after accounting for the variation explained by flower abundance, which suggests that the effect of *I. glandulifera* goes beyond its flower abundance.

Visitation web data: the direct and indirect effects on native species

Because plant–plant interactions for pollination are pollinator mediated, the way in which *I. glandulifera* affects

pollinators determines its impact on native plant species. From the insects' perspective, *I. glandulifera* is an excellent source of nectar (Chittka & Schurkens 2001) and many species appear to take advantage of it, which could enhance some insect species' populations if they are limited by floral resource availability. On the other hand, *I. glandulifera* could be displacing native plant species that might be important resources for pollinators earlier or later in the season (Rathcke 1983). Different insect species in the same community will probably be affected to different degrees by *I. glandulifera*. Specialists might be more susceptible to disturbance (Rathcke & Jules 1993; McKinney 1997; Williams *et al.* 2001; but see Vazquez & Simberloff 2002). Some specialized pollinator groups are affected by human-induced disturbances (Steffan-Dewenter & Tscharntke 2000; Goulson & Darvill 2004; Goulson *et al.* 2005; Steffan-Dewenter *et al.* 2006), and specialized bees and hoverflies are known to be declining (Biesmeijer *et al.* 2006). Invasive plants reduce the abundance of native resources by displacing native plants, which could be detrimental for specialized pollinators if they are unable to use the new resources. Indeed, generalized species were more likely to take advantage of the new resources provided by *I. glandulifera* than specialists.

Short-term experiments, such as the one reported here, assess the redistribution of insects on floral resources (attraction), which can occur at different spatial scales. Our prediction that *I. glandulifera* would redistribute insects within the plots in a way that leads to competition for visits between plants proved not to be the case. Instead, it appears that insects from further distances are attracted. Theoretically, an increasing collective floral display (total flower density) can attract pollinators to an area in an accelerating fashion, resulting in a higher number of visits to either one or more species (Rathcke 1983; Feldman *et al.* 2004). This mechanism of facilitation may be important in systems with highly mobile insects (Moeller 2004), such as *Apis mellifera* and *Bombus* spp. here (Osborne *et al.* 1999; Beekman & Ratnieks 2000; Knight *et al.* 2005). *I. glandulifera* may be attractive enough to produce this effect, thereby facilitating visitation to native species. This is supported by an experiment where floral abundance of this alien species was manipulated and visitation rates to a native plant increased with increasing numbers of *I. glandulifera* flowers (Lopezaraiza-Mikel & Memmott, unpublished data). If such a mechanism is occurring, i.e. insects are being attracted from further distances, then competition for pollinator visits could still occur at greater spatial scales among patches where *I. glandulifera* is present and patches where it is absent. This study only compared high alien floral density to no alien floral density, and floral density may account for the difference in outcome here to that reported by Chittka & Schurkens (2001). This is because it is

possible that competition occurs when the attraction of insects saturates (Rathcke 1983). Temporal and spatial variation in visitation patterns (Price *et al.* 2005), differential impact to different species, and the different scales of the experiments could also account for the difference in outcome between our experiment and that of Chittka & Schurkens (2001).

Pollen transport web data: the impact of alien pollen

At first glance, given that Hymenoptera are one of the most important pollinator groups, it seems particularly detrimental for native plants that Hymenoptera carried the largest quantity of alien pollen. Indeed, they carried significantly less native pollen at invaded plots. However, the Hymenoptera are not the only insect order responsible for the increase in visits to natives from flower removal to invaded plots, as all insect orders increase their visitation. These other orders carry considerably less alien pollen and on average more (Coleoptera) or as much (Diptera and Hemiptera) native pollen as the Hymenoptera at invaded plots. Fontaine *et al.* (2006) experimentally demonstrated the importance of functional diversity in a plant–pollinator community, defining functional groups of plants and pollinators based on morphological traits. Given the contamination of the Hymenoptera pollen loads here, functional diversity may be key for the pollination of native plants in our plots. Furthermore, the chances of alien pollen arriving to native stigmas will be affected by insect foraging behaviour. The main Hymenoptera visitors to *I. glandulifera* (*Apis mellifera* and *Bombus pascuorum*) are known to be flower constant species (e.g. Hill *et al.* 1997; Raine & Chittka 2005) and most individuals of these species were collected at *I. glandulifera*; consequently heterospecific pollen transfer may be small. In addition, the alien and native pollen carried by the Hymenoptera that were collected visiting native plants does not differ significantly between invaded and flower removal plots. Nevertheless, many individuals visiting the alien carry native pollen as well, reflecting they also visit native plants.

A sample of native stigmas from plants growing next to *I. glandulifera* revealed that while *I. glandulifera* pollen was found on native stigmas (at six of eight species analysed), it did not occur in the large amounts suggested by the pollen transport data (Lopezaraiza-Mikel, unpublished data). Thus while 71% of the 52 stigmas analysed had *I. glandulifera* pollen on the stigmatic surface, the two species hosting the largest amount of alien pollen (*Symphytum* sp., Boraginaceae and *Lythrum salicaria* L., Lythraceae) had only an average of 14% and 7%, respectively, of their stigmatic surfaces covered with alien pollen. Alien pollen has been proven to reduce seed set of native species by hand-pollinations (Brown & Mitchell 2001; Moragues & Traveset 2005).

However, no evidence of large amounts of alien pollen on native stigmas has been found under natural conditions (Moragues & Traveset 2005; Larson *et al.* 2006). Further experiments on seed set are needed to clarify the actual impact of alien pollen on pollination of native species.

Generalization of insect species and visitation to alien plant species

Our data show that generalization is important for the inclusion of the alien plant *I. glandulifera* in an insect's diet, but not in the co-dominant native plant *H. sphondylium*. Richardson *et al.* (2000) proposed that generalized pollinators, which abound in nature (Waser *et al.* 1996), readily visit alien plants, though limited evidence was available at this time. Many studies report that alien plants are visited by generalized native pollinators (e.g. Titze 2000; Chittka & Schurkens 2001; Brown *et al.* 2002; Parker & Haubensak 2002; Stout *et al.* 2006), and our data provide further support for Richardson *et al.*'s (2000) hypothesis. Moreover, using visitation networks which contained 56 alien plant species, Memmott & Waser (2002) found that visitors including alien plants in their diets were more generalized. These studies, along with the one reported here indicate that generalized native pollinator species provide a pathway for the integration of alien plant species into native visitation networks, and potentially into the pollination networks too. This not only disrupts interaction patterns as reported here, but could also lead to the facilitation of alien plants' spread via successful seed set (Parker & Haubensak 2002; Stout *et al.* 2006).

The nested structure of plant–pollinator networks (Bascompte *et al.* 2003) implies that the most generalized plant interacts with the generalized visitors in the community, along with many specialized visitors. Given that *I. glandulifera* and *H. sphondylium* are the most generalized plants in the networks, we would not expect generalized insects to have a higher probability than specialized insects of visiting these species (i.e. we would not expect generalization to be significant in the model). Interestingly this was contrary to what we found for the alien species, although the native species did as expected. In fact, very few species (18% of the species with only one interaction) were specialized on *I. glandulifera*. This suggests that *I. glandulifera*, and perhaps other alien plants, may not sustain the same variety of visitors as their native generalist equivalents and may alter structure and generalization patterns in the community.

Generalization on its own only partly explains visitation to *I. glandulifera* and our study is the first to show that taxa can differ in their likelihood of probing new species. The significance of different characteristics reflected by shared evolutionary histories among species of the same insect

order in our sample, such as insect mobility, foraging strategies, extent of dependence on floral resources and morphological features, needs further study to understand how they influence visitation to alien species. Finally, unexplained variation in our analyses indicates that other unidentified variables influence visitation to alien species. For example, Memmott & Waser (2002) found that the taxonomic affinity of alien plants to native flora is related to the number of taxa that visit alien plant species. However, in this study, no native plant belonged to the Balsaminaceae.

CONCLUDING REMARKS

This study provides the first experimental report on the impact of alien species on pollination systems in a community context, showing a facilitative effect of the alien on visitation to native species. The large amounts of alien pollen in the system may negate this positive effect if alien pollen interferes with the natives' pollination. In addition, we found evidence that generalized visitors provide a conduit for animal-pollinated alien species into visitation networks. Clearly, an alien species has the potential to alter network structure through a variety of direct or indirect pathways.

An experimental approach to food webs is essential if theoretical advances are to be tested (Proulx *et al.* 2005; de Ruiter *et al.* 2005). Experiments such as the one reported here provide unparalleled opportunities to test predictions concerning how networks respond to the removal of a highly connected species. Moreover, the removal of an alien species rarely elicits any concern from conservationists or the public; indeed such experiments could be run within eradication programmes to the advantage of network ecologists, invasion biologists and conservation biologists.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Figure S1 Treatment × site interaction plots for visitation data.

Figure S2 Visitation webs from control and experimental plots for each site.

Figure S3 Pollen transport webs from control and experimental plots for each site.

Figure S4 Species richness rarefaction curves for all visitors and visitors to native plants.

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