Combined effects of *Impatiens glandulifera* invasion and landscape structure on native plant pollination

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Summary

1. Habitat loss, land use intensification and biological invasions are all threatening pollinator communities, but the combined effects of these factors on pollinator diversity and pollination services have not been studied yet.

2. Here, we tested the hypotheses that (i) the invasive plant *Impatiens glandulifera* outcompetes native plant communities for pollinators, and (ii) pollinator abundances depend on landscape structure, but are modulated by this mass-flowering invader.

3. We selected 14 study sites in riparian habitats along a landscape gradient with decreasing proportion of natural land cover. Within each site paired invaded or non-invaded plots were studied. We performed standardized surveys of pollinators and established experimental plots by adding the native plant *Raphanus sativus* to assess the impact of *I. glandulifera* on visitation rates and seed set.

4. *Impatiens glandulifera* was well integrated in the plant–pollinator network, being visited by several native pollinators, mainly bumblebees. The invader received higher visitation rates than simultaneously flowering native riparian plants and the experimentally added native *R. sativus*. However, visitation rates to the native plant community showed no significant differences between invaded and non-invaded plots, with the exception of honeybees, which slightly increased their visits in invaded plots. With regard to the experimental setting, the presence of *I. glandulifera* reduced bumblebee visitation to *R. sativus* pots, but had no significant effects on seed set.

5. We found enhanced visitation rates of bumblebees in intensively used agricultural landscapes. However, in the presence of *I. glandulifera* this landscape effect was masked by bumblebees being highly attracted to *I. glandulifera* stands independent of the structure of the surrounding landscape. Surprisingly, wild bees and hoverflies were not affected by landscape structure, but, as also the case with bumblebees, they were principally affected by the immediate community flower abundance.

6. *Synthesis.* Our data provide no evidence that *I. glandulifera* outcompetes native plants for pollinators. However, social bees were very attracted to this late-seasonal floral resource. We conclude that both, plant invasions and landscape structure have important effects on the plant–pollinator community studied, but that they operate at different stages of the flowering season.

Key-words: alien plant, bumblebees, competition, invasive plants, landscape complexity, landscape context, mass flowering, pollination services, pollinator diversity, *Raphanus sativus*

Introduction

Destruction of natural habitats, land use intensification and biological invasions are some of the most prominent components of global change that influence biodiversity in human-dominated temperate ecosystems (Hobbs 2000; Sala *et al.* 2000). However, these components have been approached as single-factor conservation problems, rather than interacting factors. To discriminate how these global change drivers influence species diversity and biological interactions, a

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combination of approaches incorporating quantitative observational data and field experimentation is required (Kremen *et al.* 2007).

Pollinator loss is a major concern for conservation because pollination is essential for the reproduction of many wild plants and the maintenance of genetic diversity (Burd 1994; Kearns, Inouye & Waser 1998; Steffan-Dewenter, Potts & Packer 2005). Additionally, many agricultural crops depend on wild, unmanaged pollinator communities for crop pollination services (Klein *et al.* 2007). Parallel declines in pollinators and insect-pollinated plants have been reported in Great Britain and the Netherlands (Biesmeijer *et al.* 2006), presumably caused by habitat alteration and agricultural intensification.

Pollinator communities and plant–pollinator interactions depend upon the landscape structure such as land-cover type, successional age or habitat fragmentation (Steffan-Dewenter, Münzenberg & Tscharntke 2001; Aizen, Asworth & Galetto 2002; Aguilar *et al.* 2006). For instance, the local abundance and diversity of bees increases with an increasing proportion of semi-natural habitats in agricultural landscapes (Steffan-Dewenter *et al.* 2002). Moreover, each pollinator guild can be influenced by different factors acting at multiple spatial scales (Winfree & Kremen 2009). For example, key resources for wild bees with small foraging distances are nesting sites and flowering plants provided by natural or semi-natural habitats (Westrich 1996; Gathmann & Tscharntke 2002). By contrast, social bees such as bumblebees, which have broader foraging ranges, partly benefit from the occurrence of mass-flowering resources in agricultural fields at larger spatial scales (Westphal, Steffan-Dewenter & Tscharntke 2003).

Plant–pollinator interactions are sensitive to the floral composition of local neighbourhoods (Rathcke 1983). In this sense, an alien plant species could alter community plant–pollinator interactions (Traveset & Richardson 2006; Bjerknes *et al.* 2007), but there are too few empirical studies to enable us to draw general patterns. Plant invasions can decrease or increase the number of visits to particular co-flowering native plants, depending on the identities of the invader and the native species (Chittka & Schürkens 2001; Brown, Mitchell & Graham 2002; Moragues & Traveset 2005; Larson, Royer & Royer 2006; Carvalheiro, Barbosa & Memmott 2008; Cariveau & Norton 2009). However, when the presence of the invader changes visitation rates to native plants, this does not necessarily reduce seed set (Totland *et al.* 2006). Furthermore, the few existing studies analysing the effect of a plant invader on the whole plant–pollinator network have shown that the invader is very well integrated in the network (Memmott & Waser 2002) and is sometimes acting as a magnet species (Lopezaraiza-Mikel *et al.* 2007) with potentially beneficial effects for the pollination of neighbouring native species (Bartomeus, Bosch & Vilà 2008a; Bartomeus, Vilà & Santamaría 2008b). All this being said, the effects on pollinator populations have been little explored.

To date, we are not aware of empirical studies that have investigated the combined effects of landscape structure and invasion on plant–pollinator interactions. This information is, however, imperative to assess the magnitude of these impacts, to disentangle whether changes in the landscape and invasion interact additively or synergistically and also to justify mitigating actions to overcome their effects (Vane-Wright, Humphries & Williams 1991; Sala *et al.* 2000).

In our study, we combined an observational and an experimental approach along a landscape structural gradient of decreasing proportion of natural land cover with paired invaded and non-invaded sites in Central-European riparian habitats to test how these two factors influence plant–pollinator interactions. We focused on the invasive plant *Impatiens glandulifera*, a noxious invader which reduces species diversity and outcompetes native flora (Beerling & Perrins 1993; Wade *et al.* 1994; Hulme & Bremner 2006). First, we surveyed pollinator assemblages and flower visitation rates of the whole riparian plant community in invaded and non-invaded plots along a landscape structure gradient. Second, we set up experiments to expose individuals of the native plant *Raphanus sativus* in the same sites to estimate the impact of the invader on visitation rates and seed set of a native focal plant. *Raphanus sativus* is visited by a wide pollinator spectrum as the closely related wild radish, *R. raphanistrum* L. (Conner, Davis & Rush 1995). Our study therefore differs from previous works in which the focal native species potentially competing with *I. glandulifera* was more specialized (Chittka & Schürkens 2001).

We performed our experiments two times, first before and second during the flowering of *I. glandulifera* in order to disentangle landscape effects from invasion effects. Specifically, we addressed the following two hypotheses: (i) mass flowering of the invader *I. glandulifera* in riparian habitats outcompetes native plants for pollinators, and (ii) *I. glandulifera* modulates the landscape structure effects on pollinator patterns.

Materials and methods

STUDY SPECIES

Impatiens glandulifera Royle – Indian balsam – (Balsaminaceae) is a widespread invasive riparian plant from the Himalayas. It was introduced in Europe as a garden ornamental plant and has been naturalized since the 20th century (Beerling 1993). It has spread to the majority of temperate communities in Europe, growing along river and stream banks, where it forms dense linear stands (Beerling 1993; Pyšek 2005). Usually, *I. glandulifera* is the tallest (up to 2 m) annual plant in the invaded area. It bears large inflorescences with purple zygomorphic flowers (3–4 cm long). It is protandrous and self-compatible, but because it produces a large amount of nectar and pollen, it receives constant visits. Consequently, it is frequently cross-pollinated (Valentine 1978). The pollinators it attracts include several species of bumblebees, honeybees and wasps. *Impatiens glandulifera* has an impact on native plant species establishment, diversity and composition (Beerling & Perrins 1993; Hejda & Pyšek 2006).

The annual *Raphanus sativus* L. (Brassicaceae) was used as a focal native plant species. *Raphanus sativus* is visited by a wide array of pollinators including honeybees, bumblebees, wild bees, hoverflies and butterflies (Steffan-Dewenter & Tscharntke 1999; Albrecht *et al.* 2007). The plant grows fast, reaches up to 1.5 m height, and produces a large number of flowers (Ellstrand 1984; Albrecht *et al.*

2007). We used a commercially available old variety of *R. sativus* (variety Siletta; Breeding Company Schneider, Grundhof, Germany), which, in contrast to modern varieties, is self-incompatible and closely related to its wild relative *R. raphanistrum* (Steffan-Dewenter & Tscharntke 1999).

STUDY SITES

The study was performed in southern Lower Saxony, around the city of Göttingen, central Germany. The climate is temperate with a mean annual temperature of 8–8.5 °C and average annual rainfall ranging from 600 to 700 mm (http://www.wetterstation-goettingen.de/klima-bericht.htm). The study region is characterized by a highly agricultural landscape dominated by wheat and other annual crops, including mass-flowering oilseed rape, which can have a beneficial effect on social pollinators (Westphal, Steffan-Dewenter & Tscharntke 2003; Herrmann *et al.* 2007). Patches of forests and grasslands are embedded in this agricultural landscape.

In summer 2006, 14 circular sites with a radius of 3000 m were selected within a 30 × 35 km area. The centre of each site was located in riparian habitats along a river where the invader *I. glandulifera* was present. Along the riverside we selected two fixed 100-m long, 2-m wide transects, one invaded, the other one non-invaded at each study site. These two paired transects were at least 100 m apart. Invaded transects were chosen before *I. glandulifera* was at its maximum vigour. Therefore, there was a large variation in invasion degree between sites. The percentage ground cover of *I. glandulifera* in invaded transects ranged from 7% to 71% and the density of flowers from 6.2 to 45.5 flowers m^2.

The landscape structure of each site was characterized by commercially available digital maps (ATKIS-DLM 25/1, 1991–96; Landesvermessungsamt + Geobasisinformationen Niedersachen, Hannover, Germany) using the ARCVIEW GIS software in a 500–3000-m radius from the centre of each site. We computed the composition of the landscapes as the percentage of forests, grasslands (i.e. natural habitats) and agricultural land cover. The sites also included urbanized areas and water surfaces, but the total sum of the three major categories (i.e. forests, grassland and agricultural land) was always larger than 90%, and therefore we only used these three categories in our analysis. The mean area and perimeter of patches were calculated as indicators of fragmentation and shape of habitat patches in the landscapes.

The percentage of agricultural land cover within the 3000-m radii ranged from 28% to 80%. Agricultural land cover was negatively correlated with the percentage of natural habitats ($r^2 > 0.90$; $P < 0.05$ for all radii). The mean area and perimeter of the natural patches had a very small variability among all sites (mean ± SE; area: 3.10 ± 0.2 ha; perimeter: 890.1 ± 27.9 m), and therefore they were not used for the analysis.

SURVEY OF THE POLLINATOR COMMUNITY

In each site, we conducted three 30-min transect walks (one per week) along the invaded and non-invaded 100-m transects. For each flowering plant, we counted the number of floral units in the transect (Dicks, Corbet & Pywell 2002) and recorded the number and identity of all insects visiting the sexual parts of flowers (pollinators hereafter). Unknown pollinators were caught for later identification. To disentangle landscape and phenological differences in pollinator patterns from invasion effects, this protocol was repeated twice, before (20 June–15 July) and during (25 July–20 August) *I. glandulifera* flowering.

RAPHANUS SATIVUS EXPERIMENT

We grew two sets of *R. sativus* potted plants in 4 L pots filled with commercial garden soil (Einheitserde T25; Archut/Hawita, Vechta, Germany) under outdoor conditions at the Göttingen University experimental garden facilities. This permitted us to compare plant treatments with standardized soil conditions and the same origin of seeds. Before the plants started flowering we placed three pots of *R. sativus* beside each invaded and non-invaded transect (a total of 84 pots per round). To enhance their attractiveness to pollinators, the three pots were placed together. Plants were watered as needed in the experimental garden facility and in the field.

Once a week during three consecutive weeks (coinciding with surveying the pollinator community), we performed 15 min observations, recording the number and identity of all pollinator visits to *R. sativus* and counting the total number of flowers. As in the community surveys described above, pollinators that could not be identified in the field were caught for later identification. As before, we repeated the experiment twice, before and during *I. glandulifera* flowering. There was no overlap between the two experimental rounds. Overall, data are based on observations of 168 pots in 14 landscape sites and two rounds per site.

When *R. sativus* fruits were ripe, we counted the well-developed fruits in each of the two rounds. Fruit set was calculated as the proportion of flowers that set fruit. The reproductive effort was calculated as the ratio between dry fruit weight and total above-ground biomass. Fruit weight can be considered as a good estimation of plant fitness because it is highly correlated with seed set ($r^2 = 0.80$ $P < 0.001$, $n = 100$). As some plants got damaged or partially eaten in the field, we only had complete data for 10 sites at the end of the experiment.

STATISTICAL ANALYSIS

For each transect in the pollinator surveys, we calculated plant and pollinator richness (i.e. number of species) and total number of pollinator visits before and during *I. glandulifera* flowering. Because of the low sampling sizes in pollinator visits for some taxa we could not group them in different guilds; for statistical reasons we grouped pollinators as: honeybees, bumblebees, wild bees and hoverflies. For the *R. sativus* experiment, we calculated the total number of pollinator visits, visits of each pollinator group, fruit set and reproductive effort. Data were log-transformed when necessary to meet the assumptions of parametric statistical analysis.

To test whether *I. glandulifera* plants received a number of visits comparable to average visitation rates on native species, we compared the visitation frequency received by the invader with a normal distribution fitted to the number of visits received by native species for each site by means of the *Z*-statistic.

EFFECTS OF INVASION AND LANDSCAPE STRUCTURE ON POLLINATOR VISITATION TO NATIVE PLANTS

We tested whether invasion, landscape structure and local plant community characteristics influenced richness (i.e. number of pollinator species) and the number of visits of each group of pollinators to native species both in the transect walks and in the *R. sativus* experiment. For this purpose, we used Linear Mixed-Effect Models fitted by REML in R (R Development Core Team 2008). Three explanatory landscape variables (i.e. percentage of agricultural land, forests and grasslands), two invasion variables (i.e. *I. glandulifera* cover and number of *I. glandulifera* flowers) and two local plant community

variables (i.e. plant richness and number of native flowers) were sequentially added to the models following a stepwise selection (Crawley 2002). As some of our variables were highly correlated (e.g. agricultural land and natural habitats (i.e. forests + grasslands: $r^2 > 0.90$, $P < 0.05$ for all radii; *I. glandulifera* cover and number of *I. glandulifera* flowers: $r^2 = 0.89$, $P < 0.05$), we did not include these variables simultaneously in the stepwise selection. To test whether invasion and landscape structure affected the dependent variables while accounting for site clustering, we considered site as a random block factor and the other variables as fixed factors. We performed different analyses for before and during *I. glandulifera* flowering. We present here the results of the minimum adequate model with the low Akaike's Information Criterion (Akaike 1974) for each dependent variable.

Based on earlier studies in the same area on how different pollinator groups respond to land cover at different spatial scales (Steffan-Dewenter *et al.* 2002; Westphal, Steffan-Dewenter & Tscharntke 2006), we used the proportion of land covers at 3000 m radius for bumblebees and honeybees and at 500 m radius for wild bees and hoverflies. Both radii were tested for the total pollinator richness.

EFFECTS OF INVASION AND LANDSCAPE STRUCTURE ON BUMBLEBEE DENSITY

Based on the results of the Linear Mixed-Effect Models, we constructed a hypothetical causal link between the effects of landscape structure, invasion and plant community characteristics on bumblebee densities by using structural equation modelling (SEM). We conducted this analysis only for bumblebees because they accounted for almost half of total visits to the community and were shared greatly between native plants and *I. glandulifera*.

Structural equation modelling is particularly useful for confirmatory analysis and thus more suitable for the evaluation of our multivariate hypothesis (Grace 2006). First, we constructed a theory-driven model of how landscape structure, invasion and plant community affects bumblebee densities. However, we had not measured all the parameters of interest. For example, we measured the number of bumblebee visits instead of bumblebee densities because of the reliability of the former. Secondly, we created latent variables (oval boxes) that link the concepts that we intended to test with the measures that we took (square boxes), including an error term (see path diagram in Fig. 1).

We measured the overall fit of our proposed model with a chi-squared test, but because the chi-squared test of absolute model fit is sensitive to sample size and non-normality in the underlying distribution of the input variables, we also investigated the Tucker–Lewis Index (TLI) statistic to assess the overall fit of the model to the data. Values higher than 0.95 confirmed a good model fit. This index compares the absolute fit of the specified model to the absolute fit of the independent model. We presented the values associated with each path as standardized regression coefficients. These values represent the amount of change in one parameter given a standard deviation unit change in the other one. We also showed the r^2 values for each dependent or mediating variable above the variable (Fig. 1). This model has several interesting features. First, it contains both latent (unobserved) and manifest (observed) variables. Secondly, it contains both causal relationships among latent variables, represented by single-headed arrows, and correlational or bi-directional relationships among some residuals. These are represented by the dual-headed arrows connecting landscape variable errors. As the three landscape variables are measured on the same map, it makes sense that they share variance due to causes not accounted for by the latent factor.
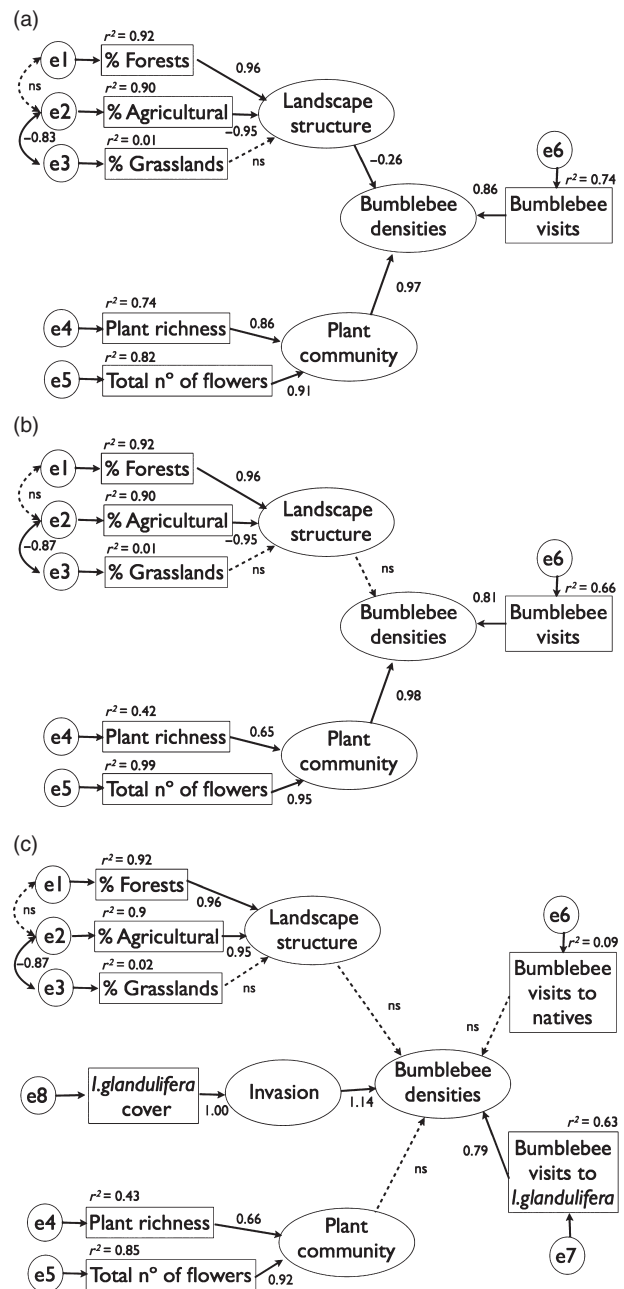

Fig. 1. Results for the structural equation models evaluating the effects of landscape structure, invasion and plant community on bumblebee densities (a) before, (b) during *Impatiens glandulifera* flowering in non-invaded sites and (c) during *I. glandulifera* flowering in invaded sites. Oval boxes are latent variables and square boxes observed variables, including an error term ('e'). The models show the standardized coefficients of each path. Dotted paths are non-significant. All models fit the data ((a) $\chi^2 = 11.7$, *P*-value $= 0.16$; (b) $\chi^2 = 22.1$, *P*-value $= 0.17$ (c); $\chi^2 = 10.98$, *P*-value $= 0.2$).

The correlations between the residuals account for that additional shared variance.

It is important to note that even though the model fits the data well and provides a theoretically consistent set of findings, there may be other equivalent models that fit the data equally well.

We used AMOS software (Arbuckle 2003) to perform the analysis. We performed three SEM diagrams: one before and the other two

during *I. glandulifera* flowering. For the first one, we used all data, using site as a grouping factor ($n = 14$). While for the other two models we used data on invaded and non-invaded sites, respectively ($n = 14$). We did not include the number of *I. glandulifera* flowers because of its linear positive dependence with *I. glandulifera* cover. We did not remove non-significant paths because the overall fit was good and this allowed us to highlight the results.

Results

EFFECTS OF *IMPATIENS GLANDULIFERA*

Flowering patterns during the pollinator survey

During the pollinator survey, the number of flowering plant species was greater before than during *I. glandulifera* flowering (Fig. 2). For example, species from the genera *Ranunculus, Rubus, Lotus* and *Stellaria* flowered early. For others, only late flowers overlapped with the flowering peak of *I. glandulifera* (e.g. *Stachis palustris, Vicia* spp. and some Apiaceae and Asteraceae). Only a few species had a flowering peak which completely overlapped with the invader (e.g. *Centaurea jacea, Epilobium angustifolium, Symphytum officinale*).

Pollinator richness during the pollinator survey

Five pollinator taxa visited *I. glandulifera*: three bumblebee species, one wasp and one honeybee species. By contrast,

during *I. glandulifera* flowering, native plants were visited by 48 pollinator taxa in total, including honeybees, 12 wild bee species, 4 bumblebee species, 7 hoverfly species and a few beetle, fly, butterfly and wasp species. However, native species received on average ($\pm$SE) 6.26 ± 1.48 pollinator taxa per plant species, a value that was not significantly different to that of *I. glandulifera* (Z-test: $P = 0.2$).

Pollinator visits during the pollinator survey

Overall, we recorded a total of 887 visits of which 464 were from bumblebees, 155 from hoverflies, 99 from honeybees and only 18 from other wild bees. No pollinator species visited exclusively *I. glandulifera* flowers.

There were more pollinator species before than during *I. glandulifera* flowering (Fig. 2). However, the total number of visits before and during *I. glandulifera* flowering was similar (Fig. 2). A great number of pollinator visits were to *I. glandulifera* (335 visits in total; 25.7 ± 4.7 visits per transect) accounting for 38% of the total visits in all invaded sites. Thus, *I. glandulifera* received on average more visits than any particular native species (before *I. glandulifera* flowering: 10.72 ± 7.2 and 9.7 ± 5.2 visits per plant species in non-invaded and invaded plots, respectively; during *I. glandulifera* flowering: 6.0 ± 1.5 and 7.64 ± 2.1 visits in non-invaded and invaded plots, respectively; Z-tests: $P < 0.001$ in all sites).
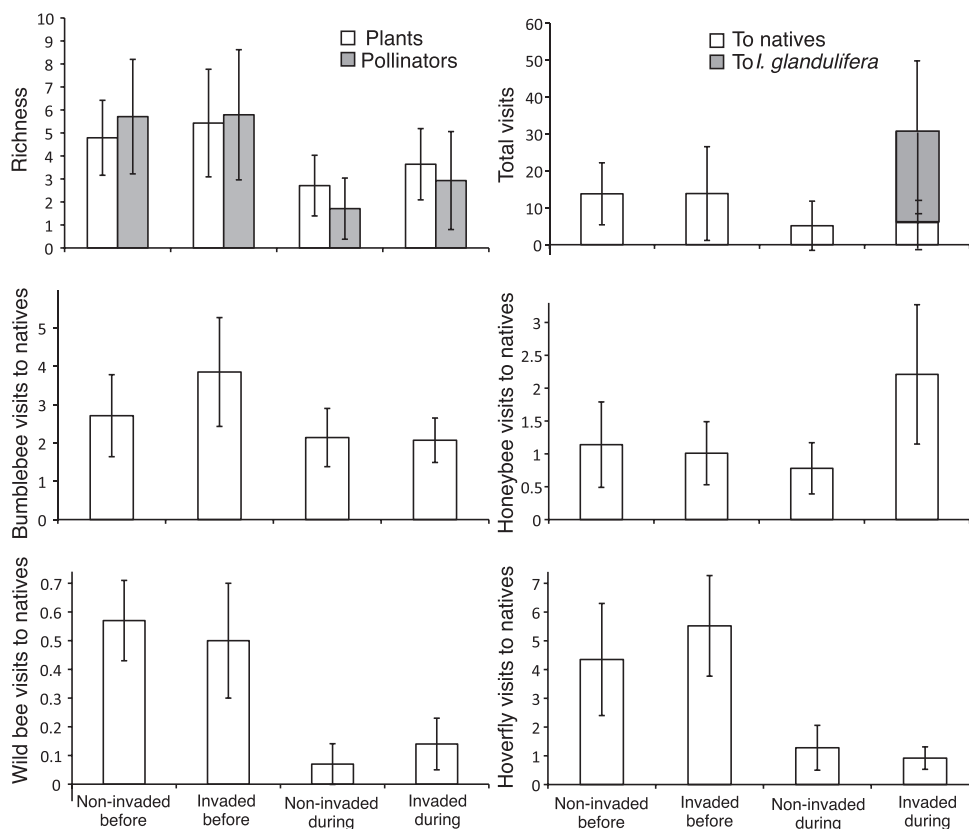

Fig. 2. Principal parameters (mean $\pm$ SE) for non-invaded and invaded transects before and during *Impatiens glandulifera* flowering.

Raphanus sativus *experiment*

Before *I. glandulifera* flowering, we recorded a total of 636 visits to *R. sativus* of which 52% were from hoverflies. During *I. glandulifera* flowering, we recorded a similar number of visits (533), also predominantly by hoverflies (58%; Fig. 3). Fruit set was not correlated with total number of observed flower visits neither before ($r^2 = 0.4$, $P = 0.6$) nor during ($r^2 = 0.04$, $P = 0.9$) *I. glandulifera* flowering.

EFFECTS OF INVASION AND LANDSCAPE STRUCTURE ON POLLINATION OF NATIVE PLANTS

Pollinator survey

There was no significant correlation between pollinator richness and any landscape parameter before *I. glandulifera* flowering (Table 1). Pollinator richness was correlated with the total number of native flowers in the transect (Table 1). During *I. glandulifera* flowering, pollinator richness depended not only on the number of native flowers, but also on the percentage of agricultural land and invader cover (Table 1).

The number of bumblebee and honeybee visits to native plants was highly correlated with the total number of native flowers both before and during *I. glandulifera* flowering. Moreover, there was a positive correlation between the number of bumblebee visits to native plants and the percentage of agricultural land before *I. glandulifera* flowering. Interestingly, this correlation disappeared during *I. glandulifera* flowering. Moreover, invasion had a non-significant effect on bumblebee visits to native plants during this period (Table 1). Landscape variables had no significant effect on honeybee visits, both before and during *I. glandulifera* flowering, but during *I. glandulifera* flowering, visits to native plants were positively correlated with *I. glandulifera* cover (Table 1). Due to the low number of wild bees recorded in the pollinator survey, we were not able to perform a statistical analysis. There was a positive correlation of visits of hoverflies with the total number of native flowers, but the visits were neither correlated with invader cover nor with any landscape variable (Table 1).

R. sativus *experiment*

The total number of flowers in the community was only correlated with honeybee visits during *I. glandulifera* flowering, and with wild bee visits both before and during *I. glandulifera* flowering (Table 2). However, bumblebee visits were marginally correlated ($P = 0.05$) with plant richness before *I. glandulifera* flowering (Table 2). The number of wild bees and hoverfly visits before *I. glandulifera* flowering was positively correlated with *R. sativus* flowers.

There was no relationship between the number of visits of any pollinator group and landscape variables except a marginal correlation ($P = 0.052$) of grasslands with hoverflies
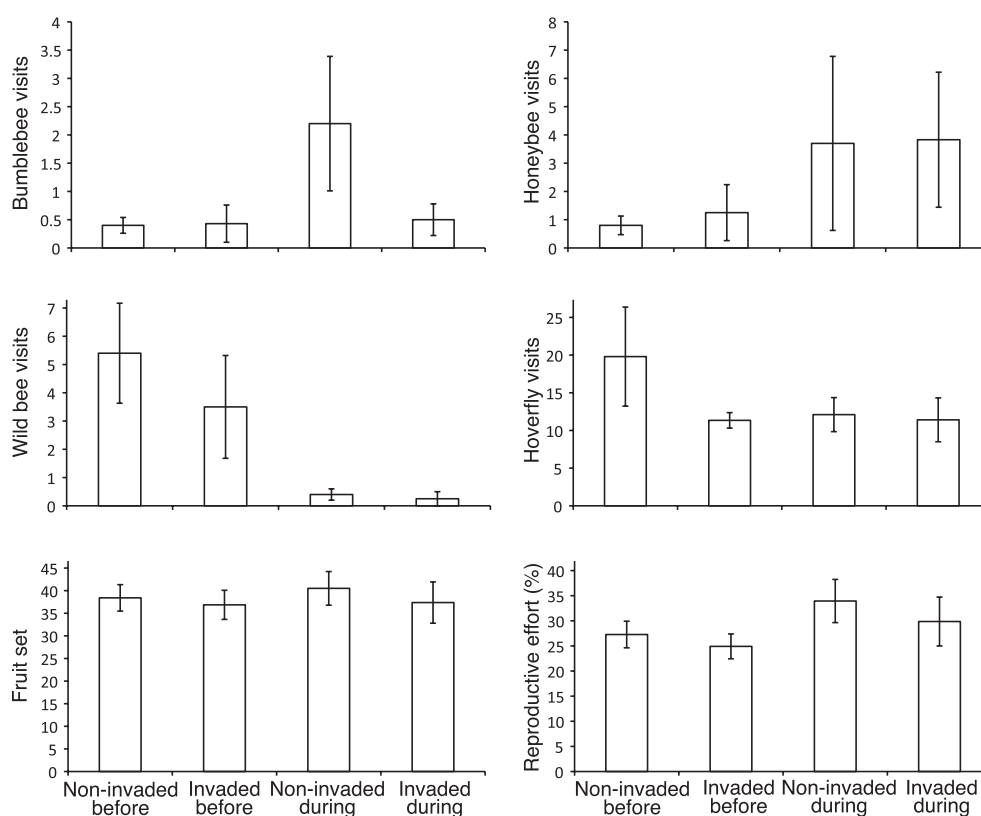

Fig. 3. Principal parameters (mean ± SE) for non-invaded and invaded *Raphanus sativus* experimental pots before and during *Impatiens glandulifera* flowering.

Table 1. Results (value ± SE) from minimum adequate mixed-effects model to the main pollinator parameters measured before and during *Impatiens glandulifera* flowering. Variables without values were removed from the model by the stepwise selection. All models included an intercept not shown in the table and site, which was included as a random effect. The *t*-values and *P*-values for each explanatory variable are shown in each cell. Significant *P*-values are shown in bold

	d.f.	No. plant species	No. native flowers	% Agricultural land	% Grasslands	*I. glandulifera* cover	AIC
Pollinator richness before	12	—	0.013 ± 0.003 $t = 1.22$; **$P = 0.01$**	0.05 ± 0.04 $t = 0.11$; $P = 0.9$	—	—	42
Pollinator richness during	12	0.08 ± 0.04 $t = 1.82$; $P = 0.09$	0.003 ± 0.001 $t = 3.48$; **$P = 0.01$**	0.016 ± 0.003 $t = 4.2$; **$P = 0.001$**	—	0.02 ± 0.002 $t = 4.21$; **$P = 0.001$**	56
Bumblebee visits before	12	0.1 ± 0.07 $t = 1.63$; $P = 0.1$	0.04 ± 0.001 $t = 4.02$; **$P = 0.001$**	0.02 ± 0.01 $t = 2.2$; **$P = 0.04$**	—	—	71
Bumblebee visits during	12	—	0.01 ± 0.01 $t = 2.42$; **$P = 0.03$**	0.01 ± 0.01 $t = 0.98$; $P = 0.3$	—	−0.01 ± 0.006 $t = 0.48$; $P = 0.6$	98
Honeybees visits before	12	—	0.002 ± 0.001 $t = 1.84$; $P = 0.08$	0.002 ± 0.01 $t = 1.77$; $P = 0.10$	—	—	85
Honeybees visits during	12	—	0.01 ± 0.001 $t = 2.21$; **$P = 0.04$**	0.008 ± 0.008 $t = 0.95$; $P = 0.3$	0.01 ± 0.01 $t = 0.25$; $P = 0.8$	0.02 ± 0.01 $t = 2.88$; **$P = 0.01$**	93
Hoverflies visits before	12	—	0.001 ± 0.001 $t = 0.78$; $P = 0.4$	—	0.02 ± 0.01 $t = 1.7$; $P = 0.11$	—	95
Hoverflies visits during	12	—	0.01 ± 0.001 $t = 3.53$; **$P = 0.003$**	—		0.001 ± 0.003 $t = 0.36$; $P = 0.72$	81

before *I. glandulifera* flowering. Finally, bumblebee visits to *R. sativus* during *I. glandulifera* flowering were negatively correlated with *I. glandulifera* cover. However, this correlation did not translate to changes in *R. sativus* fruit set or reproductive effort (Table 2).

EFFECTS OF INVASION AND LANDSCAPE STRUCTURE ON BUMBLEBEE DENSITIES

During the pollinator survey, data before and during *I. glandulifera* flowering fitted properly the proposed SEM (before: $\chi^2 = 11.72$, d.f. = 8, *P*-value = 0.16, TLI = 0.95; during non-invaded: $\chi^2 = 10.98$, d.f. = 8, *P*-value = 0.2, TLI = 0.95; during invaded: $\chi^2 = 22.14$, d.f. = 17, *P*-value = 0.17, TLI = 0.95).

The standardized coefficients revealed a stronger relationship between bumblebee densities and plant community characteristics when *I. glandulifera* flowers were not present. However, the relationship between landscape variables and bumblebee density was weak and disappeared during *I. glandulifera* flowering. In invaded sites, during *I. glandulifera* flowering, the only significant relationship was with invasion. Visits to natives did not contribute significantly to bumblebee density (Fig. 1). The high r^2 values indicate that latent variables are accounting for a large proportion of the variance in the items measured.

Discussion

Contrary to our expectations, we found no evidence for competition or facilitation between *I. glandulifera* and native plants. Our results also indicate that invasion and landscape structure have combined effects on flower visitation to native plants and on bumblebee densities. At the landscape scale bumblebee densities were associated with agricultural land only early in the season, when most of the mass-flowering crops are blooming, while at the local scale invaded plots showed enhanced bumblebee densities late in the season, when the invader is flowering. The loss of landscape-scale distribution patterns during *I. glandulifera* flowering indicates that invasions could replace the effects of landscape structure on plant–pollinator interactions.

IMPLICATIONS FOR POLLINATORS

The landscape structure of all our plots was very similar in mean patch area and perimeter of the ecotones. The principal differences were in the proportion of natural/agricultural land cover. Mass-flowering crops can provide resources for mobile pollinators such as bumblebees (Westphal, Steffan-Dewenter & Tscharntke 2003), while natural habitats offer a high diversity of flowering plants and important nesting sites for wild bees (Steffan-Dewenter *et al.* 2002). As shown in the SEM, bumblebee densities before the *I. glandulifera* flowering were correlated with the proportion of agricultural land and forests. However, the main explanatory variable was the composition of the plant community. We observed enhanced

Table 2. Results (value ± SE) from minimum adequate mixed-effects model for the main pollinator parameters measured in the *Raphanus sativus* experiment before and during *Impatiens glandulifera* flowering. Variables without values were dropped from the model by the stepwise selection. All models included an intercept not shown in the table and site, which was included as a random effect. *t*-values and *P*-values for each explanatory variable are shown in each cell. Significant *P*-values are in bold

	d.f.	No. plant species	No. native flowers	% Agricultural land	% of forests	% Grasslands	*I. glandulifera* cover	No. *R. sativus* flowers	AIC
Bumblebee visits before	10	0.11 ± 0.3 $t = 3.6$; **P = 0.05**	—	0.006 ± 0.007 $t = 0.86$; $P = 0.4$	—	—	—	—	28
Bumblebee visits during	10	0.22 ± 0.12 $t = 1.8$; $P = 0.1$	—	0.01 ± 0.01 $t = 0.1$; $P = 0.9$	—	—	−0.01 ± 0.007 $t = 2.49$; **P = 0.03**	—	75
Honeybees visits before	10	0.12 ± 0.06 $t = 1.8$; $P = 0.09$	—	0.01 ± 0.01 $t = 0.96$; $P = 0.35$	—	—	—	—	62
Honeybees visits during	10	—	0.01 ± 0.002 $t = 2.42$; **P = 0.04**	0.01 ± 0.02 $t = 0.14$; $P = 0.8$	—	—	0.01 ± 0.01 $t = 0.73$; $P = 0.48$	—	95
Wild bee visits before	10	0.22 ± 0.11 $t = 1.94$; $P = 0.09$	0.01 ± 0001 $t = 2.68$; **P = 0.03**	—	—	0.01 ± 0.01 $t = 1.08$; $P = 0.3$	—	0.004 ± 0.001 $t = 4.4$; **P = 0.03**	89
Wild bee visits during	10	—	0.002 ± 0.001 $t = 2.24$; **P = 0.05**	—	0.001 ± 0.001 $t = 0.2$; $P = 0.8$	—	−0.003 ± 0.003 $t = 1.5$; $P = 0.3$	—	59
Hoverflies visits before	10	—	—	—	—	0.03 ± 0.01 $t = 2.19$; **P = 0.05**	—	0.007 ± 0.001 $t = 5.8$; **P = 0.001**	81
Hoverflies visits during	10	—	—	—	—	0.01 ± 0.01 $t = 0.89$; $P = 0.4$	−0.01 ± 0.009 $t = 1.17$; $P = 0.3$	0.003 ± 0.001 $t = 2.02$; $P = 0.07$	87
Fruit set before	10	0.09 ± 0.03 $t = 2.57$; **P = 0.03**	0.01 ± 0.01 $t = 3.5$; $P = 0.07$	0.01 ± 0.01 $t = 1.36$; $P = 0.2$	—	—	—	—	35
Fruit set during	10	—	0.01 ± 0.01 $t = 0.78$; $P = 0.75$	0.01 ± 0.01 $t = 1.01$; $P = 0.33$	—	—	0.01 ± 0.01 $t = 0.52$; $P = 0.61$	—	67
Reproductive effort before	10	0.09 ± 0.04 $t = 1.9$; $P = 0.08$	—	0.01 ± 0.01 $t = 1.3$; $P = 0.21$	—	—	—	—	60
Reproductive effort during	10	—	−0.01 ± 0.01 $t = 0.26$; $P = 0.79$	0.02 ± 0.01 $t = 1.55$; $P = 0.15$	—	—	0.01 ± 0.01 $t = 0.79$; $P = 0.75$	—	86

bumblebee visitation to native plants in landscapes with higher proportions of agricultural land dominated by mass-flowering crops before *I. glandulifera* flowering. However, in contrast to previous studies (Steffan-Dewenter *et al.* 2002), there was no significant correlation between hoverflies and wild bee visits and landscape structure. This could be due to the fact that all our plots were located in riparian habitats and not in field margins as was the case in previous studies. For these two pollinator groups, landscape structure seems not to be as important as immediate riparian plant species richness and flower abundance, which might act as a foraging corridor. In fact, the local plant community is the most important variable that affects all groups and the overall pollinator richness. Nevertheless, late in the season, when fewer pollinators were active, the dominant invasive plant species *I. glandulifera* enhanced pollinator richness by attracting various bumblebee species.

Invaded plots also attracted a large number of bumblebees, independent from the landscape structure where they were located. Therefore, the correlation between bumblebee densities and agricultural land cover disappeared late in the season, when most of the mass-flowering crops were already harvested. At that time, pollinator composition was dependent on the cover of the invasive species *I. glandulifera*. Thus, *I. glandulifera*, by its potential to attract bumblebees over large distances, equalized landscape-related differences in bumblebee abundances late in the season, when only a few other floral resources were available. Recent studies have indicated that bumblebees also benefit from mass-flowering crops in agricultural landscapes in early spring (Westphal, Steffan-Dewenter & Tscharntke 2003; Herrmann *et al.* 2007). Presumably, the late-seasonal mass-flowering of *I. glandulifera* could have a complementary effect by increasing colony fitness in terms of the number of reproductive individuals and the size of the hibernating queen (Westphal, Steffan-Dewenter & Tscharntke 2009). Despite the fact that SEM tools gave a powerful approximation for accounting for pollinator densities, the effect of invasive plants on bumblebee population dynamics needs further empirical evaluation.

IMPLICATIONS FOR NATIVE PLANTS

Because of its flower morphology *I. glandulifera* is only accessible for rather large bees with middle sized or long tongues such as *Bombus*, *Apis* and *Vespa*. Nonetheless, *I. glandulifera* is well integrated in the plant–pollinator network, as even though it was visited by few pollinator taxa, visitations were very frequent. In fact, we saw that the presence of *I. glandulifera* increased the presence of certain pollinators, but this increase was due to the visits received by the invader, rather than to visits to natives. Native species only received slightly more visits from honeybees, but overall, *I. glandulifera* did not act as a magnet species as has been found in other regions (Lopezaraiza-Mikel *et al.* 2007), nor did it compete with native species (Bartomeus, Bosch & Vilà 2008a; Bartomeus, Vilà & Santa-maría 2008b).

Raphanus sativus receives efficient visits from various groups of pollinators, but evidence for pollination limitation has also been reported (Steffan-Dewenter & Tscharntke 1999; Albrecht *et al.* 2007). In our study, invasion by *I. glandulifera* did not increase visitation rates to *R. sativus* although more pollinators were attracted to the community. On the contrary, we found a decrease in bumblebee visits in non-invaded compared to invaded plots during the flowering period of *I. glandulifera*. However, this did not result in a decrease in fruit set or reproductive effort. Although hoverflies are less efficient pollinators than bumblebees, they may largely contribute to *R. sativus* pollination due to their high visitation frequency. We conclude that competition for pollination did not occur. In general, effective competition may only take place in certain specialist plant species with completely overlapping flowering phenology, similar flower morphologies, analogous pollinator syndromes and contrasted abundances (Rathcke 1988). Identifying such invasion-sensitive native plant species is crucial to improve conservation strategies.

With respect to a previous study, which found that the attraction of *I. glandulifera* outcompeted experimentally added potted *Stachys palustris* plants (Chittka & Schürkens 2001), we found that in natural communities the flowering peaks of these two species did not overlap and thus, in the field, competition for pollination may not occur. In fact, as for many invasive plants (Celesti-Grapow, Di Marzio & Blasi 2003; Lloret *et al.* 2005), the flowering phenology of *I. glandulifera* was different from many other native species coexisting in the community. In our plots, the number of flowering plant species during the *I. glandulifera* flowering peak was lower than before the flowering peak, indicating that *I. glandulifera* fills a late-seasonal gap in flowering phenology that native pollinators can explore.

Conclusions

Both the observational survey and the experimental setting suggest that the invasive plant *I. glandulifera* did not outcompete native plants for pollinators. As predicted, different native pollinators responded differently to the landscape structure. Bumblebee densities were higher in riparian habitats surrounded by agricultural crops as well as in invaded plots. By contrast, the number of wild bees and hoverflies was independent from landscape structure and local plant invasion. Their numbers rather depended on the characteristics of the plant community. Interestingly, the high abundance of bumblebees in invaded plots during the flowering of *I. glandulifera* could replace the effects of mass-flowering crops seen prior to flowering of the invader. These results suggest that the dynamics of native pollinators is driven by plants with rewarding flowers dominating the landscape.

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