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**Impacts of the alien invasive  
*Rhododendron ponticum* L. on native  
plants, pollinators and their interaction**

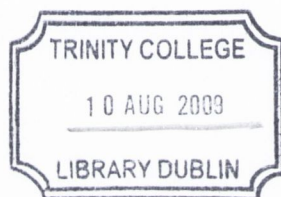
**Thesis submitted for the degree of Doctor of Philosophy (Ph.D.)**

**2009**

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# Declaration

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## Summary

This thesis addresses the impacts of an alien invasive plant, *Rhododendron ponticum* L., on pollination processes and population dynamics of native Irish plant and pollinator communities. Field studies were conducted in habitats in the West (Co. Galway) and East (Co. Wicklow and Co. Dublin).

For the plants, observational and manipulative field experiments were used to investigate both plant community responses and individual species responses of a native focal plant, *Digitalis purpurea* L., to *R. ponticum* invasion.

Flower visitation rates and nectar productivity of the entire flowering community were measured in uninvaded and invaded sites at early, peak and late flowering of *R. ponticum*. Although nectar productivity of *R. ponticum* was higher than in the majority of native plant species, there was little evidence for strong alien impacts on flower visitation of the plant community and of single species within the community. Individuals of some species revealed higher visitation rates in presence of the alien, but these differences were highly variable in space and time. Species that showed a response to alien invasion overlapped with the alien more in their pollinator assemblage and resembled the alien in high nectar production and large floral displays. They are considered to belong to the same functional group.

In a second investigation, potted *D. purpurea* plants and cut flowering branches of *R. ponticum* in water-filled buckets were used to mimic small single-species and mixed-species populations at 27 experimental field sites. Both alien and native abundances were manipulated in a replicated design. Flower visitation, nectar standing crop and seed set were measured at each field site. While native flower visitation was higher in initially larger *D. purpurea* populations, a further increase in native or alien plant abundance only marginally increased flower visitation. Conspecific individuals facilitated each others flower visitation, while alien abundance did not affect visitation. Fruit and seed set did not significantly differ for any population size treatment. *D. purpurea* is therefore assumed to display resilience towards *R. ponticum* invasion.

For a third study, pollinator visitation to *D. purpurea* was investigated in sites of different invasion levels and uninvaded sites over a two years period. Conspecific and alien pollen transfer and seed set were evaluated in the second year. Native *D. purpurea* showed a decrease in pollinator visitation in both years and lower conspecific pollen deposition on

native stigmata at high *R. ponticum* densities. In contrast, fruit and seed set were not affected by *R. ponticum* of different densities.

For the pollinators, two native Irish bumblebee species, *Bombus lucorum* L. and *B. pascuorum* Scopoti, that visit *R. ponticum* frequently were investigated.

First, a replicated laboratory feeding experiment was conducted using alien, native, two control and two differently concentrated aconitine intoxicated diets. Survival rate of individual *B. lucorum* worker bees was measured over a period of 28 days. Bee survival among the six feeding regimes did not significantly differ.

Second, mark-recapture data and DNA samples of workers of both bumblebee species were collected at two uninvaded and two invaded sites in two consecutive years. Molecular analyses were carried out using specific microsatellite primers and standard techniques.

Bumblebee abundance and colony numbers for each site and year were estimated. For both bee species, abundance estimates were subject to high deviation due to few captured bee individuals. Abundance did not significantly differ between invaded and uninvaded sites. In both bee species, colony numbers could be reproduced between years and were higher at invaded sites than at uninvaded sites. The invasion of *R. ponticum* seems to facilitate social pollinator populations in Irish habitats.

Findings are discussed in the broader context of scale dependency of impacts, community dynamics and impacts of alien plant invasion on pollination in general.

*Meinen Eltern in Liebe gewidmet*





# Table of content

|                                                                                                                              |             |
|------------------------------------------------------------------------------------------------------------------------------|-------------|
| <b>Declaration .....</b>                                                                                                     | <b>I</b>    |
| <b>Summary.....</b>                                                                                                          | <b>III</b>  |
| <b>Table of content.....</b>                                                                                                 | <b>VII</b>  |
| <b>Acknowledgements .....</b>                                                                                                | <b>X</b>    |
| <b>List of tables .....</b>                                                                                                  | <b>XII</b>  |
| <b>List of figures.....</b>                                                                                                  | <b>XIII</b> |
| <b>List of supplements .....</b>                                                                                             | <b>XIV</b>  |
| <b>List of plates .....</b>                                                                                                  | <b>XV</b>   |
| <b>1. General introduction .....</b>                                                                                         | <b>3</b>    |
| 1.1. Plants .....                                                                                                            | 4           |
| 1.1.1. Individual adaptation to pollination .....                                                                            | 4           |
| 1.1.2. Extrinsic effects on plant pollination .....                                                                          | 6           |
| 1.2. Pollinators.....                                                                                                        | 9           |
| 1.2.1. Adaptation to flower exploitation .....                                                                               | 10          |
| 1.2.2. Effects of extrinsic factors on flower visitation.....                                                                | 13          |
| 1.3. Plant-pollinator communities .....                                                                                      | 15          |
| 1.3.1. Interactions in multi-species communities .....                                                                       | 15          |
| 1.3.2. Structure of plant-pollinator networks .....                                                                          | 18          |
| 1.4. Alien species .....                                                                                                     | 19          |
| 1.4.1. General reflections .....                                                                                             | 19          |
| 1.4.2. Alien plant species in native pollination networks .....                                                              | 21          |
| 1.5. <i>Rhododendron ponticum</i> as a study system .....                                                                    | 24          |
| 1.6. Study objectives .....                                                                                                  | 26          |
| <b>2. Temporal and spatial variations of pollination services in invaded and uninvaded plant-pollinator communities.....</b> | <b>31</b>   |
| 2.1. Abstract .....                                                                                                          | 31          |
| 2.2. Introduction .....                                                                                                      | 32          |
| 2.3. Materials and methods .....                                                                                             | 34          |
| 2.3.1. Study sites .....                                                                                                     | 34          |
| 2.3.2. Insect visitation.....                                                                                                | 34          |
| 2.3.3. Nectar secretion and nectar standing crop .....                                                                       | 35          |
| 2.3.4. Data analyses .....                                                                                                   | 36          |
| 2.4. Results .....                                                                                                           | 38          |
| 2.4.1. Flower density of <i>R. ponticum</i> .....                                                                            | 38          |
| 2.4.2. Insect visitation.....                                                                                                | 39          |
| 2.4.3. Nectar secretion .....                                                                                                | 42          |



|                                                                                                                                                         |           |
|---------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| 2.4.4. Nectar standing crop.....                                                                                                                        | 43        |
| 2.4.5. Association and overlap of flower visitors.....                                                                                                  | 43        |
| 2.5. Discussion.....                                                                                                                                    | 47        |
| 2.5.1. Insect visitation.....                                                                                                                           | 49        |
| 2.5.2. Nectar secretion.....                                                                                                                            | 50        |
| 2.5.3. Nectar standing crop.....                                                                                                                        | 51        |
| 2.5.4. Overlap of flower visitors.....                                                                                                                  | 51        |
| 2.5.5. Consequences for plant and pollinator communities.....                                                                                           | 53        |
| 2.6. Conclusions.....                                                                                                                                   | 53        |
| <b>3. Intra- vs. interspecific pollination interactions between an alien invader and a native plant: insights from experimental manipulations .....</b> | <b>57</b> |
| 3.1. Abstract.....                                                                                                                                      | 57        |
| 3.2. Introduction.....                                                                                                                                  | 58        |
| 3.3. Materials and methods .....                                                                                                                        | 61        |
| 3.3.1. Study species .....                                                                                                                              | 61        |
| 3.3.2. Field sites.....                                                                                                                                 | 61        |
| 3.3.3. Study design .....                                                                                                                               | 63        |
| 3.3.4. Implementation.....                                                                                                                              | 63        |
| 3.3.5. Experimental observations .....                                                                                                                  | 64        |
| 3.3.6. Spontaneous autogamy in <i>D. purpurea</i> .....                                                                                                 | 66        |
| 3.3.7. Data analyses .....                                                                                                                              | 66        |
| 3.4. Results.....                                                                                                                                       | 68        |
| 3.4.1. Insect visitation.....                                                                                                                           | 68        |
| 3.4.2. Reproductive success .....                                                                                                                       | 72        |
| 3.4.3. Spontaneous autogamy in <i>D. purpurea</i> .....                                                                                                 | 72        |
| 3.5. Discussion.....                                                                                                                                    | 73        |
| 3.5.1. Intraspecific interactions among <i>D. purpurea</i> .....                                                                                        | 74        |
| 3.5.2. Interspecific interactions between <i>D. purpurea</i> and <i>R. ponticum</i> .....                                                               | 76        |
| 3.5.3. Pollinator response .....                                                                                                                        | 78        |
| 3.5.4. A matter of scale.....                                                                                                                           | 79        |
| 3.6. Conclusion .....                                                                                                                                   | 81        |
| <b>4. Density effects of an alien invader on native pollination processes in natural populations .....</b>                                              | <b>85</b> |
| 4.1. Abstract.....                                                                                                                                      | 85        |
| 4.2. Introduction.....                                                                                                                                  | 86        |
| 4.3. Materials and methods .....                                                                                                                        | 88        |
| 4.3.1. Study sites .....                                                                                                                                | 88        |
| 4.3.2. Insect visitation and nectar production.....                                                                                                     | 89        |
| 4.3.3. Pollen desposition.....                                                                                                                          | 90        |

|                                                                                                                          |            |
|--------------------------------------------------------------------------------------------------------------------------|------------|
| 4.3.4. Seed set .....                                                                                                    | 90         |
| 4.3.5. Statistical analysis.....                                                                                         | 90         |
| 4.4. Results .....                                                                                                       | 92         |
| 4.4.1. Field sites .....                                                                                                 | 92         |
| 4.4.2. Visitation rates .....                                                                                            | 93         |
| 4.4.3. Nectar standing crop .....                                                                                        | 93         |
| 4.4.4. Pollen desposition.....                                                                                           | 95         |
| 4.4.5. Seed set .....                                                                                                    | 98         |
| 4.5. Discussion .....                                                                                                    | 98         |
| 4.6. Conclusions .....                                                                                                   | 103        |
| <b>5. Impacts of invasive <i>Rhododendron ponticum</i> L. on survival and resource sharing in Irish bumblebees .....</b> | <b>107</b> |
| 5.1. Abstract .....                                                                                                      | 107        |
| 5.2. Introduction .....                                                                                                  | 108        |
| 5.3. Materials and methods .....                                                                                         | 111        |
| 5.3.1. Food suitability .....                                                                                            | 111        |
| 5.3.2. Resource usage .....                                                                                              | 113        |
| 5.4. Results .....                                                                                                       | 117        |
| 5.4.1. Food suitability .....                                                                                            | 117        |
| 5.4.2. Resource usage .....                                                                                              | 118        |
| 5.5. Discussion .....                                                                                                    | 123        |
| 5.5.1. Food suitability .....                                                                                            | 124        |
| 5.5.2. Resource usage .....                                                                                              | 127        |
| 5.6. Conclusions .....                                                                                                   | 129        |
| <b>6. General discussion .....</b>                                                                                       | <b>133</b> |
| 6.1. The direction and magnitude of alien impacts on native pollination .....                                            | 133        |
| 6.1.1. Impacts on plants .....                                                                                           | 133        |
| 6.1.2. Impacts on pollinators.....                                                                                       | 139        |
| 6.1.3. Impacts on pollination networks.....                                                                              | 141        |
| 6.2. Critical considerations and beyond .....                                                                            | 142        |
| 6.2.1. Methodology.....                                                                                                  | 142        |
| 6.2.2. Relevance of results and future direction .....                                                                   | 145        |
| 6.3. Concluding remarks .....                                                                                            | 148        |
| <b>7. References.....</b>                                                                                                | <b>153</b> |
| <b>8. Supplementary material.....</b>                                                                                    | <b>179</b> |



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## List of tables

|                                                                                                                                                                                                                                                                                                                                                            |     |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| <b>Table 1.1.</b> Impacts of alien invasive species on pollination of native co-flowering plant species. ....                                                                                                                                                                                                                                              | 22  |
| <b>Table 2.1.</b> Univariate ANOVA of overall pollinator visitation rates. Factor “Location” is subject to asymmetrical design.. ....                                                                                                                                                                                                                      | 39  |
| <b>Table 2.2.</b> ANOVA results of pollinator visitation rates. F-ratios with degrees of freedom are shown for main factors and their interactions in the case of a significant result at $\alpha$ -levels of 0.05 (*) or 0.01 (**). ....                                                                                                                  | 44  |
| <b>Table 3.1.</b> Experimental set up and spatial arrangement at single plots of the nine types of controls and treatments. ....                                                                                                                                                                                                                           | 62  |
| <b>Table 3.2.</b> Asymmetrical ANOVA model design according to Underwood (1997).....                                                                                                                                                                                                                                                                       | 67  |
| <b>Table 3.3.</b> ANOVA results for different variables of visitation and reproduction in experimental <i>D. purpurea</i> plants. ....                                                                                                                                                                                                                     | 70  |
| <b>Table 4.1.</b> Field site locations, their geographical position and elevation above sea level (in meters), and their percentage cover of alien <i>R. ponticum</i> . ....                                                                                                                                                                               | 89  |
| <b>Table 5.1.</b> Characteristics of paired sites with their invasion status ( <i>R. ponticum</i> coverage in %), geographical position, elevation level above sea level (in meters), habitat type description, native flowering plant species richness and the overlap in native flowering plant species between invaded and uninvaded sites (in %). .... | 113 |
| <b>Table 5.2.</b> Number of bees caught on two sampling days (first day: mark; second day: recapture) that were used to estimate bee abundance of the two bumblebee species <i>Bombus lucorum</i> and <i>B. pascuorum</i> at <i>R. ponticum</i> infested (R) and uninfested (N) sites at the two investigated locations. ....                              | 119 |



## List of figures

|                                                                                                                                                                                                                                                                                                                                                                                                |     |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| <b>Figure 1.1.</b> Native and alien habitat range of <i>Rhododendron ponticum</i> on the Eurasian continent.....                                                                                                                                                                                                                                                                               | 25  |
| <b>Figure 2.1</b> cont. Pollinator visitation rates of (A) native plant species overall and (B-G) individual native plant species.....                                                                                                                                                                                                                                                         | 41  |
| <b>Figure 2.2.</b> Mean nectar volume and sugar secretion rates of investigated plant species. Bars in grey correspond to <i>R. ponticum</i> . ....                                                                                                                                                                                                                                            | 45  |
| <b>Figure 2.3.</b> Three-dimensional ordination derived from Nonmetric Multidimensional scaling (NMS) of 87 plant species units based on the number and identity of their floral insect visitors.....                                                                                                                                                                                          | 46  |
| <b>Figure 2.4.</b> Dendrogram of hierarchical cluster analysis for pollinator visits per hour. ....                                                                                                                                                                                                                                                                                            | 48  |
| <b>Figure 3.1.</b> Mean square root transformed pollinator visitation rate (A), mean number of insect visitors per inflorescence (B), mean relative fruit set per inflorescence (C) and mean number of seeds per fruit (D) of <i>D. purpurea</i> plant individuals in response to controls, treatments and pollinator exclusion in the manipulative and spontaneous autogamy experiments. .... | 71  |
| <b>Figure 3.2.</b> Mean number of flowers visited in a single foraging bout on an inflorescence in relation to the number of open flowers on this inflorescence.....                                                                                                                                                                                                                           | 73  |
| <b>Figure 4.1.</b> Flower visitation rate (A) and number of insect visitors per inflorescence (B) of <i>D. purpurea</i> flower patches in habitats of different <i>R. ponticum</i> invasion levels in two consecutive years. ....                                                                                                                                                              | 94  |
| <b>Figure 4.2.</b> Correlation of transformed mean nectar standing crop and mean flower visitation rates. Bars show standard errors. ....                                                                                                                                                                                                                                                      | 95  |
| <b>Figure 4.3.</b> Numbers of conspecific pollen (A) and alien <i>R. ponticum</i> pollen in relation to total number of pollen grains (B) found on <i>D. purpurea</i> stigmas from different field sites that had not been or had been invaded by <i>R. ponticum</i> at different densities.....                                                                                               | 96  |
| <b>Figure 4.4.</b> Mean fruit set (in %) averaged across each field site (A) and estimated number of seeds per fruit averaged across <i>D. purpurea</i> inflorescences (B) from different field sites that had not been or had been invaded by <i>R. ponticum</i> at different densities.....                                                                                                  | 97  |
| <b>Figure 5.1.</b> Survival of <i>Bombus lucorum</i> workers over the period of 28 days in relation to their feeding regime.....                                                                                                                                                                                                                                                               | 119 |
| <b>Figure 5.2.</b> Bee abundance (number of worker bees) at investigated sites estimated with the Petersen estimator. ....                                                                                                                                                                                                                                                                     | 120 |
| <b>Figure 5.3.</b> Colony numbers of <i>B. lucorum</i> and <i>B. pascuorum</i> at different locations (A) and at invaded and uninvaded sites (B). ....                                                                                                                                                                                                                                         | 122 |
| <b>Figure 5.4.</b> Relationship between log-transformed bumblebee abundance and log-transformed colony numbers. ....                                                                                                                                                                                                                                                                           | 123 |

## List of supplements

|                                                                                                                                                                                                                                                            |     |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| <b>Supplement 1.</b> Table of locations with studied sites, if <i>Rhododendron ponticum</i> is present/absent, geographical position, elevation (“Elev” in m above sea level) and characteristics of sites. ....                                           | 179 |
| <b>Supplement 2.</b> Observed native plant species, their flowering time, if nectar standing crop and/or secretion rate was measured and where plant species were investigated (indicated by ‘x’).....                                                     | 180 |
| <b>Supplement 3.</b> Table of analyses design of pollinator visitation rates of individual native species.. ....                                                                                                                                           | 181 |
| <b>Supplement 4.</b> Table of Fligner-Policello test statistic for pairwise comparisons between each individual native plant species and <i>Rhododendron ponticum</i> . ....                                                                               | 182 |
| <b>Supplement 5.</b> Mean nectar standing crop of investigated flowering plant species.....                                                                                                                                                                | 183 |
| <b>Supplement 6.</b> Pollinator visitation rates <i>Rhododendron ponticum</i> . ....                                                                                                                                                                       | 183 |
| <b>Supplement 7.</b> Name and characteristics of study sites used for the manipulative experiment.....                                                                                                                                                     | 184 |
| <b>Supplement 8.</b> Analyses of bout length among different treatments.....                                                                                                                                                                               | 185 |
| <b>Supplement 9.</b> Taxonomic identity, absolute ( <i>N</i> ) and relative (%) frequency of pollinator species that visited <i>R. ponticum</i> and <i>D. purpurea</i> at invaded field sites and <i>D. purpurea</i> at uninvaded field sites in 2006..... | 186 |
| <b>Supplement 10.</b> Number of collected DNA samples per bumblebee species at each investigated site for each year of sampling.....                                                                                                                       | 187 |
| <b>Supplement 11.</b> Reaction cycle of performed polymerase chain reactions for different microsatellite primers.....                                                                                                                                     | 187 |
| <b>Supplement 12.</b> Example of a sister group sample distribution of <i>B. pascuorum</i> workers at the invaded Dublin site in 2005. ....                                                                                                                | 188 |



# List of plates

|                                                                                                                                                                                                                                                                                                                                                                                                  |            |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| <b>Plate 1.....</b>                                                                                                                                                                                                                                                                                                                                                                              | <b>2</b>   |
| Dense invasion of <i>Rhododendron ponticum</i> .                                                                                                                                                                                                                                                                                                                                                 |            |
| <b>Plate 2.....</b>                                                                                                                                                                                                                                                                                                                                                                              | <b>30</b>  |
| A. Example of an invaded field site with native plants and the alien <i>R. ponticum</i> growing in close vicinity.                                                                                                                                                                                                                                                                               |            |
| B. <i>Andrena</i> sp visiting <i>Rubus fruticosus</i> .                                                                                                                                                                                                                                                                                                                                          |            |
| C. <i>Vaccinium myrtillus</i> flowers bagged for nectar production measurements.                                                                                                                                                                                                                                                                                                                 |            |
| D. <i>Bombus pratorum</i> entering a <i>R. ponticum</i> flower.                                                                                                                                                                                                                                                                                                                                  |            |
| <b>Plate 3.....</b>                                                                                                                                                                                                                                                                                                                                                                              | <b>56</b>  |
| A. Example of an experimental plot with large <i>R. ponticum</i> population size and small <i>D. purpurea</i> population size.                                                                                                                                                                                                                                                                   |            |
| B. Growing <i>D. purpurea</i> plant individuals at Trintiy Botanic Gardens, Dublin.                                                                                                                                                                                                                                                                                                              |            |
| C. Flower observation at an experimental plot.                                                                                                                                                                                                                                                                                                                                                   |            |
| D. <i>Bombus pratorum</i> visiting <i>R. ponticum</i> flowers of cut branches.                                                                                                                                                                                                                                                                                                                   |            |
| E. <i>D. purpurea</i> plant individuals left for fruit maturation at Clontarf, Dublin.                                                                                                                                                                                                                                                                                                           |            |
| F. Potting <i>D. purpurea</i> plants at Trintiy Botanic Gardens, Dublin.                                                                                                                                                                                                                                                                                                                         |            |
| <b>Plate 4.....</b>                                                                                                                                                                                                                                                                                                                                                                              | <b>84</b>  |
| A. Densely invaded field site with <i>D. purpurea</i> growing close to the invasive alien.                                                                                                                                                                                                                                                                                                       |            |
| B. Bumblebee worker with pollen sacks visiting a <i>D. purpurea</i> flower.                                                                                                                                                                                                                                                                                                                      |            |
| <b>Plate 5.....</b>                                                                                                                                                                                                                                                                                                                                                                              | <b>106</b> |
| A. Marked <i>Bombus lucorum</i> worker visiting a <i>Rubus fruticosus</i> flower.                                                                                                                                                                                                                                                                                                                |            |
| B. Each <i>B. lucorum</i> worker was kept in an individual box and fed according to one of the six different feeding regims.                                                                                                                                                                                                                                                                     |            |
| C. <i>Bombus pascuorum</i> worker visiting a <i>Trifolium repens</i> inflorescence.                                                                                                                                                                                                                                                                                                              |            |
| D. Image of a polyacrylamid gel showing the two different standards (lane 1: 10 bp ladder; lane 13: M3 marker) and DNA fragments of 11 <i>B. pascuorum</i> individuals at microsatellite locus B96 (lane 2 to 12). Numbers refer to the number of base pairs of the fragments. Numbers shown in same colours refer to bee individuals that share the same genotype at this microsatellite locus. |            |
| E. Catching bees for pollen sack collection.                                                                                                                                                                                                                                                                                                                                                     |            |
| <b>Plate 6.....</b>                                                                                                                                                                                                                                                                                                                                                                              | <b>132</b> |
| Different <i>Rhododendron</i> species and hybrids planted for ornamental purposes and spread beside Howth golf course (Co. Dublin).                                                                                                                                                                                                                                                              |            |
| <b>Plate 7.....</b>                                                                                                                                                                                                                                                                                                                                                                              | <b>152</b> |
| Book page of Sprengel's <i>Das entdeckte Geheimniß der Natur im Bau und in der Befruchtung der Blumen</i> (photo from Pickhardt and Fluri, 2000).                                                                                                                                                                                                                                                |            |





# **Chapter 1**

“...Actually, science is something much broader:  
the acquisition of reliable knowledge about the world.” (Diamond, 2005)



Plate 1





## **1. General introduction**

Over millennia, human beings have observed and aimed to predict processes in their biotic environment and subsequently employed their expertise to utilise natural resources (Diamond, 2005). Thereby, knowledge about intra- and interspecific interactions between organisms was (and is) fundamental not only for agricultural and health practices, but also for explaining the evolution of life (Thompson, 1999). One fascinating class of interactions are mutualisms, in particular plant-pollinator mutualisms (Sachs and Simms, 2006). Their ecological and economical value as a key ecosystem function has long been recognised and studied (Sprengel, 1793, Darwin, 1876, Müller, 1883, Faegri and van der Pijl, 1971, Proctor et al., 1996). Pollination services not only shape and modify biotic communities (Thompson, 1982) and sustain the capacity of ecosystems (Lundberg and Moberg, 2003), they are also indispensable for 35% of the global crop production volume (Klein et al., 2007) and 84% of European crop species (Wilcock and Neiland, 2002). However, plant-pollinator mutualisms nowadays face major threats from anthropogenic activities (Kearns et al., 1998, Spira, 2001, but see Ghazoul, 2005a). In order to conserve them it is important to identify and manage factors that cause a decline in both wild flowering plants and their pollen vectors (Allen-Wardell et al., 1998, Cane and Tepedino, 2001).

A potential driver that may disrupt pollination mutualisms is the naturalisation and invasion of alien species (Traveset and Richardson, 2006). Due to the enormous problems linked to invasions, much research attention has been paid to the direct impacts and control of invasive species (Mooney et al., 2005). However, our knowledge about their effects on mutualism is still limited (Bjerknes et al., 2007). This thesis addresses the impacts of invasive plant species on native plants, pollinators and their interaction at an individual species and community level. It focuses on an alien invasive model species and different aspects of its invasiveness, which may influence its direct and indirect effects on co-flowering plant and pollinator species. In this introductory chapter, I provide some general information on the importance of, and adaptation to, plant-pollinator interactions from a plant, a pollinator and finally a community perspective. In addition I review characteristics of alien invasions and their significance for these mutualistic interactions, introduce the



reader to the ecology of the alien model plant species *Rhododendron ponticum* and finally highlight the objectives of my study.

### 1.1. Plants

#### 1.1.1. Individual adaptation to pollination

For plants, pollination is synonymous with dispersion of male gametophytes (pollen) among flowers which generates mating opportunities and is therefore essential for plant reproduction (Barrett and Harder, 1996). Angiosperms are sessile organisms and rely in most cases on a vector to transfer pollen in order to fertilise female ovaries (Traveset, 1999). Though the relative dependence on vectors for seed set can vary from complete independence in asexual and self-pollinated plants to facultative dependence in self-compatible species and obligate dependence in self-incompatible and dioecious species (Bond, 1994).

Plants are known to use abiotic agents such as wind or water for pollen transfer, but the majority (approx. 90%) of angiosperms rely on animal pollinators (Daily et al., 1997). Their abundance and efficiency regulates plant reproductive success, and the flexibility of animal vectors promotes the evolution of plant traits that manipulate pollinator behaviour (Zimmerman, 1988, Bronstein et al., 2006). Cross-fertilisation is generally the favoured mating strategy in plants (see Husband and Schemske, 1996, and Traveset, 1999, for explications and exceptions), and so plants are exposed to a strong selection on mechanisms that facilitate a reliable transfer of cross-pollen between sexual structures of flowers of the same species (Barrett and Harder, 1996). These mechanisms can be of a mechanical nature (Grant, 1994) such as the spatial separation of sexual organs within (herkogamy) or among flowers (heterostyly) (Barrett, 2002), or they can be phenological, for example temporal separation of female and male functions (dichogamy, Barrett, 2003) and synchronisation of flowering season to pollinator life cycles (Macior, 1971, Sih and Baltus, 1987, Rathcke, 1988a). Alternatively, a reduced floral display size, e.g. smaller or fewer inflorescences per plant may also result in minimised geitonogamous pollen transfer. In this respect, plant individuals face a trade-off between minimised potential selfing and maximised attractiveness to pollinators via a high number of flowers (Klinkhamer and de Jong, 1993).





These adaptations should maximise the quantity and quality of transferred pollen and in turn the number and genetic variability of seeds which is correlated to the long-term viability of plant populations (Groom, 1998, Wilcock and Neiland, 2002). However, studies suggest that qualitative and quantitative pollen limitation is common within plant populations of many species (see Burd, 1994, Ashman et al., 2004, Knight et al., 2005b for reviews). Both types of limitation are linked to individual, population and habitat characteristics (Knight et al., 2005b) (see 1.1.2 page 6). Quality limitation (i.e. increased self- or heterospecific pollen : cross-pollen ratio) is perceived as a chronic problem that particularly self-compatible plant species experience, whilst quantity limitation (i.e. a decreased receipt of adequate pollen) can be alleviated by an increase in total delivered pollen (Aizen and Harder, 2007).

Such increases in the amount of pollen may be accomplished by either attracting more pollinators (Johnson et al., 1995, Sahli and Conner, 2007, but see Jauker and Wolters, 2008) via producing more flowers or floral rewards or specialising on pollinators that transfer pollen more effectively (Stebbins, 1970, Schemske and Horvitz, 1984). Both of these evolutionary pathways depend on pollinator characteristics such as pollinator behaviour, life history and community structure. Abundance and assemblage of pollinator communities are often highly variable over the course of a plant species' flowering (Herrera, 1988) and between years (Roubik, 2001, Petanidou et al., 2008), and so it is not surprising that extreme specialisations (the second pathway) are rather the exception than the norm (Waser et al., 1996, but see also Johnson and Steiner, 2000). The rarity of tight parallel (pairwise) specialisations in plant communities corresponds to the asymmetrical nested properties that have been described for various plant-pollinator networks (Bascompte et al., 2003) (see paragraph 1.3 page 18).

Adaptation to generalist or specialist pollinators not only exerts different selection pressures on flower morphology and phenology but also on floral rewards (primarily pollen and nectar, in some cases oil, resin, food bodies, heat/shelter or deception of mating partners; Proctor et al., 1996). The quality and quantity of rewards is not exclusively correlated to species characteristics but also to abiotic factors such as temperature and humidity (Corbet et al., 1979). Still, plants with a more specialised pollination system show adjustments in flower reward production to the requirements of their specific



pollinator species or pollinator species group (Fenster et al., 2004). For example hummingbird pollinated plants tend to lack scent and secrete large volumes of low sugar-concentrated nectar (Pyke and Waser, 1981). In contrast, plant species that use generalist pollinators are subject to heterogeneous evolutionary pressures. Nevertheless they display adaptations, e.g. they produce secondary metabolites as repellents that reduce the amount of probed and robbed nectar and the number of probed flowers within plants whilst increasing generalist visitation rates (Kessler and Baldwin, 2007, Junker and Bluthgen, 2008). Such adaptations may reduce self-pollen transfer and increase pollen export (Irwin and Adler, 2008). Secondary metabolites in flower rewards may be also an adaptation of specialist plant species where illegitimate flower visitors are intoxicated or repelled to provide a maximum of rewards for specialist pollinating insects (Roulston and Cane, 2000).

### **1.1.2. Extrinsic effects on plant pollination**

Pollination of flowers is not only linked to the intrinsic (genetic or life history) factors of a plant described in paragraph 1.1.1 but also to extrinsic (environmental) factors (Herrera, 1996), namely population characteristics and habitat characteristics. The role of alien invasive plants in altering population and habitat characteristics and pollination processes will be addressed later (see paragraph 1.4.2, page 21).

### **Impact of population characteristics on pollination**

Populations can vary in their size, density and genetic structure. Each of these variables may influence individual pollination success (Ghazoul, 2005b), partly because pollinators can adjust their behaviour according to plant population sizes and/or density (Kunin, 1993, Chittka et al., 1997, Feldman, 2006, Ishii et al., 2008). This can promote the adaptation to a particular breeding system (e.g. self-compatibility is adaptive in rare species that occur at low densities over small and large ranges, Kunin and Shmida, 1997). Numerous studies have investigated the effects of these population attributes on animal pollination and plant reproductive success, particularly for small, scarce or genetically impoverished plant populations and rare species (e.g. van Treuren et al., 1994, Groom, 1998, Mustajärvi et al., 2001, Duffy and Stout, 2008; see Ghazoul, 2005b for a review). Animal pollinated plant populations are likely to experience Allee effects, that is plants within a population are





facilitated when co-occurring with other conspecific individuals (positive density dependence, Allee et al., 1949). This relationship might be reversed at very high densities or abundances when conspecific individuals compete for resources (negative density dependence, Rathcke, 1983). In a review, Ashman et al. (2004) pointed out that Allee effects are evoked by at least three mechanisms.

*Poor attraction of pollinators.* Plants growing at low abundance or density may jointly attract fewer pollinators so that their flowers may be less frequently visited, may receive less pollen and in turn set fewer seeds (e.g. Ågren, 1996, Bosch and Waser, 2001, Waite and Ågren, 2004, Spigler and Chang, 2008) (but see Mustajärvi et al., 2001). Hence, increasing density or abundance may facilitate flower visitation, but at high density or abundance a further increase in plant number may generate competition among conspecifics (Steven et al., 2003). Self-incompatible species are more sensitive to conspecific plant and pollinator variability and potential pollen limitation (Mustajärvi et al., 2001, Knight et al., 2005b). Thus, evolution of self-compatibility and autonomous selfing may be promoted if mating partners and/or pollinators are scarce or changeable (Fausto et al., 2001, Kalisz and Vogler, 2003).

*Heterospecific pollen transfer.* Conspecific plants at low density or abundance may suffer in mixed species communities from higher heterospecific – conspecific pollen ratios due to low flower constancy of generalist pollinators (Chittka et al., 1997, Groom, 1998, but see Waite and Ågren, 2004). Heterospecific pollen can prevent conspecific pollen germination in both self-compatible and self-incompatible flowers by clogging stigmata (Galen and Gregory, 1989), inducing stigma closure (Waser and Fugate, 1986) or releasing allelochemicals onto the stigmatic surface (pollen allelopathy, Sukhada and Jayachandra, 1980, Murphy and Aarssen, 1995).

*Geitonogamy.* Reduced plant abundance or density may increase the number of flowers that are visited within plant individuals or inflorescences (Field et al., 2005, Feldman, 2006), and thereby elevate the level of geitonogamous pollen transfer (de Jong et al., 1993) if plants have not adapted to this trade-off by reducing their floral display size (i.e. the number and/or size of inflorescences, Klinkhamer and de Jong, 1993). In self-incompatible plants, self-pollen can have comparable effects to heterospecific pollen (see last paragraph)





and might therefore disrupt cross-pollen performance (Waser and Price, 1991, Scribailo and Barrett, 1994). Furthermore, non-fertilising self-pollen is not exported to conspecific flowers, which reduces male fitness (Harder and Wilson, 1998). In contrast, self-compatible species can benefit from (geitonogamous) self-pollen transfer and set seed although this may also imply negative effects due to inbreeding depression (Husband and Schemske, 1996) and lost opportunities of outcrossed siring success (Harder and Barrett, 1995).

*Reduced genetic quality.* An additional fourth mechanism for Allee effects is linked to a decreased genetic variability of cross-pollen in populations of small size (Leimu et al., 2006). Even if plants in such populations receive a similar quantity of cross-pollen compared to plants in larger populations, pollen quality might be poorer due to reduced mate availability and higher genetic load (Campbell and Husband, 2007) which can translate into lower reproductive success (Paschke et al., 2002, Watanabe et al., 2003).

Obviously, pollination of plant individuals is not only shaped by the number and density of conspecific co-flowering plant individuals, but also by co-flowering heterospecific plants and pollinators and the size and assemblage of their communities. The direction of interactions between different species will be discussed in paragraph 1.3 (page 15).

### **Impact of habitat characteristics on pollination**

A habitat is primarily characterised by its abiotic conditions, e.g. sun exposure, humidity, soil depth and resource availability. These may considerably influence plant size, flower number and flower maturation and in turn reproductive success (Haig and Westoby, 1988). For example, sun exposure and soil depth can indirectly increase fruit production through a positive effect on flower number (Ågren et al., 2008). On the other hand, abiotic conditions might counteract pollination processes where low resource availability rather than pollination service limits reproductive success (Haig and Westoby, 1988, Jennersten and Nilsson, 1993).

Humans are the main cause for biological interferences of habitat characteristics.

Agricultural practice, grazing and pesticide application can all influence habitats and as a



result population characteristics of (wild and cultivated) plants and pollinators (Kearns et al., 1998). However, most prominent are fragmentation processes which may diminish plant (and pollinator) populations by reducing the size of a habitat, increasing spatial isolation between remnants and altering microclimatic conditions (Saunders et al., 1991). I have already described the restrictions to pollination in small and scarce populations in connection with Allee effects, and such constraints are similarly apparent in small habitat fragments (Aizen and Feinsinger, 1994a, Johnson et al., 2004). In addition to changes in population size and density, habitat fragmentation also increases relative isolation to other populations. Plant individuals may, with increasing isolation level, receive less pollen (Groom, 1998) and set fewer seeds (Groom, 1998, Steffan-Dewenter and Tscharnkte, 1999) even if populations among remnants are equal in size and density.

Apart from population and habitat related effects, plant pollination might be also ruled by stochastic variation in pollen receipt. Theories predict that pollen as an inexpensive investment in reproduction should be oversupplied in environments where males can adequately locate females and compete amongst themselves (Bateman's principle, Bateman, 1948). In habitats with chronically low densities of males and females and an imperfect control over pollen transfer, this strategy may require modifications (Burd, 1994). Alternatively, it is assumed that an oversupply of ovules relative to average pollen loads should be advantageous for plants in a stochastic pollination environment so that they may benefit from a 'jackpot' event, i.e. from receiving an unusually high amount of pollen or pollen of unusually high quality (bet-hedging strategy, Ashman et al., 2004).

## **1.2. Pollinators**

In contrast to plants, pollinators are highly mobile. Nevertheless, animal pollinators depend on the interaction with flowering plants for their reproduction, either directly by raising their offspring with floral resources (e.g. bees, some wasp and moth species), or indirectly by nourishing themselves with rewards (e.g. syrphids, most wasp species and vertebrates, Proctor et al., 1996). Although it is tempting to think of pollination as a truly mutualistic relationship, it is better described as a balanced mutual exploitation: flowers need pollen to be transferred among flowers and pollinators, particularly bees, need pollen as larval food. Thus, both parties compete for the same resource (Westerkamp, 1996). The majority of





pollinating species (60 – 70%) are insects (Richards, 1997), and I will restrict my further remarks to this pollinator group. Many of my examples will derive from research on bees since they are more widely studied than other taxa due to their great importance for pollination (Kevan and Baker, 1983) and the relatively easy handling of their individuals and populations in the field and laboratory (e.g. cf. Tasei, 2001, Carvell et al., 2008).

### 1.2.1. Adaptation to flower exploitation

Natural selection favours flower visitor individuals that maximise their foraging and feeding efficiency and as a result the number of offspring they procure (Levin, 1978). Maximisation can be achieved by optimising (a) the recognition of the best floral resources, (b) flower handling and (c) foraging patterns on these resources within space.

#### Resource recognition

Pollinators should choose flowers with the highest quality and quantity of rewards. However, flower discrimination can be only achieved if pollinators are sensitive to floral cues and remember them. Flower visiting insects are physiologically attuned to their habitat in vision, olfaction and taste (Kevan and Baker, 1983).

Vision is important for both diurnal insects (Dafni et al., 1997) and nocturnally active bees and moths (Brantjes, 1978, Warrant, 2007), though the latter primarily use scent for orientation. Colours and their intensity serve as floral attractants and reward guides (Kevan and Baker, 1984). For example, inexperienced hoverflies show high sensitivity to yellow colours which facilitates their search for (yellow) pollen on which they feed (Wacht et al., 1996). Flower shape, plant shape (flower height) and motion of floral parts are equally important as visual cues (reviewed by Dafni et al., 1997). Scent is another powerful cue for flower recognition. In combination with visual and gustatory cues, it can stimulate or repel pollinators in approaching and visiting flowers (Raguso, 2008b). Pollinators may even use own scent to mark visited flowers and avoid revisitation (Stout and Goulson, 2001). Secondary scent compounds in nectar may not only serve as odour attractants but may also reveal toxic properties when digested (Raguso, 2008a), and thereby influence flower visitation rate and visit duration of legitimate pollinators and in turn plant reproduction (Kessler et al., 2008). Even if these toxic compounds are not detected by pollinators via





their scent, they might change the taste of rewards. Gustatory cues can be used by pollinators to preferentially visit or avoid flowers of certain plant species (Gardener and Gillman, 2002). This may be another pathway for plants to filter reward robbers from appropriate pollinators (Johnson et al., 2006).

The ability to learn and memorise floral cues can vary considerably amongst pollinator populations and species (Raine and Chittka, 2008). Interestingly, learning ability is not only a function of taxonomic groups but also of life history traits. As an example, social bees learn better and faster to discriminate flowers by colour than do solitary bees (Dukas and Real, 1991).

### **Flower handling**

The method and speed of flower handling is primarily influenced by the complexity of the flower, morphological features of the pollinator and the latter's ability to recall handling skills.

Insect body shape, mass and tongue length often restrict access to concealed nectar and pollen (Harder, 1985, Suzuki et al., 2002). For example, insects might be too small to trigger the floral mechanisms of species with complex flowers (like *Cytisus scoparius*, Stout, 2000). Or their tongue might be too long to handle flowers with short corollas efficiently (Plowright and Plowright, 1997).

The complexity of a flower and difficulties in its handling may impact pollinator behaviour. If handling is too difficult or not economically efficient, insects may still use floral rewards but rob them (Stout et al., 2000, Zhang et al., 2007). In social species, robbing may even be socially transmitted to conspecifics as a new foraging technique (Leadbeater and Chittka, 2008). Larceny of rewards is obviously beneficial for the robbing individual, but can have negative effects on legitimate flower visitors and robbed plants (Irwin et al., 2001). Due to lower nectar volumes within flowers, legitimate pollinators might have to switch more often between flowers or even have to fly longer distances (Heinrich and Raven, 1972), which decreases foraging efficiency. However, if they do so, this can be beneficial for plant reproduction (Maloof, 2001, but Burkle et al., 2007).



If pollinators are skilled in processing complex flowers they may visit flowers legitimately but adjust their flower constancy behaviour. Flower constancy, i.e. a stronger visitation preference for one species of flower than expected by chance, is a common feature among flower visiting insects (Grant, 1950, Gross, 1992, Goulson et al., 1997, Goulson and Wright, 1998, Raine and Chittka, 2005). Yet, the reasons for flower constancy are controversial (Chittka et al., 1999). Hypotheses to explain constancy behaviour predict increased foraging efficiency in constant foragers to be a function of a decrease in handling time (interference hypothesis, Darwin, 1876, Gegear and Lavery, 1995, Goulson et al., 1997) or the limited cognitive ability of pollinators (Gegear and Thomson, 2004, Chittka and Raine, 2006). Constancy should increase with flower complexity (Lavery, 1994, Gegear and Lavery, 2005; but see Stout et al., 1998). Furthermore, pollinators should become specialists on the most abundant species, i.e. increase constancy, when resources are superabundant (Kunin and Iwasa, 1996, Chittka et al., 1997).

Learning capability of flower handling (like learning of floral cues, see above) is not only related to the taxonomical insect group but also to life history traits. For example, individuals of specialist pollinator species may be more efficient in learning to handle flowers of their specialty plant than generalist visitors are (Lavery and Plowright, 1988).

### **Spatial movement and foraging patterns**

Much modelling and research has been conducted on movement patterns of pollinators and the optimal spatial choice of flowers (Heinrich, 1975, Levin, 1978, Waddington and Heinrich, 1981, Harder, 1988). Most studies consider the energetics of movement patterns in relation to floral resources (spatial distribution and energetic value). However, there are also environmental factors such as wind force/direction (Eisikowitch, 1978, Woodell, 1978) and temperature (Heinrich, 1975, Corbet et al., 1993) that influence pollinator movement.

The quality and quantity of floral rewards within previously visited flower(s) influence pollinator foraging decision-making within inflorescences (Best and Bierzychudek, 1982) and plant patches (Pyke, 1978, Dukas and Real, 1993). Foraging distance within plant patches, which is usually not much further than to the nearest-neighbouring inflorescence,





increases when reward levels within visited flowers are low (reviewed by Waddington and Heinrich, 1981).

Some pollinator species are obligate specialists on one or very few plant species (oligolectic, Westrich and Schmidt, 1987, Groddeck et al., 2004). Thus, their individual movement patterns depend on the distribution of their speciality plants as well as their own foraging range, which in bees is positively related to body size (Gathmann and Tscharrntke, 2002, Greenleaf et al., 2007). Even generalist pollinators may show specialist foraging patterns if they behave flower constant (see above). Hence, movement patterns of generalists are linked to plant community attributes (Levin, 1978), are constrained by maximum economic foraging range (Cresswell et al., 2000), but also depend on plant species characters, such as the type of resources a plant provides. For example, foragers might need to mix near and distant pollen sources as well as visit various species if individuals of a single species only provide one component of food (i.e. only pollen but no nectar, Williams and Tepedino, 2003) or contain toxic compounds (Kessler and Baldwin, 2007).

### 1.2.2. Effects of extrinsic factors on flower visitation

As in plants (1.1.2 page 6), not only intrinsic factors, linked to individual and life history aspects of a pollinator, but also extrinsic factors, related to population and environmental attributes, determine a pollinator's foraging behaviour (Waddington, 1983, Stout, 1999).

Foraging behaviour can be constrained by the particular function of individuals. For example in many bees and wasps, feeding preferences and foraging habits differ between males that forage for self-provision and females that forage not only for self-provision but also for provision of larval offspring (Westrich, 1989, Groddeck et al., 2004). As an example, males of *Bombus pascuorum* visit clustered or compound flowers more often than workers of this species do (Prÿs-Jones and Corbet, 1991). In addition, sexual females might feed on different types and higher amounts of pollen than males to stimulate ovary development (e.g. in bumblebees, Alford, 1975, and hoverflies, Hickman et al., 1995).





Not only sex but also life cycle differences can be linked to particular foraging and flower visitation patterns. For example, solitary bees forage to provision single brood cells without accumulating excess stock, whilst eusocial bees have higher resource demands due to their long life cycle and store food in their nests to flexibly feed themselves and their offspring (Eickwort and Ginsberg, 1980, Prÿs-Jones and Corbet, 1991). Eusocial bees can sensitively evaluate this stored food and attune their individual foraging behaviour by selectively collecting a certain reward in response to its depletion rate in the nest (Landry et al., 2000). Selective reward foraging in eusocial species is further facilitated by bees' capability to communicate the location of suitable floral resources to members of the nest (Lindauer, 1971, Biesmeijer and Ermers, 1999).

Habitat characteristics shape the spatial distribution of pollinators. This distribution in relation to plant patches or plant populations in the landscape might have high impacts on flower visitation. For example, pollinators might visit plants at the edge of a monocultural stand of pollinated crops more frequently than individuals in the centre, due to a closer vicinity to their nesting sites (Cunningham and Arthur, 2008).

Distribution and abundance of pollinating insects in a habitat is food resource dependent and restricted by suitable substrates and materials for nest construction (Westrich, 1996, Potts et al., 2003). It is believed that conspecific individuals not only compete for food resources and adapt their behaviour in accordance (Thomson et al., 1987, Fontaine et al., 2008), but they also fight for nest sites (Prÿs-Jones and Corbet, 1991, Zobel, 2003). The distribution of conspecific nests within a habitat may be also related to nesting habits of other animals, if e.g. bees re-use nests of rodents (Harder, 1986). Besides such material based constraints, climatic condition, e.g. exposure to sunlight, is another determinant of nesting preference (Fussell and Corbet, 1992). These habitat requirements and therefore pollinator populations themselves may be disrupted by anthropogenic activities, such as habitat fragmentation (Brosi et al., 2008), agricultural practice (Schweiger et al., 2005, Carvell et al., 2006, Sjödin et al., 2008) or alien species introduction (Stout and Morales, submitted).



### **1.3. Plant-pollinator communities**

Under natural conditions plant and pollinator populations do not occur in isolation but in a mixed species environment, which enables not only (competitive and facilitative) interactions among *conspecific* plants and pollinators, as illustrated in the above paragraphs, but also among *heterospecific* individuals. Competition and facilitation among plant species and among insect species are central concepts to explain evolution, structure, assemblage and size of their communities (MacArthur, 1972, Wiens, 1977, Brooker et al., 2008). Equally important are competitive and facilitative interactions between plant and insect communities (Thompson, 1982, 1999, Strauss and Irwin, 2004, Bronstein et al., 2006), in particular plant and pollinator communities (Feinsinger, 1987, Potts et al., 2003, Sargent and Ackerly, 2008).

#### **1.3.1. Interactions in multi-species communities**

Factors that shape flower visitation in conspecific populations (such as morphology, density, abundance, spatial distribution, see previous paragraphs) can also influence visitation patterns in a multi-species community. Species might occur in the same habitat but they may use different interaction partners. In this case we expect little or no effects of these species on each others pollination (Goodell, 2008) as long as species do not alter other traits that might be important for pollination, e.g. density. If different species in the community use the same mutualistic partners, indirect interactions between them are likely to occur. These interactions may be neutral, facilitative or competitive (Rathcke, 1983).

Pollinators (bees) are sensitive to the quality and quantity of rewards and prefer to forage on sources that provide higher quality or quantity pollen (Robertson et al., 1999) or nectar (Corbet et al., 1984, Biesmeijer and Ermers, 1999, Cartar, 2004). If individuals of a plant species in the community produce rewards of better quality or higher quantity they may lure flower visitors which can result in fewer flower visits in individuals of other species (Rathcke, 1988b, Brown et al., 2002). Competition for pollinator visitation however might not affect seed set if other mechanisms like long-lived flowers, self-pollination or diverse pollinator assemblages ensure adequate pollen transfer (Rathcke, 1988b). If visitation rate is not altered by the presence of other species, plants might still experience a decline in reproductive success if pollinators behave as generalists, visit flowers of different species





indiscriminately and deposit disruptive heterospecific pollen (Waser and Fugate, 1986, Galen and Gregory, 1989) or insufficient amounts of conspecific pollen (Campbell and Motten, 1985, Caruso and Alfaro, 2000, Bell et al., 2005) during foraging bouts.

Alternatively, individuals of different plant species might facilitate each others visitation. They may jointly attract and share more (usually highly mobile) pollinators than they could lure on their own (Moeller, 2004, Ghazoul, 2006). The benefits of this interaction might be asymmetrical if plants that do not produce sufficient rewards receive more visits in the presence of other more rewarding species but not vice versa (magnet species effect, Laverty, 1992, Johnson et al., 2003). Facilitation might also occur if plant species bloom in succession and support long-lived pollinators, i.e. enhance pollinator abundance over time (Waser and Real, 1979). Facilitated visitation may not translate into facilitated pollination if conspecific pollen loss and heterospecific pollen deposition is high and significant for seed production (Morales and Traveset, 2008). Legitimate pollen transfer might be promoted by different floral morphology of the co-flowering species (Caruso, 2002), differences in flower handling of pollinators (Corbet et al., 1981), attraction of a greater diversity of pollinators (Bond, 1994) or temporal segregation of rewards released over the day (e.g. pollen, Stone et al., 1998).

Competition and facilitation for pollination among heterospecific individuals may result in character displacement such as a shift in flowering time (Pojar, 1974, Kevan and Baker, 1984), or in flower morphology (Caruso, 2002) to avert disruptive or promote beneficial interactions. However, in most systems, selective pressures are diffuse due to high temporal variability in interactions as well as in plant and pollinator communities themselves (Rathcke, 1983).

Pollinators may switch between flowers of different species, but they may prefer certain flower colours, shapes or sizes. For example, species with narrower and deeper flowers may restrict access to concealed rewards and therefore receive fewer pollinator species than plant species with openly accessible flowers (Stang et al., 2006). In contrast, pollinators might develop a preference for complex flowers and visit them more frequently (Stout et al., 1998). Visitation preference of pollinators for flowers of a particular plant species might be intensified if this plant is more abundant (Harder, 1985). Likewise, the





abundance of pollinators can influence their plant species preference. Pollinators may expand their diet and become more generalist if competition for floral resources among them increases due to higher pollinator densities (Fontaine et al., 2008).

Increased absolute flower density or abundance in interactive mixed-species plant neighbourhoods may facilitate pollinator foraging activity and flower visitation (Carvell et al., 2004, Hegland and Boeke, 2006). However, at very high flower density or abundance, pollinator foraging might not accelerate any more due to limits in pollinator populations (e.g. due to nest site limitation), which can result in competition for pollinators among plant individuals of different species (Ghazoul, 2006). This finding is congruent to models (Rathcke, 1983) and observed density and abundance effects in monospecific populations (see paragraph 1.1.2, page 6).

Furthermore, the relative abundance or density of a plant species within the community can also affect pollinator behaviour (Stout et al., 1998). If one species' population becomes very abundant or dense, pollinators might behave in a more flower constant manner towards this species, either as a direct consequence of its density or due to behavioural changes in the pollinator (Goulson, 1994) (see also 'Flower constancy', page 12). The resulting higher pollen loss of the rarer species (Stout et al., 1998) and increased heterospecific pollen deposition in the rarer species (Nienhuis, 2009) may disrupt its reproduction. Nevertheless, high density of one rewarding plant species may promote pollinator abundance, even if resources are restricted to a short period of time like in mass-flowering arable crops (Westphal et al., 2003). A higher abundance of pollinators can in turn facilitate pollination of other co- or subsequently flowering plants in the community.

In addition, pollinator abundance is also a function of available nesting substrates. Competition for nest sites not only affects individuals within monospecific populations (page 14), but also occurs among individuals of different species and can result in the displacement of weaker competitors (Inoue et al., 2008).

Nests are often spatially aggregated (Harder, 1986). Since pollinators are limited in their foraging range, the encounter probability between pollinators and plants is not equal across space and some plant individuals may be less frequently visited than would be expected for a well-mixed community without aggregation (Morales and Vázquez, 2008).



### **1.3.2. Structure of plant-pollinator networks**

Knowledge about the factors that influence flower visitation among co-occurring plant and pollinator species helps to characterise the general structure of plant-pollinator communities (or networks). A central aspect of network structure is each species' generalisation. In the majority of plant-pollinator communities, pairwise specialisation between a single pollinator species and a single plant species seem to be the exception rather than the rule (Waser et al., 1996, Memmott, 1999), though specialisation might be overlooked if it is based on functional groups (Fenster et al., 2004). Even if a plant is visited by many animal species or a pollinator visits various plant species, these species might be phylogenetically connected or share the same life history traits (Johnson and Steiner, 2000). In this case, we cannot assume random visitation patterns among species, i.e. generalisation, but complementarity between functional groups of plants and pollinators; this assumption is substantiated by findings that higher functional pollinator diversity can enhance plant species richness (Fontaine et al., 2006). In this context, the concept of flower syndromes, i.e. specific traits of pollinators and plants that bias their interaction, is still a useful tool to explore and predict visitation patterns (Fenster et al., 2004).

Phenotypic complementarity of functional groups can generate highly nested networks, i.e. networks that consist of a core group of generalist species, which interact with their counterpart generalists but also with specialists, however specialist species interact only with generalists (Rezende et al., 2007a). Nestedness has been agreed to be a major property of the structure of plant-pollinator communities (Petanidou and Ellis, 1993, Bascompte et al., 2003, Lewinsohn et al., 2006, Medan et al., 2007). Interaction patterns between species seem to be linked to phylogenetic history, i.e. phylogenetically related pollinator species tend to interact with a similar set of plant species and vice versa (Rezende et al., 2007b). Recently, this network approach has been broadened by the concept of modularity, which again incorporates the idea of complementary functional groups that form modules and serve as coevolutionary units (Olesen et al., 2008). In addition, modules are linked within and among each other by highly generalist key species (network hubs), mainly social bees, large solitary bees and few diptera (Olesen et al., 2007). These connectors play a critical role in maintaining the network structure and stability. In case of their extinction, the entire network may fragment into isolated modules with long-term impacts on network functioning and biodiversity (Olesen et al., 2007).





## **1.4. Alien species**

### **1.4.1. General reflections**

Alien species, also termed non-indigenous, non-native, introduced or exotic species (Colautti and MacIsaac, 2004), are species that have been deliberately or unintentionally transferred and established in areas outside their previous geographical range as a consequence of human activity (Richardson et al., 2000b, Mack and Erneberg, 2002). Such species can become naturalised, i.e. permanently establish self-sustaining populations, and finally invasive, i.e. they spread, proliferate and persist as the local dominant (Levine et al., 2004). Although only a small fraction of alien species become invasive, these can have major impacts on populations and communities of the native biota and have been recognised as a major component of global change and a threat to biodiversity (Wilcove et al., 1998, Mack et al., 2000). Health risks and economic costs that are associated with invasions can be high; estimates for the United States and European countries such as Germany amount to \$120 billion and €160 million per year, respectively (Reinhardt et al., 2003, Pimentel et al., 2005), although such estimates are not always reliable and actual cost could be even higher (Born et al., 2005).

Mediated by human transport, alien species and in particular alien plants have by now reached every biome including Antarctica (Lonsdale, 1999, Frenot et al., 2005). However, their spread and density within an area depends on habitat traits (e.g. high disturbance can enhance invasibility, Bellingham et al., 2005, Lambdon et al., 2008; but see Gilbert and Lechowicz, 2005) and biotic processes (Theoharides and Dukes, 2007) such as facilitative interactions and mutualisms (Richardson et al., 2000a, Bulleri et al., 2008). Furthermore, specific plant traits can increase the likelihood of a species' invasiveness (Milbau and Stout, 2008). The ability to use multiple reproductive strategies, and the production of fruits with multiple small seeds that are well adapted for dispersal, maximise the number of propagules and therefore the ability to spread (Sakai et al., 2001). In addition, invaders often show a superior ability than native plants to exploit resources and to tolerate environmental stress (Baker, 1965, but see Daehler, 2003). This could facilitate their adaptation and resilience towards climate change (Chornesky and Randall, 2003, Weltzin et al., 2003). The success of alien plant species in invading a new habitat may be also linked to the absence of factors that usually suppress their fitness, e.g. fungal and viral





pathogens (enemy release hypothesis, Mitchell and Power, 2003, Callaway et al., 2004, but see Colautti et al., 2004).

Invasive plant species can directly and indirectly influence co-occurring plant species (Levine et al., 2003). Most of the literature on alien invasive species report negative impacts of aliens on natives. Invaders have been shown to reduce growth and reproductive success of native plants directly by competing for light and soil moisture (Miller and Gorchov, 2004) and changing nutrient cycles in the soil (Ehrenfeld, 2003, Chapuis-Lardy et al., 2006), and indirectly by disrupting interactions between natives and their mutualistic partners (Traveset and Richardson, 2006). For example, garlic mustards (*Alliaria petiolata*) can reduce growth of native tree seedlings that depend on mutualistic arbuscular mycorrhizal fungi by phytochemically suppressing the colonisation of fungi (Stinson et al., 2006). Competition and dominance of invaders may therefore increase the likelihood that already rare local species suffer extinction from stochastic processes (Davis, 2003) although competition from alien invasions rarely cause global species extinction (Gurevitch and Padilla, 2004, Sax et al., 2007). However, in recent years, a growing body of studies caused a shift in conceptual theories on invasion processes by reporting also facilitative effects of invasive species on natives (reviewed by Rodriguez, 2006).

The intensity of both facilitative and competitive impacts of invasive plants are positively related to their range and abundance/density and may act on an individual, population, community and/or ecosystem level (Parker et al., 1999). These variables however are also important measurements for impacts of native plants on other community members within a habitat (see 1.3.1, 15). Such comparability in evaluating alien and native impacts makes it clear that we should not look at alien invasive species as ‘aliens’ but solely as additional species within a community, which display certain traits and set specific processes in motion although these processes might be far more rapid than those in purely native communities (Sakai et al., 2001). From a scientific perspective (and apart from any economic impacts), alien invasive species are valuable study objects for investigating evolutionary and ecological responses in populations and communities (Strauss et al., 2006). They make excellent models for competition-facilitation experiments although these experiments might be constrained by imperfect replication opportunities (Sax et al., 2007).



### **1.4.2. Alien plant species in native pollination networks**

Although alien plants are supposedly more likely to be self-compatible and less affected by pollinator limitation due to asexual propagation and self-pollination (Baker, 1965, but see Milbau and Stout, 2008), many alien plants require or benefit from visitation and pollen transfer by pollen vectors and as a result produce more seeds (Parker, 1997, Richardson et al., 2000a, Stout, 2007b). In most cases, these pollen vectors are animal pollinators (Goodell, 2008). Some studies found that alien invasive species can suffer from pollinator limitation (Larson et al., 2002, Parker and Haubensak, 2002), but usually alien plant species are frequently visited and well integrated into the native plant-pollinator network (Waring et al., 1993, Memmott and Waser, 2002, Olesen et al., 2002, Morales and Aizen, 2006, Lopezaraiza-Mikel et al., 2007, Bartomeus et al., 2008b). Even species that are associated with specialised pollinators in their home range (like *Fuchsia magellanica* with one pollinating hummingbird and one flycatcher species, Traveset et al., 1998) may be pollinated by generalists in the non-native habitat (in the case of *F. magellanica* by bumblebees; C. Nienhuis, unpublished data). Direct alien plant – native pollinator and indirect alien plant – native plant pollination interactions are therefore likely.

The mechanisms of interaction that have been proposed by previous studies on native pollination in invaded plant-pollinator communities (e.g. Moragues and Traveset, 2005, Lopezaraiza-Mikel et al., 2007, Jakobsson et al., 2008, Muñoz and Cavieres, 2008), match the ones for native plant-plant and plant-pollinator interactions in a community (see 1.3.1 page 15). They are specified via general characteristics of invasive plants such as high plant density and abundance, superabundant flowering and copious nectar production. It has been further proposed that native plant species should be particularly prone to facilitative or competitive effects that overlap widely (>65%) with the alien in their pollinators (Goodell, 2008).

With only 17 published papers on impacts of nine invasive plant species on native plant pollination (Table 1.1), generalisations are difficult to draw, especially because different studies investigated different aspects of pollination (visitation rate, pollen transfer and deposition and/or seed set). Half of the studies looked at pair-wise indirect interactions between one invasive and one native plant species. This provides a very limited fragment of the whole co-flowering plant community, and it is not surprising that most of these



| Reference                                        | Alien invasive species                                                | Focal native species              | Impact on       |                             |                                                              |                    |
|--------------------------------------------------|-----------------------------------------------------------------------|-----------------------------------|-----------------|-----------------------------|--------------------------------------------------------------|--------------------|
|                                                  |                                                                       |                                   | Visitation rate | Conspecific pollen transfer | Invasive pollen ÷ total pollen deposition on native stigmata | Fruit or seed set  |
| Aigner, 2004                                     | <i>Cakile maritima</i> , <i>Carpobrotus</i> spp.                      | <i>Dithyrea maritima</i>          | NS              |                             |                                                              | NS <sup>1</sup>    |
| Moragues and Traveset, 2005                      | <i>Carpobrotus edulis</i> and <i>Carpobrotus affine acinaciformis</i> | <i>Lotus cytisoides</i>           | NS/–            |                             | < 1%                                                         |                    |
|                                                  |                                                                       | <i>Anthyllis cytisoides</i>       | +/+             |                             |                                                              |                    |
|                                                  |                                                                       | <i>Cistus salviifolius</i>        | NS/+            |                             | 1%                                                           | NS <sup>2</sup>    |
|                                                  |                                                                       | <i>Cistus monspeliensis</i>       | NS/NS           |                             |                                                              | NS <sup>2</sup>    |
| Bartomeus et al., 2008a, Bartomeus et al., 2008b | <i>Opuntia stricta</i>                                                | Co-flowering community            | –               |                             |                                                              |                    |
|                                                  | <i>Carpobrotus affine acinaciformis</i>                               | Co-flowering community            | +               |                             | 65% of stigmata with 0%,<br>31% of stigmata with < 20%       |                    |
| Jakobsson et al., 2008                           | <i>Carpobrotus edulis</i> and <i>Carpobrotus affine acinaciformis</i> | <i>Asphodelus aestivus</i>        |                 |                             | 3%                                                           | NS <sup>2</sup>    |
|                                                  |                                                                       | <i>Dorycnium hirsutum</i>         |                 |                             | < 1%                                                         | NS <sup>2</sup>    |
|                                                  |                                                                       | <i>Helichrysum stoechas</i>       |                 |                             | < 1%                                                         | – <sup>2</sup>     |
|                                                  |                                                                       |                                   |                 |                             |                                                              |                    |
| Ghazoul, 2002, Ghazoul 2004                      | <i>Chromolaena odorata</i>                                            | <i>Dipterocarpus obtusifolius</i> | –               | –                           | 81.5% of flowers with 0%                                     | NS                 |
| Larson et al., 2006                              | <i>Euphorbia esula</i>                                                | <i>Campanula rotundifolia</i>     | +/–             | NS                          | < 15%                                                        |                    |
|                                                  |                                                                       | <i>Linum lewisii</i>              | NS/–            | –/–                         | < 10%/ < 10%                                                 |                    |
|                                                  |                                                                       | <i>Oxytropis lambertii</i>        | NS/NS           | –/–                         | < 1%/ < 1%                                                   |                    |
|                                                  |                                                                       | <i>Calylophus serrulatus</i>      |                 | –                           | < 10%                                                        |                    |
|                                                  |                                                                       | <i>Sphaeralcea coccinea</i>       |                 | –                           | < 20%                                                        |                    |
|                                                  |                                                                       | <i>Vicia americana</i>            |                 | NS                          | < 1%                                                         |                    |
|                                                  |                                                                       |                                   |                 |                             |                                                              |                    |
| Chittka and Schürkens, 2001                      | <i>Impatiens glandulifera</i>                                         | <i>Stachys palustris</i>          | –               |                             |                                                              | – <sup>3</sup>     |
| Lopezaraiza-Mikel 2006                           | <i>Impatiens glandulifera</i>                                         | <i>Stachys palustris</i>          | NS/+NS          |                             |                                                              |                    |
| Lopezaraiza-Mikel et al., 2007                   |                                                                       | Co-flowering community            | +               |                             |                                                              |                    |
| Nienhuis et al., 2009                            | <i>Impatiens glandulifera</i>                                         | <i>Epilobium hirsutum</i> ,       | NS              |                             |                                                              |                    |
|                                                  |                                                                       | <i>Filipendula ulmaria</i> and    |                 |                             |                                                              |                    |
|                                                  |                                                                       | <i>Stachys palustris</i>          |                 |                             |                                                              |                    |
| Grabas and Laverty, 1999                         | <i>Lythrum salicaria</i>                                              | <i>Impatiens capensis</i>         | no visits       | NS/NS                       | 0%/ 0%                                                       | –/NS <sup>3</sup>  |
|                                                  |                                                                       | <i>Eupatorium perfoliatum</i>     |                 |                             |                                                              | NS/– <sup>3</sup>  |
|                                                  |                                                                       | <i>Eupatorium maculatum</i>       | NS/+            |                             | < 15 grains/ < 30 grains                                     | NS/NS <sup>3</sup> |
| Brown and Mitchell, 2001                         | <i>Lythrum salicaria</i>                                              | <i>Lythrum alatum</i>             |                 |                             |                                                              | – <sup>2</sup>     |
| Brown et al., 2002                               | <i>Lythrum salicaria</i>                                              | <i>Lythrum alatum</i>             | –/–             |                             |                                                              | –/– <sup>3</sup>   |
| Totland et al., 2006                             | <i>Phacelia tanacetifolia</i>                                         | <i>Melampyrum pratense</i>        | –               |                             |                                                              | NS <sup>3</sup>    |
| Jones, 2004                                      | <i>Taraxacum officinale</i>                                           | <i>Delphinium nuttallianum</i>    |                 |                             |                                                              | NS <sup>1</sup>    |
| Muñoz and Cavieres, 2008                         | <i>Taraxacum officinale</i>                                           | <i>Hypochaeris thrincioides</i>   | NS/–            |                             |                                                              | +/– <sup>3</sup>   |
|                                                  |                                                                       | <i>Perezia carthamoides</i>       | NS/–            |                             |                                                              | NS/– <sup>3</sup>  |





Table 1.1 (previous page). Impacts of alien invasive species on pollination of native co-flowering plant species. Results obtained from different years (or sites – Grabas and Lavery, 1999; or densities – Lopezaraiza-Mikel 2006; Muñoz and Cavieres, 2008) are separated by (/). ‘+’ = significant facilitative effect, ‘-’ = significant competitive effect, ‘NS’ = non-significant effect on the particular aspect of pollination interaction. <sup>1</sup>Removal of alien, <sup>2</sup>Hand-pollination experiment, <sup>3</sup>Experimental introduction or manipulation.

studies (e.g. Brown and Mitchell, 2001, Chittka and Schürkens, 2001, Brown et al., 2002, Ghazoul, 2004) found negative impacts on native plant pollination which might indicate a bias towards native study species with particular traits that are more sensitive for interactions (e.g. similar flower structure). Only three studies (Lopezaraiza-Mikel et al., 2007, Bartomeus et al., 2008a, Bartomeus et al., 2008b) integrated the entire native community in their investigation. Although there is also evidence for competitive impacts of one alien species in these network studies, facilitative pollination effects on native co-flowering plant species have been detected in the other two invaders. The detected contrast in impact of e.g. *Impatiens glandulifera* on a single native species or on the community level (Chittka and Schürkens, 2001, vs. Lopezaraiza-Mikel et al., 2007, Nienhuis et al., 2009) suggests that effects of aliens do – unsurprisingly considering the high variability in pollinator communities – vary among populations and locations. Both individual and community level should be investigated if meaningful interpretations are sought. Factors such as density or abundance of the invader have been much neglected (but see Brown et al., 2002, Muñoz and Cavieres, 2008) although these population traits are particularly pronounced for invasive populations.

Impacts of alien plants on native pollinators have only been recorded indirectly in the above mentioned studies by measuring their visitation frequency to native and alien plants (see also Waring et al., 1993). Alternatively, invasive species have been stated as important food plants without quantifying the alien’s relative importance in relation to native plants (e.g. Valentine, 1978, Starý and Tkalců, 1998, Schürkens and Chittka, 2001). So far very few studies have quantified the impact of alien plants on pollinator abundance, population size or diversity in communities (Goodell, 2003, Morón et al., 2009, Nienhuis et al., 2009). They have found neutral or negative effects of invasive plants on pollinator abundance and species richness partly because study sites were very heavily invaded and did not supply other resources before or after alien flowering (Morón et al., 2009). Yet, the role of alien rewards on pollinator individuals and in turn on their population growth awaits elucidation.





Again, relative density and flowering period might be crucial for the direction of impact that a certain invader at a certain location performs on pollinators.

### 1.5. *Rhododendron ponticum* as a study system

The choice of a study object is crucial and not easy considering the numerous invasive species in Europe. The alien *Rhododendron ponticum* L is one of the “Top 20 weeds” that requires biological control (Sheppard et al., 2006) and is a severe invader in Britain and Ireland (Dehnen-Schmutz and Williamson, 2006). It is a member of the Ericaceae family and shares the genus *Rhododendron* with approx. 1000 other species worldwide. As an evergreen shrub, *R. ponticum* reaches an average height of 3 m (Erfmeier and Bruelheide, 2004) and presents a large floral display of rewarding showy purple flowers (Stout, 2007a). The five-lobed generalist flowers are incompletely protandrous, which allows self-fertilisation in this self-compatible species. However, autogamous self-pollination is partially prevented by spatial separation of anthers and stigma during stigma receptivity (Mejías et al., 2002). Outcross pollen results in the highest reproductive success if compared with self- or geitonogamous pollen (Stout, 2007b). *R. ponticum* is therefore a potential candidate for pollinator limitation, although in most Irish populations flowers are frequently visited (Stout et al., 2006). The composition of flower visitors is different from that in *R. ponticum*’s home range though where it is visited by fewer Diptera and more solitary bee species than in Ireland (Stout et al., 2006). Differences in visitor assemblage are certainly a function of the reduced number of insect species that can be found in Ireland in comparison with Britain or the European continent, in particular with Southern Europe (e.g. cf. Westrich, 1989, Fitzpatrick et al., 2006).

Although *R. ponticum* was much more widely distributed in Europe during the Pleistocene period and interglacial pollen proves its presence in Ireland (Fig. 1.1), it retreated to more southern parts of Europe during the cold glaciation stages (Coxon and Waldren, 1995). Its native range nowadays includes the Black Sea region (Western Caucasus and Eastern Balkan region as well as Asia-minor; *R. ponticum* ssp. *ponticum*) and some southern parts of the Iberian peninsula (*R. ponticum* ssp. *baeticum*, Cross, 1975) (Fig. 1.1). Individuals were first introduced to Britain in 1763 as ornamental plants (Plate 6) and game shelter (Cross, 1975). They originated from Spanish populations, and Milne and Abbott (2000)

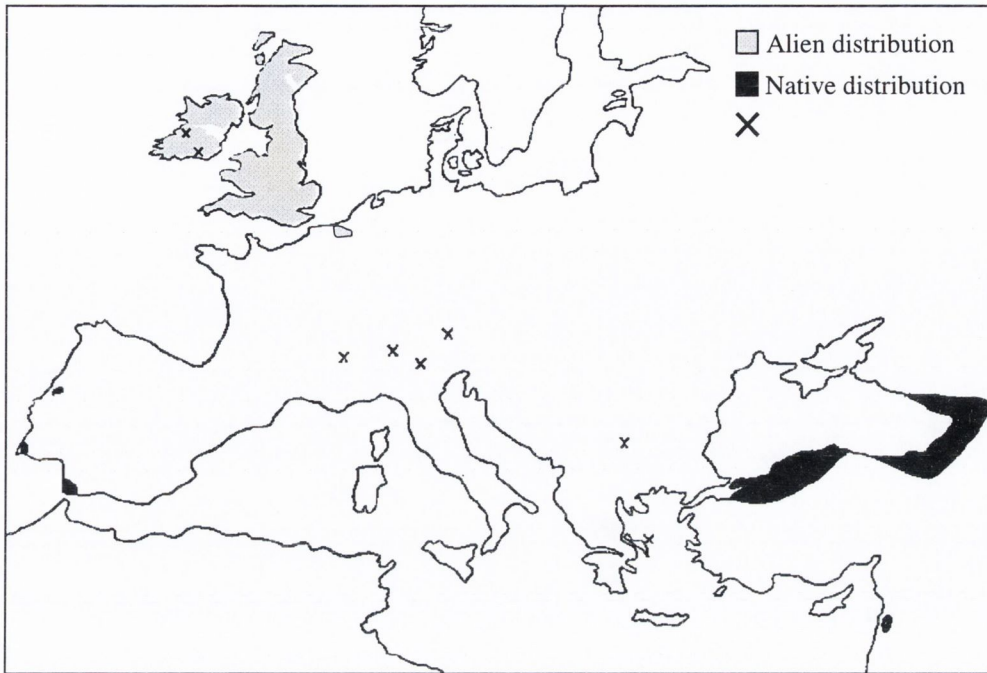


Figure 1.1. Native and alien habitat range of *Rhododendron ponticum* on the Eurasian continent. Isolated sites of established populations in France and Norway are not shown. Figure adapted from Cross (1975) and Milne and Abbott (2000).

showed that alien populations introgressed with at least two North American *Rhododendron* species during the invasion process which probably improved their cold tolerance.

The first description of *R. ponticum* in Ireland dates back to 1834 and implies that *R. ponticum* was introduced around 1774 (Dehnen-Schmutz and Williamson, 2006). Since then *R. ponticum* has expanded its distribution, especially to the oceanic climates of Western Ireland (Usher, 1986). Here, it mainly invades oak-dominated woodland, heathland and bog communities (Cross, 1982) and can form dense stands (Plate 1). Its reproductive traits facilitate invasion considerably. *R. ponticum* is not only well adapted to vegetative growth and local spread but can also display numerous flowers (over 4000 in an adult individual, cf. Cross, 1975) that may produce an uncountable number (approx. 5000 per inflorescence and over one million per adult plant) of small, wind dispersed seeds (Cross, 1981, but see Schäfer and Böcker, 2006) if pollen is transferred among flowers.





Bumblebees are the most efficient pollinators (Stout, 2007a) and are attracted to the flowers by their copious nectar production (Stout et al., 2006).

So far, only direct impacts of *R. ponticum* on native species have been measured. In particular, higher shading (Nadezhdina et al., 2004), a change in soil nutrient cycles (Ehrenfeld, 2003) and soil biota (Sutton and Wilkinson, 2007) in the presence of *R. ponticum* can affect co-occurring species negatively. However, indirect impacts of *R. ponticum* on plant pollination and pollinator communities are also imaginable. *R. ponticum* attracts generalist pollinators (Stout et al., 2006) that may also visit native co-flowering plants. Insects visit *R. ponticum* flowers frequently and alien rewards may be beneficial for pollinators' populations. However, foraging on *R. ponticum* might have negative implications too, as the alien is known to produce toxic secondary compounds not only in its vegetative parts, but also in its nectar and supposedly pollen (Koca and Koca, 2007). These diterpenes, Grayanotoxin I and III (Wong et al., 2002), which can have detrimental effects on honeybees (Carey et al., 1959) and mammals (Hotta et al., 1980) can also suppress growth and survival of neighbouring plant seedlings when released in the soil (Rotherham, 2001).

### 1.6. Study objectives

The main theme of my study is to contribute to a further understanding on the interactions of alien invasive plants with native co-flowering plant and pollinator species within a pollination network. For this approach I use *R. ponticum* as a model species of regional importance. First, I consider the botanical indirect aspect of interaction by linking the individual plant species level with the multi-species community level. I incorporate the factor 'density/abundance' of the invasive species and also manipulate this factor in the native species. Second, I shed light on the zoological direct aspect of the relationship by evaluating quality and changes in floral resources among habitats as factors of invasive impacts on the main native pollinator species.

Chapter 2 sets the initial general scheme in that it uses observational data on flower visitation, nectar production and availability and pollinator composition in *R. ponticum* invaded vs. uninvaded plant-pollinator communities in order to reveal temporal and spatial



patterns in pollination processes, changes in interactions between co-occurring species and impacts of the invader on single species and the community.

Chapter 3 uses a field experiment that artificially manipulates alien abundance as well as native abundance of one focal native plant species to distinguish between the relative importance of intraspecific and interspecific interactions for flower visitation and seed set in the native plant.

In chapter 4, this simulation of differences in plant population size is translated to the natural habitat, where I compare flower visitation, pollen transfer and seed set of the same native focal species in natural uninvaded and invaded field sites that vary in *R. ponticum* density.

My perspective changes in chapter 5 where I first feed individuals of the generalist bumblebee *Bombus lucorum* with alien and native nectar and pollen in the laboratory, to learn about the suitability of alien flower rewards for individual bumblebee survival. In a second step I estimate bumblebee abundance and colony number of two bumblebee species, *Bombus lucorum* and *B. pascuorum*, in invaded and uninvaded sites to extrapolate the relative importance of the presence of alien rewards on population characteristics of these two *R. ponticum* pollinators.

I close with chapter 6 and a synthesis of my results in the broader context of pollination and invasion biology.





## Chapter 2

“...Progress in invasion studies hinges on [...] recognition that colonist species and target communities cannot be studied independently, but that species-community interactions determine invasion success.” (Lodge, 1993)





Plate 2





## 2. Temporal and spatial variations of pollination services in invaded and uninvaded plant-pollinator communities

To be submitted as: Dietzsch, A.C. & Stout, J.C. Temporal and spatial variations of pollination services in invaded and uninvaded plant-pollinator communities. *Biological Invasion*.

### 2.1. Abstract

Alien plant species are known to alter ecosystem processes and have the potential to affect mutualistic plant-pollinator interactions. Studies that have investigated the direction of these effects often used single or few native target species for comparison and conducted observations on small temporal and spatial scales.

Here, I used a community approach to address the question whether the alien invasive plant *Rhododendron ponticum* affects native plant-pollinator interactions in Irish habitats. I surveyed invaded and uninvaded communities at a broad temporal and spatial scale and combined observational flower visitation data on all co-flowering native plant species with data on the amount and availability of rewards native plants offer.

Pollination services in both invaded and uninvaded communities revealed a high level of variability in time and space that was not consistently related to the presence of the alien invader. Positive effects of *R. ponticum* on visitation rates to some native species were site-specific and should not be generalised. The alien plant appeared to be well integrated in the native plant-pollinator network and received visits from a similar number of insect species as native co-flowering species. Plant species that had a higher number of insect visitor taxa in common with the alien were more likely to be affected in their visitation rates than plants with a smaller overlap in visitor species. They also resembled the alien in nectar production, generalist flower morphology and mass-flowering, and can be considered as members of the same functional group. *R. ponticum* integrated rather than monopolised native plant species pollination when occurring at low to medium coverage. Under these circumstances it may act as a valuable resource for native generalist pollinators, especially social bees. The variability in impacts of alien invasive species over time and space demands management decisions that incorporate temporal and spatial scales.





## 2.2. Introduction

The spread of non-native species is thought to be one of the major drivers of the worldwide decline in biodiversity (Manchester and Bullock, 2000, Perrings et al., 2000, Gurevitch and Padilla, 2004). While establishing and spreading in their new range alien species are likely to alter native species' abundance and richness (Mack et al., 2000, Ortega and Pearson, 2005). This may result in (co-)evolution between exotic and native species and promote an increase in population size or geographical range of some native species but extinction of others (Strauss et al., 2006). In plant communities, this alteration can cause shifts in multispecies plant-animal interactions such as plant-pollinator mutualisms (Traveset and Richardson, 2006).

Pollination mutualisms are not only important to assure sexual reproduction and genetic diversity of plant communities but have also a high economic value (Kearns et al., 1998, Winfree et al., 2007). In the light of decreases in both native flowering plant species and their functionally linked pollinators (Goulson et al., 2005, Biesmeijer et al., 2006) (but see Ghazoul, 2005a), alien plant invasion may have profound implications for native plant-pollinator systems.

Most studies have revealed competitive interactions between alien and native plant species for pollinator services resulting in reduced quantity or quality of native reproductive success (Chittka and Schürkens, 2001, Brown et al., 2002, but see Ghazoul, 2006).

Pollinators may prefer invasive species either as a result of higher floral abundance (Ghazoul, 2004) or more attractive rewards offered by the aliens, such as larger amounts of sugar-rich nectar (Chittka and Schürkens, 2001) and pollen (Brown and Mitchell, 2001).

The majority of these studies focused on a single native co-flowering species (e.g. Jones, 2004, Totland et al., 2006). They did not investigate entire flowering plant communities, and only a community approach can uncover the ecological and evolutionary consequences of multispecies interactions (Strauss and Irwin, 2004).

Two network studies published so far revealed facilitative effects of some invasive species and competitive effects of others on native plants as a whole (Lopezaraiza-Mikel et al., 2007, Bartomeus et al., 2008b). Those studies that investigated impacts on individual native plant species in multispecies communities have also reported diverse effects (Muñoz and Cavieres, 2008). Invasive plants facilitated visitation to some native species but



competed for pollination services with others, and some species were not affected at all (Moragues and Traveset, 2005). Effects were subject to variation; they varied temporally (between years, Larson et al., 2006) and spatially (between sites within one location, Grabas and Lavery, 1999). This illustrates the importance of incorporating time and space into further studies for better understanding invasion processes and their influences on plant-pollinator interactions (Bjerknes et al., 2007).

In this study, I investigated the impact of the severely invasive plant species *Rhododendron ponticum* on the pollination of multispecies native plant communities in Ireland. Native to the region south of the Black Sea, Bulgaria, Lebanon and the Iberian Peninsula, *R. ponticum* established and widely expanded its range since its introduction to Ireland (Cross, 1982, 2002). Its showy pink flowers assemble in large displays and attract a broad range of insects (Stout et al., 2006). Outcrossing raises its reproductive output (Stout, 2007b), and so *R. ponticum* benefits from pollinator visitation through increased seed set. This can contribute to further invasion because seeds are essential for spread and establishment of *R. ponticum* (Erfmeier and Bruehlheide, 2004).

Areas that are invaded by *R. ponticum* are habitat for a variety of different native plant species (Cross, 1982), including co-flowering species. Their individuals may potentially interact with *R. ponticum* by competing for pollination service or by benefiting from a joint attraction of larger numbers of pollinators (Rathcke, 1983, Moeller, 2004). Impacts on co-flowering species that use the same pollinators are expected to be different from impacts on species that hardly overlap in their pollinators (Waser, 1983, Morales and Aizen, 2006).

To explore spatial and temporal variation, I investigated pollination of all co-flowering native plant species within and between two locations in Ireland throughout the flowering season of *R. ponticum*. I tested whether

1. *R. ponticum* invasion affects visitation rates of native insects to native plant species?
2. Native plants that share a larger number of visiting insect taxa with *R. ponticum* are more affected than native species that overlap with the alien only in few visitor taxa?

Effects may be due to differences in reward (Corbet et al., 1984), and so I tested whether

3. Visitation rates relate to nectar standing crop and nectar secretion rates of plants in a mixed community?





The relationship of these variables has been investigated before (e.g. by Wesselingh and Arnold, 2000), but to my knowledge not for entire communities. Since species with high rewards can impact the pollination of other plants in a community (Lavery, 1992), I also wanted to know if

4. *R. ponticum* produces larger amounts of sugar-rich nectar than native plant species?

I know that nectar production in *R. ponticum* is high (Stout et al., 2006), yet its productivity in comparison to co-flowering species has not been recorded.

### 2.3. Materials and methods

#### 2.3.1. Study sites

Field work was conducted at two locations in Ireland, West Coast (Co. Galway, Plate 2 A) and East Coast (Co. Dublin) (Supplement 1). I selected two sites with *R. ponticum* and two sites without *R. ponticum* at the East Coast location to achieve local replication.

Additionally, I chose one invaded and one uninvaded site at the West Coast location to account for regional replication. Within each location, sites were a minimum of 1 km apart to minimise interferences; uninvaded sites were chosen according to similar elevation, habitat type and presence of native species (Supplement 1 and Supplement 2). At invaded sites, *R. ponticum* occurred in scattered patches at low to medium coverage (<30%).

Observations were carried out from 1<sup>st</sup> May to 22<sup>nd</sup> July 2005. Each site was visited three times (two observation days and one day of nectar secretion measurements) during each of the early, peak and late flowering season of *R. ponticum*. Flower density of the alien was measured at each flowering season by replicated counts of open flowers within 1 × 1 m<sup>2</sup> quadrats assigned randomly to *R. ponticum* shrubs.

#### 2.3.2. Insect visitation

Insect visitation was investigated for all native plant species (22 taxa) that were entomophilous and co-flowered with *R. ponticum*. In some cases different native plant species were investigated at different sites (Supplement 2). During each observation day, temperature (in the shade) was recorded with a thermometer every 10 minutes, cloud cover was estimated in percentage cover rounded to the nearest ten and wind force was recorded according to Beaufort scale. Three replicated patches of each flowering native species and



of *R. ponticum* (if present) were surveyed. At invaded sites, patches were within ten meters of the next flowering *R. ponticum* shrub. In each flower patch, I randomly selected ten focal flowers on three inflorescences and watched them for 20 min during each of three observation sessions (8am to 10.30am, 12am to 2.30pm, and 4pm to 6.30pm). The sequence of observed patches was changed at random between sessions. Inflorescences of Asteraceae and of *Trifolium pratense* were regarded as functional equivalents to focal flowers (Aizen and Feinsinger, 1994b). More than 200 hours of observations were conducted. Throughout the observations, I noted visitor identities, the number of flowers visited and the type of reward collected. Visitors were defined as (probable) pollinators when touching reproductive structures. Individuals of unknown insect species were collected in plastic tubes and killed by freezing. They were later identified to species or morphospecies with the help of taxonomists.

Visitation rate was calculated for each observed patch and observation session as number of pollinator visits per flower per 20 min (number of visited flowers per 20 min / 10 flowers). Prior to further analyses, mean visitation rates were calculated for each observed flower patch across observation sessions to balance variation between different day times. In addition, the number of individuals of each insect species visiting each flower patch was averaged across observation sessions. I then calculated the mean number of visitors per hour of each insect species across each plant species, site and season.

### 2.3.3. Nectar secretion and nectar standing crop

Nectar secretion (volume and sucrose concentration) and nectar standing crop (volume only) were recorded for the majority of native co-flowering plant species (Supplement 2). Volumes were quantified using microcapillary glass pipettes (1 and 5µl, Hirschmann, Germany) and a caliper rule. I measured sucrose concentrations (%Brix) with a pocket refractometer (CETI DIGIT-080, Medline Scientific, UK).

#### Nectar secretion

Eight inflorescences of each plant species at each site were covered with fine-meshed bridal veil to exclude visiting insects (Plate 2 C). Flowers of *Crataegus monogyna* and *Cirsium palustre* were covered with semitransparent paper bags due to their upright pointed delicate flowers susceptible to moderate rainfall. For some species, data of only five samples were analysed due to damage of flowers by vandalism and rain. After a





minimum of 24 h bagging, nectar of one flower from each inflorescence (one floret per flower head in *Cirsium palustre* and *Trifolium pratense*) was completely removed and inflorescences were immediately rebagged. Thereafter, I sampled each flower three times, with an interval of 3 hours between sampling. Sugar content (in  $\mu\text{g}$ ) was calculated according to Corbet et al. (2001) and Pr ys-Jones and Corbet (1991) and used to compute gross secretion rate ( $\mu\text{g/h}$ , B rquez and Corbet, 1991). I also calculated volume secretion rate ( $\mu\text{l/h}$ ) using nectar volume measurements.

### **Nectar standing crop**

Nectar standing crop of at least ten randomly picked unbagged flowers of each plant species was measured at each site throughout each observation day.

### **2.3.4. Data analyses**

I tested data for the assumption of homogeneity of variances and transformed data if necessary. If assumptions for parametric test procedures were not met, nonparametric statistics were employed. For certain analyses, I standardised data across each plant species with z-transformations. This procedure eradicates variation inherent to characteristics of individual species and enables analyses of native species as a unit. Computations were performed with MINITAB 13 and GMAV 5 (Underwood and Chapman, 1998).

### **Flower density of *R. ponticum***

I assumed that alien flower density was higher at peak flowering than at early or late flowering. To test this hypothesis I compared alien flower densities using the Kruskal-Wallis test and one-tailed Mann-Whitney tests for planned comparisons between seasons.

### **Insect visitation**

Visitation rates of each individual native plant species and of native plant species overall were analysed using a 5-factor mixed ANOVA design. I used the factors ‘Invasion’ (*R. ponticum* present/absent) and ‘Season’ (early, peak and late flowering of *R. ponticum*) as orthogonal, fixed factors, ‘Location’ (East and West Coast) as an orthogonal, random factor, ‘Site’ as a random factor nested within the ‘Invasion x Location’ interaction and ‘Day’ as a random factor nested within the ‘Invasion x Season x Location x Site’ interaction. For overall visitation rate, data were averaged across days, and so the factor ‘Day’ was absent. The number of factors of individual species’ analyses varied for each



plant species (Supplement 3) because not all species were present at all sites and during the whole flowering season (Supplement 2). Most analyses were constructed asymmetrically according to Glasby (1997) and Underwood (1997). In most cases, mean squares of the factors ‘Day’ and ‘Site’ could be pooled or eliminated (Winer et al., 1991). Tukey Simultaneous Tests were employed for *post hoc* comparisons (Day and Quinn, 1989). Furthermore, I tested the relationship between overall visitation rates and environmental factors using one-tailed Spearman’s rank correlations.

### **Nectar secretion**

To test for differences in nectar volume and sugar production between each native plant species and *R. ponticum* I used nonparametric one-tailed Fligner-Policello statistics for large sample sizes (Hollander and Wolfe, 1999) correcting significance levels of the multiple tests with the Dunn-Sidák method (Day and Quinn, 1989). Further, I correlated absolute and standardised values of nectar secretion rates and visitation rates using one-tailed Spearman’s rank correlations.

### **Nectar standing crop**

I averaged nectar standing crop values and nectar secretion rates across each plant species, site and flowering season and correlated them using one-tailed Spearman’s rank correlation. The same procedure was applied to standardised nectar standing crop and standardised visitation rate. I compared nectar standing crop of each native species with nectar standing crop of *R. ponticum* using one-tailed Fligner-Policello tests with Dunn-Sidák multiple-test correction.

### **Association and overlap of flower visitors**

I calculated the number of interactions (presence – absence) between each investigated plant and insect species for each site and flowering season and employed a Chi-Square test to compare the number of visiting taxa on native species at invaded sites and on *R. ponticum*. To compare the number of pollinator species between invaded and uninvaded sites and between East and West Coast I averaged the number of pollinator taxa for each plant species across invaded and uninvaded East Coast sites, respectively, and adopted a Friedman test for plant species that occurred at both the East Coast and the West Coast. Furthermore, I used multivariate techniques and PC-ORD 5.06 (McCune and Mefford, 2006) to evaluate the structure of pollinator communities. A matrix was constructed using





the identity and number of flower visitors per hour ('insect species') that visited each plant species at each site and flowering season ('plant species units'). Plant species units that were only visited by a single insect individual were deleted from the matrix to reduce noise (McCune and Grace, 2002). Similarly, insect species were deleted if observed only once during the entire field season leaving 49 insect species and 87 plant species units in the matrix. Data were fourth-root transformed to minimise the influence of absolute abundance of dominant taxa (i.e. bumblebees) on ordination and classification (Field et al., 1982, Clarke and Green, 1988). I then employed Nonmetric Multidimensional Scaling (NMS) with the Sørensen (Bray-Curtis) distance measure following a multi-step procedure (McCune and Grace, 2002) using 250 runs with real data and 250 runs with randomised data and a subsequent Monte Carlo test to calculate the final stress (square root of scaled Kruskal's stress). Subsequent classification of plant species units was based on hierarchical cluster analysis using the Sørensen distance measure and flexible beta linkage method ( $\beta = -0.25$ ). The optimum number of clusters was identified by means of Indicator Species Analysis (ISA, Dufrêne and Legendre, 1997). Thereby, the most informative level of clustering is achieved at the cluster step that maximises the average significant indicator values per cluster (Dufrêne and Legendre, 1997, McCune and Grace, 2002). Clusters were characterised by indicator insect species that were significantly closer associated with the particular cluster than expected by chance (tested with a Monte Carlo test of significance of indicator values, 4999 randomisations).

Using the cluster dendrogram, I determined the distance of each native plant species unit to temporally and spatially co-occurring *R. ponticum* units to estimate similarity in pollinator composition. Using a Mann-Whitney tests I compared similarity values of the native plant species that had been shown to be affected by *R. ponticum* in their visitation rate with similarity values of native plant species that were not significantly affected in visitation.

## 2.4. Results

### 2.4.1. Flower density of *R. ponticum*

The mean number of *R. ponticum* flowers per m<sup>2</sup> shrub surface varied significantly between flowering seasons (Kruskal-Wallis  $H_2 = 24.74$ ,  $N = 40$ ,  $P < 0.01$ ,  $x^{*0.25}$  transformed data). Flower density was lower at early ( $66.7 \pm 14.46$  flowers, range: 4 – 183) and late flowering ( $7.6 \pm 3.37$  flowers, range: 0 – 52) than at peak flowering ( $168.7 \pm 37.38$



flowers, range: 44 – 307; Mann-Whitney  $U = 169$ ,  $P = 0.008$  and  $U = 154$ ,  $P < 0.001$ , respectively).

2.4.2. Insect visitation

Native plant species were visited by a wide range of insect species, however, the most frequent visitors were bumblebees (*Bombus* spp, Hymenoptera; 77% of all observed visits), hoverflies (Syrphidae, Diptera; 12% of all observed visits) and other flies (Diptera; 5% of all observed visits). Visitation to native plant species was positively correlated with temperature (Spearman  $r = 0.216$ ,  $N = 544$ ,  $P < 0.001$ ), and negatively but weakly correlated with wind force and percentage of cloud cover ( $r = -0.093$ ,  $N = 558$ ,  $P = 0.014$  and  $r = -0.071$ ,  $N = 562$ ,  $P = 0.046$ , respectively).

Flower visitation to native plant species varied for each site among flowering times in an inconsistent way so that the interaction of these two factors was significant ('S  $\times$  Si [I  $\times$  L]' interaction,  $F_{4,18} = 5.84$ ,  $P = 0.003$ ; Table 2.1). Most of the variation was related to a significantly higher visitation rate to native plant species at the invaded West Coast site at late flowering in comparison with all other sites and flowering seasons (Fig. 2.1 A).

Table 2.1. Univariate ANOVA of overall pollinator visitation rates. Factor "Location" is subject to asymmetrical design. Data were square-root transformed to meet assumption of equal variances. Degrees of freedom (df) refer to numerators/denominators of F statistics. \*\* $P < 0.01$ .

| Source of variation          | Sum of squares | df   | F    | P        | Denominator                  |
|------------------------------|----------------|------|------|----------|------------------------------|
| Invasion I                   | 0.0534         | 1,1  | 0.37 | 0.6521   | I $\times$ L                 |
| Location L                   | 0.0816         | 1,2  | 1.26 | 0.3783   | Si (I $\times$ L)            |
| Season S                     | 0.4543         | 2,2  | 3.00 | 0.25     | L $\times$ S                 |
| Site Si (I $\times$ L)       | 0.1293         | 2,18 | 9.18 | 0.0018** | Res                          |
| I $\times$ L                 | 0.1433         | 1,2  | 2.22 | 0.2747   | Si (I $\times$ L)            |
| I $\times$ S                 | 0.4265         | 2,2  | 0.75 | 0.5714   | I $\times$ L $\times$ S      |
| L $\times$ S                 | 0.1513         | 2,4  | 1.84 | 0.2713   | S $\times$ Si (I $\times$ L) |
| I $\times$ L $\times$ S      | 0.5697         | 2,4  | 6.93 | 0.0502   | S $\times$ Si (I $\times$ L) |
| S $\times$ Si (I $\times$ L) | 0.1645         | 4,18 | 5.84 | 0.0034** | Res                          |
| Residuals                    | 0.1268         |      |      |          |                              |



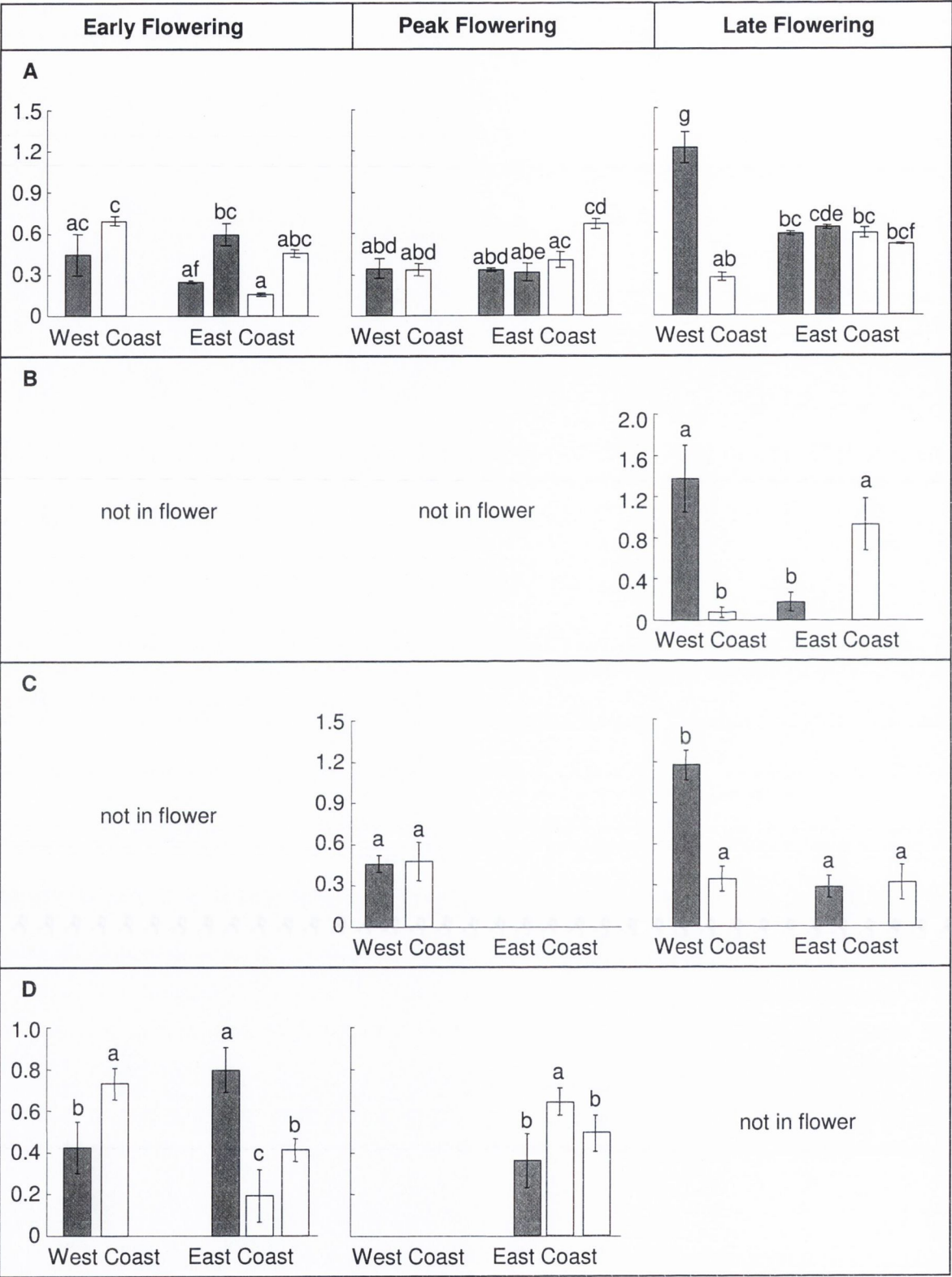


Figure 2.1.

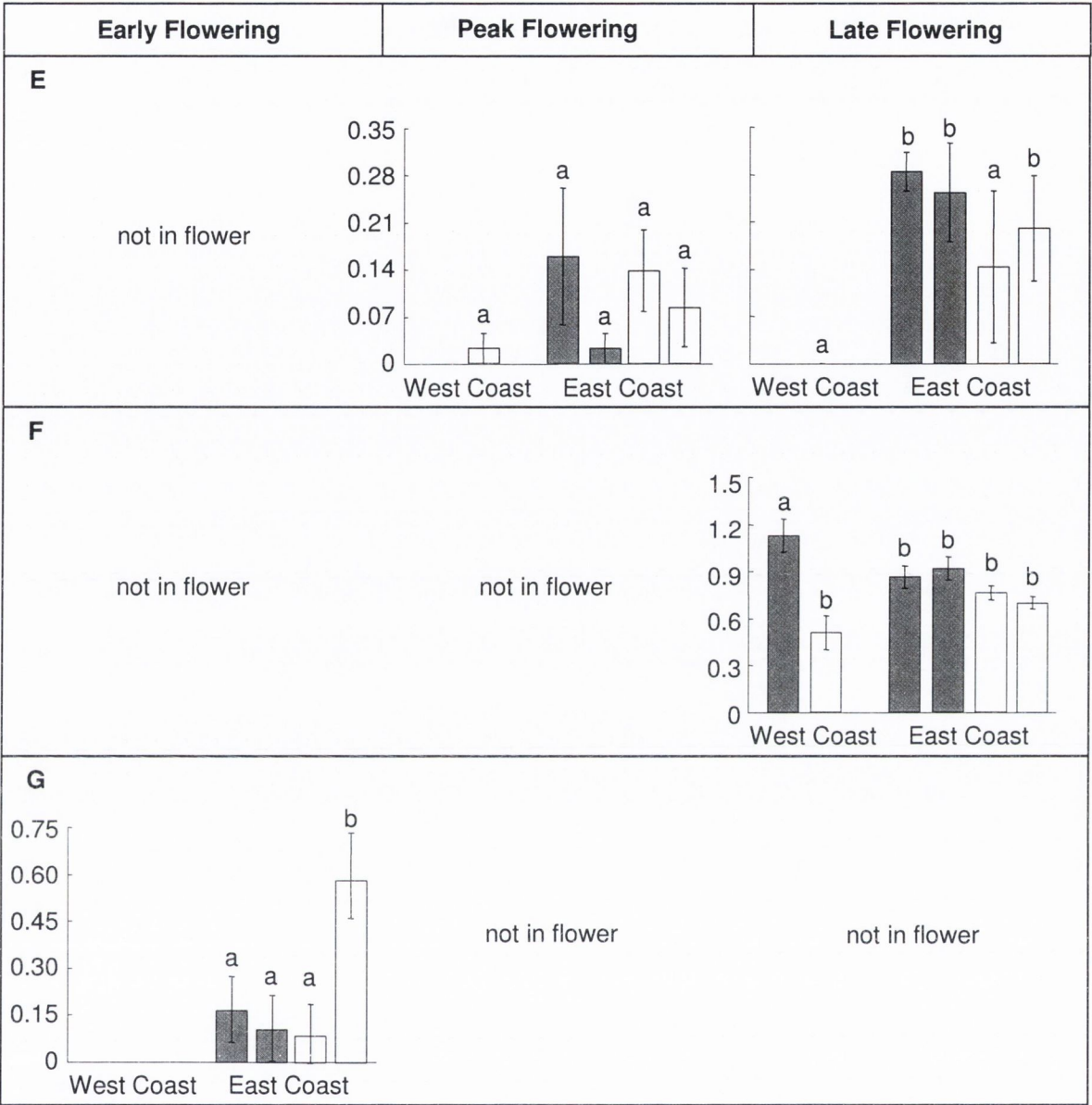


Figure 2.1 cont. Pollinator visitation rates of (A) native plant species overall and (B-G) individual native plant species. Each bar represents a specific site at a specific flowering season. X-axes separate data into different locations. Y-axes show mean number ( $\pm$ SE) of pollinator visits per flower per 20 min; in the case of transformation during analysis (Table 2.1 and Supplement 3), means of transformed data are displayed. The three columns separate data into different flowering seasons. Bars without letters in common are significantly different from each other at  $P < 0.05$ . B = *C. palustre*; C = *D. purpurea*; D = *I. aquifolium*; E = *L. corniculatus*; F = *R. fruticosus*; G = *V. myrtillus*.





Nine of the 22 native plant species occurred in at least two invaded and two uninvaded sites, and so I was able to analyse visitation rates of these species individually (Table 2.2). Visitation rates of three of these species (*Crataegus monogyna*, *Lonicera periclymenum* and *Ulex europaeus*) did not vary significantly between invaded and uninvaded sites, different locations or seasons (Table 2.2). Each of the other six native plant species showed significant differences in visitation rates (Table 2.2 and Figs. 2.1 B-G).

In most cases, these differences were revealed at interaction levels. For *Cirsium palustre*, visitation rate at the invaded East Coast site was lower than at the uninvaded East Coast site while this relation was reversed at the West Coast location (Fig. 2.1 B). For *Digitalis purpurea* and *Rubus fruticosus*, visitation rates at invaded and uninvaded East Coast sites did not vary significantly but visitation rates were higher in the invaded West Coast site than in the uninvaded West Coast site ('Invasion  $\times$  Location' interaction significant, Figs. 2.1 C and 2.1 F). In addition, for *Digitalis purpurea*, West Coast sites revealed similar visitation rates at peak flowering of *R. ponticum* but differed significantly at late flowering ('Invasion  $\times$  Season' interaction significant, Fig. 2.1 C). In *Ilex aquifolium*, visitation rate was higher at some sites, however, it also varied significantly at these sites among seasons ('S  $\times$  Si [I  $\times$  L]' interaction significant, Fig. 2.1 D). In two cases, different visitation rates were found to be related to one of the main factors. *Lotus corniculatus* was visited more often at the East Coast than at the West Coast ('Location' significant, Fig. 2.1 E). *Vaccinium myrtillus* was only found at East Coast sites and was visited significantly more often at one but not at the other sites ('Si[Invasion $\times$ Location]' significant, Fig. 2.1 G). *C. palustre*, *D. purpurea* and *U. europaeus* were not replicated within locations and so variation within and among locations could not be discriminated. Hence, variation between locations could be due to differences between sites rather than locations.

### 2.4.3. Nectar secretion

Flowers of *R. ponticum* secreted more sugar and more nectar volume per hour than flowers of the majority of native plant species (Fig. 2.2) although this difference was only significant for one third of the investigated plant species (Supplement 4). Sugar secretion rate and sugar concentration were positively correlated with visitation rate (Spearman  $r = 0.29$ ,  $P = 0.008$  and  $r = 0.307$ ,  $P = 0.005$ , respectively,  $N = 68$ ) whereas volume secretion



rate was uncorrelated with visitation rate ( $r = 0.101$ ,  $P = 0.207$ ,  $N = 68$ ). In contrast, neither standardised sugar secretion rate nor standardised volume secretion rate were correlated with standardised visitation rate ( $r = 0.184$ ,  $P = 0.066$  and  $r = 0.063$ ,  $P = 0.305$ , respectively,  $N = 68$ ).

#### 2.4.4. Nectar standing crop

Nectar standing crop was highly variable between species ranging from an average of  $2.48 \pm 0.23$   $\mu\text{l}$  per flower (*L. periclymenum*) to  $0.01 \pm 0.003$   $\mu\text{l}$  per flower (*P. erecta*) (Supplement 5). The invasive alien *R. ponticum* revealed higher overall nectar standing crop levels of  $1.67 \pm 0.12$   $\mu\text{l}$  per flower compared to all other native plant species (Fligner-Policello, corrected  $\hat{U} < -5.31$ ,  $P < 0.001$ ) except *L. periclymenum* ( $\hat{U}_{276,196} = 1.93$ ,  $P = 0.24$ ) (Supplement 4).

Standardised nectar standing crop was negatively correlated with standardised visitation rates ( $r = -0.32$ ,  $N = 153$ ,  $P < 0.001$ ). I found no significant relationship between mean nectar standing crop and sugar secretion rate or volume secretion rate ( $r = 0.148$ ,  $P = 0.128$  and  $r = 0.121$ ,  $P = 0.177$ , respectively,  $N = 61$ ).

#### 2.4.5. Association and overlap of flower visitors

Most of the 80 observed visiting insect taxa were Diptera (44 taxa, including 18 syrphid species) and Apoidea (20 bee taxa, including 7 *Bombus* species, Plate 2 D), however, almost half of the taxa were sighted once or at a single site only. Native plant species were visited on average by  $4.2 (\pm 0.64 \text{ SE})$  insect species in invaded communities and by  $4.4 (\pm 0.67 \text{ SE})$  insect species in uninvaded communities. *R. ponticum* was visited by  $6.2 (\pm 1.18 \text{ SE})$  insect taxa per flowering season but  $13 (\pm 2.0 \text{ SE})$  insect taxa across all seasons. *I. aquifolium* was the only native species that was visited by a larger number of insect taxa ( $11.8 \pm 1.44 \text{ SE}$ ) than the invasive. However, the number of insect taxa across seasons visiting *R. ponticum* and native species at invaded sites was not significantly different (Chi-Square Test;  $\chi^2 = 0.37$ ,  $\text{df} = 2$ ,  $P = 0.83$ ). Numbers of visitor species on native plant species did not differ significantly between invaded sites and uninvaded sites at East and West Coast locations (Friedman  $S = 3.21$ ,  $N = 6$ ,  $\text{df} = 3$ ,  $P = 0.36$ ).



Table 2.2. ANOVA results of pollinator visitation rates. F-ratios with degrees of freedom are shown for main factors and their interactions in the case of a significant result at  $\alpha$ -levels of 0.05 (\*) or 0.01 (\*\*). In case analyses did not reveal any significant differences according to a factor or interaction, F-values are not displayed (ns). For *D. purpurea* and *I. aquifolium*, factors “Location” and “Season” were analysed in two separate analyses due to high unbalance of data, i.e. some interactions are not calculable (N/A).

| Plant species                          | F-ratios <sub>df</sub> of main factors and interactions |                                          |                 |                                          |       |                                       |                  |                            |                                        |                           |
|----------------------------------------|---------------------------------------------------------|------------------------------------------|-----------------|------------------------------------------|-------|---------------------------------------|------------------|----------------------------|----------------------------------------|---------------------------|
|                                        | Invasion I                                              | Location L                               | Season S        | I × L                                    | L × S | I × S                                 | I × L × S        | Site Si (I × L)            | S × Si                                 | Day (I × L × S × Si)      |
| <i>Cirsium palustre</i> <sup>a</sup>   | ns                                                      | ns                                       |                 | F <sub>1,17</sub> = 24.85**              |       |                                       |                  |                            |                                        | ns                        |
| <i>Crataegus monogyna</i>              | ns                                                      | ns                                       |                 | ns                                       |       |                                       |                  | ns                         |                                        | F <sub>6,24</sub> = 3.50* |
| <i>Digitalis purpurea</i> <sup>a</sup> | ns <sup>bc</sup>                                        | F <sub>1,20</sub> = 19.82** <sup>b</sup> | ns <sup>c</sup> | F <sub>1,20</sub> = 17.59** <sup>b</sup> | N/A   | F <sub>1,4</sub> = 8.25* <sup>c</sup> | N/A              |                            |                                        | ns <sup>bc</sup>          |
| <i>Ilex aquifolium</i>                 | ns <sup>bc</sup>                                        | ns <sup>b</sup>                          | ns <sup>c</sup> | ns <sup>b</sup>                          | N/A   | ns <sup>c</sup>                       | N/A              | ns <sup>bc</sup>           | F <sub>1,28</sub> = 5.28* <sup>c</sup> | ns <sup>bc</sup>          |
| <i>Lonicera periclymenum</i>           | ns                                                      | ns                                       |                 | ns                                       |       |                                       |                  | ns                         |                                        | ns                        |
| <i>Lotus corniculatus</i>              | ns                                                      | F <sub>1,44</sub> = 11.66**              | ns              | N/A <sup>d</sup>                         | ns    | ns                                    | N/A <sup>d</sup> | ns                         | ns                                     | ns                        |
| <i>Rubus fruticosus</i>                | ns                                                      | ns                                       |                 | F <sub>1,30</sub> = 12.62**              |       |                                       |                  | ns                         |                                        | ns                        |
| <i>Ulex europaeus</i> <sup>a</sup>     | ns                                                      | ns                                       |                 | ns                                       |       |                                       |                  |                            |                                        | ns                        |
| <i>Vaccinium myrtillus</i>             | ns                                                      |                                          |                 |                                          |       |                                       |                  | F <sub>2,18</sub> = 6.30** |                                        | ns                        |

<sup>a</sup> Since this species was absent in two East Coast sites, “Site” is unreplicated and could not be nested in “Location”. Differences in “Location” and its interactions may be therefore due to inherent variation between sites.

<sup>b</sup> Results for first analysis – main factor = Location

<sup>c</sup> Results for second analysis– main factor = Season

<sup>d</sup> Interactions not calculable because species only occurred in one West Coast site.



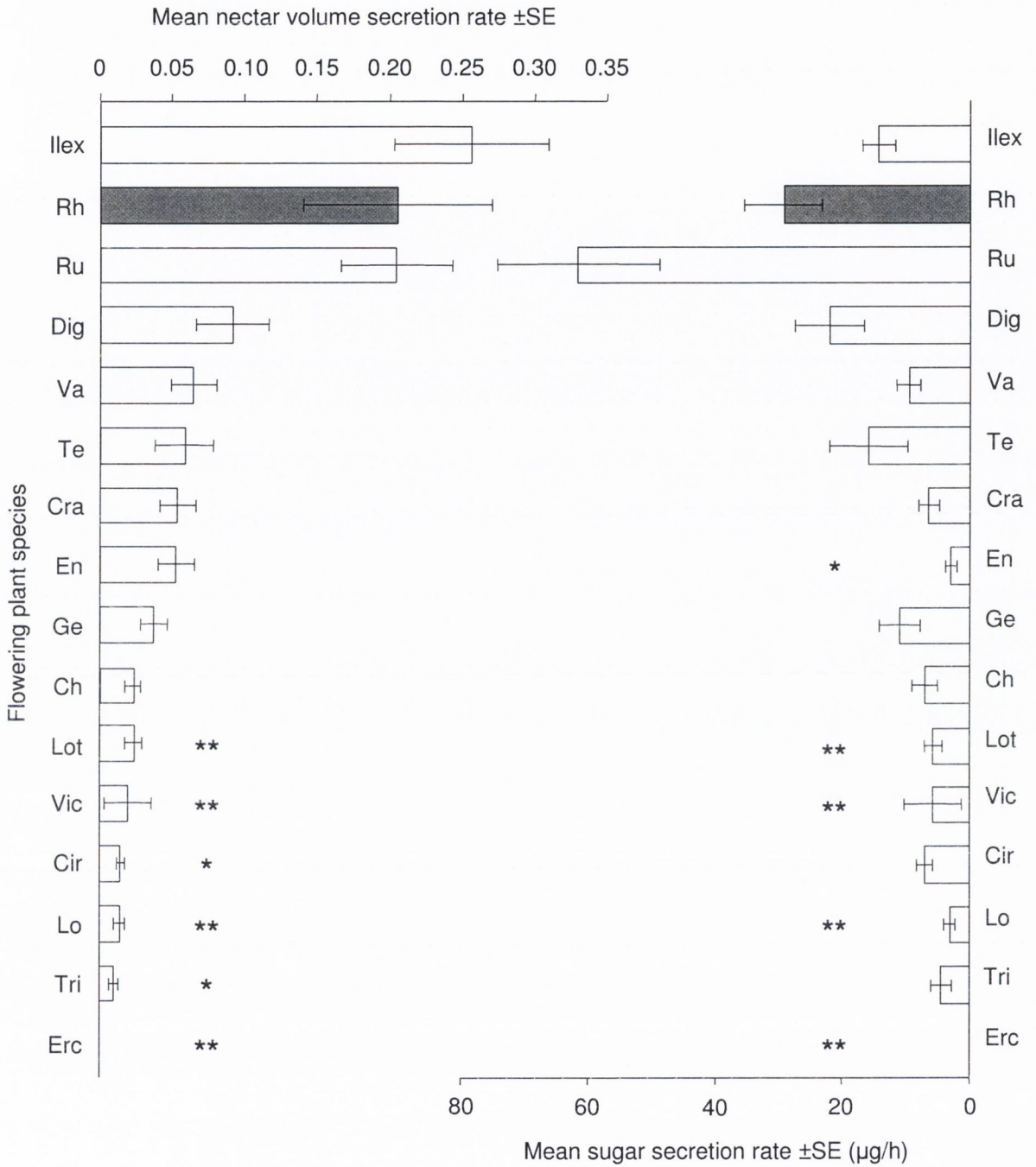


Figure 2.2. Mean nectar volume and sugar secretion rates of investigated plant species. Bars in grey correspond to *R. ponticum*. See Fig. 2.4 for abbreviations of species names. Species marked with \*\* ( $P < 0.01$ ) or \* ( $P < 0.05$ ) secreted significantly less volume or sugar per hour than the alien.



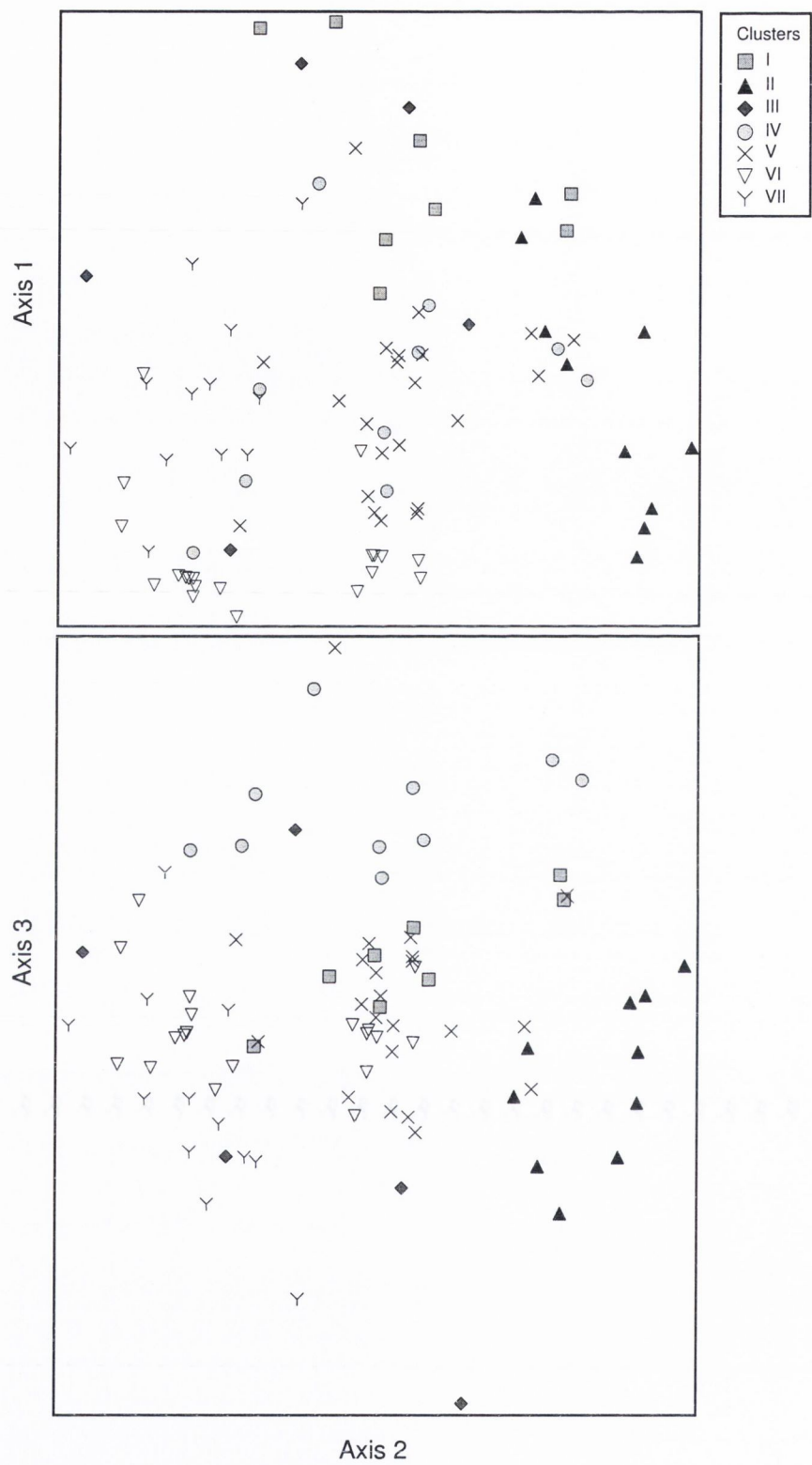


Figure 2.3. Three-dimensional ordination derived from Nonmetric Multidimensional scaling (NMS) of 87 plant species units based on the number and identity of their floral insect visitors. Each point (unit) represents a specific flowering species at a specific location and a specific flowering season in presence or absence of *R. ponticum*. The seven higher divisions identified by cluster analysis ( ) are visualised by different symbols. The three-dimensional final solution is displayed as two two-dimensional graphs (axis 2 vs axis 1 and axis 2 vs axis 3).



For NMS, the original matrix was summarised at best by a three-dimensional ordination (Fig. 2.3). The final solution represented 76.6% of the total variance in the original space (axis1: 25.5%, axis 2: 28.4%, axis 3: 22.7%) at a final stress of 17.7%. Subsequent cluster analysis resulted in a low percentage of chaining (1.96%). Two optima in clustering were identified at a cluster number of 7 and of 19. I chose 19 clusters (60% of information retained) for visualization (Fig. 2.4). Membership in a cluster was either related to plant species identity (cluster 7), spatial co-occurrence of units (cluster 2 and 6), temporal co-occurrence (cluster 9) or a combination of these factors (cluster 10) (Fig. 2.4). In few cases, membership was neither related to species identity nor to co-occurrence (cluster 15).

*R. ponticum* did not form a separate cluster but shared a similar visitor assemblage with some of the native plants, e.g. *R. fruticosus* and *I. aquifolium* (Fig. 2.4). For similarity comparisons I used the nine native species whose visitation rates had been individually analysed before (see 2.3.2). *R. ponticum* revealed higher similarity in pollinator composition with plant species that were affected by the factor 'Invasion' (*R. fruticosus*, *I. aquifolium*, *C. palustre*, *V. myrtillus* and *D. purpurea*, mean  $\pm$ SE similarity 30.4%  $\pm$ 5.0%; median, first and third quartile M = 23.5%, Q1 = 10.8%, Q3 = 46.5%) than with species that were not affected (*C. monogyna*, *L. periclymenum*, *L. corniculatus* and *U. europaeus*, mean  $\pm$ SE similarity 12.1%  $\pm$ 3.1%; M = 3, Q1 = 0, Q3 = 23) (Mann-Whitney  $U_{24,28} = 479$ ,  $P = 0.004$ ).

Most clusters revealed significant insect species associations (ISA Monte Carlo tests,  $P < 0.05$ , Fig. 2.4). Clusters that contained mainly units of invaded sites or plant species which overlapped greatly in their pollinators with *R. ponticum* were characterised by bumblebees and flies (e.g. cluster 2 and 10) whereas solitary bees were associated with plant units of uninvaded sites (cluster 18).

## 2.5. Discussion

Information about impacts of invasive plants on native plant pollination is limited (Bjerknes et al., 2007), and only two studies have investigated effects on entire plant communities so far (Lopezaraiza-Mikel et al., 2007, Bartomeus et al., 2008b). These network studies analysed native species as a whole at a local scale (a single area). Here, I



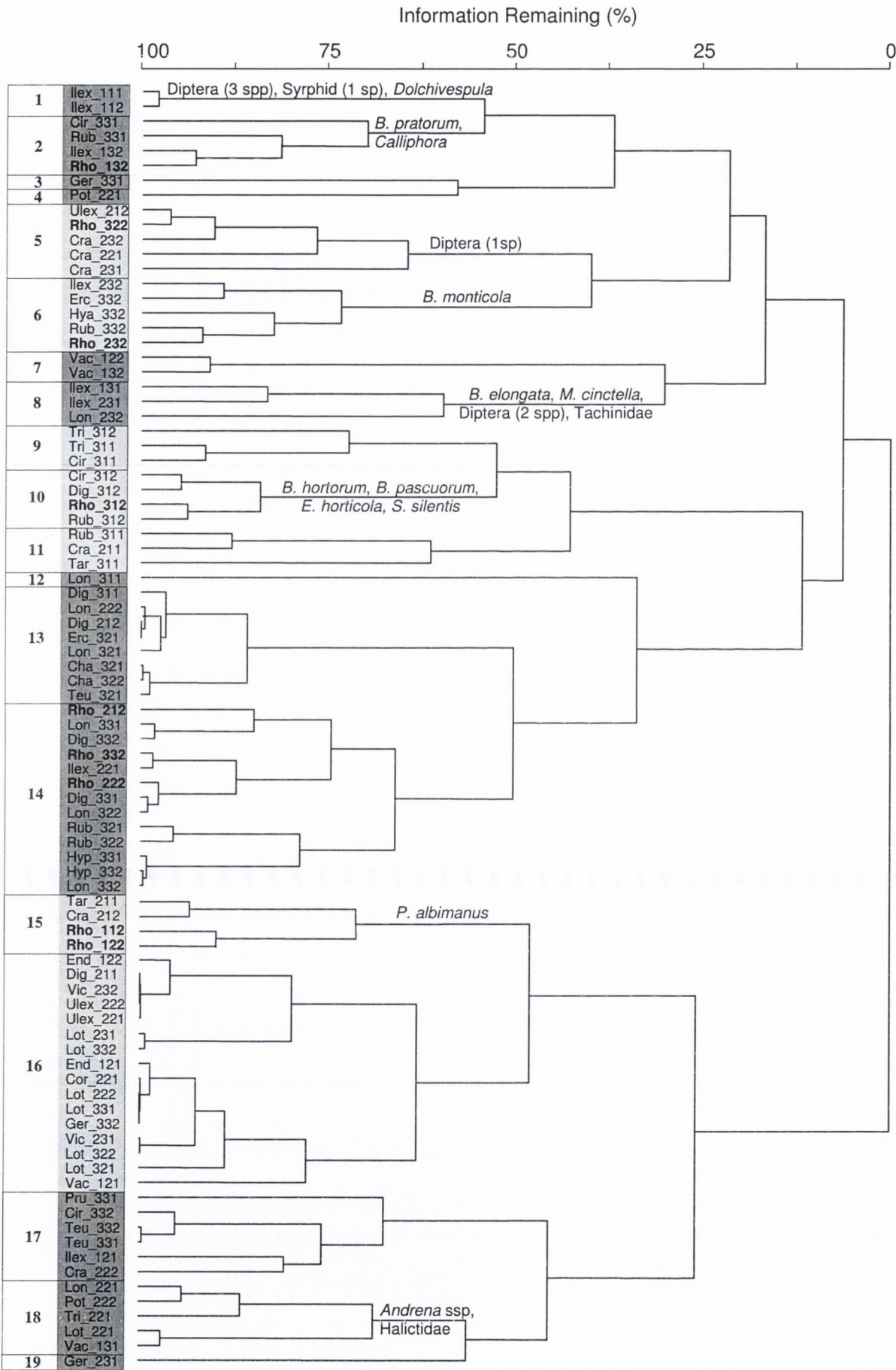




Figure 2.4 (previous page). Dendrogram of hierarchical cluster analysis for pollinator visits per hour. Each sample unit is a plant species at a specific flowering season (first digit of coding: 1=early, 2=peak, 3=late) and site (second digit: 1=West Coast, 2=East Coast A, 3=East Coast B) in an uninvaded (last digit=1) or invaded (last digit=2) habitat. Indicator insect species of clusters are attached to each cluster's base. Clusters 1 to 19 are part of seven higher divisions (I – VII from top to bottom, indicated by shaded squares). Cha = *C. angustifolium*, Cir = *C. palustre*, Cra = *C. monogyna*, Dig = *D. purpurea*, End = *E. non-scriptus*, Erc = *E. cinerea*, Ger = *G. robertianum*, Hya = *H. androsaemum*, Hyp = *H. pulchrum*, Ilex = *I. aquifolium*, Lon = *L. periclymenum*, Lot = *L. corniculatus*, Pot = *P. erecta*, Pru = *P. vulgaris*, Rho = *R. ponticum*, Rub = *R. fruticosus*, Tar = *T. officinale*, Teu = *T. scorodonia*, Tri = *T. pratense*, Ulex = *U. europaeus*, Vac = *V. myrtillus*, Vic = *V. sepium*.



investigated both individual native species and plant species overall in light of the resources they display. More importantly, by including both local and regional replication I extended the spatial scale, aiming for a better generalization of my findings.

### 2.5.1. Insect visitation

Both invaded and uninvaded native plant communities were spatially and temporally highly variable in their pollination services. Contrary to expectation, changes in visitation frequency to native plant communities did not follow a general pattern. They were neither consistently related to the presence of alien *R. ponticum*, nor to a particular season. On an individual species level, visitation rates of most analysed species remained unaffected by *R. ponticum*. However, some native species within the community revealed higher visitation rates at certain invaded sites than at comparable uninvaded sites (and vice versa), but these effects were related to individual plant communities, i.e. they were site-specific rather than general.

Site specificity was also detected on a seasonal level. Most differences in visitation rates among sites occurred in early and late flowering of *R. ponticum*. They were possibly a function of overall resource availability, and so to some degree influenced by the alien. Other manipulative studies have shown that effects of invasive species including *R. ponticum* may vary depending on their floral density (Muñoz and Cavieres, 2008, see Chapter 3). In spring and later summer, flower densities of *R. ponticum* were low or highly variable. During these periods *R. ponticum* seemed to facilitate visitation of natives at some sites. Strikingly, at late flowering higher visitation frequencies at invaded sites were revealed at the West Coast only, although this may be due to specific site differences. Environmental variables (e.g. temperature) were similar in uninvaded and invaded sites





and among locations (unpublished data), thus they cannot explain the observed differences. However, species diversity of the western plant communities was poorer than in the east. I do not know if facilitative effects of invasive species on pollination occur more likely in depauperate than in species-rich plant communities. Collective attraction and pollinator sharing have been rather found in communities with plant species at low floral density (Rathcke, 1983, Lavery, 1992).

Spatial and temporal replication within and among locations and seasons is vital to obtain a general view about impacts of invasive species on native plant pollination. Generalization of competitive or facilitative effects found by other studies so far should be done with caution. They mainly investigated populations within a single area or in a short time frame (e.g. Grabas and Lavery, 1999, Chittka and Schürkens, 2001, Lopezaraiza-Mikel et al., 2007). I detected the majority of differences at an interaction level indicating that effects are site-specific and time-specific. My findings emphasise that generalizations are difficult to draw. Predictions might be right for one location or season but wrong for another. Future studies should aim for broad observation scales that include both factors.

### 2.5.2. Nectar secretion

Sugar secretion rates in my communities were correlated with visitation rates, i.e. the more sugar a species produced the more visits it received. In contrast, there was no correlation between standardised nectar secretion rates and standardised visitation rates. Inferring from this result, insect visitors did not distinguish within each plant species between populations with lower nectar production and populations with higher nectar production (versus Cartar, 2004). The low variability observed within species did not entail a behavioral response, but visitors reacted to the higher variability among species (sensu Wesselingh and Arnold, 2000). Precise fine tuning of minimal difference detection in nectar production would not be efficient for foragers because plant species can exhibit different rates of nectar production at different times within populations and even within plant individuals (Zimmerman and Pyke, 1986).

Although *R. ponticum* secreted higher amounts of nectar (4.9  $\mu\text{l}/24\text{h}$ ) than the majority of native species it is not as nectar-rich as other alien invasive species (e.g. *Impatiens glandulifera* with  $>10 \mu\text{l}/24\text{h}$ , Nienhuis, 2009). This might partly explain the variability in impacts on native plant pollination. At a medium coverage *R. ponticum* appeared to



integrate with rather than monopolise native pollination webs. Some native species (e.g. *R. fruticosus*) produced similar nectar volumes and sugar paired with high flower numbers. One might consider these native species and the alien as members of a functional group that influence visitation of other co-flowering species in a similar way. By disrupting or facilitating pollination at both invaded and uninvaded sites these native species may have masked additional impacts of *R. ponticum* on native pollination.

### 2.5.3. Nectar standing crop

Clearly, visitors deplete nectar while foraging, and the more they forage, the less nectar remains in openly accessible flowers. This explains why nectar standing crop in my communities was negatively correlated to visitation rates. Previous studies found nectar standing crop to be highly variable within plant communities and regarded it as a feature of ongoing interactions between individual plants and flower visitors (Corbet, 2003).

*R. ponticum* bore more nectar in its accessible flowers than all other native species, except for *L. periclymenum*. Nectar of the latter could be only extracted by a minority of observed visitor taxa (Lepidoptera and long-tongued bees) with proboscis lengths comparable to the long tubular flowers. Hence, nectar standing crop remained high. For all other native species, lower nectar secretion would be an obvious explanation for their lower nectar standing crop compared to the alien. However, nectar standing crop was not correlated with nectar secretion but with visitation rates. Superabundance of the alien might explain this observation. Stout et al. (2006) related lower visitation rates in large *R. ponticum* populations to its floral superabundance. Similarly, I found *R. ponticum* flowers less or as frequently visited as flowers of native species (Supplement 6) despite their high nectar contents. Nectar secretion in *R. ponticum* was high, and so low visitation rates resulted in higher nectar standing crop. If so, the likelihood for visitors to discover nectar in alien flowers is higher than in native flowers. This, and the absence of negative impacts of *R. ponticum* nectar on e.g. bumblebee survival (see Chapter 5), makes the alien a favorable food resource for some taxa.

### 2.5.4. Overlap of flower visitors

Like other alien species (Lopezaraiza-Mikel et al., 2007, Bartomeus et al., 2008b), *R. ponticum* was well integrated in the native visitation web. It did not change the number of





insect visitors of native plants and was associated with a similar number of insect taxa per flowering season as the majority of natives (sensu Morales and Aizen, 2006, versus Olesen et al., 2006). Most pollinators were generalists and hence more likely to include aliens in their diet (Memmott and Waser, 2002). This may facilitate further spread of *R. ponticum*. Native plant species that revealed differences in visitation rates among certain invaded and uninvaded sites shared a more similar visitor community with *R. ponticum* than other native species. Visitors intermingled natives with *R. ponticum* and I can think of three explanations for their behavior. First, plant species with generalised pollination systems are usually visited by corresponding assemblages of generalist pollinators (“complementary attraction”, Moeller, 2004). Not only alien *R. ponticum* but also native *R. fruticosus*, *I. aquifolium* and *C. palustre* have generalist flowers (Faegri and van der Pijl, 1971) and presumably attract generalist pollinators collectively. Second, pollinators may use species with specialised flowers but similar floral characters as commutable resources (“floral convergence”, Schemske, 1981, Thomson, 1982) if they learn how to exploit them efficiently (Stout et al., 1998). However, most native species differed in their flower morphology from *R. ponticum*, and so floral convergence is unlikely. Third, insects often rely on high-quality rewards gained from different species (e.g. nectar from one and pollen from another species; “complementarity of resources”, Ghazoul, 2006). As seen in *D. purpurea*, visitors gathered exclusively pollen in a third of visits and exclusively nectar in another third of visits. In contrast, *R. ponticum* was visited in 72% of cases solely for nectar and in 12% of cases exclusively for pollen.

Solitary bees were mainly observed on plant species in uninvaded sites (cluster 18, Fig. 2.4) and they were absent in the majority of invaded sites. In its native range in the Iberian Peninsula, *R. ponticum* is primarily visited by small and large solitary bees (Stout et al., 2006), but I observed them on *R. ponticum* only twice. This might be a result of a generally lower diversity and abundance of solitary bees in Ireland compared to Continental Europe (cf. Westrich, 1989, and Fitzpatrick et al., 2006). However, the abundance of solitary bees is not only limited by food resources but other habitat characteristics (Potts et al., 2003). Known for transforming soil conditions and shading regimes (Ehrenfeld, 2003, Nadezhdina et al., 2004, Sutton and Wilkinson, 2007), *R. ponticum* might deteriorate nesting conditions for solitary bees at Irish habitats.



### 2.5.5. Consequences for plant and pollinator communities

Phenological matching between plant individuals and pollinators modifies the visitor assemblage of each plant species (Kudo and Suzuki, 2002). Invasive species are supposed to affect pollination of natives to a higher degree if they overlap phenologically (Morales and Aizen, 2006). Yet, in communities with a high proportion of social insect species or vertebrates, plants and pollinators are not necessarily synchronised. Distinct-flowering species could be interconnected by pollinators with long life cycles, and could influence each other indirectly (Waser and Real, 1979, Moeller, 2004). Although site-specific and not a general trend, *R. ponticum* seemed to affect pollination of early and late, sequentially flowering native species that shared similar characteristics with the invasive, e.g. produced high amounts of nectar, used similar pollinators or flowered en masse. These native species represent reliable food sources comparable to *R. ponticum* at its peak flowering.

Bumblebees especially depend on continuously offered food (Edwards and Jenner, 2004). As a result they use either long flowering (Ollerton and Lack, 1998) or sequentially flowering species (Dlusski et al., 2005). Availability of mass-flowering plants can increase bumblebee abundance (Westphal et al., 2003, Herrmann et al., 2007), population size (Chapter 5) and presumably the number of hibernating queens which then rely on native alternatives before or after *R. ponticum* blossoms. Thereby, pollinators may trigger shifts within the community (Kevan and Baker, 1984).

So far, visitation rate is regarded as a good predictor of fruit set (Aizen and Feinsinger, 1994a, Parker, 1997, Stout et al., 2006), and hence plant reproduction, but it may poorly estimate pollination efficiency in invaded plant communities. The invader may enhance pollen loss or heterospecific transfer via interspecific flower movement (Waser, 1978) and thereby limit reproduction of some species (Jakobsson et al., 2008) especially if an (over)supply of invasive pollen inhibits native pollen germination (Brown and Mitchell, 2001). Addressing the relationship between visitation rate, pollen deposition and fruit set in invaded communities would be constructive in future research.

## 2.6. Conclusions

Plant-pollinator networks show a high level of plasticity, but they are at risk if generalist pollinators are lost (Memmott et al., 2004). Their decline can cause a cascade of declines in reproduction among linked community members (Pauw, 2007). I have confirmed findings





of other community studies (Lopezaraiza-Mikel et al., 2007, Bartomeus et al., 2008b) that alien species support generalist pollinators and may be beneficial rather than disruptive when growing in mixed stands with native plants. However, in Ireland and elsewhere, native plant communities have been replaced by monocultures of *R. ponticum* (Dehnen-Schmutz et al., 2004). In these cases, pollinators that are not precisely synchronised with the alien's flowering time may not find sufficient resources due to a lack of alternative flowering plants. Preservation of mutualistic interactions can be vital for maintaining communities (Christian, 2001), and so management of *R. ponticum* to avoid monocultures is indispensable for both native plant and pollinator communities. Management decisions should be sensitive to the temporal and spatial variability that invasive species show in their impacts.

## **Chapter 3**

“...What is needed is a shift of emphasis  
and perhaps scale so that the experiments are recast  
to test explicit hypotheses about environmental disturbances.” (Underwood, 1994)





Plate 3





### **3. Intra- vs. interspecific pollination interactions between an alien invader and a native plant: insights from experimental manipulations**

To be submitted as: Dietzsch, A.C., Nienhuis, C.M. & Stout, J.C. Intra- vs. interspecific pollination interactions between an alien invader and a native plant: insights from experimental manipulations. *Oecologia*.

#### **3.1. Abstract**

Species communities are shaped by intra- and interspecific interactions among their members. These interactions can be characterised as competition, facilitation or neutral co-existence. New members within a community may alter existing interaction patterns and as a result population sizes and relative species abundance or community assemblage. Alien invasive species are candidates for such alterations as they spread rapidly and establish populations in new habitats.

I was interested in these population size effects of an alien invader *Rhododendron ponticum* on native plant-pollinator interactions and focussed on reproduction of one co-occurring focal species, *Digitalis purpurea*. I conducted a manipulative field experiment controlling an increase in both alien and native plant abundance using two different initial population sizes of the native plant.

I revealed that conspecific *D. purpurea* individuals facilitate each other's flower and inflorescence visitation at higher population sizes, however, this response did not result in higher reproductive success. Although I found evidence for an indirect interaction between the native and the alien plant species, the direction of this interaction could not be clearly identified due to high variability within treatments. However, competitive effects of the alien can be ruled out and I suggest rather neutral if not facilitative interactions between the two plant species.

I conclude that *R. ponticum* at a low population size, i.e. at an initial colonisation level, may have no or little impact on native plant pollination but benefits from native pollinator visitation which might enhance its further invasion. Further, my experiment illustrates the importance and benefits of integrating population size and density effects into pollination invasion biology by using both manipulative and observational field experiments.





### 3.2. Introduction

The potentially severe ecological and economic consequences of alien invasions in natural systems necessitate better understanding of the nature of invasion processes in order to effectively prevent or control them (Mack et al., 2000). Our knowledge of the factors shaping successful establishment, reproduction and spread of alien flowering plants and their effects on native plant species has grown (Daehler, 2003, Milbau and Stout, 2008), but is far from complete. Pollination by animal vectors has been suggested to play a considerable role in the invasion process of alien plants (Richardson et al., 2000a), because the majority of angiosperm species rely on it for reproduction (Buchmann and Nabhan, 1996). The use of mobile agents may not only secure pollen transfer among alien flowers but may also link the alien species with co-flowering native plants. This can result in competitive or facilitative interactions between the native plant community and its invader (Bjerknes et al., 2007).

Since flower visiting insects respond among other factors to floral abundance in their foraging behaviour (Goulson et al., 1998), the quality and quantity of pollination services have long been recognised to depend on plant species abundance (Ghazoul, 2005b). Two contrasting concepts linking abundance and fitness intraspecifically have been propounded. The first postulates a positive relationship between the two variables (Allee effects, Allee et al., 1949). Plant individuals that occur in low abundance are thereby more likely to suffer from a disproportionate decline in reproductive output (Hackney and McGraw, 2001), which is attributed to insufficient pollen deposition (Groom, 1998), pollinator limitation (Sih and Baltus, 1987), geitonogamous selfing and/or inbreeding depression (Ghazoul and McLeish, 2001). Although this mechanism has been mainly suggested for self-incompatible species, there is growing evidence for a more common occurrence among self-compatible species than previously suspected (Ghazoul, 2005b). The second concept predicts reproductive output as a negative function of abundance. Competition for abiotic resources and pollination services (Sowig, 1989, Fritz and Nilsson, 1994) as well as seed predation (Jennersten and Nilsson, 1993) can thereby disrupt reproductive output in large populations. These concepts are not mutually exclusive, and so their combination may explain, e.g. non-linear patterns observed within the same study system (Jennersten and Nilsson, 1993).



Within a habitat, abundance is a function of a plant species' population size and density, i.e. the number of individuals and their distance to each other within the population (Kunin and Shmida, 1997). In nature, these two components are often entangled, and so manipulative studies are essential to separate their relative importance to pollination (Ghazoul, 2005b). Furthermore, manipulative experiments minimise uneven historical, genetic and abiotic factors within natural populations by cultivating plant individuals under standardised conditions. Studies on abundance effects in artificially created plant populations mainly focussed on single species systems. In cases that manipulated both population size and density at the same time (Knight, 2003) interpretation is difficult because effects could be due to either of the two variables. However, manipulations of either density (Bosch and Waser, 2001), population size (Hackney and McGraw, 2001) or both variables independently (Kunin, 1997, Mustajärvi et al., 2001) revealed that both parameters can influence pollination and reproduction.

Under natural conditions, single species systems are rare, and in most communities plant species interact with each other for example via the same pollen vectors (Waser et al., 1996). This adds an interspecific component to the intraspecific mechanisms mentioned before. Pollinators that switch frequently between species may facilitate heterospecific pollen transfer resulting in pollen loss and pollen limitation (Waser, 1978). Alternatively, multiple species may provide a larger grand total abundance of flowers and act jointly as magnet species attracting more pollinators overall (Lavery, 1992). The system becomes disproportionately more complex, which is one reason why manipulative multiple-species experiments on abundance are rare in the pollination literature (but see e.g. Campbell, 1985, Kunin, 1993, Stout et al., 1998, Caruso, 1999, Caruso, 2002, Feldman, 2008). Among these few studies, the majority focused on interspecific (mainly competitive) interactions (but see Brown et al., 2002). Introducing heterospecific individuals to a system have been shown to influence parameters of pollination but this impact might mislead if the addition of conspecific individuals in the same manner has similar or even larger effects (Underwood, 1997). For a sensible and unconfounded interpretation, simultaneous examination of both inter- and intraspecific interactions are required (Underwood, 1986). Invasive species are known to occur in large numbers and densities in new habitats (Parker et al., 1999), and so their impact on native communities is likely to be mediated via their abundance. However, during colonisation, which is the crucial transition stage where a species moves from occurring as a casual towards forming self-perpetuating colonies





(Richardson et al., 2000b), alien population size is still very small (Theoharides and Dukes, 2007). Mimicking this initial phase I decided to focus on population size as a parameter of abundance. I tested whether a flowering alien species whose invasive character is known, has the ability to influence native pollination at an early stage of invasion. In particular, I wanted to explore the relative importance of intra- vs. interspecific interactions for (a) the number of pollinator visits to individual native plants and flowers and (b) fruit and seed set of individual native plants as a measure of reproductive success. Building on that, I was interested in the contribution of population size towards these interactions. I hypothesised that

1. Native flower visitation and reproductive success are higher in large monospecific populations than in small populations due to Allee effects and joint pollinator attraction (intraspecific facilitation).

*Alternatively:* native flower visitation and reproductive success are lower in large monospecific populations due to pollinator luring (intraspecific competition).

2. Native flower visitation and reproduction are higher in 'invaded' heterospecific populations than in native monospecific populations due to joint attraction and maintenance of pollinators (interspecific facilitation).

*Alternatively:* native visitation and reproduction are lower in the presence of an alien species due to competition for pollinators and interspecific pollen transfer (interspecific competition).

Since pollinators adapt their behaviour to the spatial distribution of foraging resources (Osborne et al., 2008), they are important keys for understanding patterns in plant pollination. Consequently I wanted to find out if they link native and alien flowers and hypothesised that

3. Native pollinators respond positively to the presence of alien flowers and switch frequently between alien and native plants or even prefer alien flowers and do not visit native species any more.

*Alternatively:* native pollinators respond negatively to the presence of alien flowers and become more flower constant toward native plants with increasing alien population size.



### 3.3. Materials and methods

#### 3.3.1. Study species

Introduced to Ireland in the 18<sup>th</sup> century, *Rhododendron ponticum* is considered an invasive alien plant species in various habitats around Britain and Ireland (Dehnen-Schmutz and Williamson, 2006). The perennial shrub produces its first flowers approximately in its 12<sup>th</sup> growing season (Robinson, 1980) and mature shrubs can bear several thousand flowers per individual (Cross, 1975). In Ireland, the pink-purple flowers emerge from May to July and produce larger amounts of sugar rich nectar than most Irish native co-flowering plant species (Chapter 2). Flowers are self-compatible but their capability for spontaneous autogamy is negligible (Stout, 2007b). They are mostly visited by bumblebees but also by other hymenopterans and dipterans, especially hoverflies (Stout et al., 2006).

The native *Digitalis purpurea* L. (foxglove) is a common facultative biannual plant species that can form large populations along road sides, forest edges and in logged forest areas. Each individual produces one or a few inflorescences with purple flowers, which are self-compatible (Stead and Moore, 1979). Basal flowers of an inflorescence bloom first and may set seed before apical flowers unfold. Flowers of *D. purpurea* are nectar-rich, and the main visitors are bumblebees although solitary bees and hoverflies also forage on the abundant pollen rewards (personal observations). Flower visitors of *D. purpurea* and *R. ponticum* overlap considerably (Chapter 2) which increases the likelihood of interaction between the two species in terms of pollination. *D. purpurea* flowers in Ireland between June and August.

#### 3.3.2. Field sites

The study was conducted in the Wicklow Mountains, Co. Wicklow, Ireland, between latitude 52°47'34.9"N to 52°58'45.4"N and longitude 06°11'04.1"W to 06°33'53.1"W. The area spans approx. 20 × 25 km<sup>2</sup>, and selection of experimental sites within it was based on the absence of *R. ponticum* in a radius of at least 1 km around sites. By choosing this distance, I maximised the likelihood that pollinators were neither experienced in visiting the alien nor influenced by *R. ponticum* flowers other than the ones at experimental sites, because 1 km exceeds the estimated average foraging distances of *R. ponticum*'s main





flower visitors, *Bombus* spp. (Knight et al., 2005a, Wolf and Moritz, 2008). Twenty-seven sites were chosen and randomly assigned to one of nine experimental treatments i.e. I spatially replicated each treatment three times (Supplement 7). Field sites were at least 1 km apart from each other to avoid interference of treatments as suspected in other studies (e.g. Bosch and Waser, 2001). Vegetation at sites was dominated by grasses but other native co-flowering species were also found. I mowed the grass cover on a small spatial scale (approx.  $3 \times 2 \text{ m}^2$ ) before setting up experimental plots to minimise influences of neighbouring co-flowering species.

Table 3.1. Experimental set up and spatial arrangement at single plots of the nine types of controls and treatments. Empty circles represent native potted *Digitalis purpurea* plant individuals, filled circles represent alien “plant individuals”, i.e. branches of *Rhododendron ponticum* in water-filled buckets. Note that Treatment 6 controls for two interspecific treatments.

|                            | Contol/<br>Treatment | Description                                                 | No. of<br>native<br>plants | No. of<br>alien<br>plants | Total<br>no. of<br>plants | Controls<br>for             | Arrangement<br>○ = native plant<br>● = alien plant |
|----------------------------|----------------------|-------------------------------------------------------------|----------------------------|---------------------------|---------------------------|-----------------------------|----------------------------------------------------|
| Control                    | Control 1            | Small native<br>population size                             | 3                          | –                         | 3                         | Treatment 1,<br>Treatment 2 |                                                    |
|                            | Control 2            | Large native<br>population size                             | 12                         | –                         | 12                        | Treatment 3,<br>Treatment 4 |                                                    |
| Interspecific interactions | Treatment 1          | Small native pop.<br>size + small pop.<br>size of alien     | 3                          | 3                         | 6                         |                             |                                                    |
|                            | Treatment 2          | Small native pop.<br>size + large pop.<br>size of alien     | 3                          | 12                        | 15                        |                             |                                                    |
|                            | Treatment 3          | Large native pop.<br>size + small pop.<br>size of alien     | 12                         | 3                         | 15                        |                             |                                                    |
|                            | Treatment 4          | Large native pop.<br>size + large pop.<br>size of alien     | 12                         | 12                        | 24                        |                             |                                                    |
| Intraspecific interactions | Treatment 5          | Small native pop.<br>size + small pop.<br>size of native    | 6                          | –                         | 6                         | Treatment 1                 |                                                    |
|                            | Treatment 6          | Small pop. size<br>of native + large<br>pop. size of native | 15                         | –                         | 15                        | Treatment 2,<br>Treatment 3 |                                                    |
|                            | Treatment 7          | Large native pop.<br>size + large pop.<br>size of native    | 24                         | –                         | 24                        | Treatment 4                 |                                                    |



### **3.3.3. Study design**

At each of the 27 sites, three plots of the same experimental treatment type were established 5 to 10 meters apart from each other. In all experimental treatments, plant individuals within each plot were placed at constant distances from each other (ca. 30 cm from centre to centre of each pot or bucket). Two levels of *D. purpurea* population sizes (small: three plant individuals; large: 12 individuals) were used as a base, i.e. as controls (Table 3.1). Small natural foxglove populations have been found to sometimes contain as few as three co-occurring plant individuals (personal observations), however, large populations can consist of several hundred individuals. I was constrained to 12 individuals for the larger population size as it was impossible to use a greater number of individuals in such a replicated experimental study. I then added either alien plants or conspecific plants to test for interspecific (alien) versus intraspecific (native) interactions in small and large foxglove populations, respectively. The number of added plant individuals was either three (small added population) or 12 (large added population) for both alien and conspecific species, respectively. I had to establish four treatment types of interspecific interactions where *R. ponticum* was present (Treatment 1 to 4, Table 3.1) as well as the corresponding three treatment types of intraspecific interactions without the invasive species (*D. purpurea* only; Treatment 5 to 7, Table 3.1). In total, 810 *D. purpurea* and 270 *R. ponticum* individuals were used.

### **3.3.4. Implementation**

One-year old foxglove plants were removed from natural populations of the Wicklow Mountains, Ireland, in April 2006 and potted in black 3 liter Goettinger pots (19 cm diameter, Plate 3 F) with self-blended compost (a mix of 6 parts wood-based compost, 1 part native soil and 1 part horticulture grit, enriched with 20 ml long-term fertiliser and 10 ml water-storing granulate). Plant survival was >99%. Plants were kept outdoors at Trinity College Botanic Gardens, Dublin (Plate 3 B), until first flowers opened in mid June 2006. Five days before the start of the experiment individual plants were relocated and randomly assigned to experimental treatments, field sites and plots where they were kept in pots and watered every third day. Towards the end of the experiment, approx. 10% of all plant individuals finished flowering and were replaced with foxglove inflorescences in water-filled bottles to keep the flower abundance at an appropriate level in relation to treatment





or control type. Flowers of bottled inflorescences still produced nectar and were replaced as soon as first signs of wilting were noticed. Potted individuals that had finished flowering were kept close to experimental plots to assure the same environmental conditions as other potted individuals in the same plot.

*R. ponticum* individuals at the age of first flowering grow to a size that is unsuitable for potting, transportation and easy manipulation of flower numbers. I therefore had to imitate living individuals of *R. ponticum* by using *R. ponticum* branches with two to seven inflorescences in water-filled black buckets (ca. 30 cm diameter; Plate 3 A). Branches were taken from *R. ponticum* infested natural field sites of the Wicklow Mountains area. Flowers of cut branches still produced nectar and revealed similar nectar standing crop values as *R. ponticum* flowers in natural field sites at peak flowering (mean  $\pm$  SE:  $2.71 \pm 2.35$   $\mu$ l in cut branches,  $2.75 \pm 2.71$   $\mu$ l in natural sites; Two-sample t-test,  $t_{209} = -0.11$ ,  $P = 0.916$ ). Branches were regularly replaced with freshly cut branches. The number of inflorescences and the number of flowers was homogeneously allocated among buckets. Every morning before observations started, flower numbers of *R. ponticum* were counted and adjusted to experimental treatment in order to keep relative flower abundances at the appropriate level. For example, in a treatment of small population size of the native (3 plant individuals) and large population size of the alien (12 buckets with *R. ponticum* branches), the number of flowers of *R. ponticum* was adjusted to the fourfold number of *D. purpurea* flowers. The number of *D. purpurea* flowers was not adjusted. The mean number of flowers ( $9.3 \pm 0.22$  SE) that were simultaneously open on *D. purpurea* inflorescences did not significantly differ between treatments and controls (ANOVA,  $F_{8,233} = 0.92$ ,  $P = 0.50$ ), and so the number of overall flowers at each experimental plot was closely correlated with the number of plants of treatment/control type (Spearman rank correlation  $r = 0.896$ ,  $N = 162$ ,  $P < 0.001$ ).

### 3.3.5. Experimental observations

#### Insect visitation

Each field site was visited twice between 28<sup>th</sup> June and 19<sup>th</sup> July 2006. During each visit, *D. purpurea* (and *R. ponticum* if present) in all three experimental plots per site were consecutively investigated in a random order. At each plot, I randomly chose three focal plant individuals of *D. purpurea*. To assure independence of observations, different focal



plants were chosen during the first and the second visit of the plot except at plots where only three plants of *D. purpurea* were present. Before observations started, I counted the number of inflorescences and open flowers of each focal plant. In addition, I recorded the total number of inflorescences and flowers of both species at each plot. I then monitored pollinator visitation to focal plant inflorescences and flowers in 10 min observation sessions (Plate 3 C). Observations were only conducted in sunny weather conditions with wind force less than 3 according to Beaufort scale. I noted the identity of each flower visitor; individuals of unknown species were collected when flying off and killed by freezing to identify them later in the laboratory. If possible, I also recorded the species identity of plant individuals from which flower visitors had arrived and/or to which they departed after visiting one of the focal plants. This provided a measure of the visitors' flower constancy.

Pollinator visitation rate was calculated as number of visited flowers of focal plants per 10 min / total number of open flowers of focal plants. In addition, the number of visitors per inflorescence was calculated as number of visitors to focal plants per 10 min / total number of inflorescences of focal plants. I also calculated bout length within each visited inflorescences as the number of flowers visited by a single insect in a single inflorescence visit.

### Reproductive success

All open but not yet receptive flowers of three randomly chosen *D. purpurea* individuals per plot were counted and marked after experimental plots were installed. In the small native population treatments, all three *D. purpurea* plants present were used. After completion of experimental observations when flowers had withered, all marked plant individuals were relocated to Dublin (my private back garden, Plate 3 E), where they were kept under common environmental conditions. I collected and counted all marked matured fruit capsules and measured their maximum length and maximum diameter with a digital calliper (Moore & Wright, UK; precision 0.01 mm). In total, 2224 capsules were measured. I randomly picked one fruit of each of 79 plant individuals and counted the number of seeds enclosed in the capsules under a dissecting microscope (Leica, UK). Since seed number was more closely related to the product of fruit length and fruit diameter than to fruit volume [volume =  $1/3\pi \times \text{length} \times (\text{diameter}/2)^2$ ], fruit length or fruit diameter (Pearson's product moment correlation: length\*diameter  $r = 0.902$ ,  $t_{75} = 17.82$ ,  $P < 0.001$ ;





volume  $r = 0.788$ ,  $t_{75} = 16.62$ ; length  $r = 0.818$ ,  $t_{75} = 12.47$ ; diameter  $r = 0.821$ ,  $t_{75} = 12.62$ ;  $P < 0.001$  for all), seed numbers of all harvested fruits were estimated as  $11.93 \times (\text{length} \times \text{diameter}) - 337.37$  (linear regression,  $R^2 = 0.813$ ). Capsules that contained at least one seed were defined as fruits. I then calculated the fruit/flower ratio (relative fruit set) as well as the average number of seeds per fruit across each inflorescence. One plant individual had been vandalised, and so I had to substitute it in analyses with means of the two other plant individuals in this plot.

### 3.3.6. Spontaneous autogamy in *D. purpurea*

Since *D. purpurea* is a self-compatible plant species, a measure of fruit and seed production in absence of any pollinators improves the evaluation of the above manipulative experiment. I therefore transferred ten potted *D. purpurea* individuals to a glasshouse before flower buds had opened, and kept them constantly enclosed to prevent insect flower visitation. At peak flowering, all open flowers of each individual were marked. Matured fruits were collected, and maximum length as well as maximum diameter, were measured with a digital calliper. I then counted seed numbers in each of the 155 fruits. In a few cases, some seeds were lost during harvest, and so I estimated seed numbers of these fruits using the linear regression model as stated above.

### 3.3.7. Data analyses

Before computations of ANOVA, data were tested for the assumption of homogeneity of variances employing Cochran's tests. If necessary, I transformed the data. Analyses were computed with the software packages MINITAB 13 (Minitab Inc., 2000), SPSS 15 (SPSS Inc., 2006) and GMAV 5 (Underwood and Chapman, 1998).

### Insect visitation and reproductive success

Two variables of insect visitation (pollinator visitation rate per flower and visitors per inflorescence) and two variables of reproductive success (relative fruit set and number of seeds per fruit) were included in analyses. I analysed all variables using asymmetrical 5-factor mixed ANOVA models (Table 3.2) described by Underwood (1997) and used in other similar studies before (e.g. Boaventura et al., 2003). The construction of asymmetrical models is based on a combination of values of sum of squares from



Table 3.2. Asymmetrical ANOVA model design according to Underwood (1997). Small letters represent the number of levels of the particular factor. The random factor 'Plot' ( $f = 3$ ) is nested within the random factor 'Site' ( $e = 3$ ) which is nested within the interaction of the fixed, orthogonal factors 'Size of *D. purpurea* population' ( $a = 2$ ) and 'Experimental treatment' ( $b = 5$ ) (Zar, 1999). The factor 'Experimental treatment' is partitioned into the control and the treatments. Treatments are distinguished by two fixed, orthogonal factors: 'Species added' ( $c = 2$ ) and 'Population size of added species' ( $d = 2$ ) with their interaction. Observations within each plot were replicated ( $n = 2$  for pollinator visitation data;  $n = 3$  for fruit/seed set data). The design for each part of the ANOVA is balanced, i.e. displayed degrees of freedom did not require any adjustment.

| Source of variation                                                                                  | df                        | F                                                                               |
|------------------------------------------------------------------------------------------------------|---------------------------|---------------------------------------------------------------------------------|
| Among all                                                                                            | $(abefn - 1) - abef(n-1)$ |                                                                                 |
| Size of <i>D. purpurea</i> population (3 vs. 12 individuals)                                         | $a - 1$                   | $MS_{Size} / MS_{Site}$                                                         |
| Experimental treatment                                                                               | $b - 1$                   |                                                                                 |
| Control vs. Treatments                                                                               | $(b - 1) - \alpha$        | $MS_{Control} / MS_{Site}$                                                      |
| Among Treatments                                                                                     | $\alpha$                  |                                                                                 |
| Species added ( <i>D. purpurea</i> vs. <i>R. ponticum</i> added)                                     | $c - 1$                   | $MS_{Species} / MS_{Site}$                                                      |
| Population size of added species (3 vs. 12 individuals added)                                        | $d - 1$                   | $MS_{Pop\ size} / MS_{Site}$                                                    |
| Species $\times$ Pop size                                                                            | $(c - 1)(d - 1)$          | $MS_{Species \times Pop\ size} / MS_{Site}$                                     |
| Size $\times$ Exp treatment                                                                          | $(a - 1)(b - 1)$          |                                                                                 |
| Size $\times$ Control vs. Treatments                                                                 | $(a - 1)(b - 1) - \beta$  | $MS_{Size \times Control} / MS_{Site}$                                          |
| Size $\times$ Among Treatments                                                                       | $\beta$                   |                                                                                 |
| Size $\times$ Species                                                                                | $(a - 1)(c - 1)$          | $MS_{Size \times Species} / MS_{Site}$                                          |
| Size $\times$ Pop size                                                                               | $(a - 1)(d - 1)$          | $MS_{Size \times Pop\ size} / MS_{Site}$                                        |
| Size $\times$ Species $\times$ Pop size                                                              | $(a - 1)(c - 1)(d - 1)$   | $MS_{Size \times Species \times Pop\ size} / MS_{Site}$                         |
| Site (Size $\times$ Exp. treatment)                                                                  | $ab(e - 1)$               |                                                                                 |
| Site (Size $\times$ Control)                                                                         | $\gamma$                  | $MS_{Site\ (Size \times Control)} / MS_{Plot}$                                  |
| Site (Among Treatments) = Site (Size $\times$ Species $\times$ Pop size)                             | $acd(e - 1)$              | $MS_{Site\ (Size \times Species \times Pop\ size)} / MS_{Plot}$                 |
| Plot (Size $\times$ Exp. treatment $\times$ Site)                                                    | $abe(f - 1)$              |                                                                                 |
| Plot (Size $\times$ Control $\times$ Site)                                                           | $\delta$                  | $MS_{Plot\ (Size \times Control \times Site)} / MS_{Residual}$                  |
| Plot (Size $\times$ Among Treatments) = Plot (Size $\times$ Species $\times$ Pop size $\times$ Site) | $acde(f - 1)$             | $MS_{Plot\ (Size \times Species \times Pop\ size \times Site)} / MS_{Residual}$ |
| Residual                                                                                             | $abef(n - 1)$             |                                                                                 |

$$\alpha = (c - 1) + (d - 1) + (c - 1)(d - 1) \quad \beta = (a - 1)(c - 1) + (a - 1)(d - 1) + (a - 1)(c - 1)(d - 1) \quad \gamma = ab(e - 1) - acd(e - 1) \quad \delta = abe(f - 1) - acde(f - 1)$$





separately computed analyses of variance within a single ANOVA model. *Post-hoc* comparisons were performed using Student-Newman-Keuls (SNK) tests or Dunnett's tests. Since one type of treatment (15 foxglove individuals) served as intraspecific control for two interspecific treatments and was included twice in the analysis (Table 3.1), data of different observation sessions as well as fruit and seed production of different flowers were randomly assigned to one or the other treatment group to assure independence of data. I furthermore used G-tests (adjusted with William's correction factor in case of  $2 \times 2$  contingency tables; Fowler et al., 1998) to compare the abundance of the main *D. purpurea* visiting insect taxa as well as their constancy among experimental treatments. In cases where observational data were used more than once in different analyses, significance levels were adjusted with the Bonferroni method (Rice, 1989).

Bout length (small integer values) was analysed using a 2-factor Generalised Linear Model (GLIM, Poisson distribution and log link, Crawley, 1993) with 'Size of population' and 'Species added' as fixed factors, their interaction term and 'Number of flowers on inflorescence' as a covariate. However, the number of flower visits on an inflorescence in relation to the open flowers on an inflorescence may not be well described by a linear model because the number of flowers available is limited to a minimum and maximum (here 1-23 flowers per *D. purpurea* inflorescence), and so data were also fitted to a non-linear logistic regression model corresponding to the one described by Kunin (1993) using the sequential quadratic programming method in SPSS.

### **Spontaneous autogamy in *D. purpurea***

I used ANOVA and *posthoc* Dunnett's tests to compare relative fruit set and number of seeds per fruit between plants kept apart from pollinator visitation and plants of all treatments and controls of the manipulative experiment.

## **3.4. Results**

### **3.4.1. Insect visitation**

The size of the experimental *D. purpurea* populations (factor 'Size of population') significantly affected both native flower visitation rate by pollinators and the number of visitors per inflorescence. Floral pollinator visitation rates of *D. purpurea* were significantly lower in small populations than in large populations of *D. purpurea* (square



root transformed mean rate  $\pm$ SE in small populations:  $0.110 \pm 0.020$ ; in large populations:  $0.190 \pm 0.024$ ; Table 3.3 A, Fig. 3.1 A). Similarly, *D. purpurea* inflorescences in small populations received fewer visitors than inflorescences in large populations (mean number of visitors per 10 min  $\pm$ SE in small populations:  $0.150 \pm 0.029$ ; in large populations:  $0.238 \pm 0.031$ ; Table 3.3 B, Fig. 3.1 B). Despite the fact that insect visitation was influenced by *D. purpurea* population size, I observed only a marginal positive effect of the number of added plant individuals on native flower visitation rate ( $P = 0.092$ ; Table 3.3 A) and the number of insects visiting *D. purpurea* inflorescences ( $P = 0.078$ ; Table 3.3 B).

The identity of the added plant individuals (factor 'Species added'), i.e. the species composition of the created plant arrangement, did not significantly affect flower visitation rates (Table 3.3 A), but it had an impact on the number of insect visitors per *D. purpurea* inflorescence. Inflorescences were approached by a significantly smaller number of visitors in populations with the alien *R. ponticum* present than in populations where only *D. purpurea* had been added (mean visitor number per 10 min  $\pm$ SE in populations with *R. ponticum* added:  $0.124 \pm 0.025$ ; in populations with *D. purpurea* added:  $0.256 \pm 0.041$ ; Table 3.3 B, Fig. 3.1 B). However, neither visitation rates nor visitor numbers of mixed-species treatments or single-species treatments differed significantly from controls (*posthoc* Dunnett  $P > 0.10$  for each treatment – control comparisons).

I recorded 16 different insect taxa on *D. purpurea* flowers, including members of the orders Hymenoptera, Diptera and Hemiptera. The most frequent visitors were bumblebees (*Bombus* spp.; 66.4 % of all flower visiting insects), solitary bees (Andrenidae and Halictidae; 16.8 %) and syrphid flies (Syrphidae; 10.3 %). An investigation of the assemblage of these main visitor groups showed that foxglove plants received fewer syrphid fly visitors than expected in plots with added *R. ponticum* (G-test,  $G = 9.27$ ,  $df = 2$ ,  $P < 0.01$ ). In mixed species plots with *R. ponticum* added (Treatment 1 – 4), I observed 80 flower visitor movements between different plant individuals. 81.3 % of these switches occurred between conspecific plant individuals whereas 18.7 % of switches were interspecific movements. I never observed a pollinator switch between *D. purpurea* and *R. ponticum* individuals in small foxglove populations which was significantly scarcer than expected (G-test,  $G_{adj} = 11.05$ ,  $df = 1$ ,  $P_{Bonferroni} < 0.01$ ). In these plots, pollinators left the patch and flew further away after visiting one or the other plant species. The population size of





Table 3.3. ANOVA results for different variables of visitation and reproduction in experimental *D. purpurea* plants. Data for visitation rates were square root transformed before analysis. Nested factors were pooled or eliminated from the model when  $P > 0.25$  (Winer et al., 1991). Degrees of freedom for numerator ( $df_{\text{Nom}}$ ) and denominator ( $df_{\text{Den}}$ ) and  $F$ -values (if applicable) are displayed for analyses after pooling or eliminating from the model.

| Source of variation                 | A) Visitation rate |                   |                   | B) Visitors/inflorescence |                   |                   | C) Fruits/flower <sup>1</sup> |                   |                | D) Seeds/fruit <sup>1</sup> |                   |               |
|-------------------------------------|--------------------|-------------------|-------------------|---------------------------|-------------------|-------------------|-------------------------------|-------------------|----------------|-----------------------------|-------------------|---------------|
|                                     | $df_{\text{Nom}}$  | $df_{\text{Den}}$ | $F$               | $df_{\text{Nom}}$         | $df_{\text{Den}}$ | $F$               | $df_{\text{Nom}}$             | $df_{\text{Den}}$ | $F$            | $df_{\text{Nom}}$           | $df_{\text{Den}}$ | $F$           |
| <b>Size of population</b>           | 1                  | 80                | <b>6.41*</b>      | 1                         | 150               | <b>5.24*</b>      | 1                             | 20                | 0.74           | 1                           | 20                | 1.03          |
| Experimental treatment              | 4                  |                   |                   |                           |                   |                   |                               |                   |                |                             |                   |               |
| Control vs. Treatments              | 1                  | 80                | 0.40              | 1                         | 150               | 0.03              | 1                             | 20                | 0.07           | 1                           | 20                | 0.01          |
| Among Treatments                    | 3                  |                   |                   |                           |                   |                   |                               |                   |                |                             |                   |               |
| <b>Species added</b>                | 1                  | 80                | 0.28              | 1                         | 150               | <b>8.56**</b>     | 1                             | 20                | 1.86           | 1                           | 20                | 1.02          |
| Pop size                            | 1                  | 80                | 2.91 <sup>#</sup> | 1                         | 150               | 3.15 <sup>#</sup> | 1                             | 20                | 1.05           | 1                           | 20                | 1.07          |
| Spp × Pop size                      | 1                  | 80                | 0.07              | 1                         | 150               | 0.18              | 1                             | 20                | 1.21           | 1                           | 20                | 0.29          |
| Size × Exp. treatment               | 4                  |                   |                   |                           |                   |                   |                               |                   |                |                             |                   |               |
| Size × Control vs. Treatments       | 1                  | 80                | 1.69              | 1                         | 150               | 2.25              | 1                             | 20                | 0.88           | 1                           | 20                | 0.26          |
| Size × Among Treatments             | 3                  |                   |                   |                           |                   |                   |                               |                   |                |                             |                   |               |
| Size × Spp                          | 1                  | 80                | 0.02              | 1                         | 150               | 0.01              | 1                             | 20                | 1.01           | 1                           | 20                | 1.39          |
| Size × Pop size                     | 1                  | 80                | 1.09              | 1                         | 150               | 0.16              | 1                             | 20                | < 0.01         | 1                           | 20                | 1.31          |
| Size × Spp × Pop size               | 1                  | 80                | < 0.01            | 1                         | 150               | 1.94              | 1                             | 20                | 0.10           | 1                           | 20                | 0.98          |
| Site (Size × Exp. treatment)        | 20 <sup>‡</sup>    |                   |                   | 20 <sup>‡</sup>           |                   |                   | 20                            |                   |                | 20                          |                   |               |
| <b>Site (Size × Control)</b>        | 4                  | 60                | 0.15              | 4                         | 150               | 0.40              | 4                             | 179               | <b>12.43**</b> | 4                           | 179               | <b>9.78**</b> |
| <b>Site (Size × Spp × Pop size)</b> | 16                 | 60                | 1.19              | 16                        | 150               | 1.39              | 16                            | 179               | <b>7.44**</b>  | 16                          | 179               | <b>4.67**</b> |
| Plot (Size × Exp. treatment × Site) | 60                 |                   |                   | 60 <sup>‡</sup>           |                   |                   | 60 <sup>‡</sup>               |                   |                | 60 <sup>‡</sup>             |                   |               |
| Plot (Size × Control × Site)        | 12                 | 90                | 1.66              | 12                        | 90                | 1.23              | 12                            | 179               | 0.85           | 12                          | 179               | 0.34          |
| Plot (Size × Spp × Pop size × Site) | 48                 | 90                | 0.92              | 48                        | 90                | 1.13              | 48                            | 179               | 1.14           | 48                          | 179               | 0.98          |
| Residuals                           | 90                 |                   |                   | 90                        |                   |                   | 179                           |                   |                | 179                         |                   |               |

<sup>‡</sup> pooled with denominator factor    <sup>‡</sup> eliminated from analysis    <sup>#</sup> $P < 0.1$     \* $P < 0.05$     \*\* $P < 0.01$

<sup>1</sup> df of residual term reduced by 1 due to substitution of a missing value by a mean (see also method section)

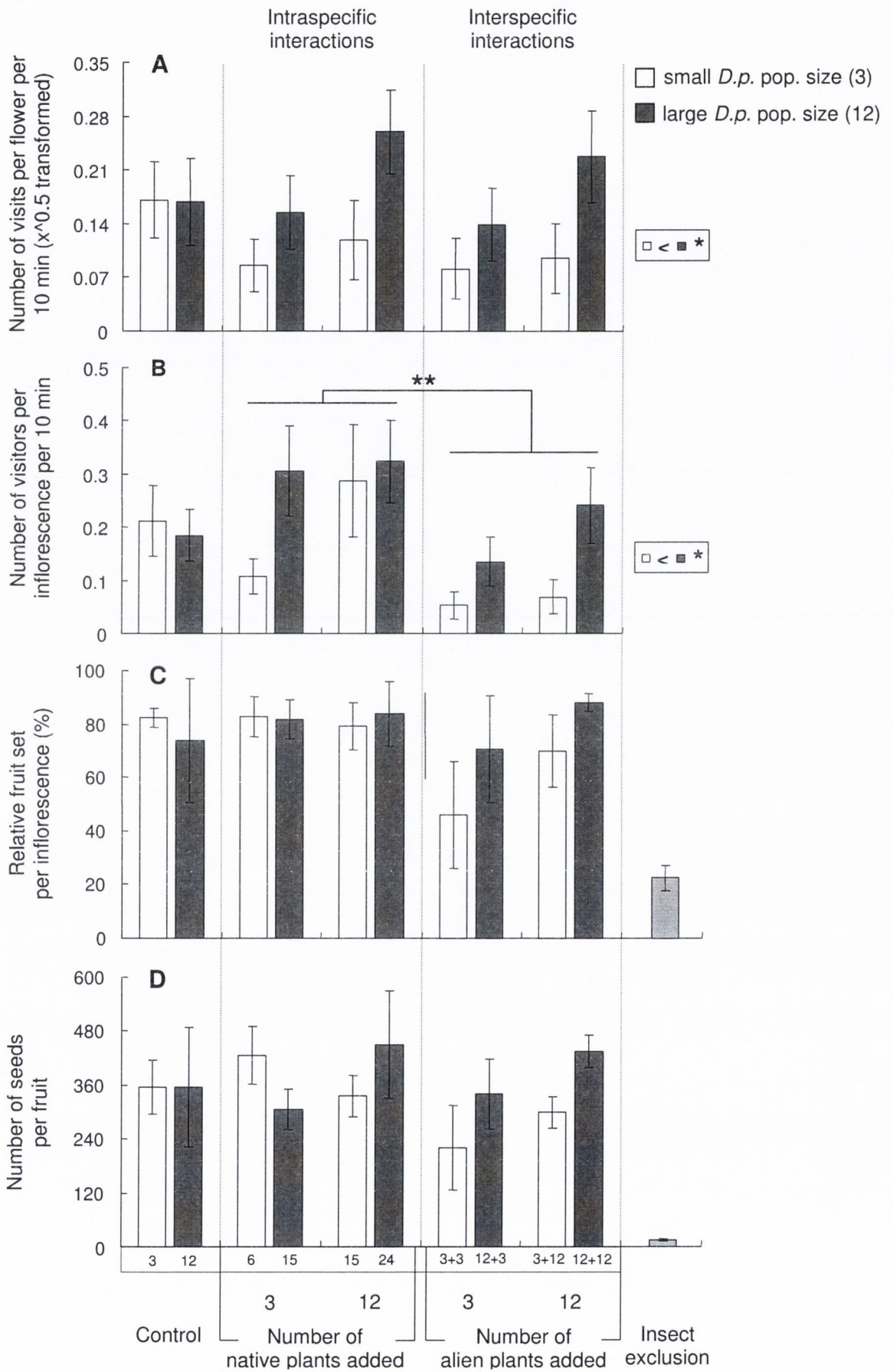






Figure 3.1 (previous page). Mean square root transformed pollinator visitation rate (A), mean number of insect visitors per inflorescence (B), mean relative fruit set per inflorescence (C) and mean number of seeds per fruit (D) of *D. purpurea* plant individuals in response to controls, treatments and pollinator exclusion in the manipulative and spontaneous autogamy experiments. Empty bars represent results for control and treatments of small *D. purpurea* population size (3 plant individuals) and dark grey bars represent results for control and treatments of large population size (12 *D. purpurea* individuals). Bars in light grey represent results from the spontaneous autogamy experiment. Error bars correspond to standard errors calculated across means of subgroups (Quinn and Keough, 2002).

*R. ponticum* at the plots did not affect pollinator movement patterns between plant individuals, i.e. flower constancy (G-test,  $G_{adj} = 0.52$ ,  $df = 1$ ,  $P_{Bonferroni} > 0.05$ ). Insects visited on average  $1.94 \pm 0.11$  SE flowers per inflorescence before they switched to the next plant individual. Bout length on an inflorescence was neither influenced by the size of population (GLIM;  $\chi^2 = 0.60$ ,  $df = 1$ ,  $P = 0.44$ ) nor by the presence of *R. ponticum* (GLIM;  $\chi^2 = 0.002$ ,  $df = 1$ ,  $P = 0.97$ ) or an interaction of the two factors (GLIM;  $\chi^2 = 0.54$ ,  $df = 1$ ,  $P = 0.46$ ). Hardly surprising, pollinators visited more flowers on larger than on smaller inflorescences (GLIM;  $\chi^2 = 7.30$ ,  $df = 1$ ,  $P > 0.01$ ). This result was corroborated by the non-linear logistic regression models (Supplement 8) that showed an increase in bout length with increasing inflorescence size (Fig. 3.2). Regression parameters did not significantly differ between treatments (Supplement 8).

### 3.4.2. Reproductive success

Relative fruit set and number of seeds per fruit were highly variable among sites of the same treatment or control and therefore the nested factor 'Site' was significant in both cases (Table 3.3 C and D). Sites within the mixed-species treatments 1, 2 and 3 did significantly differ from each other (*post-hoc* SNK,  $P < 0.01$ ) as well as sites within the single-species treatment 7 (SNK,  $P < 0.05$ ) and control 2 (SNK,  $P < 0.01$ ). As a result of the high variability among sites, fruit and seed set did not significantly differ between different treatments (Table 3.3 C and D, Fig. 3.1 C and D).

### 3.4.3. Spontaneous autogamy in *D. purpurea*

Plants of *D. purpurea* in the glasshouse that were excluded from pollinator visitation displayed low fruit and seed set (mean relative fruit set per inflorescences  $\pm$ SE: 22.4 %  $\pm$ 4.86; mean number of seeds per fruit  $\pm$ SE: 13.0  $\pm$ 2.41). Protected glasshouse plants

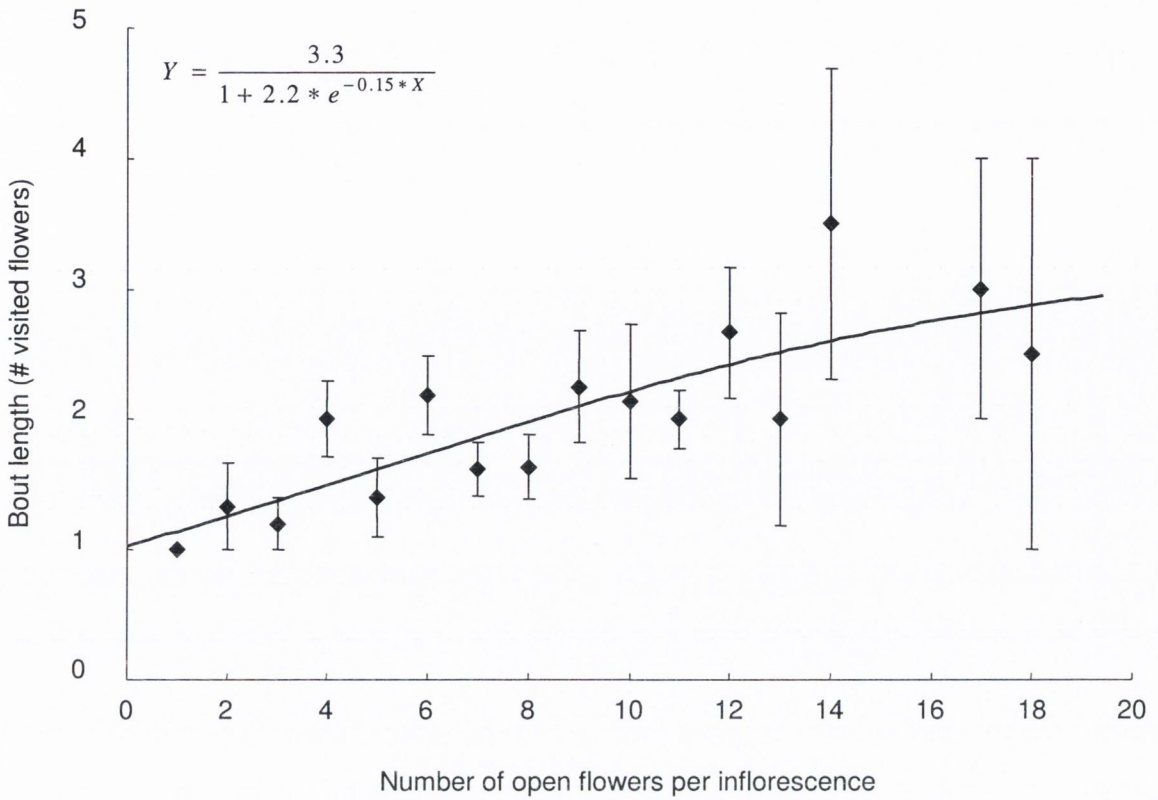


Figure 3.2. Mean number of flowers visited in a single foraging bout on an inflorescence in relation to the number of open flowers on this inflorescence. The observed relationship can be predicted by a non-linear logistic regression model ( $R^2 = 0.68$ , solid curve). Error bars correspond to standard errors.

produced significantly fewer fruits per inflorescence than experimental plants from all treatments and controls (ANOVA,  $F_{10,269} = 6.74$ ,  $P < 0.001$ ; *posthoc* Dunnett's test  $P < 0.001$ ) except for plants of treatment 1 (small population size of native and invasive species, respectively) which did not significantly differ in their relative fruit set from the plants in the glasshouse (Fig. 3.1 C). Similarly, protected glasshouse plants produced a significantly lower number of seeds per fruit than manipulative plants in the field (ANOVA,  $F_{10,269} = 6.32$ ,  $P < 0.001$ ; *posthoc* Dunnett's test for all treatments and controls:  $P < 0.001$ , except for treatment 1:  $P < 0.05$ ; Fig. 3.1 D).

### 3.5. Discussion

My experiment evaluated the effects of population size on intra- and interspecific plant-pollinator interactions between a native self-compatible plant species and an alien invasive





species and its consequences for native plant reproduction. I could demonstrate for the *D. purpurea* – *R. ponticum* system that population size of the native species influenced the plant-pollinator relationship, however, this response was not translated into reproductive success. I also showed that the alien did not clearly disrupt or facilitate pollination processes; nevertheless it had an impact on flower visitation and pollinators that I will address.

### 3.5.1. Intraspecific interactions among *D. purpurea*

For the visitation of *D. purpurea* flowers and inflorescences, the number of individuals of the surrounding plant arrangement mattered. Flowers and inflorescences in smaller populations were less frequently visited by pollinators than in larger populations. In combination with the detected (though not significant) trend that the number of added individuals had a positive impact on flower and inflorescence visitation rate, I can assume that *D. purpurea* individuals facilitate each others visitation.

The finding that an increase in plant numbers, i.e. inflorescences and flowers, attracted more pollinators is in line with my earlier hypothesis of conspecific facilitation and has been demonstrated for various other systems (e.g. Sih and Baltus, 1987, Cresswell and Osborne, 2004, Wailes and Ågren, 2004, Zorn-Arnold and Howe, 2007; but see Kunin, 1997, Mustajärvi et al., 2001). In addition, distances between plant individuals in my populations were relatively short and plants could be perceived by flower visitors as dense populations. Increasing densities on a local scale (e.g. in a 10 m radius) can affect pollinator visitation responses to plant patches positively (e.g. Thomson, 1982, Jennersten, 1988, Field et al., 2005, Kirchner et al., 2005, Feldman, 2006; but see Mustajärvi et al., 2001), and an increase in the number of plant individuals in my small populations did also increase flower density at this small scale. Increases in conspecific population size or density have been shown to also facilitate fruit and seed set (Jennersten and Nilsson, 1993, Bosch and Waser, 2001, Spigler and Chang, 2008), however, higher abundance of *D. purpurea* did not result in higher reproductive success here. This is surprising since the generality of a positive relationship between population size and female fitness can be assumed for most plant species (Leimu et al., 2006). However, it has also been pointed out



that an effect is more evident in self-incompatible and rare species (Mustajärvi et al., 2001, Leimu et al., 2006; but see Oostermeijer et al., 1998, for a self-compatible species).

In my experimental populations, fruit and seed set was lower than observed reproductive success in other *D. purpurea* communities (Buxton and Darlington, 1932, Chapter 4). Site effects within treatments were highly significant and the variability of fruit and seed set among sites was not attributed to any systematic pattern (i.e. variable fruit set was independently detected for mixed-species/single-species populations or small/large populations). Although there were no large wild *D. purpurea* populations growing in the neighbourhood (30 meter diameter) of any of the plots, I did not monitor *D. purpurea* abundances in a farther distance. Site effects might therefore be a cause of additional pollen sources in the farther surrounding area. Surely, weather is an important factor for variability in flower visitation too (Corbet et al., 1993, Peat and Goulson, 2005) and reproductive success (Alexandersson and Ågren, 1996, Kirchner et al., 2005); however, my sites should have experienced similar weather conditions at a regional scale. Furthermore, I potted plants and therefore standardised the effects of nutrients and water availability that are usually assumed to limit reproductive success (Mustajärvi et al., 2001, Ågren et al., 2008). Differences in fruit and seed set are likely to be primarily a result of different pollination success and pollen limitation which in *D. purpurea* is both linked to pollinator limitation; plants excluded from pollinator visitation revealed very low fruit and seed set. Since some plants gained full fruit set and high seed set and others in adjacent plots did not, pollination success is likely to depend on stochastic visitation patterns (e.g. bet hedging, Ashman et al., 2004). Variability in plant and pollinator communities is a well known phenomenon (Herrera, 1988, Roubik, 2001), and unpredictable pollination patterns can be common, especially in temperate environments (e.g. Totland, 1994, Alexandersson and Ågren, 1996, Waites and Ågren, 2004).

Nevertheless, individual fruit set of 80% was common even in small populations. Unlike some other study systems, *D. purpurea* did not show a dramatically lower quantitative reproductive performance or breakdown in connection with pollinator limitation at very small population sizes (Ågren, 1996, Waites and Ågren, 2004, Bell et al., 2005). This is probably due to its self-compatible breeding system where self-pollen may provide a back up mechanism to ensure reproduction even at low flower visitation (Barrett, 2003, see also Kalisz and Vogler, 2003), although autogamous selfing is negligible in *D. purpurea*.





Hence, even with very few pollinator visits an individual might set full seed, which may explain the absence of a linear relation between visitation and observed fruit/seed set. Pollinator visitation still occurred, even at very low plant abundances. This might have been facilitated by co-flowering plant species other than *R. ponticum* at a local or landscape scale (Ghazoul, 2006, Heard et al., 2007) although I chose experimental plots with low local flower abundance for this study. Effects on flower visitation that are not expressed as effects on subsequent quantitative seed set, have been reported before (e.g. Caruso, 1999, Feldman, 2008) and seem to be common among plants in variable environments. Still, in small natural populations there might be other genetic patterns that could affect seed set in *D. purpurea* in a different way.

### 3.5.2. Interspecific interactions between *D. purpurea* and *R. ponticum*

In my experiment, flower visitation rates of *D. purpurea* in mixed-species populations appeared to be similar to flower visitation in single-species populations. Neither of the treatment groups differed significantly from the control groups in their flower visitation rate, and so competitive or facilitative effects mediated by small scale increases in abundance of either species are not substantiated. However, population size of the added species tended to affect visitation rate positively, and so a positive effect of abundance in general, which would also imply a facilitative effect of *R. ponticum* on *D. purpurea* flower visitation, might have been masked by the high variation in flower visitation rates that I observed within treatments.

Although I observed more visitors on inflorescences in populations where *D. purpurea* plants had been added than in populations where *R. ponticum* had been the addition, again neither of the two treatment groups differed significantly from the control groups in which I observed an intermediate rate of *D. purpurea* inflorescence visitation. Hence, I cannot explain unequivocally if this difference in inflorescence visitation rate between single- and multi-species treatments was linked to an intraspecific facilitative effect among *D. purpurea* inflorescences in single-species stands, an interspecific competitive effect of *R. ponticum* on *D. purpurea* in mixed-species stands or a combination of both. Since I also found a trend for a positive effect of population size of the added species on inflorescence



visitation, intraspecific facilitation among *D. purpurea* inflorescences with increasing population size is more likely than interspecific competition by *R. ponticum*.

Considering the current knowledge on impacts of alien plants on native flower visitation (Bjerknes et al., 2007), a lack in impact is not surprising; impacts have been shown to vary widely from competitive (Chittka and Schürkens, 2001, Totland et al., 2006) to neutral (Aigner, 2004, Moragues and Traveset, 2005) and facilitative (Moragues and Traveset, 2005, Bartomeus et al., 2008b). Pollinators frequently visited *R. ponticum* at my field sites (unpublished data) and also in natural populations (Stout et al., 2006). They could have preferred to visit the alien in mixed stands, yet visitation rate to the native was not affected. Pollinators might have conducted longer bouts and included both the alien and the native plants in their foraging (Feldman, 2006). I observed interspecific flower movement, and so pollinators might have been attracted by both species indiscriminately. This could have also resulted in a higher number of pollinators to the overall display (Laverly, 1992) that had to be shared by both plant species. Since I know that both species are visited by the same pollinators (Chapter 1 and 4), it is less likely that alien and native plants attracted different pollinator species, which ignored the other plant. Plant species that overlap little in their pollinators are obviously less likely to impact each others' pollination (Goodell, 2008).

The tendency towards a higher visitation when either of the two plant species was added might indicate that a higher number of replicated field sites might have decreased the effect of high variability within treatments and unmasked an underlying treatment effect. However, an even larger number of study sites would have been logistically impossible. Furthermore, the plant abundances used might have been too small to reveal a statistically significant difference. Again, I was limited by human and monetary resources. This shows that although manipulative experiments are ideal for answering complex questions and control important factors in an experiment, they may be limited by practicability (see last paragraph).

With a lack of any clear impacts of the alien species on native visitation, it was not surprising that fruit and seed set of *D. purpurea* was not significantly affected by the alien





either. I conclude that effects of conspecific abundance play a more important role in *D. purpurea* pollination and reproduction than effects of co-flowering *R. ponticum*.

### 3.5.3. Pollinator response

Plant individuals of both *D. purpurea* and *R. ponticum* received flower visitors of the local insect community immediately after setting up the plots (Plate 3 D). In mixed-specific treatments, pollinators were observed to switch directly between *D. purpurea* and *R. ponticum* individuals and vice versa. Interspecific movements occurred on average in one out of five cases. This endorses other studies of multispecific populations where intraspecific switches occurred in 75% to 95% of flights between individuals (e.g. Stout et al., 1998, see Morales and Traveset, 2008 for review and exceptions). Pollen usually travels not further than to a near neighbour (Price and Waser, 1979, Waser, 1988, Wilcock and Neiland, 2002), and switches within mixed-species plots are an indication for heterospecific pollen transfer and deposition between the alien and the native species. In various studies heterospecific pollen has been shown to interfere with fertilisation of plants (e.g. Waser and Fugate, 1986, Petanidou et al., 1995, Brown and Mitchell, 2001), however, even when *R. ponticum* pollen was transferred to *D. purpurea* stigmata it did not significantly reduce the latter's reproductive success (Chapter 4). The observed higher flower constancy in small *D. purpurea* populations may have also prevented alien pollen transfer at least in the small populations. Heterospecific switches within my experimental plant populations show that insects use *R. ponticum* at low abundances, probably indiscriminately to natives, which may enhance seed set and further dispersal of the alien.

In natural populations of *D. purpurea*, Grindeland et al. (2005) found that dense patches attracted more bees but bees probed fewer flowers per plant than in scarce populations. This pattern has been also supported by other studies (Klinkhamer and de Jong, 1990, Waites and Ågren, 2004). A higher attractiveness of dense patches for pollinators could result in less available resources per flower and hence shorter bouts within dense populations (Ågren, 1996). In my study, abundance but not density differed between plots, i.e. distance to the nearest neighbour was the same in all treatments. This might be one explanation why bout lengths on single inflorescences did not differ between populations of different sizes although floral visitation was more frequent in larger than in smaller *D.*



*purpurea* populations. Another explanation may be a high nectar standing crop, which was revealed in all treatments and which exceeded measured amounts of the previous year (unpublished data). Hence, resource levels remained high and encouraged pollinator visitation overall. The only significant predictor for bout length was the size of inflorescence visited. Pollinators usually probe more flowers in individuals with larger display sizes due to reduced travelling and flower handling costs (Robertson and Macnair, 1995, Goulson et al., 1998, Grindeland et al., 2005). Yet on an individual flower level this might not be advantageous due to intra-individual floral competition for pollinator visitation (Grindeland et al., 2005) and higher geitonogamous pollen transfer (de Jong et al., 1993). *Digitalis purpurea* inflorescences of larger sizes were evenly distributed among treatments and I did not investigate such proposed negative effects here.

Combining my observed results suggests that population size provided a long distance cue for pollinators in my system with plants in larger populations being more attractive certainly due to better visual perception (Dafni et al., 1997). Once in a patch the behaviour of a pollinator may be fine-tuned by parameters associated with the attractiveness of within plant and nearest neighbouring plant characteristics, e.g. resource levels or inter-plant distances (Hodges, 1985). *R. ponticum* can contribute to an increase in overall abundance and may raise attractiveness to co-flowering plants even if my data only showed a tendency for this. In turn, the alien may support local pollinators (see also Chapter 5).

#### **3.5.4. A matter of scale**

To my knowledge, four studies have addressed the abundance of an alien invasive plant species on native pollination and reproduction so far (Grabas and Laverty, 1999, Brown et al., 2002, Lopezaraiza-Mikel, 2006, Muñoz and Cavieres, 2008). Muñoz and Cavieres (2008) reported positive effects at a small (1 individual) and negative effects at a larger (5 individuals) alien population size on native pollination and seed set. Grabas and Laverty (1999) observed some negative effects on seed set without any link to effects on other pollination parameters. Neither of the two studies investigated uninvaded control sites or tested for intraspecific interactions among the natives, and so I do not know if the observed patterns are due to a general density effect or the competitive characteristics of the invader. In contrast, Brown et al. (2002) and Lopezaraiza-Mikel (2006) conducted manipulative





experiments. Lopezaraiza-Mikel (2006) added only alien flowers (0 to 384 *Impatiens glandulifera* flowers) to experimental plots and found an increase in flower visitation which she interpreted as facilitative effects, although it is not clear if addition of native flowers would have yield even more visits. Brown et al. (2002) investigated both interspecific and intraspecific interactions by using an initial population size of eight individuals. Visitation parameters were negatively influenced by both the presence of the alien and overall abundance, however, reproductive success of the self-incompatible native *Lythrum alatum* was solely (negatively) affected by the presence of the invasive species. Brown et al. (2002) manipulated population size, but not flower numbers per individual, and so the detected impacts on visitation are likely to be due to the larger floral display of the alien. In my study I controlled abundance and flower numbers and I did not detect any response of reproductive success to the presences of the alien *R. ponticum* at the two initial population levels used.

These contrasting results for impacts of highly pollinator attractive invasive plants accentuate the need for controlled manipulative experiments which incorporate abundance into the equation of invasion and pollination. I can infer from a comparison of the four studies that effects of alien abundance on pollination are not only species but also scale dependant. Even at very small population sizes, Muñoz and Cavieres (2008) revealed effects of *Taraxacum officinale* on female fitness of native Asteraceae. Analogously, *R. ponticum* may impact *D. purpurea*'s pollination but this impact might be only detectable at higher abundances. In natural field sites *R. ponticum* revealed negative effects at higher alien densities on floral visitation and conspecific pollen transfer to *D. purpurea* (Chapter 4). However, native fruit and seed set remained unaffected. By combining these results, I assume that *D. purpurea* is well buffered against variable pollination environments since its reproductive success is not linearly related to changes in flower visitation. Traits like self-compatibility may enhance partial immunity to changes in the pollination environment (Larson and Barrett, 2000) and therefore to alien invaders at different scales. However, artificial construction of sufficiently replicated populations at different scales with e.g. 100 individuals or more is impractical for most species (but see Kunin, 1993, 1997). The use of manipulations is therefore limited, and a balance between manipulative approaches and observational field studies is essential, especially for investigating invasive plant species at higher abundances (Bjerknes et al., 2007).



### 3.6. Conclusion

My results indicate that *R. ponticum* is likely to integrate into native pollination processes from early initial colonisation onwards. At this stage the alien would not disrupt native pollination mutualisms. Due to neutral effects on native flower visitation and reproduction, *R. ponticum* could be maintained within the network and form larger populations using native pollinators. Here, I did not incorporate alien visitation data into my considerations because appropriated replication of intra- and interspecific interactions among *R. ponticum* ‘individuals’ would have been beyond the scope of this study. Nevertheless, it would be interesting to see if *D. purpurea* in turn enhances visitation to *R. ponticum* and therefore facilitates further spread of the alien. However, we understand from models and field studies (Rathcke, 1983, Ghazoul, 2004) that flowering species at high abundance are likely to cause shifts in interaction patterns of co-flowering species. At such abundances, manipulations are difficult to conduct, and field observations of naturally occurring populations might give further insights into successive invasion processes.





## **Chapter 4**

“...All that because of a few small plants whose dangers were mostly unappreciated at the time, and some of whose seeds arrived unnoticed!” (Diamond, 2005)





Plate 4





## 4. Density effects of an alien invader on native pollination processes in natural populations

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Data in 2007 was collected by Dara A. Stanley as part of her final year B.A.(Mod) project.

### 4.1. Abstract

One major characteristic of alien invasive species is their occurrence at high abundances and densities in their new habitat. Flowering invasive plant species that are visited by native insects and overlap with native plant species in their pollinators may facilitate or disrupt native flower visitation and fertilisation by means of forming large, dense populations with high numbers of flowers and copious rewards.

I investigated the direction of such a proposed effect for the alien invasive *Rhododendron ponticum* in Irish habitats. Flower visitation, conspecific and alien pollen deposition as well as fruit and seed set was measured in a self-compatible native focal plant, *Digitalis purpurea*, and compared between field sites that were uninvaded, sparsely invaded or densely invaded by *R. ponticum*.

Flower visitation to *D. purpurea* was significantly lower at high alien densities than at low densities or in absence of the alien. Native flowers experienced a weak but significant decrease in conspecific pollen deposition with increasing alien density. Heterospecific pollen transfer was very low in all treatments but increased significantly with increasing *R. ponticum* density. However, lower flower visitation and lower conspecific pollen transfer did not alter reproductive success; neither fruit nor seed set of *D. purpurea* were correlated to alien density.

My study shows that indirect interactions between alien and native species for pollination can be similarly modified by population characteristics (such as density) as interactions among native plant species. In *D. purpurea*, only certain aspects of native pollination and reproduction were affected by high alien densities which is probably a result of high resilience due to a self-compatible breeding system. Other (abiotic) factors rather than pollen dispersal might have limit fitness in this system. However, species that are more susceptible to pollen limitation might experience fitness disadvantages under high alien density conditions.





## **4.2. Introduction**

Exotic plant species can be found in almost all continents and biomes on earth (Vitousek et al., 1997). Their impact is known to be related to their range and abundance (density) (Parker et al., 1999). Some alien invasive plants have the ability to change the characteristics of their new habitats substantially at a large scale and transform conditions for co-occurring native plants by, for example, overexploiting physical resources and/or suppressing resource usage of other species (Richardson et al., 2000b). As well as abiotic resources such as water, nutrients, space and/or light which are crucial for plant survival and reproduction, an important biotic resource that many flowering plant species depend on are pollinators for pollen transfer (Palmer et al., 2003). Thus, it is not surprising that pollination services have been suggested to shape the relationship between native and alien invasive plant species (Traveset and Richardson, 2006).

The impact of invasive aliens on native pollination services can be of direct or indirect nature. If the alien species uses the same flower visitors for pollen transfer as native co-flowering plants, it might act as a direct competitor or facilitator (Lopezaraiza-Mikel et al., 2007, Bartomeus et al., 2008b, Chapter 2). Even if there is no overlap in pollinator species or if the invasive plant species is pollinated by abiotic vectors, invasion of an alien can cause displacement or replacement of native plants or a change in their colonisation patterns (Parker et al., 1999, Yurkonis and Meiners, 2004) which might indirectly alter native pollination processes. Since the absolute and relative abundance of each plant species within a community structures the complex network of mutualistic interactions (Stang et al., 2006, Olesen et al., 2008), it is not only the presence of an alien invader but also its abundance which is likely to affect pollination services of the co-occurring plant species (Muñoz and Cavieres, 2008).

Few observational and experimental studies have addressed the effect of abundance on pollination and reproductive success in multi-species assemblages. In native communities it is assumed that the direction of this effect can be opposing (Rathcke, 1983). Individuals of more abundant species can either facilitate pollinator visitation of heterospecific individuals via pollinator sharing (magnet species effect, Thomson, 1978, Johnson et al., 2003, Moeller, 2004), or they can monopolise flower constant pollinators due to the relatively more abundant rewards they offer (Smithson and Macnair, 1996, Chittka et al., 1997). Within the same species assemblage, facilitation of pollinator visitation can even shift to competition and vice versa as flower abundances of the species involved change



along a continuum (Feinsinger, 1987, Ghazoul, 2006). Beside this quantitative measure of pollinator visitation, the quality of visitation, i.e. the amount of heterospecific pollen deposited and the level of conspecific pollen deposition and wastage, may also change according to the abundance and density of the plant species (Feinsinger et al., 1986, Feinsinger and Tiebout, 1991, Caruso, 2002, but see Waites and Ågren, 2004). Changes in quantity and quality can translate into changes in reproductive success (Feinsinger et al., 1991). Indeed, there also have been examples where the presence or increased density of a native co-flowering species did not affect conspecific pollen deposition or fitness of individuals of a target species at all (Feinsinger et al., 1986, Feldman, 2008).

We can predict that similar mechanisms of competition and facilitation also characterise the pollination interaction between alien and native plants (Brown et al., 2002, Ghazoul, 2004, Nienhuis, 2009). This might not only have implications for native plant populations but also for pollinator communities (Bjerknes et al., 2007). High local population densities and the rapidity of range expansion which invasive plant species often exhibit in their new habitats, might even expose these mechanisms more rapidly and clearly in invaded than in purely native systems (Sakai et al., 2001). Invaded communities therefore seem to offer significant opportunities to investigate basic population processes like pollination interactions (Sax et al., 2007). Understanding these processes is essential if we want to assess and manage impacts of alien invasions (Levine et al., 2003).

One of the most severe invaders in Britain and Ireland that requires sustainable management is *Rhododendron ponticum* L. (Ericaceae), an evergreen, perennial shrub that has invaded Irish heaths, bogs and woodlands over the last two centuries (Cross, 1975). Its pink-purple flowers are held in large inflorescences of 9 to 21 flowers and can form impressive floral displays; each flower produces large volumes of sugar-rich nectar which attracts a wide range of native flower visitors (Stout et al., 2006, Chapter 2). In its invaded habitat, *R. ponticum* co-flowers with a variety of 20 and more native plant species and can therefore impact native pollination (Chapter 2). Depending on the habitat and environmental conditions, *R. ponticum* occurs as scattered single individuals but can also form large clumps or pure stands (Cross, 1975); these differences in plant densities likely characterise the impact *R. ponticum* has on its invaded community. In this study, I investigated the role of *R. ponticum* density on the pollination of a native target species *Digitalis purpurea* L. (Scrophulariaceae). *D. purpurea* is a biennial herb with pink-purple, nectar-rich flowers that often co-occurs with *R. ponticum* and shares a similar visitor





assemblage (Chapter 2). My objectives were to determine the effect of flower density of *R. ponticum* on

1. The number of insect visits to individual *D. purpurea* plants and flowers
2. Conspecific and heterospecific (alien) pollen deposition on *D. purpurea* stigmata and
3. Seed set of the native target species.

### 4.3. Materials and methods

#### 4.3.1. Study sites

Field observations were conducted during 2006 and 2007 at nine sites in the Wicklow Mountains area, Co. Wicklow, Ireland (Plate 4 A), that all occurred in similar habitats over a range of altitudes (Table 4.1). Invaded sites with the native target species *D. purpurea* (Plate 4 B) were selected according to the presence of the alien *R. ponticum* in different densities (Table 4.1). Coverage estimates of *R. ponticum* were obtained using 20 m × 20 m quadrats. Since floral abundance per m<sup>2</sup> *R. ponticum* shrub surface (measured by counting the number of open flowers in four randomly assigned 1 m × 1 m quadrats per invaded site) did not differ between density categories (ANOVA,  $F_{1,4} = 0.19$ ;  $P = 0.69$ ), alien plant density is considered to be an estimate for alien flower density. Six invaded sites were chosen in total. In addition, I selected three control sites that did not contain any *R. ponticum* individuals in a radius of at least 1 km. This distance assured that pollinator visitation to native plant species at these sites was little or not affected by alien *R. ponticum* growing elsewhere because most pollinators forage at a range that is on average smaller than this distance (Walther-Hellwig and Frankl, 2000, Gathmann and Tschardtke, 2002, Knight et al., 2005a, Wolf and Moritz, 2008).

In 2006, I also estimated native flowering species coverage and floral abundance by counting plant individuals, number of inflorescences and number of open flowers as well as estimating percentage cover of each species in ten 1 m × 1 m quadrats which were placed along a 100 m transect every 10 meters. Thereby, *R. ponticum* thickets were not sampled. Vegetation data were used to obtain density estimates for the native target species *D. purpurea* as well as to calculate diversity indices for each site.



Table 4.1. Field site locations, their geographical position and elevation above sea level (in meters), and their percentage cover of alien *R. ponticum*.

| Location                             | Site description        | Longitude    | Latitude    | Elev (m) | Alien cover (%) |
|--------------------------------------|-------------------------|--------------|-------------|----------|-----------------|
| Woodend Hill, Kilbridge*             | forest edge/river bank  | 53°09'48.0"N | 6°28'10.1"W | 256      | 0               |
| Cushbawn Mountain, Aughrim           | forest clearing         | 52°52'09.4"N | 6°17'15.9"W | 184      | 0               |
| Pollahoney, Arklow                   | forest edge/river bank  | 52°48'01.1"N | 6°12'19.1"W | 54       | 0               |
| Avenmore Valley, Rathdrum            | forest edge/river bank  | 52°56'41.4"N | 6°14'09.9"W | 105      | 6               |
| Avoca Valley, Killeagh               | forest clearing         | 52°49'27.8"N | 6°12'47.8"W | 18       | 22              |
| Glenmalure Valley, Baravore          | forest edge/river bank  | 52°59'10.6"N | 6°24'58.8"W | 216      | 43              |
| Coolboy Valley, Coolboy              | forest edge/river bank  | 52°45'16.3"N | 6°28'50.8"W | 82       | 70              |
| Avonbeg Valley, Greenan <sup>†</sup> | field margin/river bank | 52°56'08.9"N | 6°18'32.1"W | 125      | 78              |
| Shankill Valley, Cloghleagh          | forest edge/river bank  | 53°11'36.0"N | 6°25'37.8"W | 284      | 98              |

\* Estimates on native plant species cover not available.

<sup>†</sup> No observational data, pollen deposition or seed set collected in 2007.

#### 4.3.2. Insect visitation and nectar production

Field sites were visited once in each of 2006 and 2007 during peak *R. ponticum* flowering (June). Observations were conducted in fine weather conditions only (no rain and wind force <4 according to Beaufort Scale). During each visit, four flowering plant patches of *D. purpurea* in the vicinity (<10 m) to flowering *R. ponticum* individuals were investigated. The number of inflorescences and the number of open flowers on each inflorescence was counted, and patches were observed for 15 min between 8 am and 8 pm. The identity of flower visitors, the number of inflorescences and flowers visited on each inflorescence were recorded. I calculated visitation rate (i.e. the number of pollinator visits per 15 min / total number of observed flowers) and the number of visitors per inflorescences (i.e. the number of pollinators at the patch per 15 min / total number of inflorescences) for each observed patch.

After each observation sessions I randomly picked 10 openly accessible *D. purpurea* flowers and measured their nectar volume (nectar standing crop) with microcapillary glass pipettes (1 and 5µl, Hirschmann Laborgeräte, Germany) and callipers.





### 4.3.3. Pollen desposition

In 2007, I collected receptive stigmata from one flower from each of  $9 \pm 1.0$  SE (total 69) randomly chosen *D. purpurea* individuals at each site except for one dense site (Table 4.1) and stored them in vials filled with 70% ethanol. Stigmata were transferred to the laboratory, stained with a Safranin solution (0.5% Safranin in 50% ethanol) and individually placed on microscopic slides. Stigmata were gently squashed to allow inspection of the entire stigmatic surface. Both native and alien pollen on the stigmatic surface was counted using the 10 fold magnification of a microscope (Leica, UK).

### 4.3.4. Seed set

In 2007, I picked 7 to 9 *D. purpurea* inflorescences at each of the sites (except for one dense site, Table 4.1) and counted and marked all their open flowers. Some of the marked inflorescences were vandalised or destroyed due to bad weather conditions, and so I sampled on average  $6 \pm 0.6$  SE inflorescences at each site. Inflorescences were monitored and fruit capsules were harvested when mature. I measured their maximum length and maximum diameter with digital callipers (Moore & Wright, UK; precision 0.01 mm). From the total of 211 collected capsules, I randomly picked a subset of 55 fruits and counted the enclosed seeds. Seed numbers were most closely correlated to fruit diameter (Pearson's correlation,  $r = 0.896$ ,  $t_{52} = 14.4$ ,  $P < 0.001$ ; two outliers were excluded from analysis), and so I estimated seed numbers of all marked fruits as  $202 \times \text{fruit diameter} - 666$  (linear regression, adjusted  $R^2 = 0.799$ ). Fruit/flower ratios (relative fruit set) and the average number of seeds per fruit in each inflorescence were then calculated whereby each capsule with at least one seed was considered to be a fruit.

### 4.3.5. Statistical analysis

#### Field sites

I calculated the mean coverage and the mean number of inflorescences across all ten  $1 \text{ m}^2$  quadrats for each entomophilous native species; this gave a density estimate for the area of each site not covered by *R. ponticum*. I then corrected these estimates by including the density estimates and floral abundance data of *R. ponticum* to obtain the overall mean coverage and overall number of inflorescences for each native species at each site. Using



these corrected estimates and including *R. ponticum*, I calculated the Shannon diversity index  $H' = -\sum p_i \log_e(p_i)$  for each site with plant species coverage and number of inflorescences as importance values ( $p_i$ ). Spearman rank correlation was employed to correlate these diversity values and floral abundance (number of inflorescences) with *R. ponticum* density. Additionally, I correlated *R. ponticum* density with both corrected and uncorrected *D. purpurea* estimates of percentage cover, number of inflorescences and number of open flowers using Spearman rank correlations in MINITAB 13 (Minitab Inc., 2000).

### **Insect visitation**

I square root transformed data of both variables (flower visitation rate and number of insect visitors per inflorescences) after using Cochran's and Anderson-Darling tests to ensure homogeneity of variances and normal distribution of the data. One extreme outlier (outside the range of mean by 3.3 standard deviations) of one observed patch at one site was identified for both variables and was therefore emitted from the data. Visitation data were missing for one dense site in 2007 (Table 4.1) due to unfavourable weather conditions, and so I used Generalised Estimation Equations (GEEs) in order to do justice to the unbalanced data and the nested sampling design (Quinn and Keough, 2002). I analysed transformed data within an independent working correlation matrix due to its best fit to the model (i.e. lowest Quasi-likelihood under Independence Model Criterion QIC, an extension of the Akaike information criteria) in SPSS 15.0 (SPSS Inc., 2006). A normal distribution model with an identity link function was used (Agresti, 1996). In the models, 'Year' was incorporated as a factor, 'Density' as a covariate and individual field sites as subjects (i.e. units from which I repeatedly took replicated samples). I also included the interaction term (Year  $\times$  Density) and the intercept in the models. The best-fit model according to the lowest QIC was "Error + Year + Density" for both variables.

To obtain an estimate of pollinator sharing between the native and the invasive species, I calculated the niche overlap index (NOI, Pianka, 1973) which has been used by other studies on pollination before (Muñoz and Cavieres, 2008) for the field season 2006. The range of the index from 0 (no overlap) to 1 (absolute overlap) mirrors both visitor identities and visit frequencies of the used pollinator guild.





### Nectar standing crop

Nectar standing crop data were  $\log+1$  transformed to homogenise variances and allow for a normal distribution of data. I then analysed the relation between mean flower visitation rates and mean transformed nectar standing crop using Pearson product-moment correlation. I used MINITAB 13 (Minitab Inc., 2000) for analysis.

### Pollen deposition and seed set

I modelled the linear dependence of pollen deposition and seed set on *R. ponticum* density with Generalised Estimation Equations (GEEs) in order to take into account the unbalanced data and to incorporate the nested sampling design. *R. ponticum* density was used as a covariate and individual field sites as subjects, and I included the intercept in the models. I analysed conspecific pollen counts assuming a Poisson distribution and Log link function (Crawley, 1993). Seed set per fruit followed a Normal distribution with an Identity link function. Proportions of alien pollen number/total pollen number and of fruit number/flower number (relative fruit set) were analysed using binomial distribution and Logit link function (Agresti, 1996). I used an independent working correlation structure and performed analyses with SPSS 15.0 (SPSS Inc., 2006). A *posthoc* power analysis was conducted according to Quinn and Keough (2002).

## 4.4. Results

### 4.4.1. Field sites

Percentage cover (mean  $\pm$ SE =  $6.7 \pm 2.32$ ), number of inflorescences (mean  $\pm$ SE =  $2.0 \pm 0.84$ ) and number of open flowers (mean  $\pm$ SE =  $4.5 \pm 1.84$ ) of *D. purpurea* in the 1-m<sup>2</sup> quadrats decreased but were not significantly correlated with increasing *R. ponticum* densities (Spearman  $r_{\text{cover}} = -0.16$ ,  $r_{\text{infloresc}} = -0.43$ ,  $r_{\text{flowers}} = -0.27$ ,  $N = 8$ ,  $P > 0.28$  for all), i.e. density of *D. purpurea* in patches next to *R. ponticum* did not significantly differ from *D. purpurea* densities at control sites. However, when including the density estimates of *R. ponticum* into the calculation, these variables significantly decreased or showed a trend of decrease with increasing *R. ponticum* density (cover: mean  $\pm$ SE =  $5.1 \pm 2.37$ ,  $r = -0.71$ ,  $P = 0.05$ ; inflorescences: mean  $\pm$ SE =  $1.6 \pm 0.82$ ,  $r = -0.84$ ,  $P = 0.01$ ; flowers: mean  $\pm$ SE =  $3.5 \pm 1.86$ ,  $r = -0.70$ ,  $P = 0.052$ ;  $N = 8$  for all), i.e. overall density of *D. purpurea* within a site declined where the invasive usurped more space.



Although overall floral abundance (inflorescence number per m<sup>2</sup>, mean  $\pm$ SE = 11.8  $\pm$ 3.31) did not show any significant correlation with density of *R. ponticum* (Spearman  $r$  = 0.15,  $P$  = 0.73), overall floral diversity ( $0.13 \leq H' \leq 1.39$ ) and diversity of entomophilous plant species ( $0.05 \leq H' \leq 1.11$ ) decreased with increasing *R. ponticum* density, i.e. at sites where the invasive became more dominant (Spearman  $r$  = -0.83,  $P$  = 0.011 and  $r$  = -0.85,  $P$  = 0.008).

#### 4.4.2. Visitation rates

Flower visitation rate did not significantly differ between years (Wald  $\chi^2$  = 0.41, df = 1,  $N_{\text{total}}$  = 67,  $N_{\text{units}}$  = 9,  $P$  = 0.521), but was negatively influenced by an increase in *R. ponticum* density (Wald  $\chi^2$  = 3.97, df = 1,  $N_{\text{total}}$  = 67,  $N_{\text{units}}$  = 9,  $P$  = 0.046;  $\beta_{\text{density}}$  = -0.002  $\pm$ 0.0010 SE; Fig. 4.1 A).

Similarly, the numbers of insect visitors per inflorescence did not significantly vary between years (Wald  $\chi^2$  = 0.08, df = 1,  $N_{\text{total}}$  = 67,  $N_{\text{units}}$  = 9,  $P$  = 0.777), but inflorescences received significantly fewer flower visitors in densely invaded habitats than in sparsely or uninvaded habitats (Wald  $\chi^2$  = 5.03, df = 1,  $N_{\text{total}}$  = 67,  $N_{\text{units}}$  = 9,  $P$  = 0.025;  $\beta_{\text{density}}$  = -0.003  $\pm$ 0.0013 SE; Fig. 4.1 B).

*D. purpurea* at uninvaded control sites was only visited by bumblebees whereas *D. purpurea* at invaded sites received also visits from flies and syrphids (Supplement 9). Still, *D. purpurea* at uninvaded sites and *D. purpurea* at invaded sites overlapped largely in their pollinators (NOI = 0.881). Overlap in flower visitors between *R. ponticum* and *D. purpurea* increased by approx. 50% when *D. purpurea* co-occurred with *R. ponticum* at invaded sites (NOI = 0.402) in comparison to *D. purpurea* at uninvaded sites (NOI = 0.269, Supplement 9).

#### 4.4.3. Nectar standing crop

Nectar standing crop of *D. purpurea* varied between 0  $\mu$ l and 21.9  $\mu$ l with a mean standing crop of 1.0  $\mu$ l  $\pm$  0.18 SE in 2006 and 2.6  $\mu$ l  $\pm$  0.60 SE in 2007, and was negatively related to flower visitation rates of *D. purpurea* (Pearson correlation;  $r$  = -0.805;  $N$  = 16;  $P$  < 0.001; Fig. 4.2). Low flower visitation rates at some of the study sites are therefore unlikely to be a result of a lack of floral nectar rewards.



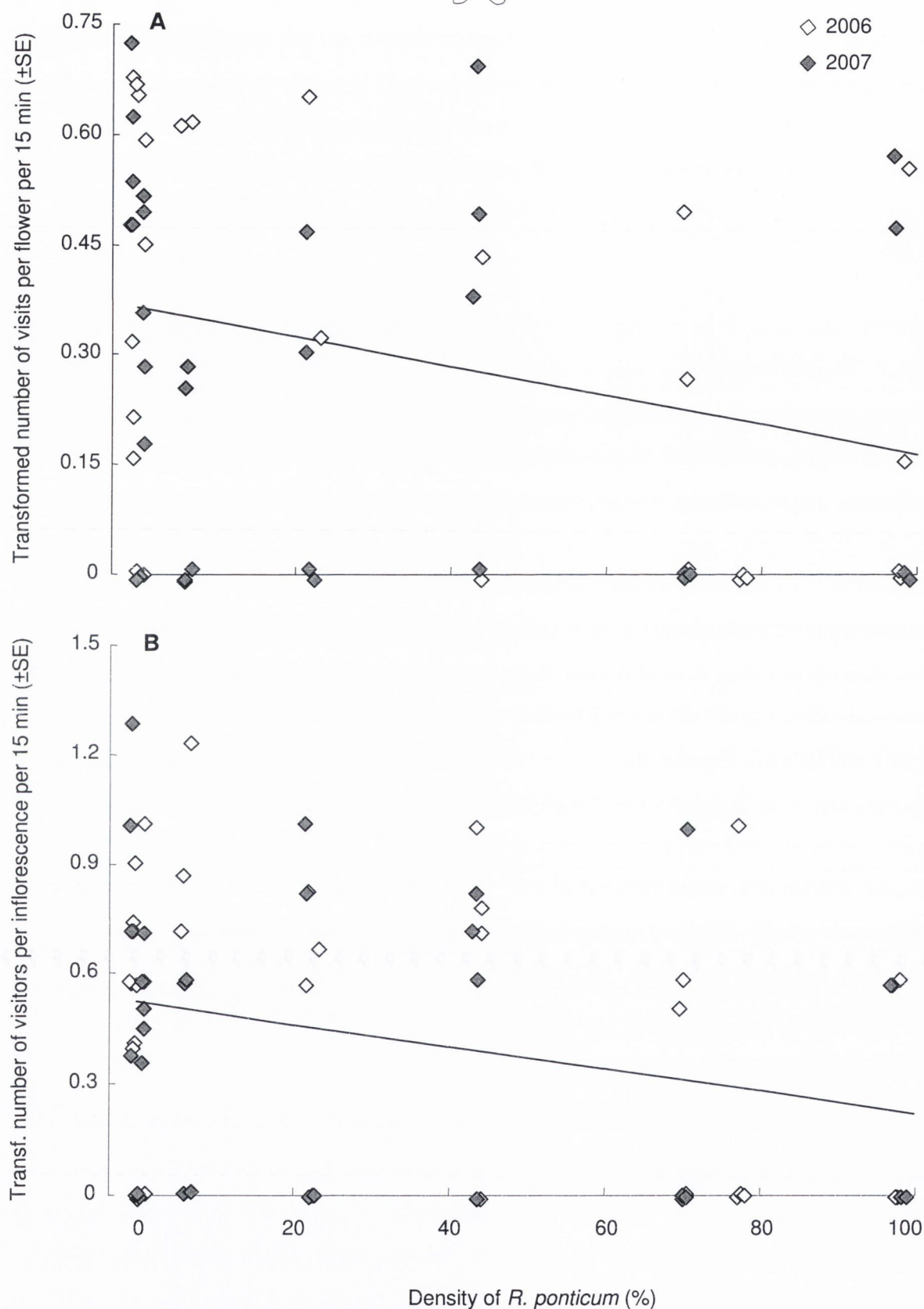


Figure 4.1. Flower visitation rate (A) and number of insect visitors per inflorescence (B) of *D. purpurea* flower patches in habitats of different *R. ponticum* invasion levels in two consecutive years. Data of both variables were square root transformed. For visual display, data were jittered along the x- and y-dimension so that overlapping data points are visible.

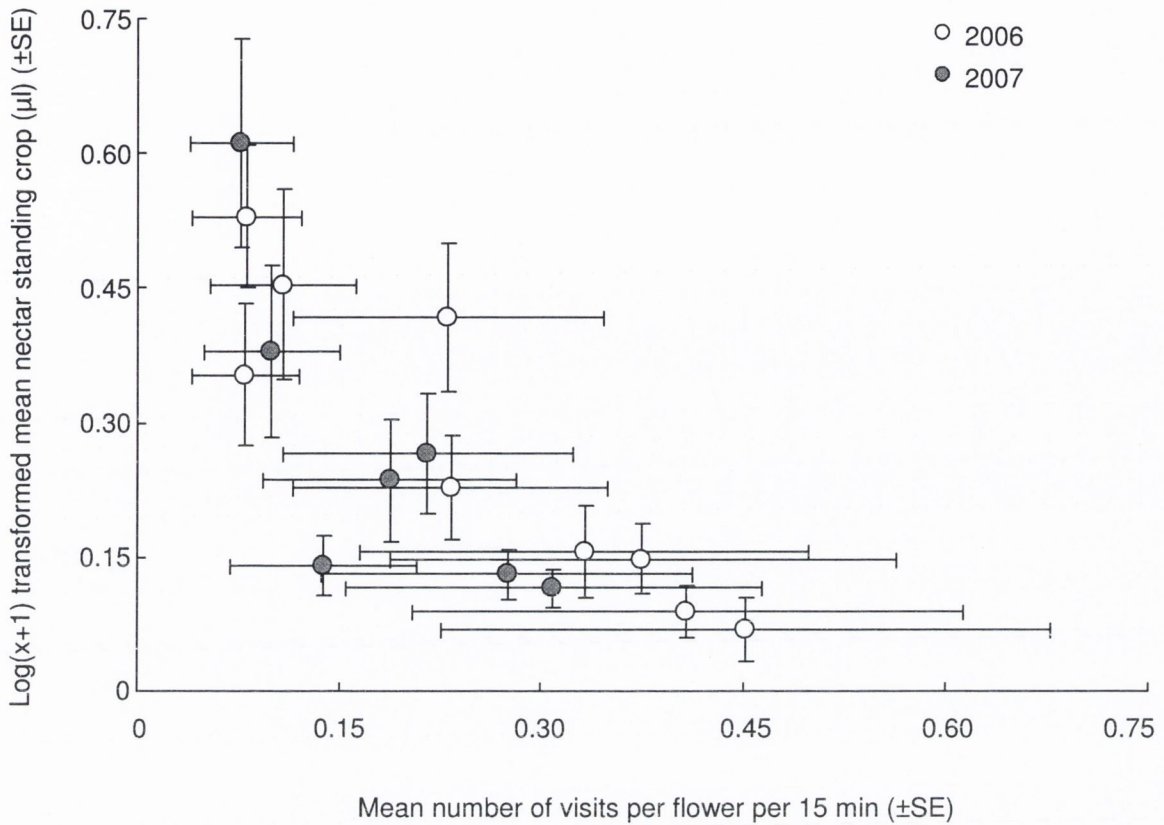


Figure 4.2. Correlation of transformed mean nectar standing crop and mean flower visitation rates. Bars show standard errors.

#### 4.4.4. Pollen deposition

Deposition of conspecific pollen varied considerably from 24 to 3034 conspecific pollen grains per individual *D. purpurea* stigma with a mean of 1238 ( $\pm 129$  SE) grains per stigma in control sites, 849 ( $\pm 106$  SE) in sparsely invaded and 644 ( $\pm 123$  SE) in densely invaded sites. I observed a weak, negative relationship between conspecific pollen deposition and the density of alien *R. ponticum* (Wald  $\chi^2 = 5.46$ ,  $df = 1$ ,  $N_{\text{total}} = 68$ ,  $N_{\text{units}} = 8$ ,  $P = 0.019$ ;  $\beta_{\text{density}} = -0.006 \pm 0.0025$  SE; Fig. 4.3 A).

In contrast, pollen deposition of alien pollen on native *D. purpurea* stigmata was very low and ranged from zero to nine pollen grains per stigma. I did not find any *R. ponticum* pollen on stigmata of *D. purpurea* in uninvaded field sites. Alien pollen in relation to the total amount of pollen found on *D. purpurea* stigmata was positively related to *R. ponticum* density (Wald  $\chi^2 = 62.25$ ,  $df = 1$ ,  $N_{\text{total}} = 65$ ,  $N_{\text{units}} = 8$ ,  $P < 0.001$ ;  $\beta_{\text{density}} = 0.057 \pm 0.0072$  SE; Fig. 4.3 B).



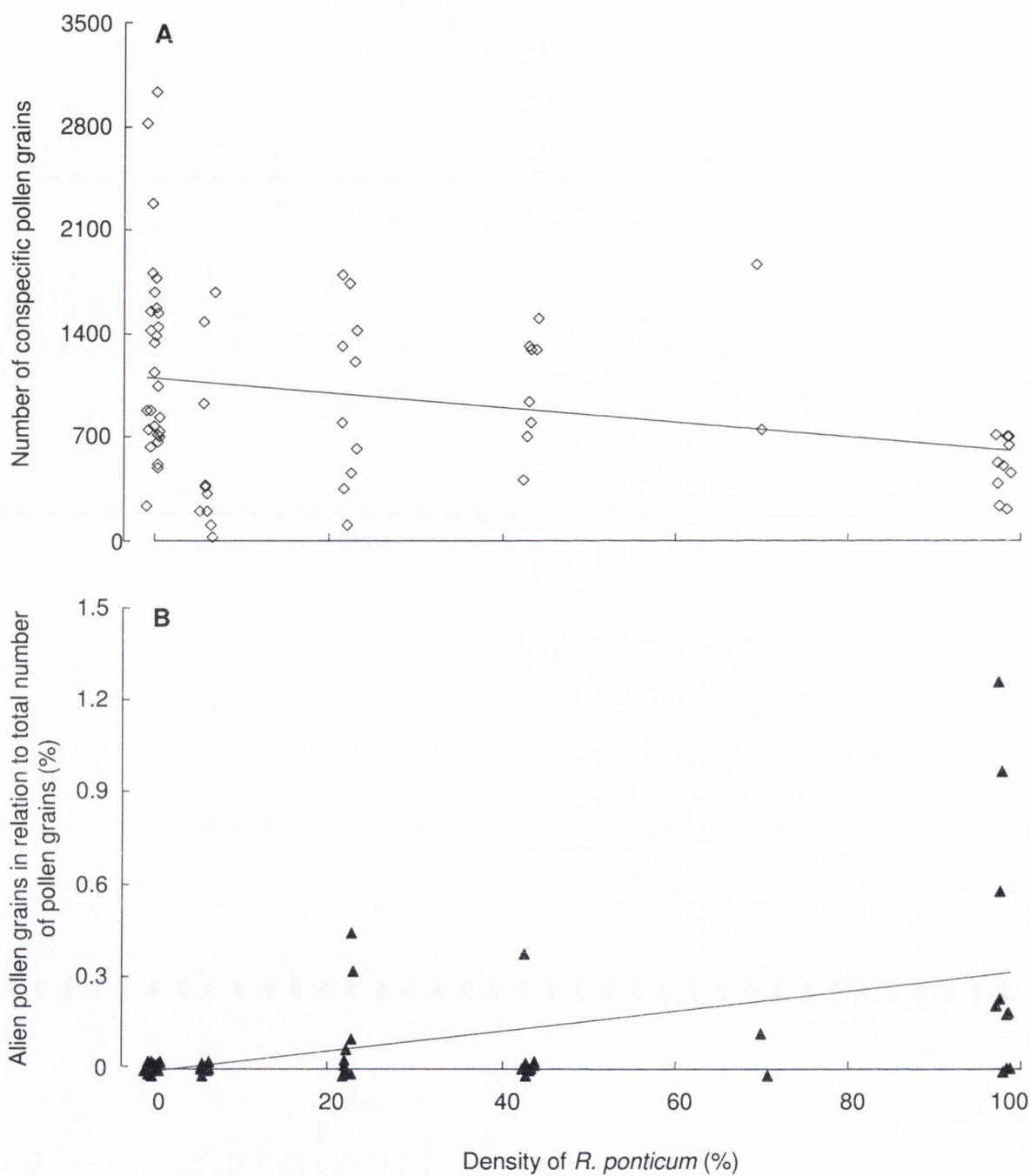


Figure 4.3. Numbers of conspecific pollen (A) and alien *R. ponticum* pollen in relation to total number of pollen grains (B) found on *D. purpurea* stigmas from different field sites that had not been or had been invaded by *R. ponticum* at different densities. For visual display, data were jittered along the x-dimension so that overlapping data points are visible.

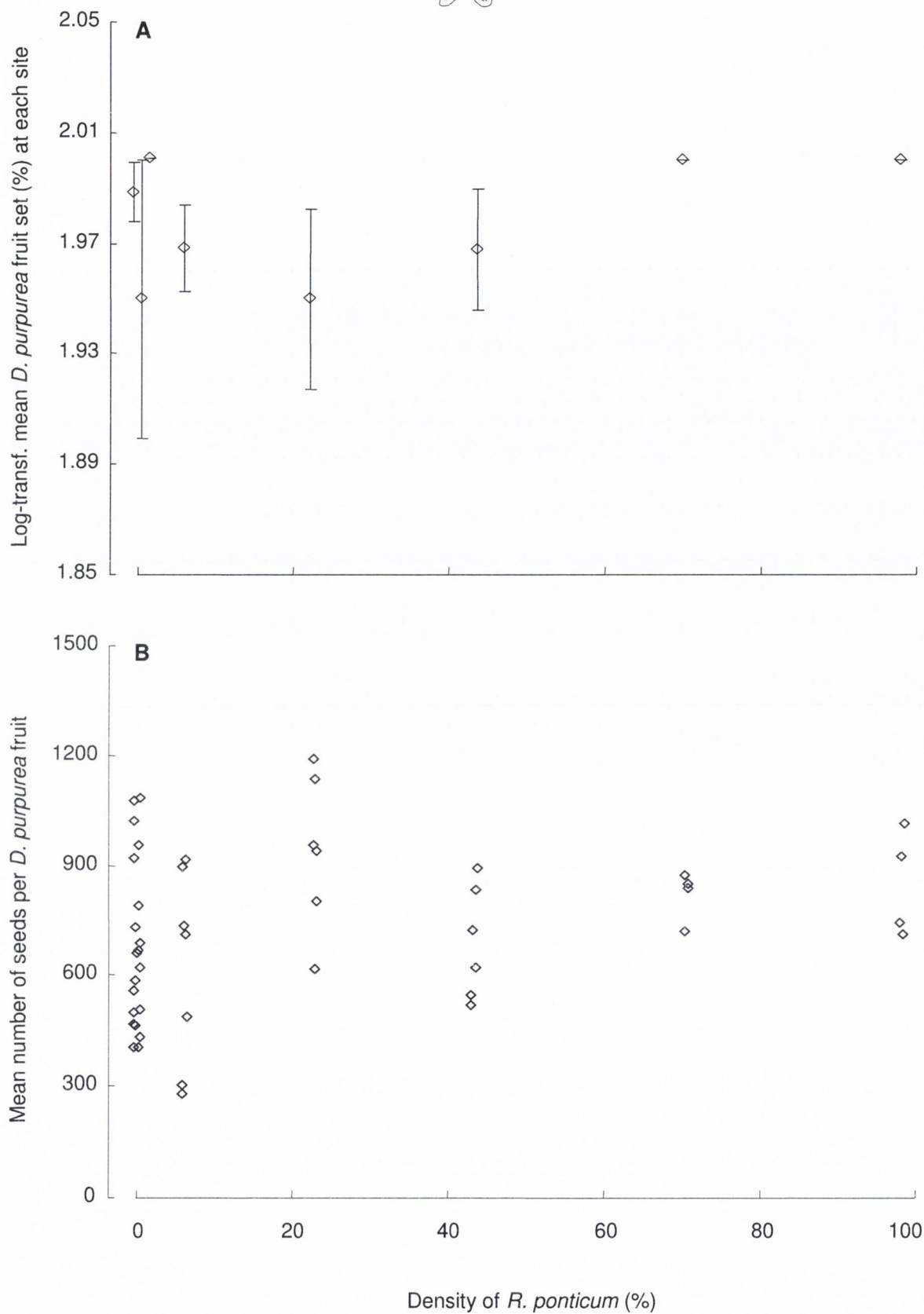


Figure 4.4. Mean fruit set (in %) averaged across each field site (A) and estimated number of seeds per fruit averaged across *D. purpurea* inflorescences (B) from different field sites that had not been or had been invaded by *R. ponticum* at different densities (in % *R. ponticum* coverage). For visual display, the predictor variable (Density of *R. ponticum*) was jittered so that overlapping data points are visible.





#### 4.4.5. Seed set

Fruit set was high in all three density treatments (control: 96.5%  $\pm$  2.64 SE; sparse: 92.5%  $\pm$  2.54 SE; dense: 100%  $\pm$  0 SE). Individual fruits contained between 1 and 1455 (estimated) seeds with an average of 739  $\pm$  21.2 seeds per fruit. Neither fruit set nor the estimated mean number of seeds per fruit were related to *R. ponticum* density (number of fruits per flower: Wald  $\chi^2 = 0.47$ , df = 1,  $N_{\text{total}} = 48$ ,  $N_{\text{units}} = 8$ ,  $P = 0.494$ ; Fig. 4.4 A; mean number of seeds per fruit: Wald  $\chi^2 = 3.66$ , df = 1,  $N_{\text{total}} = 48$ ,  $N_{\text{units}} = 8$ ,  $P = 0.056$ ; Fig. 4.4 B).

I detected low power ( $\alpha = 0.094$ ) for the analysis on seed set per fruit, and increasing the number of sites by a factor of five (overall  $N = 45$ ) did increase power but not greatly ( $\alpha = 0.426$ ).

#### 4.5. Discussion

*Rhododendron ponticum* has occupied various habitats in Ireland and occurs often in high densities (Usher, 1986). Here I demonstrated that these high densities can impact both the diversity of entomophilous plant communities as well as the pollination of a self-compatible native plant species but that they ultimately did not influence its reproductive success.

Pollinator attraction is one of the first steps of several stages that a plant has to administer in order to successfully produce offspring. Flower visitors of *D. purpurea* were more similar to *R. ponticum* visitors in the presence of the invader than in its absence, that is the presence of *R. ponticum* modified the identity of *D. purpurea* flower visitors. Although the species similarity of flower visitors between *R. ponticum* and *D. purpurea* was smaller than has been observed for alien-native species pairs in other systems (Muñoz and Cavieres, 2008), both species overlapped considerably in their visitors, which could foster direct interspecific interactions between the two plant species. If their generalist pollinators foraged randomly and did not distinguish between flowers of different species, individual visitation rates would not change across various relative densities of a species (Levin and Anderson, 1970). In reality, pollinators assess and distinguish between individual rewards of each species (Heinrich, 1975, Goulson et al., 1997, Hill et al., 2001), incorporating information on species' flower complexity (Lavery, 1994, Stout et al., 1998) as well as the relative density of each species (Feinsinger et al., 1991, Stout et al., 1998) and may refuse



or prefer to visit a species beyond what is expected by its relative frequency. Therefore it is not surprising that I observed lower visitation rates of *D. purpurea* in densely invaded field sites. Studies on other invasive species revealed similar disruptive effects on native visitation at high alien density (Ghazoul, 2004, Muñoz and Cavieres, 2008; but see Grabas and Lavery, 1999).

The decrease in visitation cannot be fully attributed to an interaction between relative frequency and reward levels or flower complexity of the two species because both *R. ponticum* and *D. purpurea* have relatively easily accessible flowers that secrete similar nectar volumes and sugar concentration (Chapter 2). Lower visitation rates in *D. purpurea* are more likely explained by flower preference and higher constancy of pollinators towards the relatively more abundant *R. ponticum* at high density sites. Such a monopolisation would suggest the occurrence of interspecific competition and has been observed in other invasive species before (Chittka and Schürkens, 2001, Ghazoul, 2004). Indeed, models predict that pollinators behave as specialists on the most common plant species in habitats where resources occur in superabundance (Goulson, 1994, Kunin and Iwasa, 1996).

Alternatively, *D. purpurea* at densely invaded sites might have attracted fewer insect pollinators to its flowers without experiencing any influence of *R. ponticum*'s presence or higher density. This could have been a function of the reduced overall *D. purpurea* density at densely invaded habitats. Plants are likely to suffer from pollinator limitation at low abundances due to decreased mutual attraction of pollinators (Sih and Baltus, 1987, Kasagi and Kudo, 2003, Aizen and Harder, 2007). Lower intraspecific facilitation among *D. purpurea* individuals is therefore a possible explanation for the observed pattern.

A third proposition is that flower visitation by bees can be positively associated with the diversity of flowering communities (Petanidou and Ellis, 1996, but see Hegland and Boeke, 2006) implying that *D. purpurea* might have encountered lower interspecific facilitation by other native species due to the reduced native entomophilous plant diversity at densely invaded sites.

I unfortunately cannot distinguish between the three mechanisms, i.e. between higher interspecific alien competition, lower intraspecific facilitation and lower interspecific native facilitation, because in the investigated habitats the density and diversity of natives was greatly affected by the density of the alien. In natural field sites, densities of co-occurring species are often correlated (Ågren, 1996, Kunin and Shmida, 1997), especially in invaded communities where an alien can simply displace native plants (Parker et al.,





1999). Manipulative experiments are required to disentangle these components (Kunin, 1997, see Chapter 3). Although the precise mechanism that suppressed visitation of *D. purpurea* inflorescences and flowers is in doubt, this study nevertheless shows that heavy invasion by *R. ponticum* may disrupt pollinator visitation to native plants. Clearly, pollen transfer is a function of flower visitation rate (Wilcock and Neiland, 2002), and this disrupted visitation rate is likely to impact pollen deposition in *D. purpurea*. Frequency of pollen transfer events (i.e. the quantity of visitation) alone is not necessarily a strong correlative of successful pollination if the composition of pollen loads (i.e. the quality of visitation) is variable (Rathcke, 1983).

Considering that the two plant species used, in almost half of the cases, the same pollinator species, I expected interspecific flower movement to occur. As a consequence, heterospecific pollen may be transferred to and interact with native stigmata (Morales and Traveset, 2008). Although heterospecific pollen ratios increased with increasing *R. ponticum* density, I still found very little foreign pollen on native *D. purpurea* stigmata. This falls in line with other studies that found low alien pollen deposition on native stigmata in natural communities (Larson et al., 2006, Bartomeus et al., 2008a, Jakobsson et al., 2008; but see Ghazoul, 2002). Flower constancy exhibited by bees and hoverflies (Free, 1970, Goulson and Wright, 1998) was not measured in this study, but it might plausibly explain my observations at high densities, when alien pollen should be highly abundant in the pollen transport web (Lopezaraiza-Mikel et al., 2007). Even if pollinators moved between heterospecific flowers, alien pollen deposition might be low due to segregated pollen placement on the pollinators' bodies (mechanical isolation, Grant, 1994). Mechanical isolation is likely because *D. purpurea* flowers place and collect pollen very precisely on the dorsal surface of most visiting insects (personal observation). *R. ponticum* pollen is more diffusely placed on the visitors' bodies because the long viscin threads that adhesively join the pollen grain tetrads can easily stick to and drape over the insects' bodies (King and Buchmann, 1995). Furthermore, pollinator behaviour (e.g. grooming, Harder and Wilson, 1998) may restrict placement and transfer of heterospecific pollen. Bumblebees, the main pollinators in my study system, often do not touch the reproductive parts of *R. ponticum* due to size constraints (Stout, 2007a) and therefore might not pick up large amounts of alien pollen. Due to the low heterospecific pollen loads on *D. purpurea* stigmata it is unlikely that alien pollen interfered negatively with the fertilisation in *D. purpurea* individuals.



Increased conspecific pollen loss in the presence of an invasive species is thought to be one of the mechanisms for native pollination disruption (Morales and Traveset, 2008).

However, the proposed higher pollinator flower constancy at high alien densities makes *D. purpurea* pollen loss rather unlikely. The weak decrease in deposited conspecific *D. purpurea* pollen with increasing *R. ponticum* density is probably directly related to lower visitation rates. Similarly, Ghazoul (2002) and Larson et al. (2006) observed a parallel decrease in native visitation rate and conspecific pollen deposition for some native species in different invaded communities (but see Grabas and Lavery, 1999).

Nevertheless, conspecific pollen loads on *D. purpurea* stigmata were relatively high even in sites with lower visitation rates. Since *D. purpurea* flowers that were less visited held more nectar, this surplus in rewards might have triggered a change in pollinator behaviour resulting in longer visits within each flower (Pappers et al., 1999) which can increase successful pollen transfer (Thomson, 1986, Harder, 1990). Muñoz and Cavieres (2008) showed that longer native visits at low alien densities were positively related to an increase in native seed set implying higher native pollen deposition caused by longer visitation. Here, I did not measure visitation time at a flower level, and so I cannot prove an alteration in pollinator behaviour.

Native pollen deposition might also have been high because *D. purpurea* flowers offer large amounts of pollen (sixfold or more than other native species, Nienhuis, 2009). This may increase pollen loads transferred and deposited by each pollinator so that even a single visit might be sufficient to fertilise the majority of ovules.

Visitation rates and pollen deposition are usually known to be good predictors for reproductive success (Bosch and Waser, 2001, Waite and Ågren, 2004, Kirchner et al., 2005). So why does *D. purpurea* seed set not mirror the differences in visitation and pollen deposition?

Negative impacts of alien species on native seed set predominate in studies of self-incompatible species (Chittka and Schürkens, 2001, Brown et al., 2002, Bjerknes, 2008, Nienhuis, 2009). In self-compatible plants, interspecific effects of density on reproductive success may be weaker (Feinsinger et al., 1991, Caruso, 1999). In *D. purpurea*, small amounts of conspecific cross-pollen could be counterbalanced by a higher amount of self-pollen (Kalisz and Vogler, 2003) although an animal vector is still needed to transfer this





pollen (spontaneous autogamy in *D. purpurea* results in no or very low seed set, Chapter 3). Higher selfing rates are likely to occur due to more flowers visited per inflorescence or longer visits per flower in less frequently visited plants (see above). *D. purpurea* is mainly pollinated by bumblebees, i.e. pollinators that are able to fly long distances when foraging (Osborne et al., 2008). Negative effects of low local conspecific density, e.g. pollen limitation or self-pollination, might be outweighed by the high mobility of pollen vectors that may import cross-pollen from more distant populations (Somanathan et al., 2004). A high conspecific pollen/seed number ratio especially in control sites suggests that *D. purpurea* was not pollen limited. Still, average seed set was only half as large as the maximum seed set and lower than average seed set estimated by other studies (Buxton and Darlington, 1932). Although pollination limitation has been considered to play a more important role for reproductive success than previously suspected even in self-compatible species (Burd, 1994, Ashman et al., 2004, Ghazoul, 2005b), and although the power of my seed set analysis was very low so that I might have failed to detect an effect of alien density on native seed set, my results may indicate limitation by factors other than pollen (e.g. water, nutrients or hosts) that do not correlate with the level of *R. ponticum* invasion. Such limitations can overshadow the abundant availability of pollen (Goulson et al., 1998, Parker and Haubensak, 2002, Totland et al., 2006). In my case, plants could not capitalise from higher pollen loads in e.g. uninvaded sites because other resources seemed to have restricted their reproduction (Haig and Westoby, 1988).

Bee and fly visitation has been described as a function of floral density at a habitat scale (100 m<sup>2</sup> to 1 ha, Thomson, 1981, Johnson et al., 2003) but fitness can be also related to densities at a local scale (2 – 10 m radii, Steven et al., 2003, Kirchner et al., 2005). Hence choosing a habitat scale might have concealed alien density effects on native reproduction that only operate within close neighbourhood (as in Muñoz and Cavieres, 2008). However, *R. ponticum* invasion manifests at a habitat scale with single individuals covering on average 4 m<sup>2</sup> (Erfmeier and Bruelheide, 2004) and forming large clumps. In this field study I was therefore more interested in alien impacts at a larger scale. However, manipulations of the abundance of *R. ponticum* and *D. purpurea* at a local scale did not reveal any response of *D. purpurea*'s reproductive success to *R. ponticum* abundance either (Chapter 3).



## **4.6. Conclusions**

Individuals of native species can display substantial plasticity that allows the integration of an alien species in the existing plant-pollinator web without affecting their fitness (Memmott et al., 2004). My study indicates that an impact of *R. ponticum* on native plant populations seems to arise rather from its vegetative characteristics of direct competition for space than from alteration of native pollinator-mediated reproductive success, although I did observe an effect of alien density on native flower visitation and conspecific pollen deposition. Individuals of species with self-incompatible breeding systems or low pollen production might respond to such decreased visitation rates and pollen deposition more sensitively than *D. purpurea* (Ashman et al., 2004). Since disruptive impacts of *R. ponticum* on native pollination were mainly apparent at high alien densities, the establishment of pure *R. ponticum* stands should be impaired. In this context, management of *R. ponticum* invasions at the beginning of naturalisation and density reduction of existing populations are indispensable.





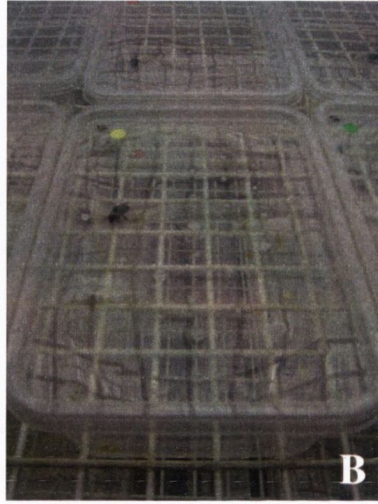
## Chapter 5

“...Thus I can understand how a flower and a bee might slowly become [...] modified and adapted to each other in the most perfect manner...” (Darwin, 1859)

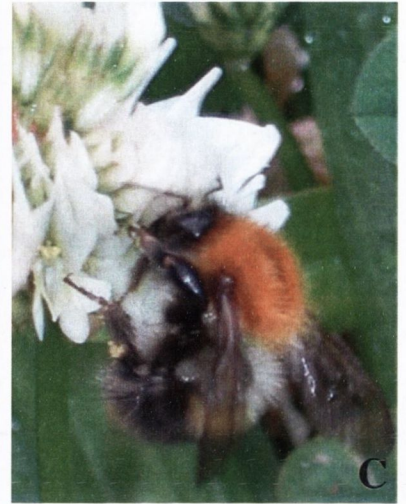




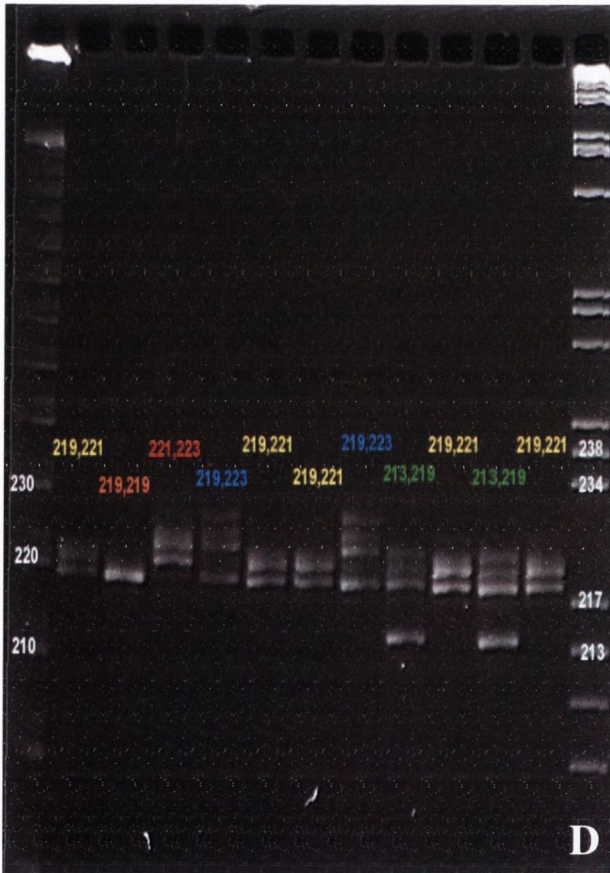
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E

Plate 5





## 5. Impacts of invasive *Rhododendron ponticum* L. on survival and resource sharing in Irish bumblebees

To be submitted as: Dietzsch, A.C., Brown, M.J.F. & Stout, J.C. Impacts of invasive *Rhododendron ponticum* L. on survival and resource sharing in Irish bumblebees. *Environmental Entomology*.

### 5.1. Abstract

Although many alien invasive plant species are proposed to disrupt native plant biodiversity and abundance and in turn to affect the native pollinator community negatively, they may also provide valuable food rewards for flower visitors. However, very little information is available on impacts of invasive plants on pollinator individuals and populations.

Here I tested the suitability of floral rewards produced by the Irish alien invasive *Rhododendron ponticum* on individuals of its main bumblebee pollinators and investigated the effects that the presence of the alien has on abundance and population size of these bees. I conducted controlled feeding experiments on individuals of the generalist bumblebee *Bombus lucorum* using alien, native, artificially intoxicated and control diets. Furthermore, I estimated abundance and colony numbers of the two frequent *R. ponticum* visitors *B. lucorum* and *B. pascuorum* in invaded sites using a mark-recapture method and molecular analysis and compared these variables with estimates obtained from uninvaded habitats.

Survival of individual *B. lucorum* workers did not differ in relation to diet. Although I did not detect any significant difference in abundance of the two bumblebee species between uninvaded and invaded habitats, colony numbers of both bee species were higher at sites invaded by *R. ponticum* than at uninvaded sites.

I assume that *R. ponticum* rewards can be considered a harmless suitable food resource for bumblebees and that the alien invader is a valuable dietary addition for these social insects in Irish habitats and can enhance their population size. Although the toxicity of *R. ponticum* rewards might harm other functional pollinator groups, complete eradication of the alien might not be considered an appropriate management strategy for the conservation of wild social bees in Ireland.





## 5.2. Introduction

For a long time, mutualistic plant-pollinator interactions have been of scholarly interest in answering questions on ecological and evolutionary processes (Sprengel, 1793, Darwin, 1876). In recent years this key ecosystem service attracted special scientific and economic attention due to an alarming decline in native (European) bees (Westrich, 1996, Biesmeijer et al., 2006, Goulson et al., 2008) which provide the majority of pollination services in natural and agricultural systems (Klein et al., 2007). Anthropogenic activities that result in habitat transformation and fragmentation as well as climatic change are proposed to be the drivers of this loss (Memmott et al., 2007, Steffan-Dewenter and Westphal, 2008). One type of habitat transformation that has raised concerns, particularly over the last decade, is linked to the introduction of alien species that become invasive (Mack et al., 2000, Levine, 2008).

Alien invaders can have impacts on existing plant-pollinator interaction, which have been deemed to be disruptive in most cases (e.g. Chittka and Schürkens, 2001, Brown et al., 2002, Lach, 2007, Abe et al., 2008, but see Bartomeus et al., 2008b, Chapter 2; see Traveset and Richardson, 2006 for review). The greater body of studies so far has focussed on invasive flowering plants and their direct and indirect effects on native plant pollination (Bjerknes et al., 2007), however, there is very little known about impacts of aliens on native pollinators, in particular bees (Goodell, 2008). Survival and reproduction of bees are primarily linked to two factors: the availability and quality of (a) nesting sites and (b) food resources (Westrich, 1989). While the quality and quantity of nesting sites influence reproductive success, the quality and quantity of food resources can impact both the foraging behaviour and survival of adult bee individuals as well as the number of larvae which in turn affects abundance, population size and structure of bee communities. Invasive alien plants may shape these two factors either negatively or positively (Stout and Morales, submitted).

Although breeding systems of alien invasive species are biased towards wind-pollination when compared with the general trend among angiosperms, the majority of invasive plant species are visited and pollinated by animal vectors (Goodell, 2008), which requires a display of rewarding flowers that attract pollinators. Furthermore, invasive species occur by definition in large quantities or high densities (Parker et al., 1999), and so they might be



considered a valuable addition to an insect's foraging source and diet. However, this assumption proves wrong if the alien offers inaccessible flowers to native bees (Stout et al., 2002), if its rewards are of low quality, inedible or even toxic for pollinators (Génissel et al., 2002, Del Lama and Peruquetti, 2006), or if it displaces alternative foraging sources of higher value to pollinators (Goodell, 2008). Alternatively, if high quality resources are available for a long period of time or in very large quantities, alien plants might enhance pollinator populations (Graves and Shapiro, 2003, Tepedino et al., 2008). On an individual insect level, invasive plant species have been shown to modify foraging behaviour by luring visitors and changing their foraging preference (Chittka and Schürkens, 2001), flower constancy (Nienhuis, 2009) or nectar robbing behaviour (Bjerknes, 2008). In contrast, the very few studies that conducted research at a pollinator population level have found neutral (Nienhuis et al., 2009) or negative (Goodell, 2003, Morón et al., 2009) effects of the invader on pollinator abundance, species richness or both, although the aliens (*Lythrum salicaria*, *Solidago* spp., *Impatiens glandulifera*) were frequently visited by pollinators for their rewards. Goodell (2003) and Morón et al. (2009) proposed that the detected disruption was driven by native plant species displacement which caused a shift in flowering phenology of the plant communities, i.e. in narrower peaks of flowering time. Even if invasive species produced large quantities of rewards, these might be less useful because they are available at such a short time frame. Hence, alien impacts on individual foraging behaviour do not reliably predict impacts on a population level. Predictions are further complicated because we may not clearly differentiate between the relative importance of alien rewards and bees' nesting behaviour for bee abundance and communities. Invasive plants are known to modify soil and shading characteristics of their new habitats (Ehrenfeld, 2003, Reinhart and Callaway, 2006, Reinhart et al., 2006), and such transformations are likely to impact nesting behaviour of pollinators. However, this hypothesis has not been addressed so far.

In Ireland, one of the most severe invasive species is the evergreen shrub *Rhododendron ponticum* L. Due to its showy, extensive floral display it was introduced to the British Isles in the 18<sup>th</sup> century as an ornamental plant (Dehnen-Schmutz and Williamson, 2006) and has ever since spread widely. *R. ponticum* is visited by a large range of native insect species (Stout et al., 2006) offering them higher nectar volumes and more sugar in its nectar than most other co-flowering plant species (Chapter 2). As a perennial, the alien





represents a reliable foraging source over years, although its annual flowering period is relatively short and early in the season (one month of peak flowering between early May and early July, depending on geographic location). Nevertheless, quality of its rewards and value to insect visitors are questionable because nectar and pollen of Irish *R. ponticum* (supposedly a hybrid, Milne and Abbott, 2000) may contain grayanotoxins that can be detrimental or even lethal to honeybees and mammals (Carey et al., 1959, Koca and Koca, 2007).

The most dominant flower visitors that not only serve as pollinators of *R. ponticum* but also of numerous native plant species in Ireland are bumblebees (*Bombus* spp: Apoidea, Hymenoptera) (Stout, 2007a, Chapter 2). Bumblebees can travel long distances while foraging (Chapman et al., 2003, Osborne et al., 2008), and their abundance fluctuates with resource availability at a landscape level (Westphal et al., 2003, Hines and Hendrix, 2005, Herrmann et al., 2007). However, bee abundance may serve only as a poor predictor of reproductive success and effective population size (a correlative of long-term population viability) of eusocial, haplodiploid hymenopteran (Chapman and Bourke, 2001, Knight et al., 2005a). The number of colonies accommodated by a habitat estimates effective population size more reliably (Darvill et al., 2004, Knight et al., 2005a). Both worker abundance and colony numbers are likely to respond to the presence of a mass-flowering species such as *R. ponticum*.

The aim of this study was to evaluate the impacts of alien *R. ponticum* on native Irish bumblebees, that is, to clarify if

1. *R. ponticum* nectar and pollen is a suitable food resource for Irish bumblebees, i.e. if bumblebee survival is affected by a *R. ponticum* diet.
2. *R. ponticum* alters bumblebee population characteristics, i.e. if bumblebee abundance and the number of nests differ between habitats uninvaded and invaded by *R. ponticum*.



## 5.3. Materials and methods

### 5.3.1. Food suitability

#### Bumblebee management

For the feeding experiment, I used individuals of the native Irish bumblebee species *Bombus lucorum* L. which is known to frequently visit *R. ponticum* in invaded habitats (Stout, 2007a). 20 nest-searching queens were caught with a net at various locations in the Wicklow Mountains (Co. Wicklow, Ireland) in March 2007 and reared by a commercial bumblebee rearing company (Biobest, Westerlo, Belgium). In May 2007, four colonies each containing approx. 30 workers were transferred to a glass house at Trinity College Dublin, Ireland. Eighteen individual worker bees of each colony were individually placed in plastic tubes, cooled for immobilisation and weighed. I equally split individuals of the same colony into three weight categories: light (mean 98.7 mg, 95% CI = [89.6,107.7]), medium (mean 136.0 mg, 95% CI = [127.9,144.1]) and heavy (mean: 165.8 mg, 95% CI = [154.6,176.9]). Similarly sized (weighted) individuals of ‘pollen-storer’ species like *B. lucorum* have been shown to derive from the same brood batch, i.e. to have hatched at a similar time, with smaller bees generally produced early in the season (Plowright and Jay, 1968, reviewed in Alford, 1975). Since I did not know the exact time of individual hatching, size served in my study as a correlative for bee age. I assigned bees to one of six feeding regimes (see below) by sampling in a stratified manner from the three weight categories. Hence, three bees of each colony (i.e. one from each weight category) received the same type of food and each feeding regime was replicated 12 times with 72 bee individuals in total. Workers were individually kept in transparent plastic containers (15 cm × 9.5 cm × 6 cm, Plate 5 B) in a climate cabinet at 28°C, 65% RH with a 8:16h L:D photoperiodic regime over a period of 28 days, because *R. ponticum* peak flowering is approx. 4 weeks (personal observation) and individual worker bees of bumblebee species from temperate climates live on average  $22.9 \pm 3.0$  days (calculated from da Silva-Matos and Garófalo, 2000). Nectar, water and pollen were supplied ad libitum in 0.5 ml plastic tube feeders (nectar and water) and 1 cm diameter plastic dishes (pollen). I daily registered bee mortality and recorded the identity of colony origin and feeding regime (see below) of each bee that died. I then calculated survival statistics in SPSS 15.0 (SPSS Inc., 2006) fitting a Cox Regression model for censored data with ‘Feeding regime’





and ‘Colony identity’ and their interaction as categorical covariates and ‘Weight category’ as strata.

### Feeding regimes

The six feeding regimes included four treatments and two control diets: 1. a *R. ponticum* pollen and nectar diet (alien diet), 2. a *Digitalis purpurea* pollen and nectar diet (native diet), 3. a diet of commercial pollen and sucrose solution that contained aconitine at a low concentration (weakly toxic diet, see below), 4. a diet of commercial pollen and sucrose solution that contained aconitine at a high concentration (highly toxic diet, see below), 5. a diet of commercial pollen and pure sucrose solution (control diet) and 6. a diet of a commercial food solution (BIOGLUC<sup>®</sup>, Biobest, Belgium) and commercial pollen (commercial control diet).

For regimes 1 and 2, I collected flower rewards of wild Irish plant individuals. Nectar was hand extracted with a micropipette from 6794 *R. ponticum* flowers and 7799 *D. purpurea* flowers from inflorescences that were covered with veil for two to three days, cut and transferred to the laboratory in water filled buckets. In total I collected approx. 43 ml *R. ponticum* nectar (30 – 45% Brix sucrose) and approx. 67 ml *D. purpurea* nectar (20 – 28% Brix sucrose). I gathered *R. ponticum* pollen by collecting pollen sacs of bumblebees that foraged in monocultural stands of *R. ponticum* (Plate 5 E). Such pollen sacs primarily contain *R. ponticum* pollen ( $99.2\% \pm 0.36\text{SE}$ , Nienhuis, 2009). Pollen of *D. purpurea* was collected from anthers of the same *D. purpurea* flowers that I used for nectar collection, and was pressed into small pellets with soft tweezers. For regimes 3 to 6, commercial pollen was offered in the form of pollen sacs collected by honeybees (Biobest, Belgium). Sucrose solutions and the commercial food solution contained 45% and 60% Brix sucrose, respectively (measured with a pocket refractometer; CETI DIGIT-080, Medline Scientific Limited, UK). For the toxic diets 3 and 4, I used aconitine (Sigma Aldrich, Ireland) as an available substitute for grayanotoxin. Both toxins are plant derivatives and depolarise excitable membranes by persistently activating sodium ion channels (Sperelakis et al., 2001). They bind at the same single binding site (Denac et al., 2000). Studies on their toxicity have shown contrary results; mammals responded similarly on intravenous injections when molar aconitine concentrations were 10 times smaller than grayanotoxin concentrations (Schneider and Enders, 1955, vs. Hotta et al., 1980), whereas in other studies an equivalent response was achieved by three to five times smaller molar



grayanotoxin concentrations in comparison to aconitine concentrations (Takahashi et al., 1999, Buschmann et al., 2007). I used similar concentrations of aconitine as other studies on honey bee response had used for grayanotoxin before (Carey et al., 1959), i.e. sucrose solutions with either 10 µg aconitine/ml (15.5 µM, weakly toxic diet) or 100 µg aconitine/ml (154.9 µM, highly toxic diet; for comparison a solution of grayanotoxin at a concentration of 100 µg/ml is 241.3 µM). All nectar, pollen and other food solutions were stored in plastic tubes at –20°C until needed.

5.3.2. Resource usage

Bumblebee abundance

I chose two native Irish bumblebee species, *Bombus pascuorum* Scopoti and *B. lucorum* L. (Plate 5 A and C) that are frequent visitors of *R. ponticum* (Stout, 2007a), but differ in some of their characteristics, to compare their abundance in invaded and uninvaded habitats. *B. lucorum* is short-tongued and nests below ground, while the long-tongued *B. pascuorum* uses above ground nests (Fussell and Corbet, 1992, Edwards and Jenner, 2004). Field work was carried out at two different locations (Dublin and Glencullen Valley), each containing one site with *R. ponticum* and one site without *R. ponticum* (Table 5.1). Sites

Table 5.1. Characteristics of paired sites with their invasion status (*R. ponticum* coverage in %), geographical position, elevation level above sea level (in meters), habitat type description, native flowering plant species richness and the overlap in native flowering plant species between invaded and uninvaded sites (in %).

| Location   | Studied site           | <i>R. ponticum</i> cover (%) | Position                | Elev (m) | Site characteristics                                | Plant spp richness | Plant spp overlap (%) |
|------------|------------------------|------------------------------|-------------------------|----------|-----------------------------------------------------|--------------------|-----------------------|
| Dublin     | Howth Head, Co. Dublin | 13                           | 53°22'36"N<br>6°04'12"W | 130      | Gentle NW facing slope, coastal habitat, heath      | 12                 | 80                    |
|            | Bray Head, Co. Wicklow | 0                            | 53°11'22"N<br>6°05'00"W | 180      | Steep NE facing slope, coastal habitat, heath       | 15                 |                       |
| Glencullen | Ballybrack, Co. Dublin | 8                            | 53°13'49"N<br>6°15'56"W | 330      | Steep SW facing slope, edge of blanket bog & heath  | 15                 | 75                    |
|            | Tibradden, Co. Dublin  | 0                            | 53°14'21"N<br>6°17'38"W | 300      | Gentle W facing slope, edge of blanket bog & forest | 13                 |                       |





were situated at least 1 km apart to maximise the probability that sampled individuals were representative of the sampled site only; bumblebees travel on average smaller distances than 1 km while foraging although they can flight much farther (Walther-Hellwig and Frankl, 2000, Darvill et al., 2004, Knight et al., 2005a). Invaded sites had an average *R. ponticum* cover of 10.5% (estimated by aerial photography and GPS data) and overlapped greatly in their native species composition compared to the uninvaded sites (Table 5.1). Sampling was conducted after the flowering season of *R. ponticum* between 7<sup>th</sup> and 16<sup>th</sup> August 2006 using a mark-recapture method. At each site, two plots of bumblebee-visited native plants (*Cirsium palustre*, *Chamaenerion angustifolium*, *Erica cinerea*, *Rubus fruticosus*, *Ulex europaeus* and *Teucrium scorodonia*) were selected 40 to 80 meters apart from each other to later pool data. Each plot was sampled for 60 min during both capture and recapture sessions, respectively. During capture sessions, all foraging bumblebee workers of the two species were caught with a net, placed in individual plastic tubes and cooled down to immobilise them. I discriminated *B. lucorum* workers from similar *B. terrestris* workers by their more lemon-like coloured bands and the absence of both a buff abdominal tip band and a distinct buff line between the abdominal tip band and the neighbouring black band (Prŷs-Jones and Corbet, 1991, Chapman et al., 2003). Some of the sampled workers might have been *B. magnus* or *B. cryptarum* individuals since these species are morphologically indistinguishable from *B. lucorum* and can be only detected by special molecular techniques (Murray et al., 2008). However, integrating individuals of these two species into my data set should have been equally likely among paired sites. Bees were counted, marked on the thorax using permanent markers (UNIPOSCA, Mitsubishi Pencil Co, Japan) and released at the point of capture where they immediately foraged again (personal observation). Marks usually remain visible for five days and longer (personal observations on freely foraging workers from colonies in captivity). I revisited each plot 48 hours later (except for two plots that were revisited after 72 hours due to persistent rain on the day of scheduled re-sampling), caught all bumblebee workers encountered and placed them in tubes for cooling them. After the sessions, the number of marked and unmarked bee individuals was recorded and bees were released again.

I obtained an estimate of bee abundance for each site using the Chapman hypergeometric model for the Petersen estimator (Seber, 1982), its standard error and asymptotic 95% confidence interval (based on an inverse cube root transformation of the estimate, Arnason



et al., 1991). I chose an estimate for closed populations because I assumed that migration, recruitment and mortality were negligible (Seber, 1986) due to the affiliation of bumblebee workers to a resident colony and the short period between mark and recapture (Seber, 1992). Computation was achieved with SPAS 1.2 (Arnason et al., 1996). Estimates were compared using Generalised Estimating Equations (GEE) with an unstructured working correlation matrix and a log-linear model (Burton et al., 1998) including 'Location' and 'Bee species' as between-subjects factors and 'Invasion' as a within-subjects factor in SPSS 15 (SPSS Inc., 2006). I chose the model with the lowest Quasi-Likelihood under Independence Model Criterion (an extension of the Akaike information criteria) as the best fit:  $\text{Abundance} = \text{Error} + \text{Bee species} + \text{Invasion} + \text{Location} + (\text{Bee species} \times \text{Invasion}) + (\text{Bee species} \times \text{Location}) + (\text{Invasion} \times \text{Location}) + (\text{Bee species} \times \text{Invasion} \times \text{Location})$ .

### Colony numbers

*Bombus pascuorum* and *B. lucorum* individuals were sampled at the same sites that were used for estimating bumblebee abundance (see above, Table 5.1). Each site was visited on two or three days between 15<sup>th</sup> July and 1<sup>st</sup> August 2005 and between 7<sup>th</sup> August and 17<sup>th</sup> August 2006, respectively. Hence, sampling took place after the flowering season of *R. ponticum* (personal observation) and at the seasonal peak of the bumblebees' colony cycles (Prÿs-Jones and Corbet, 1991). At each site, five to eight flower patches of native plant species (*Cirsium palustre*, *Chamaenerion angustifolium*, *Erica cinerea*, *Rubus fruticosus*, *Ulex europaeus* and *Teucrium scorodonia*) along an approx. 175 meter transect were monitored for an average of 69 min ( $\pm 6$  min SE). A mean of 40 ( $\pm 6.5$  SE) *B. pascuorum* and 32 ( $\pm 5.3$  SE) *B. lucorum* individuals were caught per site while foraging at these patches (Supplement 10). I took a tarsal sample of each individual using a non-lethal sampling method (Holehouse et al., 2003) and stored it in 70% ethanol at  $-20^{\circ}\text{C}$ .

Samples were cleansed in distilled water, pulverised and their DNA was extracted using standard methods (Walsh et al., 1991). For a subset of 22 workers of each species, 12 microsatellite loci (B10, B11, B96, B100, B101, B116, B118, B119, B121, B124, B131 and B132) were analysed, and I genotyped the remaining bee individuals at the six most polymorphic loci: B10, B11, B100, B118, B121, B132 for *B. lucorum* and B11, B96, B118, B124, B131, B132 for *B. pascuorum* (Estoup et al., 1995, Estoup et al., 1996). Polymerase chain reactions (PCRs) were carried out following standard protocols by Estoup et al. (1995, 1996) (Supplement 11). All 3456 PCR products were electrophoresed





on high resolving polyacrylamide sequencing gels (Spreadex<sup>®</sup> 400, 500 and 600, Elchrom Scientific, Switzerland) together with two scoring standards (10 bp ladder and M3 marker, Elchrom Scientific, Switzerland). Gels were afterwards stained with SYBR<sup>®</sup> Gold nucleic acid gel stain (Molecular Probes Inc., USA) and photographed using an AlphaImager 3300<sup>™</sup> Imaging System (Alpha Innotech Co., USA) with a green filter (537/535 nm). Microsatellite fragment lengths were then manually scored using the AlphaEaseFC<sup>™</sup> software (Alpha Innotech Co., 2002)(Plate 5 D). In uncertain cases of fragment scores, amplification and electrophoresis were rerun, and I excluded samples from further analysis if scoring repeatedly failed.

I identified kinship among sampled workers of the same bee species at each site for each year using the maximum likelihood method of COLONY 1.2 (Wang, 2004). COLONY takes into account that *B. pascuorum* and *B. lucorum* workers of the same nest share on average a genetic relatedness of 0.75 due to their haplodiploidy and monandry (Estoup et al., 1995, Schmid-Hempel and Schmid-Hempel, 2000). After removing sisters and leaving only one sister individual per nest in the data set I checked for scoring errors and null alleles with the software MICROCHECKER (van Oosterhout et al., 2004). I further tested for linkage disequilibrium (LD) and departures from Hardy-Weinberg equilibrium (HWE) in GENEPOP 3.4 (Raymond and Rousset, 1995). To minimise type I errors, significance levels were adjusted by constructing individual sequential Bonferroni tables (Rice, 1989) for both populations across all loci and loci across all populations.

The total number of nests at each site can be estimated by summing the number of detected sister groups, i.e. the number of detected groups in each sister category (1 sister, 2 sisters, 3 sisters etc.) and the number of colonies that remained undiscovered due to limited sampling effort. This latter 'zero' category was extrapolated using two different procedures. First, I fitted a Poisson distribution to the observed sample distribution using the program FITTING (Abramson and Gahlinger, 2001) and thereby estimated the unsampled nests (FITTING method; cf. Chapman et al., 2003, Darvill et al., 2004, Ellis et al., 2006). Second, I corrected the observed sampled nest number for the limited sample size using a modified version of the equation proposed by Cornuet and Aries (1980,  $E|X(m)| = k - k(1 - 1/k)^m$ ) that links the total number of colonies ( $k$ ) with the number of colonies ( $E$ ) expected within a drawn sample of small size ( $m$ ) via a probability distribution (EQUATION method; cf. Herrmann et al., 2007, Moritz et al., 2007). I



calculated standard errors for the estimates with a jackknife resampling method across each site for each year (Crowley, 1992, Quinn and Keough, 2002, cf. Moritz et al., 2008).

I analysed data by GEE in SPSS 15.0 (SPSS Inc., 2006) using an unstructured working correlation matrix (Horton and Lipsitz, 1999) and a log-linear model (Agresti, 1996) to evaluate the influence of 'Bee species', 'Invasion' and 'Location' on the number of estimated colonies. The best-fit model according to the lowest QIC was Colony number = Error + Bee species + Invasion + Location + (Bee species  $\times$  Invasion) + (Bee species  $\times$  Location) + (Invasion  $\times$  Location) + (Bee species  $\times$  Invasion  $\times$  Location), with Bee species and Location as between subject factors and Invasion as within subject factor. Pairwise contrasts were calculated for significant factors and interactions whereby significance levels were adjusted with the sequential Bonferroni procedure.

### Relationship between bumblebee abundance and colony number

Both the estimated bumblebee abundance and number of colonies at each site were log-transformed and one extreme outlier which was more than one standard deviation smaller than the lower bound of the 95% confidence interval (*B. lucorum* abundance at the invaded Glencullen site) was excluded from further analyses. Data conformed to the assumption of normal distribution (Kolmogorov-Smirnov test,  $P > 0.2$ ) and I correlated the transformed variables of overall abundance and colony numbers (*B. lucorum* + *B. pascuorum*) using one-tailed Pearson product-moment correlation.

## 5.4. Results

### 5.4.1. Food suitability

After a feeding period of 28 days, 51 bee individuals (70.8%) were still alive, and I included them in analyses as censored data. Although I counted more dead light bees (11 bees or 45.8% within this stratum) than dead medium weight or heavy bees (5 bees or 20.8% in either stratum) by the end of the experiment, this difference in survival between weight strata was not significant ( $G^2$  statistics with Yates' correction,  $\chi^2 = 4.69$ ,  $df = 2$ ,  $P = 0.096$ ).

When analysing the main factors, neither the full model ('Feeding regime', 'Colony identity' and their interaction) nor the reduced model ('Feeding regime' and 'Colony identity') were significantly different from the Null model ( $G^2$  statistics, full model:  $\chi^2_{\log-}$





likelihood = 32.72,  $df = 23$ ,  $P = 0.086$ ; reduced model:  $\chi^2_{\log\text{-likelihood}} = 6.71$ ,  $df = 8$ ,  $P = 0.579$ ). That is survival of individual workers did not significantly differ between the six feeding regimes (Wald statistic,  $t_W = 2.324$ ,  $df = 5$ ,  $P = 0.803$ , Fig. 5.1) or between the four colonies (Wald statistic,  $t_W = 0.03$ ,  $df = 3$ ,  $P = 0.999$ ), and in regard to bee survival I could not detect any significant interaction between feeding regime and colony identity either (Wald statistic,  $t_W = 3.688$ ,  $df = 15$ ,  $P = 0.999$ ). The majority of bees fed with a *R. ponticum* diet survived (Fig. 5.1), i.e. I did not detect a toxic effect of the alien rewards.

### 5.4.2. Resource usage

#### Bumblebee abundance

The number of bees caught at both mark and recapture days was very low for *B. lucorum* for the majority of sites and higher for *B. pascuorum* (Table 5.2). Abundance estimates were therefore constrained to large standard errors (Table 5.2). These standard errors estimated by the Petersen estimator were significantly higher than standard errors estimated by GEE (Paired *T*-test,  $t_7 = -2.531$ ,  $P = 0.039$ ). I therefore cast doubt upon the validity of the GEE results and only evaluated differences between sites using confidence intervals of the Petersen estimator adjusted with the sequential Bonferroni procedure (Fig. 5.2). There was no significant impact of the presence of the alien on bee abundance (Fig. 5.2). Worker bee abundance of *B. pascuorum* was significantly higher in the invaded Dublin site than in Glencullen, however, this difference was not revealed for *B. lucorum* abundance (Fig. 5.2).

Similar results were revealed when discarding the Petersen procedure and calculating simply means and confidence intervals of census data across the two sampling days.

#### Colony numbers

In *B. pascuorum*, no locus across populations and no population across loci showed any significant deviation from HWE in either 2005 or 2006 after significance levels had been adjusted with the sequential Bonferroni method (table-wide  $P > 0.05$ ). A global test across all loci and populations did not reveal any significant deviation from HWE either (Fisher's method; 2005:  $\chi^2 = 45.8$ ,  $df = 44$ ,  $P = 0.396$ ; 2006:  $\chi^2 = 54.5$ ,  $df = 48$ ,  $P = 0.242$ ). Further, I could not detect a significant linkage disequilibrium between any two loci pairs in any of the two years (table-wide  $P > 0.75$ , respectively). In contrast, I found for

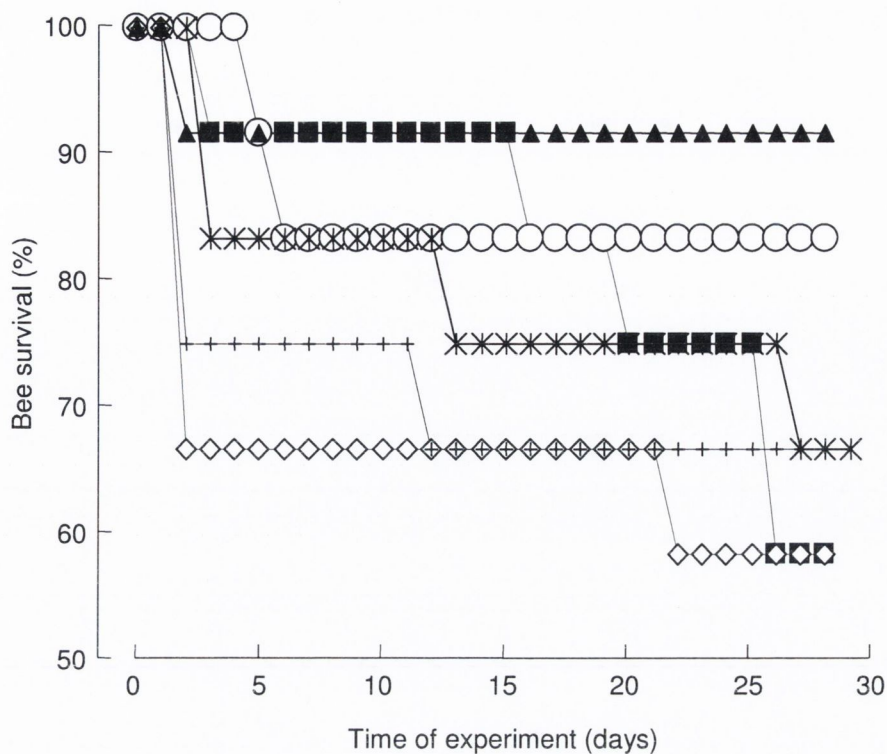


Figure 5.1. Survival of *Bombus lucorum* workers over the period of 28 days in relation to their feeding regime. The percentage survival of each feeding regime is displayed for each experimental day. Bees were offered either an alien diet (○), a native diet (■), a weakly toxic diet (+), a highly toxic diet (\*), a control diet (◇) or a commercial control diet (▲); 12 bees were used as replicates for each treatment.

Table 5.2. Number of bees caught on two sampling days (first day: mark; second day: recapture) that were used to estimate bee abundance of the two bumblebee species *Bombus lucorum* and *B. pascuorum* at *R. ponticum* infested (R) and uninfested (N) sites at the two investigated locations.

| Bee species         | Invasion  | Location   | No. of bees marked on first day | Total no. of bees caught after 48 h | No. of marked bees caught after 48 h | Petersen estimator | SE of Petersen estimator |
|---------------------|-----------|------------|---------------------------------|-------------------------------------|--------------------------------------|--------------------|--------------------------|
| <i>B. lucorum</i>   | Invaded   | Glencullen | 19                              | 16                                  | 3                                    | 84                 | 29.7                     |
|                     |           | Dublin     | 7                               | 8                                   | 0                                    | 71                 | 44.9                     |
|                     | Uninvaded | Glencullen | 0                               | 1                                   | 0                                    | 1                  | 1                        |
|                     |           | Dublin     | 7                               | 5                                   | 0                                    | 47                 | 29.0                     |
| <i>B. pascuorum</i> | Invaded   | Glencullen | 7                               | 6                                   | 2                                    | 18                 | 5.6                      |
|                     |           | Dublin     | 43                              | 51                                  | 20                                   | 108                | 13.0                     |
|                     | Uninvaded | Glencullen | 5                               | 7                                   | 3                                    | 11                 | 2.2                      |
|                     |           | Dublin     | 11                              | 34                                  | 3                                    | 101                | 35.0                     |



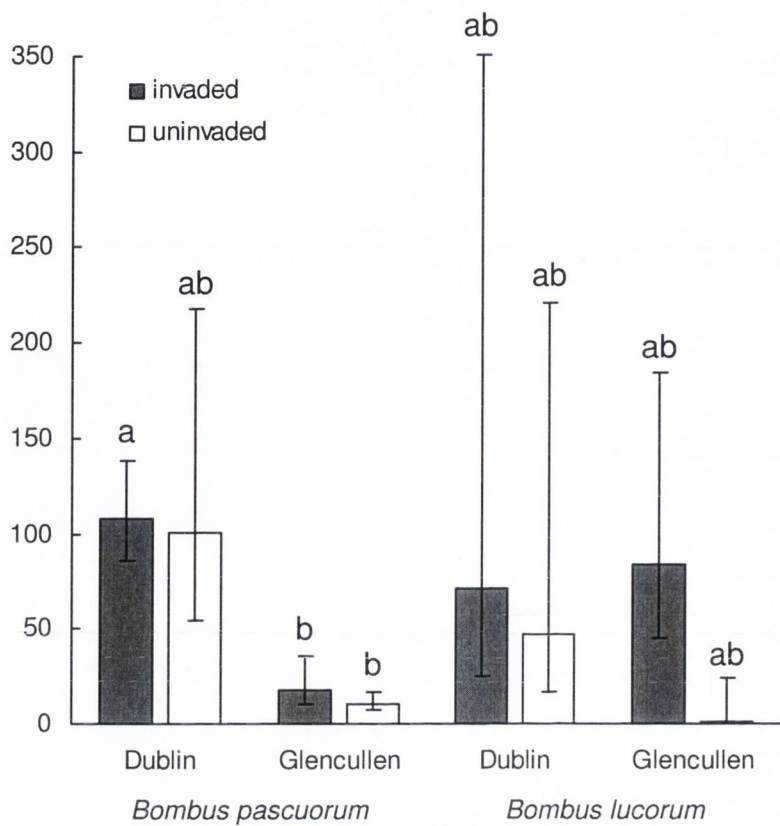


Figure 5.2. Bee abundance (number of worker bees) at investigated sites estimated with the Petersen estimator. Abundance estimates are displayed with asymptotic 95% confidence intervals. Different letters indicate a significant difference at a table-wide  $P = 0.05$  level after I adjusted the 95% CI with the sequential Bonferroni procedure.

*B. lucorum* populations a significant linkage of locus B121 with B100 in 2005 as well as locus B121 with B100 and B118 in 2006 ( $P < 0.01$  for these loci pairs after sequential Bonferroni; for all other pairs  $P > 0.05$ ). I consequently analysed *B. lucorum* data sets including and excluding B121. Resulting estimates of the two analyses did not significantly differ from each other (Paired  $T$ -test,  $t_7 = 1.64$ ,  $P = 0.145$ ); for further analyses I used estimates excluding B121. Data sets of both years showed significant deviation from HWE for the invaded site at Glencullen and for locus B132 in both years and additionally for locus B100 in 2005 linked to higher than expected frequencies of homozygotes (Markov chain method;  $P < 0.05$  for both years). Analyses in MICROCHECKER also suggested the presence of two null alleles. COLONY assumes HWE (Wang, 2004), and so I estimated the number of colonies anew after removing data of the two loci. These estimates were lower for each site, however, differences in results were



minor and non-significant and did not influence the outcome of further tests. Hence, I included the loci in my analyses.

The evaluation of the genetic data revealed that 214 individuals (66.7%) among the 321 sampled *B. pascuorum* bees and 105 individuals (41%) among the 255 sampled *B. lucorum* bees were sisters of at least one other bee individual in the sample. When using the FITTING method for nest number estimation, truncated Poisson distributions fitted the distributions of detected sister groups closely ( $G^2$  statistics; range between  $\chi^2 = 2.71$ ,  $df = 3$ ,  $P = 0.438$  and  $\chi^2 < 0.01$ ,  $df = 2$ ,  $P = 0.999$ ). Hence, detected nest numbers of each bee species at each site for each year were corrected with the number of unsampled colonies using the zero categories of these Poisson distributions (e.g. Supplement 12). I then compared these estimates of total nest numbers with total colony numbers obtained with the EQUATION methSpearman  $r = 0.985$ ,  $P < 0.001$ ), and so I used results of the latter method for further calculations because it provides error terms for the estimates. Individual estimates of colony numbers were associated with relatively small standard errors (mean 3.0) except for two estimates of *B. lucorum* colony numbers at an uninvaded site (SE of 13 and 22 respectively) where sample sizes were very low (20 and 13 caught individuals respectively). Since estimates of 2005 resembled estimates of 2006 (Paired  $T$ -test,  $t_7 = 1.04$ ,  $P = 0.333$ ), I averaged colony numbers across years to obtain a better total nest number value for each site and bee species. In addition, I added both years' variances and calculated a mean standard error for the mean colony number estimate of each site. These standard errors did not significantly differ from standard errors estimated by GEE (Paired  $T$ -test,  $t_7 = -0.017$ ,  $P = 0.987$ ); hence, I assumed that GEE results (see below) are valid.

Nest number estimates varied considerably between the two bee species with  $65 \pm 11.4$  SE *B. lucorum* colonies and  $36 \pm 6.5$  SE *B. pascuorum* colonies on average per site. Nest numbers between the two bee species differed between locations investigated (Bee species  $\times$  Location, Wald  $\chi^2 = 133.9$ ,  $df = 1$ ,  $P < 0.001$ ; Bee species, Wald  $\chi^2 = 93.3$ ,  $df = 1$ ,  $P < 0.001$ ; Location, Wald  $\chi^2 = 9.5$ ,  $df = 1$ ,  $P = 0.002$ ; Fig. 5.3 A). They were significantly larger at invaded sites than at uninvaded sites (Invasion, Wald  $\chi^2 = 15.3$ ,  $df = 1$ ,  $P < 0.01$ ; Fig. 5.3 B). I did not reveal any other significant interaction between factors (Bee species  $\times$  Invasion  $\times$  Location, Wald  $\chi^2 = 3.56$ ,  $df = 1$ ,  $P = 0.06$ ; Bee species  $\times$  Invasion, Wald  $\chi^2 = 0.52$ ,  $df = 1$ ,  $P = 0.47$ ; Invasion  $\times$  Location, Wald  $\chi^2 = 0.35$ ,  $df = 1$ ,  $P = 0.55$ ).



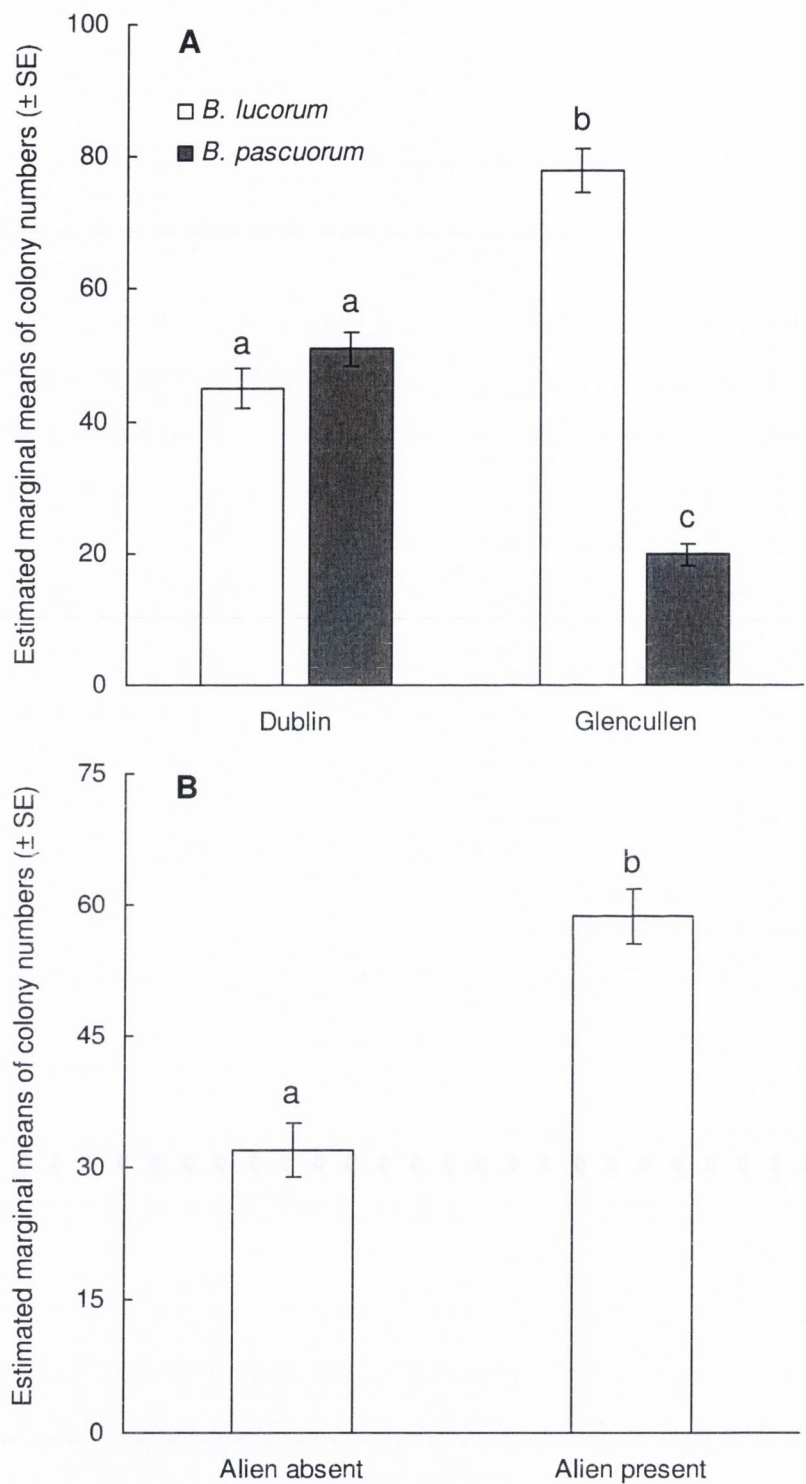


Figure 5.3. Colony numbers of *B. lucorum* and *B. pascuorum* at different locations (A) and at invaded and uninvaded sites (B). Marginal means estimated by Generalised Estimating Equations (GEE) are displayed with their standard errors. Different letters indicate a significant difference at a sequential Bonferroni adjusted  $P = 0.05$  level.

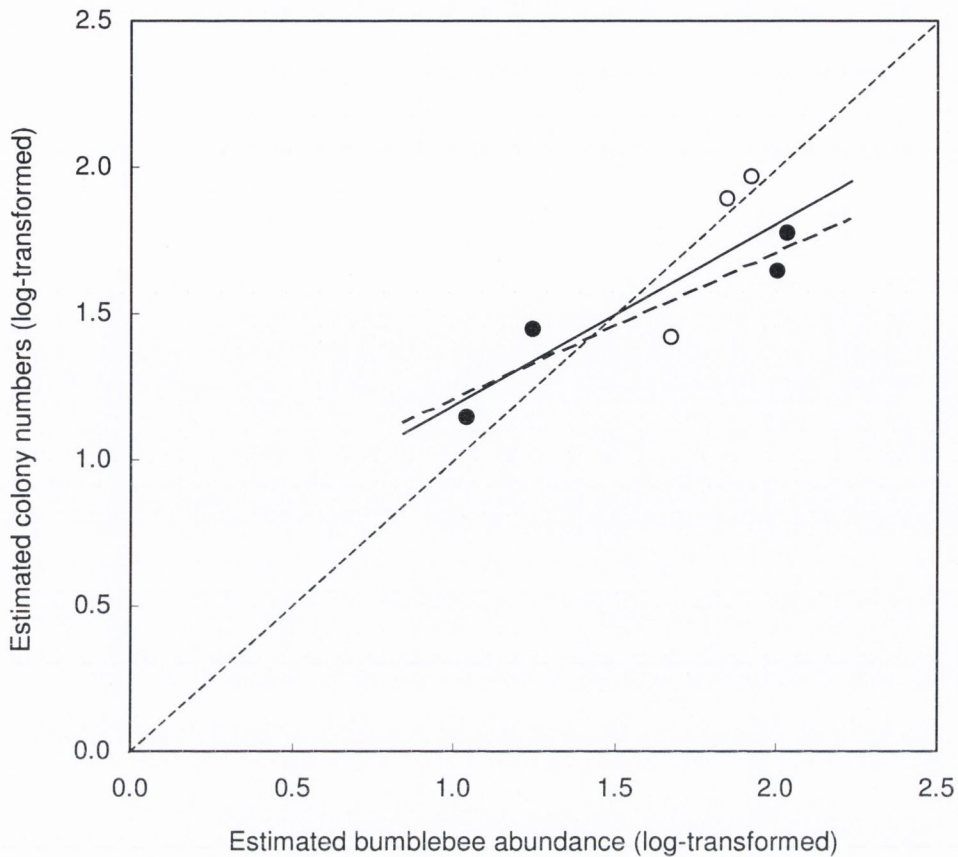


Figure 5.4. Relationship between log-transformed bumblebee abundance and log-transformed colony numbers. The dotted bisect line represents an ideal relationship of  $\text{Log (Abundance)} = \text{Log (Colony number)}$ .  $\circ$  *B. lucorum*;  $\bullet$  *B. pascuorum*; — *B. lucorum* + *B. pascuorum* linear trend, ---- *B. pascuorum* linear trend..

### Relationship between bumblebee abundance and colony number

For the combined analysis of *B. lucorum* and *B. pascuorum* data, the log-transformed number of *Bombus* spp colonies increased significantly with increasing log-transformed *Bombus* spp abundance (Pearson correlation,  $r = 0.832$ ,  $N = 7$ ,  $P = 0.010$ ; Fig. 5.4).

## 5.5. Discussion

My study is the first of its kind that links investigations on the suitability of alien rewards for pollinators with observations on impacts of an alien on population characteristics of its main pollinator group. Even though, I have to interpret the present results on population characteristics cautiously because, although I minimised the likelihood of temporal





inconsistencies by sampling over a two years period and could show that data were temporarily replicable, observations were only conducted at two different locations.

This lack of replication is the result of two problems. First, the difficulty to find comparable invaded and uninvaded sites without confounding samples with large scale biogeochemical variation is inherent to studies on invasive organisms (Stout and Morales, submitted). If initial naturalisation and invasion took place several decades or even centuries ago, potentially uninvaded sites that resemble a standard invaded site in their abiotic characteristics and species composition are usually already invaded by the alien. Thus, establishment of suitable sampling sites was extremely difficult and restricted.

Second, I initially chose and investigated two more replicated locations than the two presented here, however, I was unable to collect sufficient bee samples for a sound genetic analysis (especially at uninvaded sites) and had to discard these additional sites. The problem of low bee abundance is also mirrored in the low, and therefore analytically problematic, number of marked and recaptured bees. Studies on bees conducted in Ireland are likely to encounter this problem because generally poorer weather conditions in comparison to continental Europe not only entail lower bee diversity (cf. Westrich, 1989, and Fitzpatrick et al., 2006) but also lower bee abundance.

Conclusions drawn from limited data sets still have proven to be reliable (Fleishman et al., 2005), and so I am convinced that despite any study restrictions my results give interesting insights in a much neglected aspect of invasion biology.

### **5.5.1. Food suitability**

The majority of bees collect and rely on nectar and pollen for their own consumption and provisioning of progeny (exceptions are e.g. oil collecting bees, Vogel, 1974, Pauw, 2007). While nectar serves as an energy-rich “fuel” for adult bees which is primarily composed of amino acids and carbohydrates such as sucrose, glucose and fructose (Percival, 1961), pollen insures the supply with proteins, free amino acids and other nutrients (Roulston and Cane, 2000) and is mainly used as larval food (Pereboom, 2000, Roulston and Cane, 2002, but see Smeets and Duchateau, 2003). Surprisingly, nectar and pollen of some plant species contain secondary compounds such as alkaloids, terpenes or glycosides that can



also induce intoxication of their pollinators (Detzel and Wink, 1993). Much thought has been given to the ecological significance of secondary compounds in floral rewards (e.g. Rhoades and Bergdahl, 1981, Adler and Irwin, 2005, Singaravelan et al., 2005, Johnson et al., 2006, Irwin and Adler, 2008, Junker and Bluthgen, 2008) suggesting that they serve as adaptation to specialist pollinators, deterrence of reward robbers, preservatives against microbial degradation, (narcotic) modifiers of flower constancy or just a pleiotropic constraint (see Adler, 2000 for review).

Although *R. ponticum*'s floral rewards usually contain grayanotoxins (Koca and Koca, 2007) which can cause lethal reactions in some bee species (e.g. honeybees, Carey et al., 1959), I did not detect any differences in survival rates between *B. lucorum* workers that received a *R. ponticum* diet and workers that were fed any of the control diets (native *D. purpurea*, commercial control and sucrose control). Since this result is certainly related to the low statistical power of the tests to detect an effect (low number of replication), it is still obvious that *R. ponticum* does not harm the bumblebees because I found one of the highest survival rates when feeding an alien diet. I can think of three non-mutually exclusive theories for this observation.

First, Irish invasive *R. ponticum* might lack grayanotoxins in its nectar. Milne and Abbott (2000) detected evidence of introgression in invasive *R. ponticum* on the British Isles, and hybridisation of *Rhododendron* species can result in loss of toxic nectar properties (Carey et al., 1959). However, Wong et al. (2002) reported the presence of grayanotoxin in British invasive *R. ponticum* material. Since invasive *R. ponticum* in Britain and Ireland is likely to be derived from the same populations (Milne and Abbott, 2000), I expect that Irish invasive *R. ponticum* also produces grayanotoxins. Chemical analyses are currently being conducted, and first results for nectar samples of one Irish population indicate the presence of the toxin in low concentrations (7  $\mu$ M, Geraldine Wright, personal communication). Such low toxic concentrations might have in turn a weak or undetectable toxic effect on feeding insects in general (Carey et al., 1959).

Second, the toxin might have broken down into components of non-toxic properties by the time of feeding. Grayanotoxin decomposes only when heated (von Malottki and





Wiechmann, 1996), and my samples were immediately frozen after nectar extraction. Disintegration of the toxin is therefore unlikely.

Third, bumblebees might have evolved a detoxification or sequestration mechanism. Flowers of *Rhododendron* species are regarded as bumblebee flowers that are not very attractive to honeybees (Barker, 1978). Contrasts in foraging preference among different insect taxa could be explained by differences in tolerance to secondary plant metabolites. A tolerance to toxic compounds is not exceptional; bees and their larvae have been found indifferent to other secondary compounds before (Stephenson, 1981, 1982, Elliott et al., 2008). The tolerance hypothesis is further supported by my finding that *B. lucorum* workers did not show decreased survival when fed an aconitine diet, either. Aconitine generally has strong insecticidal properties (González-Coloma et al., 2004), however, other bumblebee species also tolerate this neurotoxin (e.g. *B. consobrinus*, Pierre Rasmont, personal communication). Grayanotoxin and aconitine reveal similar toxic characteristics (Denac et al., 2000), and it is likely that bumblebees use the same metabolic pathway to neutralise either of the two substances.

High survival rate of individual workers fed with a *R. ponticum* diet and high floral nectar volume and sugar production of the alien (Chapter 2) imply that invasive *R. ponticum* can be regarded a suitable food resource for bumblebee species in Ireland. However, it is not clear if bumblebee larvae inherited the same tolerance to the toxin. Since studies on other bee species showed a discrepancy in tolerance among adult bees and larval offspring (Elliott et al., 2008), I cannot assume similar impacts on larvae without experimental backup.

Although *R. ponticum* rewards can be considered beneficial for bumblebees, their impact on other pollinator groups remains obscure. It is possible that e.g. solitary bees lack – like honeybees (Carey et al., 1959) – a tolerance to grayanotoxin. In the absence of a less or non-toxic alternative resource, polylectic bees still utilise toxic rewards in a similar manner as non-toxic resources (Detzel and Wink, 1993, Gegear et al., 2007, Tan et al., 2007).

Thus, if an invasive plant species with toxic nectar and pollen like *R. ponticum* dominates the floral community, bees might come to forage on its flowers with lethal results. Earlier studies on *R. ponticum* suggested a lack of solitary bees in most of the investigated invaded



habitats (Stout et al., 2006, Chapter 2), although *R. ponticum* is frequently visited by solitary bees in its Spanish native habitat (Stout et al., 2006). Further investigations on food tolerance of frequent *R. ponticum* visitors (solitary bees, hoverflies) are advisable.

### 5.5.2. Resource usage

Given that a temporarily mass-flowering species – such as *R. ponticum* or many other invaders and crop species – offers suitable, accessible rewards, bumblebee populations may respond to its presence in different ways. If nesting sites are not a limiting factor and the plant species in question flowers at the time of colony initiation, queens themselves might profit from a surplus of resources resulting in a larger number of queens that successfully found and/or maintain a colony (Alford, 1975). This would cause an increase in effective population size in the habitat. Alternatively, a raised availability of foraging sources may accelerate the growth of already founded colonies, i.e. an increase in bumblebee abundance (Westphal et al., 2003). Hence, in contrast to solitary insect species (Collier et al., 2008, Larsson and Franzén, 2008), population size of eusocial insects is not necessarily correlated to the abundance of individuals (Herrmann et al., 2007).

Although invasive alien plant species in Ireland are biased towards late flowering (Milbau and Stout, 2008), *R. ponticum* is an exception with flowers emerging from late April to early July (depending on the location). Due to its early flowering season, I expected and suggested that the alien positively impacted population characteristics of its bumblebee visitors probably by providing resources for nest founding queens during colony establishment. Invaded sites were used by a larger number of colonies of both species although colony numbers showed variation according to the investigated bumblebee species and location. That is, spatial characteristics such as altitude or soil properties may influence the establishment of young queens of different bumblebee species in different ways. Nevertheless, in both species and locations the presence of adequate alien supplies of nectar and pollen at a time when native flowering plants are still scarce (Edwards and Jenner, 2004) seemed to increase the number of colonies which implies food resource rather than nesting site limitations for bumblebee population growth in Irish habitats.

In contrast, I did not detect any consistent differences in bumblebee abundance in relation to habitat invasion status for either bee species. Potential differences in my study might





have been obscured by the very high standard deviations of my estimates due to the very small sample sizes I had to rely on. The power of the test is very low and a second study is needed to reliably draw conclusions. Despite this limitation and the low replication, the significant positive relationship between log-transformed combined *B. lucorum* and *B. pascuorum* abundance and colony numbers indicates that colony size may be similar at all sites in the two species or even decrease slightly in sites where a high number of colonies can be found. In this case, *R. ponticum* rewards would have lost their facilitative influence on individuals and colonies of the two species after the initial colony establishment. Indeed, peak flowering of *R. ponticum* lasts for scarcely more than one month and climaxes just when the first worker bees emerge (cf. Prŷs-Jones and Corbet, 1991, Stout et al., 2006).

My results contrast to studies on *B. pascuorum* in landscapes dominated by mass-flowering crops (Westphal et al., 2003, Herrmann et al., 2007) which report increased bee abundance in conjunction with unaltered colony numbers. Herrmann et al. (2007) stated that although bees profited from mass-flowering crop rewards, fields that were subject to crop cultivation represented unfavourable nesting sites due to a rapid change in land use after crop maturation. In such landscapes, suitable nesting sites may therefore be limited. Although *R. ponticum* potentially changes shading (Nadezhdina et al., 2004) and soil conditions (Ehrenfeld, 2003, Sutton and Wilkinson, 2007) of invaded habitats and could in turn interfere with both above and below ground nesting Irish bees, constraints in nesting sites were not evident in my study (see also Hines and Hendrix, 2005). However, we have to keep in mind that sites in this study were not heavily invaded (10% alien cover only), and bees may be restricted to very few, limited nesting sites when entering an area with massive invasion (80-90%). Average nest numbers of *B. pascuorum* at my study sites ( $36 \pm 6.5$  SE) resemble colony numbers ( $37 \pm 3$  SE) observed by Herrmann et al. (2007) who used a similar sampling method (but see Chapman et al., 2003 with 66 nests per site). *B. pascuorum* responses in the two – crop and invader – study systems appear modified to slightly different flowering phenologies and site characteristics.

Although *B. lucorum* and *B. pascuorum* differ in their morphological and behavioural characteristics, e.g. found their colonies below (*Bl*) or above (*Bp*) ground, possess a short (*Bl*) or long (*Bp*) tongue and exhibit high (*Bl*) or low (*Bp*) flower constancy (Fussell and



Corbet, 1992, Gegear and Lavery, 2005), the two *Bombus* species both responded to *R. ponticum* invasion with an increase in colony numbers. Hence, invasive *R. ponticum* with its freely accessible generalist flowers (Mejías et al., 2002) seems to accommodate these generalist pollinators. Invasive plants have been proposed to increase population sizes of generalists bees before (Tepedino *et al.*, 2008). Such shifts in bee abundance and colony number of selectively facilitated generalists might entail a shift in the pollinator community (Strauss et al., 2006). Bumblebees demonstrate a flexible foraging strategy by utilising short-term available food resources of invasive plants and substituting them at other times with site internal and more distant native rewards due to their ability to travel long distances (Walther-Hellwig and Frankl, 2000, Knight et al., 2005a, Osborne et al., 2008). Foraging distances of the usually smaller solitary bee species are shorter (Gathmann and Tscharntke, 2002, Greenleaf et al., 2007), and so solitary bees might not be able to adapt to a displacement of native species easily. This can result in a lower abundance of solitary bees in invaded habitats than in uninvaded habitats (Goodell, 2003, 2008, cf. also Chapter 2). Flight distance of a pollinator species might be therefore a sensible predictor for facilitative or disruptive effects of aliens on abundance and population size of this species.

## 5.6. Conclusions

*R. ponticum* was found to benefit populations of social bees in Ireland. Similar to *Bombus* populations in other European countries (Goulson *et al.*, 2008), Irish bumblebees are in decline (Fitzpatrick *et al.*, 2007), and in future this decrease could be accelerated by ongoing habitat transformation. In a lot of places, the diversity of native foraging plants declines at different scales (Carvell et al., 2006); invasive flowering plants like *R. ponticum* might fill a gap by providing rewards for generalist pollinators when resources are scarce (Starý and Tkalců, 1998). On the other hand they may create a gap while invading and becoming dominant in an area. Although I cannot rule out that the invasive is harmful to more specialised flower visitors like solitary bees, the maintenance of the plant-pollinator network relies on the social bees which link species and species groups within the network (network hubs, Olesen et al., 2007). Invasive alien flowering plants may support these generalists at the expense of more specialist bees. Indisputably, invasive species require management, however, rapid elimination actions might have unintended negative effects





on social bees and their populations (Goodell, 2008). Management measures should abandon the single-sided view of alien species as solely noxious nuisances (Tepedino et al., 2008) and sustainably control alien invasive species and associated native plants and pollinators.

## Chapter 6

“...Where science scores over alternative world views is that we know our uncertainty, we can often measure its magnitude, and we work optimistically to reduce it.” (Dawkins, 2004)





Plate 6





## 6. General discussion

This thesis aimed to shed light on the direction and magnitude of impact that an alien invasive plant can have on native plant pollination and pollinating insects. By using both an individual and a community level approach, I characterised interactions between plant and insect species that are linked to the alien within a plant-pollinator network. I also considered the influence of alien population size and density on pollination and fertilisation of a native focal plant at a smaller experimental and a larger natural scale. I finally investigated the suitability of alien rewards for the main native pollinator species and looked at their population response to alien invasion. In this final section, I will summarise my results in light of other studies that investigated impacts of alien plants on native pollination systems. I will consider the relevance of my research for invasion and pollination biology in a broader scheme and give suggestions for future research.

### 6.1. The direction and magnitude of alien impacts on native pollination

Irish invasive *Rhododendron ponticum* was found to be an alien that provided copious nectar rewards and was highly attractive to insect visitors. In comparison to most of the native flowering plant species it produced a higher nectar volume and more sugar per flower and time unit (Chapter 2). Up to now, resources of alien and native plants at a community level have not been investigated, so it is reassuring that the often hypothesised higher nectar productivity of alien invasive plants relative to their native counterparts proved valid in this case. Due to its plentiful rewards, I suspected *R. ponticum* to impact pollinator behaviour and therefore visitation to native plant species in its neighbourhood.

#### 6.1.1. Impacts on plants

For plants such impacts can be apparent at three different stages: 1. a flower visitation level, 2. a pollen deposition level and 3. a seed set level. Most pollination ecology studies investigate flower visitation (e.g. Lopezaraiza-Mikel et al., 2007, Bartomeus et al., 2008b, Chapter 2), because it is often easier to conduct on a community level. However, it might not appropriately mirror impacts on plant reproduction which is usually the ultimate factor of interest (see below).





Surprisingly, flower visitation to most of the plant species investigated here was not significantly affected by the presence of *R. ponticum*, with few exceptions (Chapters 2 and 3). Some native plants, and the co-flowering community as a whole, showed a positive response in floral visitation rate when *R. ponticum* was present at low densities (Chapter 2). In some cases, the impact of the alien could not be interpreted unequivocally, although a competitive effect was unlikely (e.g. in *D. purpurea*, Chapter 3; Chapter 2). Such lack of clear direction of impacts was mainly a result of high variability among locations and times (Chapter 2). Plant-pollinator interactions in general are highly variable due to alteration in community structure of plants and pollinators (Herrera, 1988, Strauss and Irwin, 2004, Petanidou et al., 2008). Some newly integrated alien plant species may be more attractive to native pollinators than residents and may impact native flower visitation patterns. However, we might not be able to detect an impact due to high variability among replicated sites (cf. also Grabas and Lavery, 1999, Bjerknes, 2008, Nienhuis et al., 2009). It can be assumed that plant species within communities are adapted to variability in pollinator services by various traits (e.g. a long flowering season, see also Chapter 1). These may also enhance resilience towards the invasion of alien species, i.e. an ultimate impact of the alien on native reproduction may not be detectable because it is not present. Even if an impact is present, it may not be detectable due to the general problem of lack of statistical power when using a limited number of replicates in a variable environment. Many of the visitation patterns of native species in invaded communities are characterised by such high variability between locations or time (e.g. Grabas and Lavery, 1999, Aigner, 2004, Moragues and Traveset, 2005). In this light, it is not surprising to also find contrasting results of different studies on the same invasive species. For example, the presence of *Impatiens glandulifera* influenced native flower visitation negatively (Chittka and Schürkens, 2001), positively (Lopezaraiza-Mikel et al., 2007), or had no effect (Nienhuis et al., 2009). Hence, generalisations about impacts of alien invasive species on flower visitation patterns should not be made on the basis of single studies.

Plant species, whose visitation was affected at some sites or some seasons, overlapped more with the alien in their pollinators than unaffected species did (Chapter 2). Although this is not surprising and has been proposed (Goodell, 2008) and indicated by meta-analysis (Morales and Traveset, 2008) before, this is the first study that showed such a relationship on an entire community level. Furthermore, affected native species also



resembled the alien in their characteristics. They were mass-flowering generalists and produced high nectar quantities, i.e. were members of the same functional group (Chapter 2). This supports the growing recognition of functional groups (guilds) as important subdivisions within communities (Williams et al., 2001, Fontaine et al., 2006, Rezende et al., 2007a, Hoehn et al., 2008).

Alien plant effects on flower visitation may change in relation to plant abundance. The impact of a plant species may be greater (and therefore better detectable) when its abundance within the community changes to extreme dominance (Parker et al., 1999). Density effects however are dependent on the plant species involved and present at different scales (Kunin and Shmida, 1997). In the case of *R. ponticum* higher alien abundance at a fine spatial scale (12 plant “individuals” in a patch) marginally increased native flower visitation (Chapter 3). However, a change in density at a larger community scale affected native flower visitation negatively (Chapter 4). Lopezaraiza-Mikel (2006) also found increased visitation rates at a small scale (96 *Impatiens glandulifera* flowers), while Muñoz and Cavieres (2008) revealed contrasting impacts at an even smaller scale. In their study, the presence of one *Taraxacum officinale* individual increased visit duration to one of their native focal species, but an addition of five alien individuals disrupted visitation rates of both investigated native plant species (Muñoz and Cavieres, 2008). The scale at which an impact occurs is hardly predictable, although a larger overlap in pollinator species may give an indication for a higher magnitude (Goodell, 2008). Direction and magnitude of impacts are certainly linked to the availability and limitation of pollinators. Flower visitors in Muñoz’ and Cavieres’ (2008) study were mainly solitary bees and flies whose increase in abundance in response to higher resource availability only takes effect in the next generation, i.e. in the next flowering season (Westrich, 1989, Potts et al., 2003). Pollinators in such a system are likely to be a limiting resource for plant pollination. In Ireland, social bees such as bumblebees conduct the majority of flower visits in native and invasive plant species (Chapters 2 and 4). Social species can adapt their behaviour and also their population size and abundance to changes in resource availability within the same season (Coffey and Breen, 1997, Westphal et al., 2003, Herrmann et al., 2007). Hence, alien luring of pollinators at moderate alien abundances may be counterbalanced by a higher overall abundance of pollinators.





Although impacts in Muñoz' and Cavieres' (2008), Lopezaraiza-Mikel's (2006) and my studies occurred at different alien abundances, there is a similarity in pattern that has been theoretically proposed by Rathcke (1983) and demonstrated in native systems (Steven et al., 2003). Rathcke (1983) predicted that co-occurring plant individuals can facilitate each others visitation at lower abundances, and may compete for pollination interactions at higher abundances. Her theory also points to a factor that is often neglected in density studies on plant pollination: the magnitude of intraspecific effects and interspecific effects (but see Brown et al., 2002). I have shown that higher conspecific abundance in the initial population facilitated native flower visitation and that the addition of further individuals of both alien and native species revealed marginal facilitation of native visitation (Chapter 3). If alien addition had caused less facilitation than native addition, I could have interpreted alien addition as an indirect competitive effect because alien presence could have prevented conspecific native facilitation by physically displacing the native. Such indirect effects may be modelled at a small experimental scale, but they are difficult to detect at a large scale since alien and native abundances at natural sites are often interlinked with each other. Nevertheless, ecological experiments on density/abundance should lay more emphasis on both inter- and intraspecific aspects of interactions than they have done so far (Underwood, 1997).

As mentioned earlier, pollinator visitation is only one aspect of the impact of aliens on native pollination. Pollen transfer and direct seed set measures might be in many cases a better method. Particularly heterospecific alien pollen transfer has been assumed to disrupt native reproduction (Traveset and Richardson, 2006). Alien pollen potentially interferes with native pollen performance (Brown and Mitchell, 2001, Jakobsson et al., 2008) or increases the likelihood of hybridisation (Ellstrand, 2003). Although an impact of alien pollen may also depend on the number of ovules per ovary, alien pollen deposition on native stigmata seems to be a minor pathway of impact on native pollination (e.g. Bartomeus et al., 2008a). In my study, alien pollen loads on stigmata of native *D. purpurea* in natural systems were negligible (Chapter 4). Likewise, in two thirds of the studies that investigated pollen transfer the proportion of alien pollen was less than 5% (and often less than 1% as here) of the overall pollen deposited on native stigmata (Table 1.1). In the remaining third of studies transferred alien pollen did not exceed 20% of the overall pollen (Table 1.1).



At the first glance, this seems surprising because studies on pollen transport webs strongly suggest that pollinators are swamped with alien pollen (95.5% and 73% of pollen carried by pollinators was alien; Lopezaraiza-Mikel et al., 2007, and Bartomeus et al., 2008a, respectively), yet they do not transfer alien pollen efficiently to native stigmata. This could be a result of a higher intraspecific pollen-stigma affinity where plants have stigmata with features that increase their affinity to conspecific pollen while decreasing their affinity to heterospecific pollen (Kunin and Gaston, 1993). A negligible transfer of alien pollen to native stigmata may also be due to morphological or behavioural constraints. Pollinators may be better adapted to native species in terms of morphological fit (Adler and Irwin, 2006), or alien pollen may be placed distinctively on certain body parts of the pollinator where native stigmata do not pick it up (Totland et al., 2006, cf. also native systems, Yang et al., 2007). The latter is related to differences in alien and native flower morphologies and is indication for a different phylogenetic history which decreases the likelihood of their interaction (Rezende et al., 2007b). Alternatively, pollinators may groom pollen to “safe sites” where it is unavailable for deposition (Harder and Wilson, 1998) or they may behave with high flower constancy, which minimises heterospecific pollen transfer in general (Grant, 1994). I could not observe any distinct placement of *R. ponticum* pollen on pollinator bodies. Pollinator behaviour and morphological modifications of native plants (e.g. of *D. purpurea*, Chapters 3 and 4) are a more plausible explication for low alien pollen transfer in the *R. ponticum* system.

If low alien pollen transfer is a general trait in invaded plant-pollinator networks, I infer that any impacts of alien species on native pollination success, i.e. native plant reproduction, is more likely a result of an alteration of conspecific pollen transfer among native plants. Indeed, (native and alien) co-flowering species in general have been shown to disproportionately impact conspecific rather than heterospecific pollen transfer in their neighbouring species (Table 1.1, see also review by Morales and Traveset, 2008). Pollen limitation can be best studied by investigating conspecific pollen transfer in combination with seed set.

Due to the shortage of studies that quantified both conspecific pollen transfer and seed set, I cannot extrapolate the magnitude of impact that alien invasive species may have on pollen limitation in native species. Ghazoul (2002, 2004) is the only researcher who





measured both variables in native *Dipterocarpus obtusifolius* at habitats of high alien density. He detected a decrease in conspecific pollen transfer, but assumed that resource limitation was the driver for low native reproduction because seed set was not affected by a lower native pollen transfer. I found similar patterns in *D. purpurea* where a (weak) decrease in conspecific pollen at high alien densities did not affect seed set (Chapter 4). Habitat characteristics such as soil mutualisms (Stinson et al., 2006, Cahill et al., 2008), nutrient limitation (Bos et al., 2007) or below-ground interactions between native species and invader (Orr et al., 2005, Kueffer et al., 2007) may affect reproduction more strongly than changes in pollen transfer do. This can obscure competitive or facilitative effects on pollination between species (Bos et al., 2007).

Such habitat effects have been also assumed for many of the studies that found effects of alien invaders on native flower visitation, but not a resulting change in seed set (Table 1.1). Impacts on seed set were also variable between communities. For example, Muñoz and Cavieres (2008) revealed facilitative and disruptive effects of invasive *T. officinale* at different abundances on native seed set, while Jones (2004) did not detect any impact of the alien on native reproduction. In my studies, native *D. purpurea* whose visitation was affected by conspecific individuals at a small scale (Chapter 3) and by alien *R. ponticum* at a large scale (Chapter 4), did not respond to treatment differences with an alteration in fruit or seed set. The native grew, at least in the small scale experiment, in a controlled soil environment (pots with equivalent compost mixtures). I therefore suspect *D. purpurea* to be primarily buffered against changeable pollination environments by means of its self-compatible breeding system. Other native plants that are self-incompatible might be prone to the negative effects of *R. ponticum* on flower visitation at high densities. This still has to be tested.

The relatively little evidence for *R. ponticum* impacts on native plant pollination and reproduction imply that *R. ponticum*'s disruptive properties on plant communities (Cross, 1982, Manchester and Bullock, 2000) are primarily realised by vegetative displacement of native species and allelopathic interactions (Mitchell et al., 1997, Sutton and Wilkinson, 2007). *R. ponticum* may potentially affect plant reproduction at high densities if plants are sensitive to pollen limitation (e.g. due to a self-incompatible breeding system).



In general, alien species may impact native plant reproduction via alteration in pollination far less often than proposed, which is indicated by non-significant results in seed set data found in the majority of studies on this topic (Table 1.1). Vegetative growth of invaders and their ability to change soil cycles may be far more important for the reproduction of native plant individuals in the community (Daehler, 2003, Reinhart et al., 2003, Reinhart et al., 2006, Vilà et al., 2006).

### **6.1.2. Impacts on pollinators**

So far impacts of alien invasive species on pollinators is a short story to tell. Besides models and general predictions made on this topic (Bjerknes et al., 2007, Goodell, 2008), I cannot extrapolate major patterns because very few studies have been published to date (Moroñ et al., 2009, Nienhuis et al., 2009; see also Goodell, 2003, Tepedino et al., 2008). This is surprising, since native pollinators are, by directly interacting with alien plant species (see Stout et al., 2002, for an exception), more likely to be affected by the presence of an invasive plant than co-occurring native plants which are only indirectly linked to aliens by pollination.

The few studies I know of that addressed alien impacts on pollinators, mostly found no (Nienhuis et al., 2009) or negative impacts (Goodell, 2003, Moroñ et al., 2009) on pollinator abundance or diversity. However, *R. ponticum*'s impact appeared to be positive, at least for the main Irish pollinator group (bumblebees, Chapter 5). This could be due to *R. ponticum*'s perennial characteristics. Plant abundance and flower density of an annual plant like *I. glandulifera* might be unpredictable and its benefits for pollinators may vary among years or sites (i.e. no alien impact is apparent, Nienhuis et al., 2009), whilst floral food resources produced by shrubs like *R. ponticum* are more reliable within a habitat over years. I showed that alien rewards had no harmful effect on bumblebees in the laboratory (Chapter 5). Since the alien produced more rewards than most of the native plant species (Chapter 2), I considered it a valuable resource in natural habitats. I could indirectly demonstrate this resource benefit for two of the main (bumblebee) pollinator species by observing higher colony numbers in invaded habitats (Chapter 5). Such a combination of reward productivity within the community (Chapter 2) and reward suitability for single pollinator species (Chapter 5) was here conducted for the first time but may be a suitable approach for various pollinator species in other studies.





While bumblebees increased their population size in the presence of the alien, I found fewer solitary bees in invaded habitats than in uninvaded habitats (Chapter 2). I did not measure population sizes of solitary bees, mainly because their abundance was generally very low in all investigated habitats (cf. Chapter 4). Solitary bees frequently forage on *R. ponticum* in its native habitat (Stout et al., 2006). Although bees of the same genera can be found in Ireland (Fitzpatrick et al., 2006), they seem to reject *R. ponticum* flowers here. The toxicity of *Rhododendron* nectars for honeybees (Carey et al., 1959) indicates that not all pollinating insects may benefit from *R. ponticum* rewards. Alternative native resources might be more important for these insect groups. A lack of locally available native sources, especially at high alien densities (cf. decreased native plant abundance, Chapter 4), may disrupt pollinators with short, less flexible foraging ranges (Moroń et al., 2009). Furthermore, some alien plants (e.g. *R. ponticum*) can disrupt soil biota due to allelopathic properties (Sutton and Wilkinson, 2007) or change shading (Nadezhdina et al., 2004) which may also influence nest site quality. This awaits further investigation.

Certainly, research on pollinator communities is in general not an easy task due to long foraging distances of many pollinators (e.g. bees, Gathmann and Tschardtke, 2002, Osborne et al., 2008) and the potential separation of foraging habitats and nesting sites (Westrich, 1996). In solitary organisms, low abundance and difficulties in identification under field conditions challenge an estimation of abundance and population size (but see Beil et al., 2008, Holzschuh et al., 2008, Larsson and Franzén, 2008, for suggestions). So far pollinator communities have been studied in agricultural systems (Westphal et al., 2003, Herrmann et al., 2007, Hoehn et al., 2008, Holzschuh et al., 2008) and fragmented semi-natural landscapes (Steffan-Dewenter and Tschardtke, 1999, Steffan-Dewenter, 2002). Their dynamics in invaded habitats is worth investigating.

To summarise, I consider *R. ponticum* beneficial at least for *Bombus* species in Irish habitats of low invasion levels. This direction of impact may reverse with increasing dominance of the alien (cf. Moroń et al., 2009) and may be in general disruptive for other pollinator groups. The impact of the alien on the main pollinators seems to be, expectedly, more unequivocal than on the investigated plant community.



### **6.1.3. Impacts on pollination networks**

Impacts on one or the other partner of a mutualistic relationship may not appropriately describe the relationship itself. The patterns just described can have contrasting effects on the network structure and functioning itself due to synergistic and antagonistic amplifications.

If functional groups that include the alien as a member experience higher impacts than the rest of the community, species within these groups may be more likely to be facilitated or disrupted (*sensu* Fontaine et al., 2006). In the long term, this could cause a shift in community assemblage and diversity depending on the function of these native species within the network. If they act as network hubs that maintain the structure of the web (Olesen et al., 2007), disruptive or facilitative effects may impact wide parts of the community. Network hubs are usually generalists which connect different modules with the rest of the network. A higher dominance or abundance of these native generalist species may facilitate more specialised or rarer species in the long term (Bascompte et al., 2003). If these generalist species decline/are removed from the web, the web disintegrates into isolated modules. This may not have major impacts on the internal structure of each individual module, however disturbances are expected to spread through and impact the resulting structures much faster than a modular network structure (Olesen et al., 2007). To maintain modularity, i.e. conserve generalist network hub species, may therefore decrease the risk of other species' extinctions and loss of biodiversity (Memmott et al., 2004).

If alien species are super-generalists themselves and usurp most of the interaction links within the network, connectivity among native species may decrease and natives may become dependent on the alien (Aizen et al., 2008). This may open different pathways for evolutionary and ecological processes. Individuals of some species may be disrupted in their reproduction which could cause adaptation to new interaction partners or traits, whilst others may be facilitated (Aizen et al., 2008). Alien super-generalisation can be observed in highly invaded networks where aliens occur in high abundance/density. This may explain the presence of an alien impact on pollination processes at higher invasion levels (Chapter 4), but a lack of impact at lower alien abundance (Chapter 3). A shift of interaction links and dependence of many native species on the alien invader within the network has implications for management too. The eradication or radical diminishing of an





alien super-generalist species in such webs could be detrimental for many of its native associates (Goodell, 2008), at least in the short term.

In the case of *R. ponticum*, pollination systems in its invasive range (primarily Britain and Ireland) are regarded highly generalised pollination systems in most cases (Lopezaraiza-Mikel, 2006, Stout et al., 2006; but see Proctor, 1978, for exceptions in the Burren). The level of generalisation refers to the number of species interactions (Olesen et al., 2002) and can be measured as “connectance” (the actually established interactions in proportion to all possible interactions, Jordano, 1987) at a network level. If a higher level of generalisation is already a trait of the network, super-generalisation may establish in this case even more quickly. A comparison between networks of different initial generalisation levels could clarify if generalisation level of a network is a good predictor for the level of its invasibility.

Although here I laid the main emphasis on alien invasive species in pollination networks, native species may be regarded as “invasive” in a comparable way. Species that share similar characteristics with the alien (Chapter 2) are often called “weeds” themselves because they can become locally super-abundant (e.g. *Rubus fruticosus*) often due to anthropogenic activities. Such native species fulfil the same function in native networks as a superabundant alien in invaded webs. This illustrates that alien invasive species are examples of successful competitors perhaps by means of absence of their herbivores (Ebeling et al., 2008) or other regulating interaction partners (Reinhart et al., 2003).

## 6.2. Critical considerations and beyond

When reviewing a study, it is appropriate to evaluate the suitability of the methodology employed and the relevance of results. In my case, this may help to channel future research into directions which aid a further understanding of pollination and invasion ecology in particular and of ecosystem functioning in general.

### 6.2.1. Methodology

In my studies, I used various methods to investigate the impacts of a specific alien model species on the native pollination network. This variety of methods to approach one



question was in my opinion very useful. Since impacts are likely to occur at different scales, it makes sense to use not only an individual species level (Chapters 3, 4 and 5), but also a population and community level (Chapters 2 and 5). In addition, variable factors such as density may modify an impact at different levels, and so investigations at a smaller (Chapter 3) and a larger (Chapter 4) scale were necessary. Moreover, the incorporation of manipulative (Chapter 3) and laboratory (Chapter 5) experiments, the use of molecular techniques (Chapter 5) and the consideration of both plants *and* pollinators are the strength of this thesis.

Without doubt, we need observational field data in natural communities to understand interactions between species and their embedding into the community *a priori* (Chapter 2). The information gained helps to choose subsequent study species or to focus on particular aspects of a complex process. In an ideal world, observed communities would contain the same species, be structured in the same way and be exposed to similar environmental conditions as each other. In reality, natural communities come in tandem with high variability of abiotic and biotic factors that are difficult if not impossible to control. The usual technique to filter this variability and to detect general patterns other than temporal and spatial variability is to increase the number of replicates (Underwood, 1997). This would have helped in my community study (Chapter 2) to draw a clearer picture and would have improved bumblebee abundance and population size estimates (Chapter 5). In many situations further replication is very difficult to achieve. Field sites that are comparable in their properties and suitable as replicates are difficult to find. In invasion studies uninvaded habitats are particularly a scarcity if they are to have the same characteristics as invaded locations because alien invasive species spread extensively. Furthermore, a single researcher cannot grow 16 hands and eyes to handle and observe an eightfold increase in the number of field sites. This dilemma is eased by using manipulative and laboratory experiments which allow control of a variety of factors such as distance between and properties of field sites, soil conditions, climatic regions, species identity and genotype, abundance and density of investigated species and screened maturation of progeny. The downside of such methods is their limited range and size. For example, population size experiments as in Chapter 3 will hardly mimic a comparable natural population size of the investigated species. Its applicability in interpreting natural processes is therefore limited to specific situations such as very early invasion. However,





the strength of my approach is a balanced combination of both field observations and manipulations/laboratory methods. With the help of these two elements I can draw conclusions about natural pollination dynamics in *R. ponticum* invaded communities.

In addition to the methodology, an evaluation of the parameters measured seems important too. The summary of my results as well as findings of other studies in the previous section point out that investigating both seed set and conspecific pollen transfer is preferable for estimating female fitness in plants. Flower visitation rates can indicate pollination success and are especially helpful in assessing resource usage of pollinators. However, pollinator efficiency in transferring pollen is highly variable within and between flower visitor species (Sahli and Conner, 2007, Stout, 2007a), and so visitation data are often unsuitable for extrapolating conspecific pollen deposition (Kandori, 2002). My community study (Chapter 2) lacks both pollen transfer and seed set data. Therefore it may not adequately reflect reproductive success, particularly since flower visitation, pollen transfer and seed set of *D. purpurea* was not linearly related (Chapters 3 and 4). Such a discrepancy between flower visitation data and reproductive success should be kept in mind when interpreting published studies that state facilitative or competitive effects between plant species in pollination networks (e.g. Lopezaraiza-Mikel et al., 2007, Bartomeus et al., 2008b). Nevertheless, my community study laid the foundations of the single-species studies (Chapters 3 and 4) and especially aided in choosing important pollinator species to investigate in detail (Chapter 5).

Was it appropriate to have only a single model species, in particular *R. ponticum*?

Clearly, the selection of a model species is critical for the relevance of results, but is also restricted by the number of available invasive study organisms in a region. In addition, data are only relevant for further application in management and conservation if the study species is of local or regional importance. *R. ponticum* as a model was therefore a good choice in an Irish context. From a more scientific point of view, the *R. ponticum* system had some advantages and some disadvantages. It is favourable to study communities invaded by a perennial plant species because an impact, if present, on native pollination dynamics is likely to be constant over years due to lower variability in alien population size in comparison to annual invaders. Furthermore, the generalist pollination system of *R. ponticum* may allow conclusions that are relevant for generalist invaders in other systems too. However, its manipulation is challenging, e.g. removal of a whole population or of the



entire floral display within an invaded community is nearly impossible and growing potted flowering individuals can be only achieved if one has 12 years patience to wait for the first flowering of the shrub. Furthermore, *R. ponticum*'s population dynamics as a shrub species are quite particular in that *R. ponticum* mostly occurs patchily scattered at low densities in contrast to more evenly distributed annual or perennial herbs (e.g. *Solanum elaeagnifolium* or *Lythrum salicaria*). This complicates comparisons of impacts between different study systems but also opens a new perspective on invasive plant species other than herbs. Parallel investigations on different invasive species in separated habitats can in theory help to generalise results enormously. Combining my results with findings of studies on *I. glandulifera* in Ireland by Caroline Nienhuis (Trinity College Dublin) may be a promising approach to face this lack of multiple study systems.

### **6.2.2. Relevance of results and future direction**

Evaluation of the relevance of my results depends on the angle of the reader: Are they relevant for a better understanding of invasion dynamics? Are they relevant for broadening the knowledge about pollination dynamics? Are they relevant for a better comprehension of ecosystem processes? I think I can affirm the latter two questions, but I have doubts about answering the first.

Invaded systems are useful examples of communities in which the relative abundance of species has shifted due to embedding of a new colonising (alien) species. They are therefore good models to address ecological and evolutionary questions in general (Sakai et al., 2001, Strauss et al., 2006) and interaction patterns between plants and pollinators in particular (Bjerknes et al., 2007, Sargent and Ackerly, 2008). However, pollination interactions can only partly explain overall invasion patterns of plant species and impacts on the co-occurring native communities (cf. Lodge, 1993, Richardson et al., 2000a, Levine et al., 2003, Strauss et al., 2006). Beyond doubt, pollination is important for the establishment and spread of many alien species (Richardson et al., 2000a). Still, other factors like vegetative and allelopathic properties of the alien or alien habitat modifications (e.g. fire regime or hydrology) may impact native communities even stronger than changes in pollination patterns (Levine et al., 2003). The latter is more subtle and might impact communities over a longer time period, e.g. by increasing the likelihood of inbreeding depression or hybridisation (Ellstrand, 2003).





In the case of *R. ponticum*, I could draw conclusions about the impact of the invader at different invasion levels (Chapters 3 and 4), but I cannot say anything about the overall dynamics of *R. ponticum* in the community. For long term management and predictions it is still useful to integrate pollination ecology into the equation of invasion (Traveset and Richardson, 2006).

It is preferable to perceive invasive plant species and invaded communities as a model for communities that are characterised by dynamic changes. A change in abundance or density of one member within the community may not only be a result of invasion of a species, but could be for example caused by habitat fragmentation (Saunders et al., 1991, Benitez-Malvido, 1998). Hence, studying systems with changing community structures has broader applications for various habitats. Results on intra- and interspecific pollination effects (Chapter 3), on density effects (Chapter 4) and on impacts of a species' attractiveness and dominance on pollinator populations (Chapter 5) are transferable. They have a relevance for pollination ecology in general. From this perspective, future research in invaded habitats may head in various directions. I will give two examples that, in my opinion, can further contribute to understand pollination interactions and ecosystem processes.

### **Future research 1: the narrower angle**

As illustrated in my review of the literature, much work has been carried out on plant pollination (population dynamics, density effects etc.) and pollinator foraging behaviour (flower constancy, preference and robbing, communication in social insects etc.). Yet, our knowledge about pollinator population dynamics and community responses to changes in plant community structure and relative abundance of single plant species is still relatively limited (Cane and Tepedino, 2001). Plant and pollinator species diversity seems to be strongly interlinked and can decline in relation to each other (Rathcke and Jules, 1993, Biesmeijer et al., 2006). Enlightening studies on pollinator abundance in relation to foraging plant abundance and diversity derive mainly from research on agricultural systems (but see Petanidou and Ellis, 1996, Steffan-Dewenter, 2002, Potts et al., 2003, Hines and Hendrix, 2005, for other examples). Here, the abundance of pollinators plays an important role for crop pollination (Klein et al., 2007). For example, increased density of mass-flowering crops at a landscape scale have been shown to correlate with an increased abundance of social bumblebee pollinators (Westphal et al., 2003, Herrmann et al., 2007;



but see Mänd et al., 2002), but can also increase their susceptibility for parasites (Carvell et al., 2008). Changes in management of field margins or in agricultural practices that increase the diversity and/or abundance of foraging plants in the landscape can also enhance bumblebee abundance (Carvell et al., 2004) or bee density and diversity in general (Holzschuh et al., 2008). Most of these studies focused on social bees (Hatfield and LeBuhn, 2007, Rundlöf et al., 2008b) and there are very few studies that included pollinators such as solitary bees, hoverflies or butterflies (but see Steffan-Dewenter and Tscharnkte, 1999, Brosi et al., 2008, Rundlöf et al., 2008a).

Pollinators are a key factor for plant reproduction and therefore not only worthy of conserving in their own right. Knowledge about their population and community structure as well as methods to enhance their abundance and population sizes can be crucial in securing future crop production and conserving plant biodiversity in natural habitats (Ricketts et al., 2008). However, it is not enough to solely study their foraging behaviour. Linking their needs for nesting sites and food resources is a more comprehensive approach. Studying pollinator communities in habitats invaded by alien plant species may help to understand how relative abundance of species within natural plant communities affects pollinator communities. Are generalist pollinator species more supported than specialists? Does increasing dominance of a plant species change nest site or larval host plant availability for pollinator species? Do floral resources of the dominant (alien) plant species have effects on larval growth of (solitary) bees? Answers to these scientific questions may also facilitate management decisions on the appropriate abundance level of (alien) dominant plant species in a habitat.

### **Future research 2: a broader outlook**

One of the major concerns among scientists and non-scientists alike are the ongoing anthropogenic induced changes in global climate that result in an increase in (global) temperatures and elevated CO<sub>2</sub> levels. Climate change already affects and will further affect ecosystems and ecosystem functions (Brooker, 2006). Biotic interactions are based on phenological overlap of interacting species. In plant-pollinator interactions the flowering season of plants in relation to the abundance of pollinating animals is important for plant reproduction (e.g. Totland, 1994, Kudo and Suzuki, 2002) but also for the maintenance of pollinator communities (cf. Tepedino et al., 2008). Changes in flower





phenology due to increased global temperatures may alter this overlap. Models on impacts of climate change on pollination networks predict a reduction in overlap between flowering time and pollinator activity and thereby a disruption of networks (Memmott et al., 2007). Pollination networks may change more drastically in response to a predicted alteration in the composition of plant and pollinator communities themselves. Changes in temperature may entail migration which selects highly mobile and opportunistic species with higher migrational capabilities (Malcolm et al., 2002). Even if former interaction partners still overlap in their phenology and habitat, the strength of interaction may vary with the changing characteristics of the interaction partners. For example, certain plant species may produce more nectar under elevated CO<sub>2</sub> concentrations (Erhardt et al., 2005) and could be more attractive to flower visitors or vice versa.

One of the predicted results of climate change is an increase in biological invasions and increased ranges of invasive species (Willis and Hulme, 2002, Kriticos et al., 2003, Weltzin et al., 2003; but see also Hulme, 2003). The outcome of such an increase in invasions combined with a mismatch in native interaction links is difficult to predict. On the one hand, pollinator species with narrow climatic niches may respond more sensitively than other species to a change in climate and could be disrupted (Williams et al., 2007). On the other hand, they might benefit from newly invading plant species that offer resources at times when native plants are not available (anymore) (Tepedino et al., 2008). A challenge for future research is to disentangle these components of an environment in change. Both prediction of plant-pollinator dynamics by modelling and experimental manipulations of single species or even community responses can add to our knowledge. Knowing what we might face in the near future gives us time for alternative actions.

### 6.3. Concluding remarks

The literature published on invasion biology in recent years reveals a slowly shift from an often biased judgement of “aliens as the bad guys *per se*” towards a more comprehensive view. Impacts of invasive (plant) species should not be generalised because they are linked to a specific scale. Whether an alien plant disrupts its community members or interaction partners depends on its own and the counterpart’s individual and population characteristics. Direct partners (some of the pollinators) in the mutualistic network were facilitated, while



indirect interaction partners (the plants) did not show such a clear response in pollination and reproduction. Their resilience towards variable pollination environments in a temperate climate like Ireland seems to be strong and may buffer individuals of single plant species and the entire community alike.

All efforts should be made to impede establishment of new alien organisms and to control population dynamics of alien invasive plant species in invaded habitats. At the same time, management should take recent scientific voices (Lopezaraiza-Mikel, 2006, Goodell, 2008, Tepedino et al., 2008) to heart which acknowledge the facilitative aspects alien plants can offer to members of the native plant and pollinator communities. Our knowledge about mutualistic interaction patterns in communities that are highly dynamic due to invasion is rapidly growing. We should use our chances and take advantage of these systems in addressing and investigating further ecological and evolutionary questions in a broader community context.





## **Chapter 7**



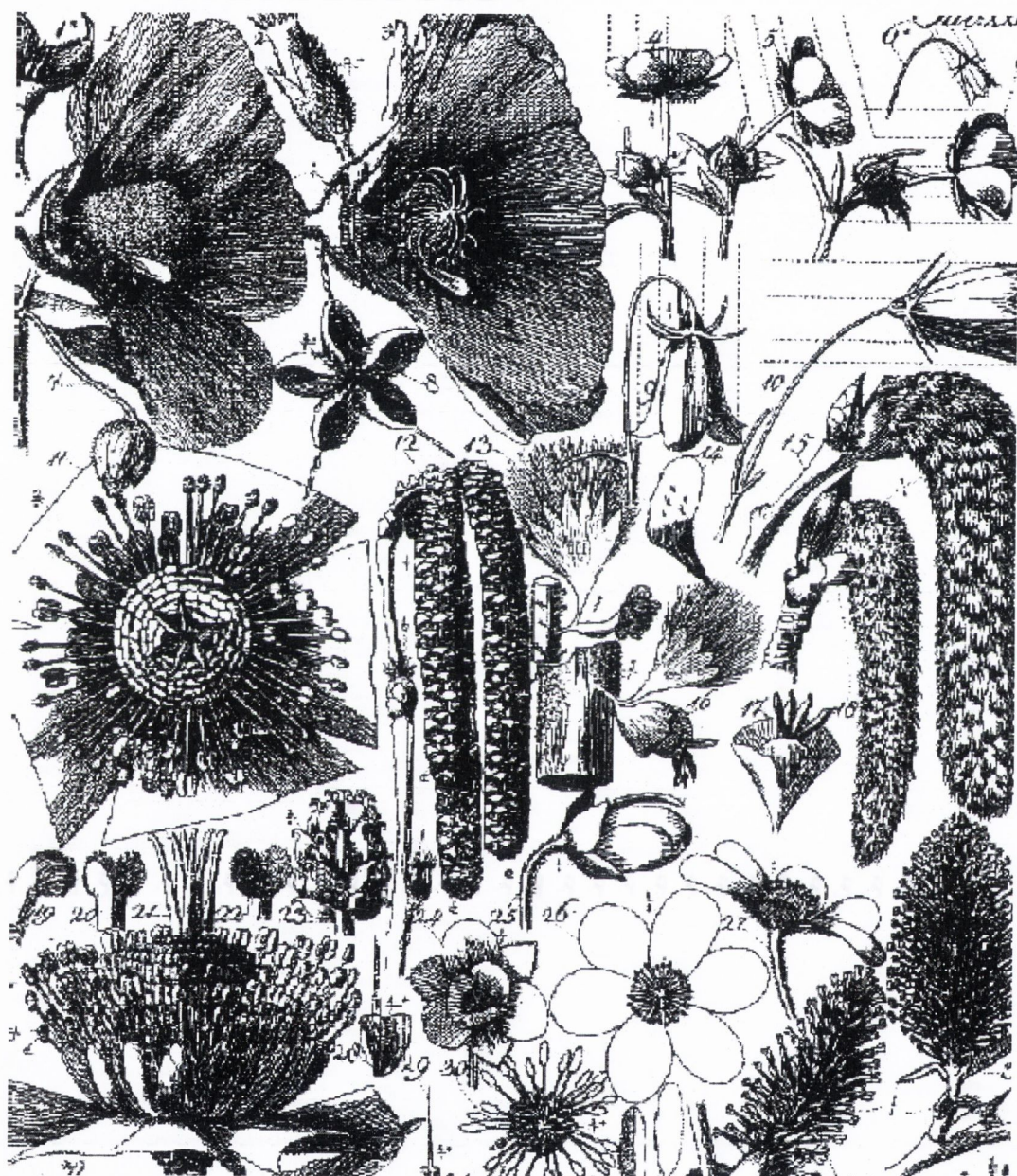


Plate 7





## 7. References

- Abe, T., Makino, S. i. & Okochi, I. (2008) Why have endemic pollinators declined on the Ogasawara Islands? *Biodiversity and Conservation*, **17**, 1465-1473.
- Abramson, J. H. & Gahlinger, P. M. (2001) Computer programs for epidemiologists: PEPI version 4.0. Sagebrush Press, Salt Lake City, Utah.
- Adler, L. S. (2000) The ecological significance of toxic nectar. *Oikos*, **91**, 409-420.
- Adler, L. S. & Irwin, R. E. (2005) Ecological costs and benefits of defenses in nectar. *Ecology*, **86**, 2968-2978.
- Adler, L. S. & Irwin, R. E. (2006) Comparison of pollen transfer dynamics by multiple floral visitors: experiments with pollen and fluorescent dye. *Annals of Botany*, **97**, 141-150.
- Ågren, J. (1996) Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. *Ecology*, **77**, 1779-1790.
- Ågren, J., Ehrlén, J. & Solbreck, C. (2008) Spatio-temporal variation in fruit production and seed predation in a perennial herb influenced by habitat quality and population size. *Journal of Ecology*, **96**, 334-345.
- Agresti, A. (1996) *An Introduction to Categorical Data Analysis*. John Wiley & Sons, New York.
- Aigner, P. A. (2004) Ecological and genetic effects on demographic processes: pollination, clonality and seed production in *Dithyrea maritima*. *Biological Conservation*, **116**, 27-34.
- Aizen, M. A. & Feinsinger, P. (1994a) Forest fragmentation, pollination, and plant reproduction in a Chaco Dry Forest, Argentina. *Ecology*, **75**, 330-351.
- Aizen, M. A. & Feinsinger, P. (1994b) Habitat fragmentation, native insect pollinators, and feral honey-bees in Argentine Chaco Serrano. *Ecological Applications*, **4**, 378-392.
- Aizen, M. A. & Harder, L. D. (2007) Expanding the limits of the pollen-limitation concept: effects of pollen quantity and quality. *Ecology*, **88**, 271-281.
- Aizen, M. A., Morales, C. L. & Morales, J. M. (2008) Invasive mutualists erode native pollination webs. *PLoS Biology*, **6**, e31.
- Alexandersson, R. & Ågren, J. (1996) Population size, pollinator visitation and fruit production in the deceptive orchid *Calypso bulbosa*. *Oecologia*, **107**, 533-540.
- Alford, D. V. (1975) *Bumblebees*. Davis-Poynter, London.
- Allee, W. C., Emerson, A. E., Park, O., Park, T. & Schmidt, K. P. (1949) *Principals of Animal Ecology*. Saunders, Philadelphia, Pennsylvania.
- Allen-Wardell, G., Bernhardt, P., Bitner, R., Burquez, A., Buchmann, S., Cane, J., Cox, P. A., Dalton, V., Feinsinger, P., Ingram, M., Inouye, D., Jones, C. E., Kennedy, K., Kevan, P., Koopowitz, H., Medellin, R., Medellin-Morales, S., Nabhan, G. P., Pavlik, B., Tepedino, V., Torchio, P. & Walker, S. (1998) The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conservation Biology*, **12**, 8-17.
- Alpha Innotech Co. (2002) AlphaEaseFC™ Software, Version 3.1.2. Alpha Innotech Co., San Leandro, California.
- Arnason, A. N., Schwarz, C. J. & Gerrard, J. M. (1991) Estimating closed population size and number of marked animals from sighting data. *Journal of Wildlife Management*, **55**, 716-730.
- Arnason, N., Kirby, C., Shar, L., Schwarz, C. J. & Irvine, J. R. (1996) Stratified Population Analysis System, Release 1.2. Winnipeg, Manitoba.
- Ashman, T.-L., Knight, T. M., Steets, J. A., Amarasekare, P., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mazer, S. J., Mitchell, R. J., Morgan, M. T. & Wilson, W. G. (2004) Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology*, **85**, 2408-2421.
- Baker, H. G. (1965) Characteristics and modes of origin of weeds. *The Genetics of Colonizing Species* (eds H. G. Baker & G. L. Stebbins), pp. 147-172. Academic Press, New York.





- Barker, R. J. (1978) Poisoning by plants. *Honey Bee Pests, Predators, and Diseases* (ed R. A. Morse), pp. 275-296. Cornell University Press, London.
- Barrett, S. C. H. (2002) The evolution of plant sexual diversity. *Nature Reviews Genetics*, **3**, 274-284.
- Barrett, S. C. H. (2003) Mating strategies in flowering plants: the outcrossing-selfing paradigm and beyond. *Philosophical Transactions: Biological Sciences*, **358**, 991-1004.
- Barrett, S. C. H. & Harder, L. D. (1996) Ecology and evolution of plant mating. *Trends in Ecology and Evolution*, **11**, A73-A79.
- Bartomeus, I., Bosch, J. & Vilà, M. (2008a) High invasive pollen transfer, yet low deposition on native stigmas in a *Carpobrotus*-invaded community. *Annals of Botany*, **102**, 417-424.
- Bartomeus, I., Vilà, M. & Santamaría, L. (2008b) Contrasting effects of invasive plants in plant-pollinator networks. *Oecologia*, **155**, 761-770.
- Bascompte, J., Jordano, P., Melián, C. J. & Olesen, J. M. (2003) The nested assembly of plant-animal mutualistic networks. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 9383-9387.
- Bateman, A. J. (1948) Intra-sexual selection in *Drosophila*. *Heredity*, **2**, 349-368.
- Beil, M., Horn, H. & Schwabe, A. (2008) Analysis of pollen loads in a wild bee community (Hymenoptera: Apidae) - a method for elucidating habitat use and foraging distances. *Apidologie*, **39**, 456-467.
- Bell, J. M., Karron, J. D. & Mitchell, R. J. (2005) Interspecific competition for pollination lowers seed production and outcrossing in *Mimulus ringens*. *Ecology*, **86**, 762-771.
- Bellingham, P. J., Tanner, E. V. J. & Healey, J. R. (2005) Hurricane disturbance accelerates invasion by the alien tree *Pittosporum undulatum* in Jamaican montane rain forests. *Journal of Vegetation Science*, **16**, 675-684.
- Benitez-Malvido, J. (1998) Impact of forest fragmentation on seedling abundance in a tropical rain forest. *Conservation Biology*, **12**, 380-389.
- Best, L. S. & Bierzychudek, P. (1982) Pollinator foraging on foxglove (*Digitalis purpurea*): a test of a new model. *Evolution*, **36**, 70-79.
- Biesmeijer, J. C. & Ermers, M. C. W. (1999) Social foraging in stingless bees: how colonies of *Melipona fasciata* choose among nectar sources. *Behavioral Ecology and Sociobiology*, **46**, 129-140.
- Biesmeijer, J. C., Roberts, S. P. M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers, A. P., Potts, S. G., Kleukers, R., Thomas, C. D., Settele, J. & Kunin, W. E. (2006) Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science*, **313**, 351-354.
- Bjerknes, A.-L. (2008) *Indirect and direct effects of invasive alien plant species on the pollination of co-occurring species: a literature review and experimental studies*. PhD thesis, Norwegian University of Life Sciences, Ås.
- Bjerknes, A.-L., Totland, Ø., Hegland, S. J. & Nielsen, A. (2007) Do alien plant invasions really affect pollination success in native plant species? *Biological Conservation*, **138**, 1-12.
- Boaventura, D., Cancela da Fonseca, L. & Hawkins, S. J. (2003) Size matters: competition within populations of the limpet *Patella depressa*. *Journal of Animal Ecology*, **72**, 435-446.
- Bond, W. J. (1994) Do mutualisms matter? Assessing the impact of pollinator and disperser disruption on plant extinction. *Philosophical Transactions: Biological Sciences*, **344**, 83-90.
- Born, W., Rauschmayer, F. & Brauer, I. (2005) Economic evaluation of biological invasions - a survey. *Ecological Economics*, **55**, 321-336.
- Bos, M. M., Veddeler, D., Bogdanski, A. K., Klein, A. M., Tschardtke, T., Steffan-Dewenter, I. & Tylianakis, J. M. (2007) Caveats to quantifying ecosystem services: fruit abortion blurs benefits from crop pollination. *Ecological Applications*, **17**, 1841-1849.
- Bosch, M. & Waser, N. M. (2001) Experimental manipulation of plant density and its effect on pollination and reproduction of two confamilial montane herbs. *Oecologia*, **126**, 76-83.
- Brantjes, N. B. M. (1978) Sensory responses to flowers in night-flying moths. *The Pollination of Flowers by Insects* (ed A. J. Richards), pp. 13-19. Academic Press, London.





- Bronstein, J. L., Alarcón, R. & Geber, M. (2006) The evolution of plant-insect mutualisms. *New Phytologist*, **172**, 412-428.
- Brooker, R. W. (2006) Plant-plant interactions and environmental change. *New Phytologist*, **171**, 271-284.
- Brooker, R. W., Maestre, F. T., Callaway, R. M., Lortie, C. L., Cavieres, L. A., Kunstler, G., Liancourt, P., Tielbörger, K., Travis, J. M. J., Anthelme, F., Armas, C., Coll, L., Corcket, E., Delzon, S., Forey, E., Kikvidze, Z., Olofsson, J., Pugnaire, F., Quiroz, C. L., Saccone, P., Schiffers, K., Seifan, M., Touzard, B. & Michalet, R. (2008) Facilitation in plant communities: the past, the present, and the future. *Journal of Ecology*, **96**, 18-34.
- Brosi, B. J., Daily, G. C., Shih, T. M., Oviedo, F. & Durán, G. (2008) The effects of forest fragmentation on bee communities in tropical countryside. *Journal of Applied Ecology*, **45**, 773-783.
- Brown, B. J. & Mitchell, R. J. (2001) Competition for pollination: effects of pollen of an invasive plant on seed set of a native congener. *Oecologia*, **129**, 43-49.
- Brown, B. J., Mitchell, R. J. & Graham, S. A. (2002) Competition for pollination between an invasive species (purple loosestrife) and a native congener. *Ecology*, **83**, 2328-2336.
- Buchmann, S. L. & Nabhan, G. P. (1996) *The Forgotten Pollinators*. Island Press, Washington, D.C.
- Bulleri, F., Bruno, J. F. & Benedetti-Cecchi, L. (2008) Beyond competition: incorporating positive interactions between species to predict ecosystem invasibility. *PLoS Biology*, **6**, 1136-1140.
- Burd, M. (1994) Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *The Botanical Review*, **60**, 83-139.
- Burkle, L. A., Irwin, R. E. & Newman, D. A. (2007) Predicting the effects of nectar robbing on plant reproduction: implications of pollen limitation and plant mating system. *American Journal of Botany*, **94**, 1935-1943.
- Búrquez, A. & Corbet, S. A. (1991) Do flowers reabsorb nectar? *Functional Ecology*, **5**, 369-379.
- Burton, P., Gurrin, L. & Sly, P. (1998) Extending the simple linear regression model to account for correlated responses: an introduction to generalized estimating equations and multi-level mixed modelling. *Statistics in Medicine*, **17**, 1261-1291.
- Buschmann, H. H., Noheda Marín, P., Vela Hernández, J. M. & Cándenas, L. (2007) Use of sodium channel blockers for the treatment of preterm labor. Wex Pharmaceuticals Inc., Vancouver, <http://www.freepatentsonline.com/EP1844781A1.html>.
- Buxton, B. H. & Darlington, C. D. (1932) Crosses between *Digitalis purpurea* and *Digitalis ambigua*. *New Phytologist*, **31**, 225-240.
- Cahill, J. F., Elle, E., Smith, G. R. & Shore, B. H. (2008) Disruption of a belowground mutualism alters interactions between plants and their floral visitors. *Ecology*, **89**, 1791-1801.
- Callaway, R. M., Thelen, G. C., Rodriguez, A. & Holben, W. E. (2004) Soil biota and exotic plant invasion. *Nature*, **427**, 731-733.
- Campbell, D. R. (1985) Pollinator sharing and seed set of *Stellaria pubera*: competition for pollination. *Ecology*, **66**, 544-553.
- Campbell, D. R. & Motten, A. F. (1985) The mechanism of competition for pollination between two forest herbs. *Ecology*, **66**, 554-563.
- Campbell, L. G. & Husband, B. C. (2007) Small populations are mate-poor but pollinator-rich in a rare, self-incompatible plant, *Hymenoxys herbacea* (Asteraceae). *New Phytologist*, **174**, 915-925.
- Cane, J. H. & Tepedino, V. J. (2001) Causes and extent of declines among native North American invertebrate pollinators: detection, evidence, and consequences. *Conservation Ecology*, **5**, 1. [online] URL: <http://www.consecol.org/vol5/iss1/art1>.
- Carey, F. M., Lewis, J. J., MacGregor, J. L. & Martin-Smith, M. (1959) Pharmacological and chemical observations on some toxic nectars. *Journal of Pharmacy and Pharmacology*, **11**, 269T-274T.
- Cartar, R. V. (2004) Resource tracking by bumble bees: responses to plant-level differences in quality. *Ecology*, **85**, 2764-2771.





- Caruso, C. M. (1999) Pollination of *Ipomopsis aggregata* (Polemoniaceae): effects of intra- vs. interspecific competition. *American Journal of Botany*, **86**, 663-668.
- Caruso, C. M. (2002) Influence of plant abundance on pollination and selection on floral traits of *Ipomopsis aggregata*. *Ecology*, **83**, 241-254.
- Caruso, C. M. & Alfaro, M. (2000) Interspecific pollen transfer as a mechanism of competition: effect of *Castilleja linariaefolia* pollen on seed set of *Ipomopsis aggregata*. *Canadian Journal of Botany*, **78**, 600-606.
- Carvell, C., Meek, W. R., Pywell, R. F. & Nowakowski, M. (2004) The response of foraging bumblebees to successional change in newly created arable field margins. *Biological Conservation*, **118**, 327-339.
- Carvell, C., Rothery, P., Pywell, R. F. & Heard, M. S. (2008) Effects of resource availability and social parasite invasion on field colonies of *Bombus terrestris*. *Ecological Entomology*, **33**, 321-327.
- Carvell, C., Roy, D. B., Smart, S. M., Pywell, R. F., Preston, C. D. & Goulson, D. (2006) Declines in forage availability for bumblebees at a national scale. *Biological Conservation*, **132**, 481-489.
- Chapman, R. E. & Bourke, A. F. G. (2001) The influence of sociality on the conservation biology of social insects. *Ecology Letters*, **4**, 650-662.
- Chapman, R. E., Wang, J. & Bourke, A. F. G. (2003) Genetic analysis of spatial foraging patterns and resource sharing in bumble bee pollinators. *Molecular Ecology*, **12**, 2801-2808.
- Chapuis-Lardy, L., Vanderhoeven, S., Dassonville, N., Koutika, L.-S. & Meerts, P. (2006) Effect of the exotic invasive plant *Solidago gigantea* on soil phosphorus status. *Biology and Fertility of Soils*, **42**, 481-489.
- Chittka, L., Gumbert, A. & Kunze, J. (1997) Foraging dynamics of bumble bees: correlates of movements within and between plant species. *Behavioral Ecology*, **8**, 239-249.
- Chittka, L. & Raine, N. E. (2006) Recognition of flowers by pollinators. *Current Opinion in Plant Biology*, **9**, 428-435.
- Chittka, L. & Schürkens, S. (2001) Successful invasion of a floral market - an exotic Asian plant has moved in on Europe's river-banks by bribing pollinators. *Nature*, **411**, 653-653.
- Chittka, L., Thomson, J. D. & Waser, N. M. (1999) Flower constancy, insect psychology, and plant evolution. *Naturwissenschaften*, **86**, 361-377.
- Chornesky, E. A. & Randall, J. M. (2003) The threat of invasive alien species to biological diversity: setting a future course. *Annals of the Missouri Botanical Garden*, **90**, 67-76.
- Christian, C. E. (2001) Consequences of a biological invasion reveal the importance of mutualism for plant communities. *Nature*, **413**, 635-639.
- Clarke, K. R. & Green, R. H. (1988) Statistical design and analysis for a 'biological effects' study. *Marine Ecology-Progress Series*, **46**, 213-226.
- Coffey, M. F. & Breen, J. (1997) Seasonal variation in pollen and nectar sources of honey bees in Ireland. *Journal of Apicultural Research*, **36**, 63-76.
- Colautti, R. I. & MacIsaac, H. J. (2004) A neutral terminology to define 'invasive' species. *Diversity and Distributions*, **10**, 135-141.
- Colautti, R. I., Ricciardi, A., Grigorovich, I. A. & MacIsaac, H. J. (2004) Is invasion success explained by the enemy release hypothesis? *Ecology Letters*, **7**, 721-733.
- Collier, N., Mackay, D. A. & Benkendorff, K. (2008) Is relative abundance a good indicator of population size? Evidence from fragmented populations of a specialist butterfly (Lepidoptera: Lycaenidae). *Population Ecology*, **50**, 17-23.
- Corbet, S. A. (2003) Nectar sugar content: estimating standing crop and secretion rate in the field. *Apidologie*, **34**, 1-10.
- Corbet, S. A., Bee, J., Dasmahapatra, K., Gale, S., Gorringer, E., La Ferla, B., Moorhouse, T., Trevail, A., van Bergen, Y. & Vorontsova, M. (2001) Native or exotic? Double or single? Evaluating plants for pollinator-friendly gardens. *Annals of Botany*, **87**, 219-232.
- Corbet, S. A., Cuthill, I., Fallows, M., Harrison, T. & Hartley, G. (1981) Why do nectar-foraging bees and wasps work upwards on inflorescences? *Oecologia*, **51**, 79-83.





- Corbet, S. A., Fussell, M., Ake, R., Fraser, A., Gunson, C., Savage, A. & Smith, K. (1993) Temperature and the pollinating activity of social bees. *Ecological Entomology*, **18**, 17-30.
- Corbet, S. A., Kerslake, C. J. C., Brown, D. & Morland, N. E. (1984) Can bees select nectar-rich flowers in a patch? *Journal of Apicultural Research*, **23**, 234-242.
- Corbet, S. A., Unwin, D. M. & Prŷs-Jones, O. E. (1979) Humidity, nectar and insect visits to flowers, with special reference to *Crataegus*, *Tilia* and *Echium*. *Ecological Entomology*, **4**, 9-22.
- Cornuet, J.-M. & Aries, F. (1980) Number of sex alleles in a sample of honeybee colonies. *Apidologie*, **11**, 87-93.
- Coxon, P. & Waldren, S. (1995) The floristic record of Ireland's Pleistocene temperate stage. *Island Britain: A Quaternary Perspective* (ed R. C. Preece), pp. 243-267. Geological Society Special Publication No. 96, London.
- Crawley, M. J. (1993) *GLIM for Ecologists*. Blackwell, Oxford.
- Cresswell, J. E. & Osborne, J. L. (2004) The effect of patch size and separation on bumblebee foraging in oilseed rape: implications for gene flow. *Journal of Applied Ecology*, **41**, 539-546.
- Cresswell, J. E., Osborne, J. L. & Goulson, D. (2000) An economic model of the limits to foraging range in central place foragers with numerical solutions for bumblebees. *Ecological Entomology*, **25**, 249-255.
- Cross, J. R. (1975) *Rhododendron ponticum* L. *Journal of Ecology*, **63**, 345-364.
- Cross, J. R. (1981) The establishment of *Rhododendron ponticum* in the Killarney oakwoods, S.W. Ireland. *Journal of Ecology*, **69**, 807-824.
- Cross, J. R. (1982) The invasion and impact of *Rhododendron ponticum* in native Irish vegetation. *Journal of Life Sciences of the Royal Dublin Society*, **3**, 209-220.
- Cross, J. R. (2002) The invasion and control of *Rhododendron ponticum* L. in native Irish vegetation. *Biologische Invasionen: Herausforderung zum Handeln?* (eds I. Kowarik & U. Starfinger), pp. 329-338. Berlin.
- Crowley, P. H. (1992) Resampling methods for computation-intensive data analysis in ecology and evolution. *Annual Review of Ecology and Systematics*, **23**, 405-447.
- Cunningham, S. A. & Arthur, A. D. (2008) Effect of landscape context on introduced and native bee visitors to canola in southeast Australia (abstract). *XXIII International Congress of Entomology*. Durban.
- da Silva-Matos, E. V. & Garófalo, C. A. (2000) Worker life tables, survivorship, and longevity in colonies of *Bombus (Fervidobombus) atratus* (Hymenoptera: Apidae). *Revista De Biologia Tropical*, **48**, 657-663.
- Daehler, C. C. (2003) Performance comparisons of co-occurring native and alien invasive plants: implications for conservation and restoration. *Annual Review of Ecology, Evolution, and Systematics*, **34**, 183-211.
- Dafni, A., Lehrer, M. & Kevan, P. G. (1997) Spatial flower parameters and insect spatial vision. *Biological Reviews*, **72**, 239-282.
- Daily, G. C., Alexander, S., Ehrlich, P. R., Goulder, L., Lubchenco, J., Matson, P. A., Mooney, H. A., Postel, S., Schneider, S. H., Tilman, D. & Woodwell, G. M. (1997) Ecosystem services: benefits supplied to human societies by natural ecosystems. *Issues in Ecology*, **2**, 1-16.
- Darvill, B., Knight, M. E. & Goulson, D. (2004) Use of genetic markers to quantify bumblebee foraging range and nest density. *Oikos*, **107**, 471-478(8).
- Darwin, C. D. (1859) *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*. John Murray, London.
- Darwin, C. D. (1876) *On the Effects of Cross- and Self-fertilization in the Animal Kingdom*. John Murray, London.
- Davis, M. A. (2003) Biotic globalization: Does competition from introduced species threaten biodiversity? *Bioscience*, **53**, 481-489.
- Dawkins, R. (2004) *The Ancestor's Tale: A Pilgrimage to the Dawn of Life*. Weidenfeld & Nicolson, London.





- Day, R. W. & Quinn, G. P. (1989) Comparisons of treatments after an analysis of variance in ecology. *Ecological Monographs*, **59**, 433-463.
- de Jong, T. J., Waser, N. M. & Klinkhamer, P. G. L. (1993) Geitonogamy: the neglected side of selfing. *Trends in Ecology and Evolution*, **8**, 321-325.
- Dehnen-Schmutz, K., Perrings, C. & Williamson, M. (2004) Controlling *Rhododendron ponticum* in the British Isles: an economic analysis. *Journal of Environmental Management*, **70**, 323-332.
- Dehnen-Schmutz, K. & Williamson, M. (2006) *Rhododendron ponticum* in Britain and Ireland: social, economic and ecological factors in its successful invasion. *Environment and History*, **12**, 325-350.
- Del Lama, M. A. & Peruquetti, R. C. (2006) Mortalidade de abelhas visitantes de flores de *Caesalpinia peltophoroides* Benth (Leguminosae) no estado de São Paulo, Brasil. *Revista Brasileira De Entomologia*, **50**, 547-549.
- Denac, H., Mevissen, M. & Scholtysik, G. (2000) Structure, function and pharmacology of voltage-gated sodium channels. *Naunyn-Schmiedeberg's Archives of Pharmacology*, **362**, 453-479.
- Detzel, A. & Wink, M. (1993) Attraction, deterrence or intoxication of bees (*Apis mellifera*) by plant allelochemicals. *Chemoecology*, **4**, 8-18.
- Diamond, J. (2005) *Collapse: How Societies Choose to Fail or Succeed*. Viking Penguin, New York, New York.
- Dlusski, G. M., Glazunova, K. P. & Perfilieva, K. S. (2005) Mechanisms that limit pollinator range in Ericaceae. *Žurnal obščej biologii*, **66**, 224-238.
- Duffy, K. J. & Stout, J. C. (2008) The effects of plant density and nectar reward on bee visitation to the endangered orchid *Spiranthes romanzoffiana*. *Acta Oecologica*, **34**, 131-138.
- Dufrêne, M. & Legendre, P. (1997) Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs*, **67**, 345-366.
- Dukas, R. & Real, L. A. (1991) Learning foraging tasks by bees: a comparison between social and solitary species. *Animal Behaviour*, **42**, 269-276.
- Dukas, R. & Real, L. A. (1993) Effects of recent experience on foraging decisions by bumble bees. *Oecologia*, **94**, 244-246.
- Ebeling, S. K., Hensen, I. & Auge, H. (2008) The invasive shrub *Buddleja davidii* performs better in its introduced range. *Diversity and Distributions*, **14**, 225-233.
- Edwards, M. & Jenner, M. (2004) *Field Guide to the Bumblebees of Great Britain and Ireland*. Countryside and Garden Conservation Series. Ocelli, UK.
- Ehrenfeld, J. G. (2003) Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems*, **6**, 503-523.
- Eickwort, G. C. & Ginsberg, H. S. (1980) Foraging and mating in Apoidea. *Annual Review of Entomology*, **25**, 421-446.
- Eisikowitch, D. (1978) Insect visiting of two subspecies of *Nigella arvensis* under adverse seaside conditions. *The Pollination of Flowers by Insects* (ed A. J. Richards), pp. 125-132. Academic Press, London.
- Elliott, S. E., Irwin, R. E., Adler, L. S. & Williams, N. M. (2008) The nectar alkaloid, gelsemine, does not affect offspring performance of a native solitary bee, *Osmia lignaria* (Megachilidae). *Ecological Entomology*, **33**, 298-304.
- Ellis, J. S., Knight, M. E., Darvill, B. & Goulson, D. (2006) Extremely low effective population sizes, genetic structuring and reduced genetic diversity in a threatened bumblebee species, *Bombus sylvarum* (Hymenoptera: Apidae). *Molecular Ecology*, **15**, 4375-4386.
- Ellstrand, N. C. (2003) Current knowledge of gene flow in plants: implications for transgene flow. *Philosophical Transactions: Biological Sciences*, **358**, 1163-1170.
- Erfmeier, A. & Bruelheide, B. (2004) Comparison of native and invasive *Rhododendron ponticum* populations: growth, reproduction and morphology under field conditions. *Flora*, **199**, 120-133.
- Erhardt, A., Rusterholz, H. P. & Stocklin, J. (2005) Elevated carbon dioxide increases nectar production in *Epilobium angustifolium* L. *Oecologia*, **146**, 311-317.





- Estoup, A., Scholl, A., Pouvreau, A. & Solignac, M. (1995) Monoandry and polyandry in bumble bees (Hymenoptera: Bombinae) as evidenced by highly variable microsatellites. *Molecular Ecology*, **4**, 89-93.
- Estoup, A., Solignac, M., Cornuet, J.-M., Goudet, J. & Scholl, A. (1996) Genetic differentiation of continental and island populations of *Bombus terrestris* (Hymenoptera: Apidae) in Europe. *Molecular Ecology*, **5**, 19-31.
- Faegri, K. & van der Pijl, L. (1971) *The Principles of Pollination Ecology*, 2<sup>nd</sup> edn. Pergamon Press, Oxford.
- Fausto, J. A., Eckhart, V. M. & Geber, M. A. (2001) Reproductive assurance and the evolutionary ecology of self-pollination in *Clarkia xantiana* (Onagraceae). *American Journal of Botany*, **88**, 1794-1800.
- Feinsinger, P. (1987) Effects of plant species on each other's pollination: Is community structure influenced? *Trends in Ecology and Evolution*, **2**, 123-126.
- Feinsinger, P., Murray, K. G., Kinsman, S. & Busby, W. H. (1986) Floral neighborhood and pollination success in four hummingbird-pollinated cloud forest plant species. *Ecology*, **67**, 449-464.
- Feinsinger, P. & Tiebout, H. M., III (1991) Competition among plants sharing hummingbird pollinators: laboratory experiments on a mechanism. *Ecology*, **72**, 1946-1952.
- Feinsinger, P., Tiebout, H. M., III & Young, B. E. (1991) Do tropical bird-pollinated plants exhibit density-dependent interactions? Field experiments. *Ecology*, **72**, 1953-1963.
- Feldman, T. S. (2006) Pollinator aggregative and functional responses to flower density: does pollinator response to patches of plants accelerate at low-densities? *Oikos*, **115**, 128-140.
- Feldman, T. S. (2008) The plot thickens: does low density affect visitation and reproductive success in a perennial herb, and are these effects altered in the presence of a co-flowering species? *Oecologia*, **156**, 807-817.
- Fenster, C. B., Armbruster, W. S., Wilson, P., Dudash, M. R. & Thomson, J. D. (2004) Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics*, **35**, 375-403.
- Field, D. L., Ayre, D. J. & Whelan, R. J. (2005) The effect of local plant density on pollinator behavior and the breeding system of *Persoonia bargoensis* (Proteaceae). *International Journal of Plant Sciences*, **166**, 969-977.
- Field, J. G., Clarke, K. R. & Warwick, R. M. (1982) A practical strategy for analysing multispecies distribution patterns. *Marine Ecology-Progress Series*, **8**, 37-52.
- Fitzpatrick, Ú., Murray, T. E., Byrne, A., Paxton, R. J. & Brown, M. J. F. (2006) Regional red list of Irish bees. pp. 1-38. National Parks & Wildlife Service; Environment & Heritage Service, Dublin, Belfast.
- Fitzpatrick, Ú., Murray, T. E., Paxton, R. J., Breen, J., Cotton, D., Santorum, V. & Brown, M. J. F. (2007) Rarity and decline in bumblebees - a test of causes and correlates in the Irish fauna. *Biological Conservation*, **136**, 185-194.
- Fleishman, E., Mac Nally, R. & Murphy, D. D. (2005) Relationship among non-native plants, diversity of plants and butterflies, and adequacy of spatial sampling. *Biological Journal of the Linnean Society*, **85**, 157-166.
- Fontaine, C., Collin, C. L. & Dajoz, I. (2008) Generalist foraging of pollinators: diet expansion at high density. *Journal of Ecology*, **96**, 1002-1010.
- Fontaine, C., Dajoz, I., Meriguet, J. & Loreau, M. (2006) Functional diversity of plant-pollinator interaction webs enhances the persistence of plant communities. *PLoS Biology*, **4**, 129-135.
- Fowler, J., Cohen, L. & Jarvis, P. (1998) *Practical Statistics for Field Biology*, 2<sup>nd</sup> edn. John Wiley & Sons, Chichester.
- Free, J. B. (1970) The flower constancy of bumblebees. *Journal of Animal Ecology*, **39**, 395-402.
- Frenot, Y., Chown, S. L., Whinam, J., Selkirk, P. M., Convey, P., Skotnicki, M. & Bergstrom, D. M. (2005) Biological invasions in the Antarctic: extent, impacts and implications. *Biological Reviews*, **80**, 45-72.
- Fritz, A.-L. & Nilsson, L. A. (1994) How pollinator-mediated mating varies with population size in plants. *Oecologia*, **100**, 451-462.





- Fussell, M. & Corbet, S. A. (1992) The nesting places of some British bumble bees. *Journal of Apicultural Research*, **31**, 32-41.
- Galen, C. & Gregory, T. (1989) Interspecific pollen transfer as a mechanism of competition: consequences of foreign pollen contamination for seed set in the alpine wildflower, *Polemonium viscosum*. *Oecologia*, **81**, 120-123.
- Gardener, M. C. & Gillman, M. P. (2002) The taste of nectar - a neglected area of pollination ecology. *Oikos*, **98**, 552-557.
- Gathmann, A. & Tschardt, T. (2002) Foraging ranges of solitary bees. *Journal of Animal Ecology*, **71**, 757-764.
- Gegeer, R. J. & Laverty, T. M. (1995) Effect of flower complexity on relearning flower-handling skills in bumble bees. *Canadian Journal of Zoology*, **73**, 2052-2058.
- Gegeer, R. J. & Laverty, T. M. (2005) Flower constancy in bumblebees: a test of the trait variability hypothesis. *Animal Behaviour*, **69**, 939-949.
- Gegeer, R. J., Manson, J. S. & Thomson, J. D. (2007) Ecological context influences pollinator deterrence by alkaloids in floral nectar. *Ecology Letters*, **10**, 375-382.
- Gegeer, R. J. & Thomson, J. D. (2004) Does the flower constancy of bumble bees reflect foraging economics? *Ethology*, **110**, 793-805.
- Génissel, A., Aupinel, P., Bressac, C., Tasei, J.-N. & Chevrier, C. (2002) Influence of pollen origin on performance of *Bombus terrestris* micro-colonies. *Entomologia Experimentalis Et Applicata*, **104**, 329-336.
- Ghazoul, J. (2002) Flowers at the front line of invasion? *Ecological Entomology*, **27**, 638-640.
- Ghazoul, J. (2004) Alien abduction: disruption of native plant-pollinator interactions by invasive species. *Biotropica*, **36**, 156-164.
- Ghazoul, J. (2005a) Buzziness as usual? Questioning the global pollination crisis. *Trends in Ecology and Evolution*, **20**, 367-373.
- Ghazoul, J. (2005b) Pollen and seed dispersal among dispersed plants. *Biological Reviews*, **80**, 413-443.
- Ghazoul, J. (2006) Floral diversity and the facilitation of pollination. *Journal of Ecology*, **94**, 295-304.
- Ghazoul, J. & McLeish, M. (2001) Reproductive ecology of tropical forest trees in logged and fragmented habitats in Thailand and Costa Rica. *Plant Ecology*, **153**, 335-345.
- Gilbert, B. & Lechowicz, M. J. (2005) Invasibility and abiotic gradients: the positive correlation between native and exotic plant diversity. *Ecology*, **86**, 1848-1855.
- Glasby, T. M. (1997) Analysing data from post-impact studies using asymmetrical analyses of variance: a case study of epibiotas on marinas. *Australian Journal of Ecology*, **22**, 448-459.
- González-Coloma, A., Reina, M., Medinaveitia, A., Guadaño, A., Santana, O., Martínez-Díaz, R., Ruiz-Mesía, L., Alva, A., Grandez, M., Díaz, R., Gavín, J. A. & De La Fuente, G. (2004) Structural diversity and defensive properties of norditerpenoid alkaloids. *Journal of Chemical Ecology*, **30**, 1393-1408.
- Goodell, K. (2003) Structure of bee communities in calcareous fens invaded by purple loosestrife compared to uninvaded fens (abstract). *Ecological Society of America Annual Meeting*. Allen Press, Savannah, Georgia.
- Goodell, K. (2008) Invasive exotic plant-bee interactions. *Bee pollination in agroecosystems* (eds R. R. James & T. L. Pitts-Singer), pp. 166-183. Oxford University Press, Oxford.
- Goulson, D. (1994) A model to predict the influence of insect flower constancy on interspecific competition between insect pollinated plants. *Journal of Theoretical Biology*, **168**, 309-314.
- Goulson, D., Hanley, M. E., Darvill, B., Ellis, J. S. & Knight, M. E. (2005) Causes of rarity in bumblebees. *Biological Conservation*, **122**, 1-8.
- Goulson, D., Lye, G. C. & Darvill, B. (2008) Decline and conservation of bumble bees. *Annual Review of Entomology*, **53**, 191-208.
- Goulson, D., Stout, J. C. & Hawson, S. A. (1997) Can flower constancy in nectaring butterflies be explained by Darwin's interference hypothesis? *Oecologia*, **112**, 225-231.





- Goulson, D., Stout, J. C., Hawson, S. A. & Allen, J. A. (1998) Floral display size in comfrey, *Symphytum officinale* L. (Boraginaceae): relationships with visitation by three bumblebee species and subsequent seed set. *Oecologia*, **113**, 502-508.
- Goulson, D. & Wright, N. P. (1998) Flower constancy in the hoverflies *Episyrphus balteatus* (Degeer) and *Syrphus ribesii* (L.) (Syrphidae). *Behavioral Ecology*, **9**, 213-219.
- Grabas, G. P. & Lavery, T. M. (1999) The effect of purple loosestrife (*Lythrum salicaria* L.; Lythraceae) on the pollination and reproductive success of sympatric co-flowering wetland plants. *Ecoscience*, **6**, 230-242.
- Grant, V. (1950) The flower constancy of bees. *Botanical Review*, **16**, 379-398.
- Grant, V. (1994) Modes and origins of mechanical and ethological isolation in angiosperms. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 3-10.
- Graves, S. D. & Shapiro, A. M. (2003) Exotics as host plants of the California butterfly fauna. *Biological Conservation*, **110**, 413-433.
- Greenleaf, S. S., Williams, N. M., Winfree, R. & Kremen, C. (2007) Bee foraging ranges and their relationship to body size. *Oecologia*, **153**, 589-596.
- Grindeland, J. M., Sletvold, N. & Ims, R. A. (2005) Effects of floral display size and plant density on pollinator visitation rate in a natural population of *Digitalis purpurea*. *Functional Ecology*, **19**, 383-390.
- Groddeck, J., Mauss, V. & Reinhold, K. (2004) The resource-based mating system of the Mediterranean pollen wasp *Ceramius fonscolombei* Latreille 1810 (Hymenoptera, Vespidae, Masarinae). *Journal of Insect Behavior*, **17**, 397-418.
- Groom, M. J. (1998) Allee effects limit population viability of an annual plant. *The American Naturalist*, **151**, 487-496.
- Gross, C. L. (1992) Floral traits and pollinator constancy: foraging by native bees among three sympatric legumes. *Australian Journal of Ecology*, **17**, 67-74.
- Gurevitch, J. & Padilla, D. K. (2004) Are invasive species a major cause of extinctions? *Trends in Ecology and Evolution*, **19**, 470-474.
- Hackney, E. E. & McGraw, J. B. (2001) Experimental demonstration of an Allee effect in American Ginseng. *Conservation Biology*, **15**, 129-136.
- Haig, D. & Westoby, M. (1988) On limits to seed production. *The American Naturalist* **131**, 757-759.
- Harder, L. D. (1985) Morphology as a predictor of flower choice by bumble bees. *Ecology*, **66**, 198-210.
- Harder, L. D. (1986) Influences on the density and dispersion of bumble bee nests (Hymenoptera: Apidae). *Holarctic Ecology*, **9**, 99-103.
- Harder, L. D. (1988) Choice of individual flowers by bumble bees: interaction of morphology, time and energy. *Behaviour*, **104**, 60-77.
- Harder, L. D. (1990) Pollen removal by bumble bees and its implications for pollen dispersal. *Ecology*, **71**, 1110-1125.
- Harder, L. D. & Barrett, S. C. H. (1995) Mating cost of large floral displays in hermaphrodite plants. *Nature*, **373**, 512-515.
- Harder, L. D. & Wilson, W. G. (1998) Theoretical consequences of heterogeneous transport conditions for pollen dispersal by animals. *Ecology*, **79**, 2789-2807.
- Hatfield, R. G. & LeBuhn, G. (2007) Patch and landscape factors shape community assemblage of bumble bees, *Bombus* spp. (Hymenoptera: Apidae), in montane meadows. *Biological Conservation*, **139**, 150-158.
- Heard, M. S., Carvell, C., Carreck, N. L., Rothery, P., Osborne, J. L. & Bourke, A. F. G. (2007) Landscape context not patch size determines bumble-bee density on flower mixtures sown for agri-environment schemes. *Biology Letters*, **3**, 638-641.
- Hegland, S. J. & Boeke, L. (2006) Relationships between the density and diversity of floral resources and flower visitor activity in a temperate grassland community. *Ecological Entomology*, **31**, 532-538.





- Heinrich, B. (1975) Energetics of pollination. *Annual Review of Ecology and Systematics*, **6**, 139-170.
- Heinrich, B. & Raven, P. H. (1972) Energetics and pollination ecology. *Science*, **176**, 597-602.
- Herrera, C. M. (1988) Variation in mutualisms: the spatio-temporal mosaic of a pollinator assemblage. *Biological Journal of the Linnean Society*, **35**, 95-125.
- Herrera, C. M. (1996) Floral traits and plant adaptation to insect pollinators: a devil's advocate approach. *Floral Biology. Studies on Floral Evolution in Animal-pollinated Plants* (eds D. G. Lloyd & S. C. H. Barrett), pp. 65-87. Chapman and Hall, New York, New York.
- Herrmann, F., Westphal, C., Moritz, R. F. A. & Steffan-Dewenter, I. (2007) Genetic diversity and mass resources promote colony size and forager densities of a social bee (*Bombus pascuorum*) in agricultural landscapes. *Molecular Ecology*, **16**, 1167-1178.
- Hickman, J. M., Lovei, G. L. & Wratten, S. D. (1995) Pollen feeding by adults of the hoverfly *Melanostoma fasciatum* (Diptera: Syrphidae). *New Zealand Journal of Zoology*, **22**, 387-392.
- Hill, P. S. M., Hollis, J. & Wells, H. (2001) Foraging decisions in nectarivores: unexpected interactions between flower constancy and energetic rewards. *Animal Behaviour*, **62**, 729-737.
- Hines, H. M. & Hendrix, S. D. (2005) Bumble bee (Hymenoptera: Apidae) diversity and abundance in tallgrass prairie patches: effects of local and landscape floral resources. *Environmental Entomology*, **34**, 1477-1484.
- Hodges, C. M. (1985) Bumble bee foraging: energetic consequences of using a threshold departure rule. *Ecology*, **66**, 188-197.
- Hoehn, P., Tschardtke, T., Tylianakis, J. M. & Steffan-Dewenter, I. (2008) Functional group diversity of bee pollinators increases crop yield. *Proceedings of the Royal Society of London Series B*, **275**, 2283-2291.
- Holehouse, K. A., Hammond, R. L. & Bourke, A. F. G. (2003) Non-lethal sampling of DNA from bumble bees for conservation genetics. *Insectes Sociaux*, **50**, 277-285.
- Hollander, M. & Wolfe, D. (1999) *Nonparametric Statistical Methods*, 2<sup>nd</sup> edn. John Wiley & Sons, New York.
- Holzschuh, A., Steffan-Dewenter, I. & Tschardtke, T. (2008) Agricultural landscapes with organic crops support higher pollinator diversity. *Oikos*, **117**, 354-361.
- Horton, N. J. & Lipsitz, S. R. (1999) Review of software to fit generalized estimating equation regression models. *The American Statistician*, **53**, 160-169.
- Hotta, Y., Takeya, K., Kobayashi, S., Harada, N., Sakakibara, J. & Shirai, N. (1980) Relationship between structure, positive inotropic potency and lethal dose of grayanotoxins in guinea pig. *Archives of Toxicology*, **44**, 259-267.
- Hulme, P. E. (2003) Biological invasions: winning the science battles but losing the conservation war? *Oryx*, **37**, 178-193.
- Husband, B. C. & Schemske, D. W. (1996) Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution*, **50**, 54-70.
- Inoue, M., Yokoyama, J. & Washitani, I. (2008) Displacement of Japanese native bumblebees by the recently introduced *Bombus terrestris* (L.) (Hymenoptera: Apidae). *Journal of Insect Conservation*, **12**, 135-146.
- Irwin, R. E. & Adler, L. S. (2008) Nectar secondary compounds affect self-pollen transfer: implications for female and male reproduction. *Ecology*, **89**, 2207-2217.
- Irwin, R. E., Brody, A. K. & Waser, N. M. (2001) The impact of floral larceny on individuals, populations, and communities. *Oecologia*, **129**, 161-168.
- Ishii, H. S., Hirabayashi, Y. & Kudo, G. (2008) Combined effects of inflorescence architecture, display size, plant density and empty flowers on bumble bee behaviour: experimental study with artificial inflorescences. *Oecologia*, **156**, 341-350.
- Jakobsson, A., Padrón, B. & Traveset, A. (2008) Pollen transfer from invasive *Carpobrotus* spp. to natives - a study of pollinator behaviour and reproduction success. *Biological Conservation*, **141**, 136-145.





- Jauker, F. & Wolters, V. (2008) Hover flies are efficient pollinators of oilseed rape. *Oecologia*, **156**, 819-823.
- Jennersten, O. (1988) Pollination in *Dianthus deltoides* (Caryophyllaceae): effects of habitat fragmentation on visitation and seed set. *Conservation Biology*, **2**, 359-366.
- Jennersten, O. & Nilsson, S. G. (1993) Insect flower visitation frequency and seed production in relation to patch size of *Viscaria vulgaris* (Caryophyllaceae). *Oikos*, **68**, 283-292.
- Johnson, S. D., Collin, C. L., Wissman, H. J., Halvarsson, E. & Ågren, J. (2004) Factors contributing to variation in seed production among remnant populations of the endangered daisy *Gerbera aurantiaca*. *Biotropica*, **36**, 148-155.
- Johnson, S. D., Hargreaves, A. L. & Brown, M. (2006) Dark, bitter-tasting nectar functions as a filter of flower visitors in a bird-pollinated plant. *Ecology*, **87**, 2709-2716.
- Johnson, S. D., Peter, C. I., Nilsson, L. A. & Ågren, J. (2003) Pollination success in a deceptive orchid is enhanced by co-occurring rewarding magnet plants. *Ecology*, **84**, 2919-2927.
- Johnson, S. D. & Steiner, K. E. (2000) Generalization versus specialization in plant pollination systems. *Trends in Ecology and Evolution*, **15**, 140-143.
- Johnson, S. G., Delph, L. F. & Elderkin, C. L. (1995) The effect of petal-size manipulation on pollen removal, seed set, and insect-visitor behaviour in *Campanula americana*. *Oecologia*, **102**, 174-179.
- Jones, K. N. (2004) Do dandelion flowers influence seed set of a native plant (*Delphinium nuttallianum*) in subalpine meadows? *The American Midland Naturalist*, **151**, 201-205.
- Jordano, P. (1987) Patterns of mutualistic interactions in pollination and seed dispersal: connectance, dependence, and coevolution. *American Naturalist*, **129**, 657-677.
- Junker, R. R. & Bluthgen, N. (2008) Floral scents repel potentially nectar-thieving ants. *Evolutionary Ecology Research*, **10**, 295-308.
- Kalisz, S. & Vogler, D. W. (2003) Benefits of autonomous selfing under unpredictable pollinator environments. *Ecology*, **84**, 2928-2942.
- Kandori, I. (2002) Diverse visitors with various pollinator importance and temporal change in the important pollinators of *Geranium thunbergii* (Geraniaceae). *Ecological Research*, **17**, 283-294.
- Kasagi, T. & Kudo, G. (2003) Variations in bumble bee preference and pollen limitation among neighboring populations: comparisons between *Phyllodoce caerulea* and *Phyllodoce aleutica* (Ericaceae) along snowmelt gradients. *American Journal of Botany*, **90**, 1321-1327.
- Kearns, C. A., Inouye, D. W. & Waser, N. M. (1998) Endangered mutualisms: the conservation of plant-pollinator interactions. *Annual Review of Ecology and Systematics*, **29**, 83-112.
- Kessler, D. & Baldwin, I. T. (2007) Making sense of nectar scents: the effects of nectar secondary metabolites on floral visitors of *Nicotiana attenuata*. *The Plant Journal*, **49**, 840-854.
- Kessler, D., Gase, K. & Baldwin, I. T. (2008) Field experiments with transformed plants reveal the sense of floral scents. *Science*, **321**, 1200-1202.
- Kevan, P. G. & Baker, H. G. (1983) Insects as flower visitors and pollinators. *Annual Review of Entomology*, **28**, 407-453.
- Kevan, P. G. & Baker, H. G. (1984) Insects on flowers. *Ecological Entomology* (eds C. B. Huffaker & R. L. Rabb), pp. 607-631. John Wiley & Sons, New York.
- King, M. J. & Buchmann, S. L. (1995) Bumble bee-initiated vibration release mechanism of *Rhododendron* pollen. *American Journal of Botany*, **82**, 1407-1411.
- Kirchner, F., Luijten, S. H., Imbert, E., Riba, M., Mayol, M., González-Martínez, S. C., Mignot, A. & Colas, B. (2005) Effects of local density on insect visitation and fertilization success in the narrow-endemic *Centaurea corymbosa* (Asteraceae). *Oikos*, **111**, 130-142.
- Klein, A. M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C. & Tscharntke, T. (2007) Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society of London Series B*, **274**, 303-313.
- Klinkhamer, P. G. L. & de Jong, T. J. (1990) Effects of plant size, plant density and sex differential nectar reward on pollinator visitation in the protandrous *Echium vulgare* (Boraginaceae). *Oikos*, **57**, 399-405.





- Klinkhamer, P. G. L. & de Jong, T. J. (1993) Attractiveness to pollinators - a plant's dilemma. *Oikos*, **66**, 180-184.
- Knight, M. E., Martin, A. P., Bishop, S., Osborne, J. L., Hale, R. J., Sanderson, R. A. & Goulson, D. (2005a) An interspecific comparison of foraging range and nest density of four bumblebee (*Bombus*) species. *Molecular Ecology*, **14**, 1811-1820.
- Knight, T. M. (2003) Floral density, pollen limitation, and reproductive success in *Trillium grandiflorum*. *Oecologia*, **137**, 557-563.
- Knight, T. M., Steets, J. A., Vamosi, J. C., Mazer, S. J., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mitchell, R. J. & Ashman, T.-L. (2005b) Pollen limitation of plant reproduction: pattern and process. *Annual Review of Ecology, Evolution, and Systematics*, **36**, 467-497.
- Koca, I. & Koca, A. F. (2007) Poisoning by mad honey: a brief review. *Food and Chemical Toxicology*, **45**, 1315-1318.
- Kriticos, D. J., Sutherst, R. W., Brown, J. R., Adkins, S. W. & Maywald, G. F. (2003) Climate change and the potential distribution of an invasive alien plant: *Acacia nilotica* ssp *indica* in Australia. *Journal of Applied Ecology*, **40**, 111-124.
- Kudo, G. & Suzuki, S. (2002) Relationships between flowering phenology and fruit-set of dwarf shrubs in alpine fellfields in northern Japan: a comparison with a subarctic heathland in northern Sweden. *Arctic, Antarctic, and Alpine Research*, **34**, 185-190.
- Kueffer, C., Schumacher, E., Fleischmann, K., Edwards, P. J. & Dietz, H. (2007) Strong below-ground competition shapes tree regeneration in invasive *Cinnamomum verum* forests. *Journal of Ecology*, **95**, 273-282.
- Kunin, W. E. (1993) Sex and the single mustard: population density and pollinator behavior effects on seed-set. *Ecology*, **74**, 2145-2160.
- Kunin, W. E. (1997) Population size and density effects in pollination: pollinator foraging and plant reproductive success in experimental arrays of *Brassica kaber*. *Journal of Ecology*, **85**, 225-234.
- Kunin, W. E. & Gaston, K. J. (1993) The biology of rarity: patterns, causes and consequences. *Trends in Ecology and Evolution*, **8**, 298-301.
- Kunin, W. E. & Iwasa, Y. (1996) Pollinator foraging strategies in mixed floral arrays: density effects and floral constancy. *Theoretical Population Biology*, **49**, 232-263.
- Kunin, W. E. & Shmida, A. (1997) Plant reproductive traits as a function of local, regional, and global abundance. *Conservation Biology*, **11**, 183-192.
- Lach, L. (2007) A mutualism with a native membracid facilitates pollinator displacement by Argentine ants. *Ecology*, **88**, 1994-2004.
- Lambdon, P. W., Lloret, F. & Hulme, P. E. (2008) Do alien plants on Mediterranean islands tend to invade different niches from native species? *Biological Invasions*, **10**, 703-716.
- Landry, F., Plowright, C. M. S. & Plowright, R. C. (2000) Behaviour of individual bumble bees (*Bombus impatiens*) on blueberry (*Vaccinium angustifolium*) flowers: the effects of pollen and nectar deprivation. *Journal of Apicultural Research*, **39**, 29-35.
- Larson, B. M. H. & Barrett, S. C. H. (2000) A comparative analysis of pollen limitation in flowering plants. *Biological Journal of the Linnean Society*, **69**, 503-520.
- Larson, D. L., Royer, R. A. & Royer, M. R. (2006) Insect visitation and pollen deposition in an invaded prairie plant community. *Biological Conservation*, **130**, 148-159.
- Larson, K. C., Fowler, S. P. & Walker, J. C. (2002) Lack of pollinators limits fruit set in the exotic *Lonicera japonica*. *The American Midland Naturalist*, **148**, 54-60.
- Larsson, M. & Franzén, M. (2008) Estimating the population size of specialised solitary bees. *Ecological Entomology*, **33**, 232-238.
- Laverty, T. M. (1992) Plant interactions for pollinator visits: a test of the magnet species effect. *Oecologia*, **89**, 502-508.
- Laverty, T. M. (1994) Costs to foraging bumble bees of switching plant species. *Canadian Journal of Zoology*, **72**, 43-47.
- Laverty, T. M. & Plowright, R. C. (1988) Flower handling by bumblebees: a comparison of specialists and generalists. *Animal Behaviour*, **36**, 733-740.





- Leadbeater, E. & Chittka, L. (2008) Social transmission of nectar-robbing behaviour in bumblebees. *Proceedings of the Royal Society of London Series B*, **275**, 1669-1674.
- Leimu, R., Mutikainen, P., Koricheva, J. & Fischer, M. (2006) How general are positive relationships between plant population size, fitness and genetic variation? *Journal of Ecology*, **94**, 942-952.
- Levin, D. A. (1978) Pollinator behaviour and the breeding structure of plant populations. *The Pollination of Flowers by Insects* (ed A. J. Richards), pp. 133-150. Academic Press, London.
- Levin, D. A. & Anderson, W. W. (1970) Competition for pollinators between simultaneously flowering species. *The American Naturalist*, **104**, 455-467.
- Levine, J. M. (2008) Biological invasions. *Current Biology*, **18**, R57-R60.
- Levine, J. M., Adler, P. B. & Yelenik, S. G. (2004) A meta-analysis of biotic resistance to exotic plant invasions. *Ecology Letters*, **7**, 975-989.
- Levine, J. M., Vilà, M., D'Antonio, C. M., Dukes, J. S., Grigulis, K. & Lavorel, S. (2003) Mechanisms underlying the impacts of exotic plant invasions. *Proceedings of the Royal Society of London Series B*, **270**, 775-781.
- Lewinsohn, T. M., Prado, P. I., Jordano, P., Bascompte, J. & Olesen, J. M. (2006) Structure in plant-animal interaction assemblages. *Oikos*, **113**, 174-184.
- Lindauer, M. (1971) *Communication among Social Bees*. Harvard University Press, Cambridge, Massachusetts.
- Lodge, D. M. (1993) Biological invasions: lessons for ecology. *Trends in Ecology and Evolution*, **8**, 133-137.
- Lonsdale, W. M. (1999) Global patterns of plant invasions and the concept of invasibility. *Ecology*, **80**, 1522-1536.
- Lopezaraiza-Mikel, M. E. (2006) *The impact of alien species on native pollination systems*. PhD thesis, University of Bristol, Bristol.
- Lopezaraiza-Mikel, M. E., Hayes, R. B., Whalley, M. R. & Memmott, J. (2007) The impact of an alien plant on a native plant-pollinator network: an experimental approach. *Ecology Letters*, **10**, 539-550.
- Lundberg, J. & Moberg, F. (2003) Mobile link organisms and ecosystem functioning: implications for ecosystem resilience and management. *Ecosystems*, **6**, 87-98.
- MacArthur, R. H. (1972) *Geographical Ecology: Patterns in the Distribution of Species*. Princeton University Press, Princeton, New Jersey.
- Macior, L. W. (1971) Co-evolution of plants and animals. Systematic insights from plant-insect interactions. *Taxon*, **20**, 17-28.
- Mack, R. N. & Erneberg, M. (2002) The United States naturalized flora: largely the product of deliberate introductions. *Annals of the Missouri Botanical Garden*, **89**, 176-189.
- Mack, R. N., Simberloff, D., Lonsdale, W. M., Evans, H., Clout, M. & Bazzaz, F. A. (2000) Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications*, **10**, 689-710.
- Malcolm, J. R., Markham, A., Neilson, R. P. & Garaci, M. (2002) Estimated migration rates under scenarios of global climate change. *Journal of Biogeography*, **29**, 835-849.
- Maloof, J. E. (2001) The effects of a bumble bee nectar robber on plant reproductive success and pollinator behaviour. *American Journal of Botany*, **88**, 1960-1965.
- Manchester, S. J. & Bullock, J. M. (2000) The impacts of non-native species on UK biodiversity and the effectiveness of control. *Journal of Applied Ecology*, **37**, 845-864.
- Mänd, M., Mänd, R. & Williams, I. H. (2002) Bumblebees in the agricultural landscape of Estonia. *Agriculture, Ecosystems and Environment*, **89**, 69-76.
- McCune, B. & Grace, J. B. (2002) *Analysis of Ecological Communities*. MjM Software Design, Gleneden Beach, Oregon.
- McCune, B. & Mefford, M. J. (2006) PC-ORD for Windows. MjM Software, Gleneden Beach, Oregon.





- Medan, D., Perazzo, R. P. J., Devoto, M., Burgos, E., Zimmermann, M. G., Ceva, H. & Delbue, A. M. (2007) Analysis and assembling of network structure in mutualistic systems. *Journal of Theoretical Biology*, **246**, 510-521.
- Mejías, J. A., Arroyo, J. & Ojeda, F. (2002) Reproductive ecology of *Rhododendron ponticum* (Ericaceae) in relict Mediterranean populations. *Botanical Journal of the Linnean Society*, **140**, 297-311.
- Memmott, J. (1999) The structure of a plant-pollinator food web. *Ecology Letters*, **2**, 276-280.
- Memmott, J., Craze, P. G., Waser, N. M. & Price, M. V. (2007) Global warming and the disruption of plant-pollinator interactions. *Ecology Letters*, **10**, 710-717.
- Memmott, J. & Waser, N. M. (2002) Integration of alien plants into a native flower-pollinator visitation web. *Proceedings of the Royal Society of London Series B*, **269**, 2395-2399.
- Memmott, J., Waser, N. M. & Price, M. V. (2004) Tolerance of pollination networks to species extinctions. *Proceedings of the Royal Society of London Series B*, **271**, 2605-2611.
- Milbau, A. N. N. & Stout, J. C. (2008) Factors associated with alien plants transitioning from casual, to naturalized, to invasive. *Conservation Biology*, **22**, 308-317.
- Miller, K. E. & Gorchov, D. L. (2004) The invasive shrub, *Lonicera maackii*, reduces growth and fecundity of perennial forest herbs. *Oecologia*, **139**, 359-375.
- Milne, R. I. & Abbott, R. J. (2000) Origin and evolution of invasive naturalized material of *Rhododendron ponticum* L. in the British Isles. *Molecular Ecology*, **9**, 541-556.
- Minitab Inc. (2000) MINITAB™ Statistical Software for Windows, Release 13.30. Minitab Inc., State College, Pennsylvania.
- Mitchell, C. E. & Power, A. G. (2003) Release of invasive plants from fungal and viral pathogens. *Nature*, **421**, 625-627.
- Mitchell, R. J., Marrs, R. H., LeDuc, M. G. & Auld, M. H. D. (1997) A study of succession on lowland heaths in Dorset, southern England: changes in vegetation and soil chemical properties. *Journal of Applied Ecology*, **34**, 1426-1444.
- Moeller, D. A. (2004) Facilitative interactions among plants via shared pollinators. *Ecology*, **85**, 3289-3301.
- Mooney, H. A., Mack, R. N., McNeely, J. A., Neville, L. E., Schei, P. J. & Waage, J. K. (2005) *Invasive Alien Species: a New Synthesis*. Island Press, Washington, D.C.
- Moragues, E. & Traveset, A. (2005) Effect of *Carpobrotus* spp. on the pollination success of native plant species of the Balearic Islands. *Biological Conservation*, **122**, 611-619.
- Morales, C. L. & Aizen, M. A. (2006) Invasive mutualisms and the structure of plant-pollinator interactions in the temperate forests of north-west Patagonia, Argentina. *Journal of Ecology*, **94**, 171-180.
- Morales, C. L. & Traveset, A. (2008) Interspecific pollen transfer: magnitude, prevalence and consequences for plant fitness. *Critical Reviews in Plant Sciences*, **27**, 221-238.
- Morales, J. M. & Vázquez, D. P. (2008) The effect of space in plant-animal mutualistic networks: insights from a simulation study. *Oikos*, **117**, 1362-1370.
- Moritz, R. F. A., Dietemann, V. & Crewe, R. (2008) Determining colony densities in wild honeybee populations (*Apis mellifera*) with linked microsatellite DNA markers. *Journal of Insect Conservation*, **12**, 455-459.
- Moritz, R. F. A., Kraus, F. B., Kryger, P. & Crewe, R. M. (2007) The size of wild honeybee populations (*Apis mellifera*) and its implications for the conservation of honeybees. *Journal of Insect Conservation*, **11**, 391-397.
- Moroń, D., Lenda, M., Skórka, P., Szentgyörgyi, H., Settele, J. & Woyciechowski, M. (2009) Pollinator communities are negatively affected by invasion of alien goldenrods in a grassland landscape. **142**, 1322-1332.
- Müller, H. (1883) *The Fertilisation of Flowers*. Macmillan, London.
- Muñoz, A. A. & Cavieres, L. A. (2008) The presence of a showy invasive plant disrupts pollinator service and reproductive output in native alpine species only at high densities. *Journal of Ecology*, **96**, 459-467.
- Murphy, S. D. & Aarssen, L. W. (1995) Reduced seed set in *Elytrigia repens* caused by allelopathic pollen from *Phleum pratense*. *Canadian Journal of Botany*, **73**, 1417-1422.





- Murray, T. E., Fitzpatrick, Ú., Brown, M. J. F. & Paxton, R. J. (2008) Cryptic species diversity in a widespread bumble bee complex revealed using mitochondrial DNA RFLPs. *Conservation Genetics*, **9**, 653-666.
- Mustajärvi, K., Siikamäki, P., Rytkönen, S. & Lammi, A. (2001) Consequences of plant population size and density for plant-pollinator interactions and plant performance. *Journal of Ecology*, **89**, 80-87.
- Nadezhdina, N., Tatarinov, F. & Ceulemans, R. (2004) Leaf area and biomass of *Rhododendron* understory in a stand of Scots pine. *Forest Ecology and Management*, **187**, 235-246.
- Nienhuis, C. M. (2009) *The interactions between native bees and alien plants*. PhD thesis, Trinity College Dublin, Dublin.
- Nienhuis, C. M., Dietzsch, A. C. & Stout, J. C. (2009) The impacts of an invasive alien plant and its removal on bees. *Apidologie*, **online**, doi: 10.1051/apido/2009005.
- Olesen, J. M., Bascompte, J., Dupont, Y. L. & Jordano, P. (2006) The smallest of all worlds: pollination networks. *Journal of Theoretical Biology*, **240**, 270-276.
- Olesen, J. M., Bascompte, J., Dupont, Y. L. & Jordano, P. (2007) The modularity of pollination networks. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 19891-19896.
- Olesen, J. M., Bascompte, J., Elberling, H. & Jordano, P. (2008) Temporal dynamics in a pollination network. *Ecology*, **89**, 1573-1582.
- Olesen, J. M., Eskildsen, L. I. & Venkatasamy, S. (2002) Invasion of pollination networks on oceanic islands: importance of invader complexes and endemic super generalists. *Diversity and Distributions*, **8**, 181-192.
- Ollerton, J. & Lack, A. (1998) Relationships between flowering phenology, plant size and reproductive success in *Lotus corniculatus* (Fabaceae). *Plant Ecology*, **139**, 35-47.
- Oostermeijer, J. G. B., Luijten, S. H., Krenova, Z. V. & Nijs, H. C. M. D. (1998) Relationships between population and habitat characteristics and reproduction of the rare *Gentiana pneumonanthe* L. *Conservation Biology*, **12**, 1042-1053.
- Orr, S. P., Rudgers, J. A. & Clay, K. (2005) Invasive plants can inhibit native tree seedlings: testing potential allelopathic mechanisms. *Plant Ecology*, **181**, 153-165.
- Ortega, Y. K. & Pearson, D. E. (2005) Weak vs. strong invaders of natural plant communities: assessing invasibility and impact. *Ecological Applications*, **15**, 651-661.
- Osborne, J. L., Martin, A. P., Carreck, N. L., Swain, J. L., Knight, M. E., Goulson, D., Hale, R. J. & Sanderson, R. A. (2008) Bumblebee flight distances in relation to the forage landscape. *Journal of Animal Ecology*, **77**, 406-415.
- Palmer, T. M., Stanton, M. L. & Young, T. P. (2003) Competition and coexistence: exploring mechanisms that restrict and maintain diversity within mutualist guilds. *The American Naturalist*, **162**, S63-S79.
- Pappes, S. M., de Jong, T. J., Klinkhamer, P. G. L. & Meelis, E. (1999) Effects of nectar content on the number of bumblebee approaches and the length of visitation sequences in *Echium vulgare* (Boraginaceae). *Oikos*, **87**, 580-586.
- Parker, I. M. (1997) Pollinator limitation of *Cytisus scoparius* (Scotch broom), an invasive exotic shrub. *Ecology*, **78**, 1457-1470.
- Parker, I. M. & Haubensak, K. A. (2002) Comparative pollinator limitation of two non-native shrubs: do mutualisms influence invasions? *Oecologia*, **130**, 250-258.
- Parker, I. M., Simberloff, D., Lonsdale, W. M., Goodell, K., Wonham, M., Kareiva, P. M., Williamson, M., Von Holle, B., Moyle, P. B., Byers, J. E. & Goldwasser, L. (1999) Impact: toward a framework for understanding the ecological effects of invaders. *Biological Invasions*, **1**, 3-19.
- Paschke, M., Abs, C. & Schmid, B. (2002) Effects of population size and pollen diversity on reproductive success and offspring size in the narrow endemic *Cochlearia bavarica* (Brassicaceae). *American Journal of Botany*, **89**, 1250-1259.
- Pauw, A. (2007) Collapse of a pollination web in small conservation areas. *Ecology*, **88**, 1759-1769.





- Peat, J. & Goulson, D. (2005) Effects of experience and weather on foraging rate and pollen versus nectar collection in the bumblebee, *Bombus terrestris*. *Behavioral Ecology and Sociobiology*, **58**, 152-156.
- Percival, M. S. (1961) Types of nectar in angiosperms. *New Phytologist*, **60**, 235-281.
- Pereboom, J. J. M. (2000) The composition of larval food and the significance of exocrine secretions in the bumblebee *Bombus terrestris*. *Insectes Sociaux*, **47**, 11-20.
- Perrings, C., Williamson, M. & Dalmazzone, S. (2000) Conclusions. *The Economics of Biological Invasions* (eds C. Perrings, M. Williamson & S. Dalmazzone), pp. 227-240. Edward Elgar, Cheltenham.
- Petanidou, T., Den Nijs, J. C. M. & Oostermeijer, J. G. B. (1995) Pollination ecology and constraints on seed set of the rare perennial *Gentiana cruciata* L. in The Netherlands. *Acta Botanica Neerlandica*, **44**, 55-74.
- Petanidou, T. & Ellis, W. N. (1993) Pollinating fauna of a phryganic ecosystem: composition and diversity. *Biodiversity Letters*, **1**, 9-22.
- Petanidou, T. & Ellis, W. N. (1996) Interdependence of native bee faunas and floras in changing Mediterranean communities. *The Conservation of Bees* (eds A. Matheson, S. L. Buchmann, C. O'Toole, P. Westrich & I. H. Williams), pp. 210-226. Academic Press, London.
- Petanidou, T., Kallimanis, A. S., Tzanopoulos, J., Sgardelis, S. P. & Pantis, J. D. (2008) Long-term observation of a pollination network: fluctuation in species and interactions, relative invariance of network structure and implications for estimates of specialization. *Ecology Letters*, **11**, 564-575.
- Pianka, E. R. (1973) The structure of lizard communities. *Annual Review of Ecology and Systematics*, **4**, 53-74.
- Pickhardt, A. & Fluri, P. (2000) *Die Bestäubung der Blütenpflanzen durch Bienen - Biologie, Ökologie, Ökonomie*. Mitteilung Nr 38. Schweizerisches Zentrum für Bienenforschung, Forschungsanstalt für Milchwirtschaft, Bern.
- Pimentel, D., Zuniga, R. & Morrison, D. (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics*, **52**, 273-288.
- Plowright, C. M. S. & Plowright, R. C. (1997) The advantage of short tongues in bumble bees (*Bombus*) - analyses of species distributions according to flower corolla depth, and of working speeds on white clover. *Canadian Entomologist*, **129**, 51-59.
- Plowright, R. C. & Jay, S. C. (1968) Caste differentiation in bumblebees (*Bombus* Latr.: Hym.) 1. The determination of female size. *Insectes Sociaux*, **15**, 171-192.
- Pojar, J. (1974) Reproductive dynamics of four plant communities of southwestern British Columbia. *Canadian Journal of Botany*, **52**, 1819-1834.
- Potts, S. G., Vulliamy, B., Dafni, A., Ne'eman, G. & Willmer, P. (2003) Linking bees and flowers: How do floral communities structure pollinator communities? *Ecology*, **84**, 2628-2642.
- Price, M. V. & Waser, N. M. (1979) Pollen dispersal and optimal outcrossing in *Delphinium nelsoni*. *Nature*, **277**, 294-297.
- Proctor, M., Yeo, P. & Lack, A. (1996) *The Natural History of Pollination*. Timber Press, Portland, Oregon.
- Proctor, M. C. F. (1978) Insect pollination syndromes in an evolutionary and ecosystemic context. *The Pollination of Flowers by Insects* (ed A. J. Richards), pp. 105-116. Academic Press, London.
- Prŷs-Jones, O. & Corbet, S. (1991) *Bumblebees* The Richmond Publishing, Slough.
- Pyke, G. H. (1978) Optimal foraging: movement patterns of bumblebees between inflorescences. *Theoretical Population Biology*, **13**, 72-98.
- Pyke, G. H. & Waser, N. M. (1981) The production of dilute nectars by hummingbird and honeyeater flowers. *Biotropica*, **13**, 260-270.
- Quinn, G. P. & Keough, M. J. (2002) *Experimental Design and Data Analysis for Biologists*, 1<sup>st</sup> edn. Cambridge University Press, Cambridge.
- Raguso, R. A. (2008a) Plant science - the "Invisible Hand" of floral chemistry. *Science*, **321**, 1163-1164.





- Raguso, R. A. (2008b) Start making scents: the challenge of integrating chemistry into pollination ecology. *Entomologia Experimentalis et Applicata*, **128**, 196-207.
- Raine, N. E. & Chittka, L. (2005) Comparison of flower constancy and foraging performance in three bumblebee species (Hymenoptera: Apidae: *Bombus*). *Entomologia Generalis*, **28**, 81-89.
- Raine, N. E. & Chittka, L. (2008) The correlation of learning speed and natural foraging success in bumble-bees. *Proceedings of the Royal Society of London Series B*, **275**, 803-808.
- Rathcke, B. (1988a) Flowering phenologies in a shrub community: competition and constraints. *Journal of Ecology*, **76**, 975-994.
- Rathcke, B. (1988b) Interactions for pollination among coflowering shrubs. *Ecology*, **69**, 446-457.
- Rathcke, B. J. (1983) Competition and facilitation among plants for pollination. *Pollination Biology* (ed L. A. Real), pp. 305-329. Academic Press, Orlando, Florida.
- Rathcke, B. J. & Jules, E. S. (1993) Habitat fragmentation and plant-pollinator interactions. *Current Science*, **65**, 273-277.
- Raymond, M. & Rousset, F. (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248-249.
- Reinhardt, F., Herle, M., Bastiansen, F. & Streit, B. (2003) Ökonomische Folgen der Ausbreitung von gebietsfremden Organismen in Deutschland. Umweltbundesamt, Berlin.
- Reinhart, K. O. & Callaway, R. M. (2006) Soil biota and invasive plants. *New Phytologist*, **170**, 445-457.
- Reinhart, K. O., Gurnee, J., Tirado, R. & Callaway, R. M. (2006) Invasion through quantitative effects: intense shade drives native decline and invasive success. *Ecological Applications*, **16**, 1821-1831.
- Reinhart, K. O., Packer, A., van der Putten, W. H. & Clay, K. (2003) Plant-soil biota interactions and spatial distribution of black cherry in its native and invasive ranges. *Ecology Letters*, **6**, 1046-1050.
- Rezende, E. L., Jordano, P. & Bascompte, J. (2007a) Effects of phenotypic complementarity and phylogeny on the nested structure of mutualistic networks. *Oikos*, **116**, 1919-1929.
- Rezende, E. L., Lavabre, J. E., Guimarães Jr., P. R., Jordano, P. & Bascompte, J. (2007b) Non-random coextinctions in phylogenetically structured mutualistic networks. *Nature*, **448**, 925-928.
- Rhoades, D. F. & Bergdahl, J. C. (1981) Adaptive significance of toxic nectar. *The American Naturalist*, **117**, 798-803.
- Rice, W. R. (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223-225.
- Richards, A. J. (1997) *Plant Breeding Systems*, 2<sup>nd</sup> edn. Chapman & Hall, London.
- Richardson, D. M., Allsopp, N., D'Antonio, C. M., Milton, S. J. & Rejmánek, M. (2000a) Plant invasions - the role of mutualisms. *Biological Reviews*, **75**, 65-93.
- Richardson, D. M., Pyšek, P., Rejmánek, M., Barbour, M. G., Panetta, F. D. & West, C. J. (2000b) Naturalization and invasion of alien plants: concepts and definitions. *Diversity and Distributions*, **6**, 93-107.
- Ricketts, T. H., Regetz, J., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., Bogdanski, A., Gemmill-Herren, B., Greenleaf, S. S., Klein, A. M., Mayfield, M. M., Morandin, L. A., Ochieng', A. & Viana, B. F. (2008) Landscape effects on crop pollination services: are there general patterns? *Ecology Letters*, **11**, 499-515.
- Robertson, A. W. & Macnair, M. R. (1995) The effects of floral display size on pollinator service to individual flowers of *Myosotis* and *Mimulus*. *Oikos*, **72**, 106-114.
- Robertson, A. W., Mountjoy, C., Faulkner, B. E., Roberts, M. V. & Macnair, M. R. (1999) Bumble bee selection of *Mimulus guttatus* flowers: the effects of pollen quality and reward depletion. *Ecology*, **80**, 2594-2606.
- Robinson, J. D. (1980) *Rhododendron ponticum*. A weed of woodlands and forest plantations seriously affecting management. *Weed Control in Forestry* pp. 89-95. Conference Proceedings.
- Rodriguez, L. F. (2006) Can invasive species facilitate native species? Evidence of how, when, and why these impacts occur. *Biological Invasions*, **8**, 927-939.





- Rotherham, I. D. (2001) *Rhododendron* gone wild. *Biologist*, **48**, 7-11.
- Roubik, D. W. (2001) Ups and downs in pollinator populations: when is there a decline? *Conservation Ecology*, **5**, 2. [online] URL: <http://www.consecol.org/vol5/iss1/art2>.
- Roulston, T. H. & Cane, J. H. (2000) Pollen nutritional content and digestibility for animals. *Plant Systematics and Evolution*, **222**, 187-209.
- Roulston, T. H. & Cane, J. H. (2002) The effect of pollen protein concentration on body size in the sweat bee *Lasioglossum zephyrum* (Hymenoptera: Apiformes). *Evolutionary Ecology*, **16**, 49-65.
- Rundlöf, M., Bengtsson, J. & Smith, H. G. (2008a) Local and landscape effects of organic farming on butterfly species richness and abundance. *Journal of Applied Ecology*, **45**, 813-820.
- Rundlöf, M., Nilsson, H. & Smith, H. G. (2008b) Interacting effects of farming practice and landscape context on bumble bees. *Biological Conservation*, **141**, 417-426.
- Sachs, J. L. & Simms, E. L. (2006) Pathways to mutualism breakdown. *Trends in Ecology and Evolution*, **21**, 585-592.
- Sahli, H. F. & Conner, J. K. (2007) Visitation, effectiveness, and efficiency of 15 genera of visitors to wild radish, *Raphanus raphanistrum* (Brassicaceae). *American Journal of Botany*, **94**, 203-209.
- Sakai, A. K., Allendorf, F. W., Holt, J. S., Lodge, D. M., Molofsky, J., With, K. A., Baughman, S., Cabin, R. J., Cohen, J. E., Ellstrand, N. C., McCauley, D. E., O'Neil, P., Parker, I. M., Thompson, J. N. & Weller, S. G. (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics*, **32**, 305-332.
- Sargent, R. D. & Ackerly, D. D. (2008) Plant-pollinator interactions and the assembly of plant communities. *Trends in Ecology and Evolution*, **23**, 123-130.
- Saunders, D. A., Hobbs, R. J. & Margules, C. R. (1991) Biological consequences of ecosystem fragmentation: a review. *Conservation Biology*, **5**, 18-32.
- Sax, D. F., Stachowicz, J. J., Brown, J. H., Bruno, J. F., Dawson, M. N., Gaines, S. D., Grosberg, R. K., Hastings, A., Holt, R. D., Mayfield, M. M., O'Connor, M. I. & Rice, W. R. (2007) Ecological and evolutionary insights from species invasions. *Trends in Ecology and Evolution*, **22**, 465-471.
- Schäfer, B. & Böcker, R. (2006) Water bound seed dispersal of *Rhododendron ponticum* L. (poster). Neobiota Conference, Wien.
- Schemske, D. W. (1981) Floral convergence and pollinator sharing in two bee-pollinated tropical herbs. *Ecology*, **62**, 946-954.
- Schemske, D. W. & Horvitz, C. C. (1984) Variation among floral visitors in pollination ability: a precondition for mutualism specialization. *Science*, **225**, 519-521.
- Schmid-Hempel, R. & Schmid-Hempel, P. (2000) Female mating frequencies in *Bombus* spp. from Central Europe. *Insectes Sociaux*, **47**, 36-41.
- Schneider, W. & Enders, A. (1955) Zur Pharmakologie von quarten Delphinin-Derivaten. *Arzneimittel-Forschung*, **5**, 324-326.
- Schürkens, S. & Chittka, L. (2001) Zur Bedeutung der invasiven Kreuzblütler-Art *Bunias orientalis* (Brassicaceae) als Nektarquelle für mitteleuropäische Insekten. *Entomologia Generalis*, **25**, 115-120.
- Schweiger, O., Maelfait, J. P., van Wingerden, W. K. R. E., Hendrickx, F., Billeter, R., Speelmans, M., Augenstein, I., Aukema, B., Aviron, S., Bailey, D., Bukacek, R., Burel, F., Diekötter, T., Dirksen, J., Frenzel, M., Herzog, F., Liira, J., Roubalova, M. & Bugter, R. (2005) Quantifying the impact of environmental factors on arthropod communities in agricultural landscapes across organizational levels and spatial scales. *Journal of Applied Ecology*, **42**, 1129-1139.
- Scribailo, R. W. & Barrett, S. C. H. (1994) Effects of prior self-pollination on outcrossed seed set in tristylous *Pontederia sagittata* (Pontederiaceae). *Sexual Plant Reproduction*, **7**, 273-281.
- Seber, G. A. F. (1982) *The Estimation of Animal Abundance*, 2<sup>nd</sup> edn. Griffin, London.
- Seber, G. A. F. (1986) A review of estimating animal abundance. *Biometrics*, **42**, 267-292.
- Seber, G. A. F. (1992) A review of estimating animal abundance II. *International Statistical Review*, **60**, 129-166.





- Sheppard, A. W., Shaw, R. H. & Sforza, R. (2006) Top 20 environmental weeds for classical biological control in Europe: a review of opportunities, regulations and other barriers to adoption. *Weed Research*, **46**, 93-117.
- Sih, A. & Baltus, M.-S. (1987) Patch size, pollinator behavior, and pollinator limitation in Catnip. *Ecology*, **68**, 1679-1690.
- Singaravelan, N., Nee'man, G., Inbar, M. & Izhaki, I. (2005) Feeding responses of free-flying honeybees to secondary compounds mimicking floral nectars. *Journal of Chemical Ecology*, **31**, 2791-2804.
- Sjödin, N. E., Bengtsson, J. & Ekbom, B. (2008) The influence of grazing intensity and landscape composition on the diversity and abundance of flower-visiting insects. *Journal of Applied Ecology*, **45**, 763-772.
- Smeets, P. & Duchateau, M. J. (2003) Longevity of *Bombus terrestris* workers (Hymenoptera: Apidae) in relation to pollen availability, in the absence of foraging. *Apidologie*, **34**, 333-337.
- Smithson, A. & Macnair, M. R. (1996) Frequency-dependent selection by pollinators: mechanisms and consequences with regard to behaviour of bumblebees *Bombus terrestris* (L) (Hymenoptera: Apidae). *Journal of Evolutionary Biology*, **9**, 571-588.
- Somanathan, H., Borges, R. M. & Chakravarthy, V. S. (2004) Does neighborhood floral display matter? Fruit set in carpenter bee-pollinated *Heterophragma quadriloculare* and beetle-pollinated *Lasiosiphon eriocephalus*. *Biotropica*, **36**, 139-147.
- Sowig, P. (1989) Effects of flowering plant's patch size on species composition of pollinator communities, foraging strategies, and resource partitioning in bumblebees (Hymenoptera: Apidae). *Oecologia*, **78**, 550-558.
- Sperelakis, N., Sunagawa, M., Nakamura, M., Yokoshiki, H. & Seki, T. (2001) Toxins that affect ion channels, pumps, and exchangers. *Cardiovascular Toxicology* (ed D. Acosta, Jr.), pp. 322-367. Taylor & Francis, London.
- Spigler, R. B. & Chang, S.-M. (2008) Effects of plant abundance on reproductive success in the biennial *Sabatia angularis* (Gentianaceae): spatial scale matters. *Journal of Ecology*, **96**, 323-333.
- Spira, T. P. (2001) Plant-pollinator interactions: a threatened mutualism with implications for the ecology and management of rare plants. *Natural Areas Journal*, **21**, 78-88.
- Sprengel, C. K. (1793) *Das entdeckte Geheimniß der Natur im Bau und in der Befruchtung der Blumen*. Friedrich Vieweg der Ältere, Berlin.
- SPSS Inc. (2006) SPSS for Windows, Release 15.0.0. SPSS Inc., Chicago, Illinois.
- Stang, M., Klinkhamer, P. G. L. & van der Meijden, E. (2006) Size constraints and flower abundance determine the number of interactions in a plant-flower visitor web. *Oikos*, **112**, 111-121.
- Starý, P. & Tkalců, B. (1998) Bumble-bees (Hym., Bombidae) associated with the expansive touch-me-not, *Impatiens glandulifera* in wetland biocorridors. *Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz*, **71**, 85-87.
- Stead, A. D. & Moore, K. G. (1979) Studies on flower longevity in *Digitalis*: pollination induced corolla abscission in *Digitalis* flowers. *Planta*, **146**, 409-514.
- Stebbins, G. L. (1970) Adaptive radiation of reproductive characteristics in angiosperms. 1. Pollination mechanisms. *Annual Review of Ecology and Systematics*, **1**, 307-326.
- Steffan-Dewenter, I. (2002) Landscape context affects trap-nesting bees, wasps, and their natural enemies. *Ecological Entomology*, **27**, 631-637.
- Steffan-Dewenter, I. & Tschardtke, T. (1999) Effects of habitat isolation on pollinator communities and seed set. *Oecologia*, **121**, 432-440.
- Steffan-Dewenter, I. & Westphal, C. (2008) The interplay of pollinator diversity, pollination services and landscape change. *Journal of Applied Ecology*, **45**, 737-741.
- Stephenson, A. G. (1981) Toxic nectar deters thieves of *Catalpa speciosa*. *American Midland Naturalist*, **105**, 381-383.
- Stephenson, A. G. (1982) Iridoid glycosides in the nectar of *Catalpa speciosa* are unpalatable to nectar thieves. *Journal of Chemical Ecology*, **8**, 1025-1034.





- Steven, J. C., Rooney, T. P., Boyle, O. D. & Waller, D. M. (2003) Density-dependent pollinator visitation and self-incompatibility in Upper Great Lakes populations of *Trillium grandiflorum*. *Journal of the Torrey Botanical Society*, **130**, 23-29.
- Stinson, K. A., Campbell, S. A., Powell, J. R., Wolfe, B. E., Callaway, R. M., Thelen, G. C., Hallett, S. G., Prati, D. & Klironomos, J. N. (2006) Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. *PLoS Biology*, **4**, e140.
- Stone, G. N., Willmer, P. & Rowe, J. A. (1998) Partitioning of pollinators during flowering in an African *Acacia* community. *Ecology*, **79**, 2808-2827.
- Stout, J. C. (1999) *The foraging ecology of bumblebees*. PhD thesis, University of Southampton, Southampton.
- Stout, J. C. (2000) Does size matter? Bumblebee behaviour and the pollination of *Cytisus scoparius* L. (Fabaceae). *Apidologie*, **31**, 129-139.
- Stout, J. C. (2007a) Pollination of invasive *Rhododendron ponticum* (Ericaceae) in Ireland. *Apidologie*, **38**, 198-206.
- Stout, J. C. (2007b) Reproductive biology of the invasive exotic shrub, *Rhododendron ponticum* L. (Ericaceae). *Botanical Journal of the Linnean Society*, **155**, 373-381.
- Stout, J. C., Allen, J. A. & Goulson, D. (1998) The influence of relative plant density and floral morphological complexity on the behaviour of bumblebees. *Oecologia*, **117**, 543-550.
- Stout, J. C., Allen, J. A. & Goulson, D. (2000) Nectar robbing, forager efficiency and seed set: bumblebees foraging on the self incompatible plant *Linaria vulgaris* (Scrophulariaceae). *Acta Oecologica*, **21**, 277-283.
- Stout, J. C. & Goulson, D. (2001) The use of conspecific and interspecific scent marks by foraging bumblebees and honeybees. *Animal Behaviour*, **62**, 183-189.
- Stout, J. C., Kells, A. R. & Goulson, D. (2002) Pollination of the invasive exotic shrub *Lupinus arboreus* (Fabaceae) by introduced bees in Tasmania. *Biological Conservation*, **106**, 425-434.
- Stout, J. C. & Morales, C. L. (submitted) Ecological impacts of invasive alien species on bees. *Apidologie*.
- Stout, J. C., Parnell, J. A. N., Arroyo, J. & Crowe, T. P. (2006) Pollination ecology and seed production of *Rhododendron ponticum* in native and exotic habitats. *Biodiversity and Conservation*, **15**, 755-777.
- Strauss, S. Y. & Irwin, R. E. (2004) Ecological and evolutionary consequences of multispecies plant-animal interactions. *Annual Review of Ecology, Evolution, and Systematics*, **35**, 435-466.
- Strauss, S. Y., Lau, J. A. & Carroll, S. P. (2006) Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? *Ecology Letters*, **9**, 357-374.
- Sukhada, D. K. & Jayachandra (1980) Pollen allelopathy - a new phenomenon. *New Phytologist*, **84**, 739-746.
- Sutton, C. A. & Wilkinson, D. M. (2007) The effects of *Rhododendron* on testate amoebae communities in woodland soils in north west England. *Acta Protozoologica*, **46**, 333-338.
- Suzuki, K., Dohzono, I., Hiei, K. & Fukuda, Y. (2002) Pollination effectiveness of three bumblebee species on flowers of *Hosta sieboldiana* (Liliaceae) and its relation to floral structure and pollinator sizes. *Plant Species Biology*, **17**, 139-146.
- Takahashi, S., Shibata, M. & Fukuuchi, Y. (1999) Role of sodium ion influx in depolarization-induced neuronal cell death by high KCl or veratridine. *European Journal of Pharmacology*, **372**, 297-304.
- Tan, K., Guo, Y. H., Nicolson, S. W., Radloff, S. E., Song, Q. S. & Hepburn, H. R. (2007) Honeybee (*Apis cerana*) foraging responses to the toxic honey of *Tripterygium hypoglaucum* (Celastraceae): changing threshold of nectar acceptability. *Journal of Chemical Ecology*, **33**, 2209-2217.
- Tasei, J. N. (2001) Effects of insect growth regulators on honey bees and non-*Apis* bees. A review. *Apidologie*, **32**, 527-545.





- Tepedino, V. J., Bradley, B. A. & Griswold, T. L. (2008) Might flowers of invasive plants increase native bee carrying capacity? Intimations from Capitol Reef National Park, Utah. *Natural Areas Journal*, **28**, 44–50.
- Theoharides, K. A. & Dukes, J. S. (2007) Plant invasion across space and time: factors affecting nonindigenous species success during four stages of invasion. *New Phytologist*, **176**, 256–273.
- Thompson, J. N. (1982) *Interaction and Coevolution*. John Wiley & Sons, New York.
- Thompson, J. N. (1999) The evolution of species interactions. *Science*, **284**, 2116–2118.
- Thomson, J. D. (1978) Effect of stand composition on insect visitation in two-species mixtures of *Hieracium*. *American Midland Naturalist*, **100**, 431–440.
- Thomson, J. D. (1981) Spatial and temporal components of resource assessment by flower-feeding insects. *Journal of Animal Ecology*, **50**, 49–59.
- Thomson, J. D. (1982) Patterns of visitation by animal pollinators. *Oikos*, **39**, 241–250.
- Thomson, J. D. (1986) Pollen transport and deposition by bumble bees in *Erythronium*: influences of floral nectar and bee grooming. *Journal of Ecology*, **74**, 329–341.
- Thomson, J. D., Peterson, S. C. & Harder, L. D. (1987) Response of traplining bumble bees to competition experiments: shifts in feeding location and efficiency. *Oecologia*, **71**, 295–300.
- Totland, Ø. (1994) Intraseasonal variation in pollination intensity and seed set in an alpine population of *Ranunculus acris* in southwestern Norway. *Ecography*, **17**, 159–165.
- Totland, Ø., Nielsen, A., Bjerknes, A.-L. & Ohlson, M. (2006) Effects of an exotic plant and habitat disturbance on pollinator visitation and reproduction in a boreal forest herb. *American Journal of Botany*, **93**, 868–873.
- Traveset, A. (1999) Ecology of plant reproduction: mating systems and pollination. *Handbook of Functional Plant Ecology* (eds F. I. Pugnaire & F. Valladares), pp. 545–588. Marcel Dekker, New York.
- Traveset, A. & Richardson, D. M. (2006) Biological invasions as disruptors of plant reproductive mutualisms. *Trends in Ecology and Evolution*, **21**, 208–216.
- Traveset, A., Willson, M. F. & Sabag, C. (1998) Effect of nectar-robbing birds on fruit set of *Fuchsia magellanica* in Tierra del Fuego: a disrupted mutualism. *Functional Ecology*, **12**, 459–464.
- Underwood, A. J. (1986) The analysis of competition by field experiments. *Community Ecology: Pattern and Process* (eds J. Kikkawa & D. J. Anderson), pp. 240–268. Blackwell Scientific Publications, Melbourne.
- Underwood, A. J. (1994) On beyond BACI: sampling designs that might reliably detect environmental disturbances. *Ecological Applications*, **4**, 3–15.
- Underwood, A. J. (1997) *Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance*, 1<sup>st</sup> edn. Cambridge University Press, Cambridge.
- Underwood, A. J. & Chapman, M. G. (1998) GMAV for Windows, Version 5. Institute of Marine Ecology, University of Sydney, Sydney.
- Usher, M. B. (1986) Invasibility and wildlife conservation: invasive species on nature reserves. *Philosophical Transactions of the Royal Society of London Series B*, **314**, 695–710.
- Valentine, D. H. (1978) The pollination of introduced species, with special reference to the British Isles and the genus *Impatiens*. *The Pollination of Flowers by Insects* (ed A. J. Richards), pp. 117–123. Academic Press, London.
- van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M. & Shipley, P. F. (2004) Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.
- van Treuren, R., Bijlsma, R., Ouborg, N. J. & Kwak, M. M. (1994) Relationships between plant density, outcrossing rates and seed set in natural and experimental populations of *Scabiosa columbaria*. *Journal of Evolutionary Biology*, **7**, 287–302.
- Vilà, M., Tessier, M., Suehs, C. M., Brundu, G., Carta, L., Galanidis, A., Lambdon, P., Manca, M., Médail, F., Moragues, E., Traveset, A., Troumbis, A. Y. & Hulme, P. E. (2006) Local and regional assessments of the impacts of plant invaders on vegetation structure and soil properties of Mediterranean islands. *Journal of Biogeography*, **33**, 853–861.





- Vitousek, P. M., D'Antonio, C. M., Loope, L. L., Rejmánek, M. & Westbrooks, R. (1997) Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology*, **21**, 1-16.
- Vogel, S. (1974) *Ölblumen und ölsammelnde Bienen*. Tropische und subtropische Pflanzenwelt 7. Akademie der Wissenschaften und der Literatur, Mainz.
- von Malottki, K. & Wiechmann, H. W. (1996) Akute lebensbedrohliche Bradykardie: Nahrungsmittelintoxikation durch türkischen Waldhonig. *Deutsche Medizinische Wochenschrift*, **121**, 936-938.
- Wacht, S., Lunau, K. & Hansen, K. (1996) Optical and chemical stimuli control pollen feeding in the hoverfly *Eristalis tenax*. *Entomologia Experimentalis Et Applicata*, **80**, 50-53.
- Waddington, K. D. (1983) Foraging behaviour of pollinators. *Pollination Biology* (ed L. A. Real), pp. 213-239. Academic Press, New York.
- Waddington, K. D. & Heinrich, B. (1981) Patterns of movement and floral choice by foraging bees. *Foraging Behaviour: Ecological, Ethological and Psychological Approaches* (eds A. C. Kamil & T. D. Sargent), pp. 215-230. Garland Press, New York.
- Waites, A. R. & Ågren, J. (2004) Pollinator visitation, stigmatic pollen loads and among-population variation in seed set in *Lythrum salicaria*. *Journal of Ecology*, **92**, 512-526.
- Walsh, P. S., Metzger, D. A. & Higuchi, R. (1991) Chelex 100<sup>®</sup> as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques*, **10**, 506-513.
- Walther-Hellwig, K. & Frankl, R. (2000) Foraging distances of *Bombus muscorum*, *Bombus lapidarius*, and *Bombus terrestris* (Hymenoptera, Apidae). *Journal of Insect Behavior*, **13**, 239-246.
- Wang, J. L. (2004) Sibship reconstruction from genetic data with typing errors. *Genetics*, **166**, 1963-1979.
- Waring, G. H., Loope, L. L. & Medeiros, A. C. (1993) Study on use of alien versus native plants by nectarivorous forest birds on Maui, Hawaii. *Auk*, **110**, 917-920.
- Warrant, E. J. (2007) Nocturnal bees. *Current Biology*, **17**, R991-R992.
- Waser, N. M. (1978) Interspecific pollen transfer and competition between co-occurring plant species. *Oecologia*, **36**, 223-236.
- Waser, N. M. (1983) Competition for pollination and floral character differences among sympatric plant species: a review of evidence. *Handbook of Experimental Pollination Ecology* (eds C. E. Jones & R. J. Little), pp. 277-293. Van Nostrand Reinhold, New York.
- Waser, N. M. (1988) Comparative pollen and dye transfer by pollinators of *Delphinium nelsonii*. *Functional Ecology*, **2**, 41-48.
- Waser, N. M., Chittka, L., Price, M. V., Williams, N. M. & Ollerton, J. (1996) Generalization in pollination systems, and why it matters. *Ecology*, **77**, 1043-1060.
- Waser, N. M. & Fugate, M. L. (1986) Pollen precedence and stigma closure: a mechanism of competition for pollination between *Delphinium nelsonii* and *Ipomopsis aggregata*. *Oecologia*, **70**, 573-577.
- Waser, N. M. & Price, M. V. (1991) Reproductive costs of self-pollination in *Ipomopsis aggregata* (Polemoniaceae): are ovules usurped? *American Journal of Botany*, **78**, 1036-1043.
- Waser, N. M. & Real, L. A. (1979) Effective mutualism between sequentially flowering plant species. *Nature*, **281**, 670-672.
- Watanabe, A., Goka, K. & Washitani, I. (2003) Effects of population spatial structure on the quantity and quality of seeds set by *Primula sieboldii* (Primulaceae). *Plant Species Biology*, **18**, 107-121.
- Weltzin, J. F., Belote, R. T. & Sanders, N. J. (2003) Biological invaders in a greenhouse world: will elevated CO<sub>2</sub> fuel plant invasions? *Frontiers in Ecology and the Environment*, **1**, 146-153.
- Wesselingh, R. A. & Arnold, M. L. (2000) Nectar production in Louisiana iris hybrids. *International Journal of Plant Sciences*, **161**, 245-251.
- Westerkamp, C. (1996) Pollen in bee-flower relations - some considerations on melittophily. *Botanica Acta*, **109**, 325-332.





- Westphal, C., Steffan-Dewenter, I. & Tscharntke, T. (2003) Mass flowering crops enhance pollinator densities at a landscape scale. *Ecology Letters*, **6**, 961-965.
- Westrich, P. (1989) *Die Wildbienen Baden-Württembergs*. Verlag Eugen Ulmer, Stuttgart.
- Westrich, P. (1996) Habitat requirements of central European bees and the problems of partial habitats. *The Conservation of Bees* (eds A. Matheson, S. L. Buchmann, C. O'Toole, P. Westrich & I. H. Williams), pp. 1-16. Academic Press, London.
- Westrich, P. & Schmidt, K. (1987) Pollenanalyse, ein Hilfsmittel beim Studium des Sammelverhaltens von Wildbienen (Hymenoptera, Apoidea). *Apidologie*, **18**, 199-214.
- Wiens, J. A. (1977) On competition and variable environments. *American Scientist*, **65**, 590-597.
- Wilcock, C. & Neiland, R. (2002) Pollination failure in plants: why it happens and when it matters. *Trends in Plant Science*, **7**, 270-277.
- Wilcove, D. S., Rothstein, D., Dubow, J., Phillips, A. & Losos, E. (1998) Quantifying threats to imperiled species in the United States. *Bioscience*, **48**, 607-615.
- Williams, N. M., Minckley, R. L. & Silveira, F. A. (2001) Variation in native bee faunas and its implications for detecting community changes. *Conservation Ecology*, **5**, 7. [online] URL: <http://www.consecol.org/vol5/iss1/art7>.
- Williams, N. M. & Tepedino, V. J. (2003) Consistent mixing of near and distant resources in foraging bouts by the solitary mason bee *Osmia lignaria*. *Behavioral Ecology*, **14**, 141-149.
- Williams, P. H., Araújo, M. B. & Rasmont, P. (2007) Can vulnerability among British bumblebee (*Bombus*) species be explained by niche position and breadth? *Biological Conservation*, **138**, 493-505.
- Willis, S. G. & Hulme, P. E. (2002) Does temperature limit the invasion of *Impatiens glandulifera* and *Heracleum mantegazzianum* in the UK? *Functional Ecology*, **16**, 530-539.
- Winer, B. J., Brown, D. R. & Michels, K. M. (1991) *Statistical Principles in Experimental Design*, 3<sup>rd</sup> edn. McGraw-Hill, New York.
- Winfree, R., Williams, N. M., Gaines, H., Ascher, J. S. & Kremen, C. (2007) Wild bee pollinators provide the majority of crop visitation across land-use gradients in New Jersey and Pennsylvania, USA. *Journal of Applied Ecology*, **45**, 793-802.
- Wolf, S. & Moritz, R. F. A. (2008) Foraging distance in *Bombus terrestris* L. (Hymenoptera: Apidae). *Apidologie*, **39**, 419-427.
- Wong, J., Youde, E., Dickinson, B. & Hale, M. (2002) Report of the *Rhododendron* feasibility study. pp. 1-73. School of Agricultural and Forest Sciences, University of Wales, Bangor.
- Woodell, S. R. J. (1978) Directionality in bumblebees in relation to environmental factors. *The Pollination of Flowers by Insects* (ed A. J. Richards), pp. 31-39. Academic Press, London.
- Yang, C. F., Gituru, R. W. & Guo, Y. H. (2007) Reproductive isolation of two sympatric louseworts, *Pedicularis rhinanthoides* and *Pedicularis longiflora* (Orobanchaceae): how does the same pollinator type avoid interspecific pollen transfer? *Biological Journal of the Linnean Society*, **90**, 37-48.
- Yurkonis, K. A. & Meiners, S. J. (2004) Invasion impacts local species turnover in a successional system. *Ecology Letters*, **7**, 764-769.
- Zar, J. H. (1999) *Biostatistical Analysis*, 4<sup>th</sup> edn. Prentice Hall, London.
- Zhang, Y. W., Robert, G. W., Wang, Y. & Guo, Y. H. (2007) Nectar robbing of a carpenter bee and its effects on the reproductive fitness of *Glechoma longituba* (Lamiaceae). *Plant Ecology*, **193**, 1-13.
- Zimmerman, M. (1988) Nectar production, flowering phenology, and strategies for pollination. *Plant Reproductive Ecology. Patterns and Strategies* (eds J. Lovett Doust & L. Lovett Doust), pp. 157-178. Oxford University Press, New York.
- Zimmerman, M. & Pyke, G. H. (1986) Reproduction in *Polemonium*: patterns and implications of floral nectar production and standing crops. *American Journal of Botany*, **73**, 1405-1415.
- Zobel, M. (2003) *Königinnen-Konkurrenz während des Nestbaus und der Nestversorgung bei der Furchenbiene Lasiglossum malachurum*. Diplom thesis, Eberhardt-Karls-Universität, Tübingen.





Zorn-Arnold, B. & Howe, H. F. (2007) Density and seed set in a self-compatible forb, *Penstemon digitalis* (Plantaginaceae), with multiple pollinators. *American Journal of Botany*, **94**, 1594-1602.

## **Chapter 8**







8. Supplementary material

Supplement 1. Table of locations with studied sites, if *Rhododendron ponticum* is present/absent, geographical position, elevation ("Elev" in m above sea level) and characteristics of sites.

| Location   | Studied site                             | <i>R. ponticum</i> | Position                 | Elev (m) | Site characteristics                                             |
|------------|------------------------------------------|--------------------|--------------------------|----------|------------------------------------------------------------------|
| West Coast | Recess,<br>Connemara,<br>Co. Galway      | present            | 53°28'00"N<br>09°44'20"W | 20       | Flat, lake site habitat, edge of open woodland                   |
|            | Bunscanniff,<br>Connemara,<br>Co. Galway | absent             | 53°27'20"N<br>09°36'40"W | 50       | Flat/gentle S facing slope, edge of blanket bog and heath        |
| East Coast | Howth Head,<br>Co. Dublin                | present            | 53°22'36"N<br>06°04'12"W | 130      | Gentle NW facing slope, coastal habitat, edge of woodland, heath |
|            | Glencullen,<br>Ballybrack,<br>Co. Dublin | present            | 53°13'49"N<br>06°15'56"W | 330      | Steep SW facing slope, edge of blanket bog and heath             |
|            | Bray Head, Co.<br>Wicklow                | absent             | 53°11'22"N<br>06°05'00"W | 180      | Steep NE facing slope, coastal habitat, heath                    |
|            | Glencullen,<br>Tibradden,<br>Co. Dublin  | absent             | 53°14'21"N<br>06°17'38"W | 300      | Gentle W facing slope, edge of woodland and blanket bog          |





Supplement 2. Observed native plant species, their flowering time, if nectar standing crop and/or secretion rate was measured and where plant species were investigated (indicated by ‘x’). Flowering time was defined due to flowering of *R. ponticum*.

| Native plant species                                       | Flowering time | Nectar standing crop/secretion measured? | West Coast                 |                           | East Coast                 |                           |                |
|------------------------------------------------------------|----------------|------------------------------------------|----------------------------|---------------------------|----------------------------|---------------------------|----------------|
|                                                            |                |                                          | <i>R. ponticum</i> present | <i>R. ponticum</i> absent | <i>R. ponticum</i> present | <i>R. ponticum</i> absent |                |
| <i>Endymion non-scriptus</i> (Liliaceae)                   | Early          | Yes/Yes                                  |                            |                           | x                          | x                         |                |
| <i>Vaccinium myrtillus</i> (Ericaceae)                     | Early          | Yes/Yes                                  |                            |                           | x                          | x                         | x              |
| <i>Ilex aquifolium</i> (Aquifoliaceae)                     | Early & peak   | Yes/Yes                                  | x <sup>1</sup>             | x <sup>1</sup>            | x                          | x                         | x              |
| <i>Corydalis claviculata</i> (Fumariaceae)                 | Peak           | Yes/No                                   |                            |                           |                            |                           | x              |
| <i>Crataegus monogyna</i> (Rosaceae)                       | Peak           | Yes/Yes                                  | x                          | x                         | x                          | x                         | x              |
| <i>Potentilla erecta</i> (Rosaceae)                        | Peak           | Yes/No                                   |                            |                           | x                          |                           | x              |
| <i>Ulex europaeus</i> (Fabaceae)                           | Peak           | No nectar                                | x                          | x                         | x                          |                           | x              |
| <i>Vicia sepium</i> (Fabaceae)                             | Peak           | Yes/Yes                                  |                            |                           |                            | x                         | x              |
| <i>Digitalis purpurea</i> (Scrophulariaceae)               | Peak & late    | Yes/Yes                                  | x                          | x                         | x <sup>3</sup>             |                           | x <sup>3</sup> |
| <i>Geranium robertianum</i> (Geraniaceae)                  | Peak & late    | Yes/Yes                                  |                            |                           | x                          |                           | x              |
| <i>Lonicera periclymenum</i> <sup>2</sup> (Caprifoliaceae) | Peak & late    | Yes/Yes                                  |                            | x <sup>3</sup>            | x                          | x                         | x <sup>3</sup> |
| <i>Lotus corniculatus</i> (Fabaceae)                       | Peak & late    | Yes/Yes                                  |                            | x                         | x                          | x                         | x              |
| <i>Taraxacum officinale</i> (Asteraceae)                   | Peak & late    | No/No                                    |                            | x                         | x                          |                           | x              |
| <i>Trifolium pratense</i> (Fabaceae)                       | Peak & late    | Yes/Yes                                  |                            | x <sup>3</sup>            | x <sup>4</sup>             | x <sup>3</sup>            | x <sup>4</sup> |
| <i>Cirsium palustre</i> (Asteraceae)                       | Late           | Yes/Yes                                  | x                          | x                         |                            | x                         | x              |
| <i>Chamaenerion angustifolium</i> (Onagraceae)             | Late           | Yes/Yes                                  |                            |                           | x                          |                           | x              |
| <i>Erica cinerea</i> (Ericaceae)                           | Late           | Yes/Yes                                  |                            | x                         | x                          | x                         | x              |
| <i>Hypericum androsaemum</i> (Hypericaceae)                | Late           | No nectar                                |                            |                           |                            | x                         |                |
| <i>Hypericum pulchrum</i> (Hypericaceae)                   | Late           | No nectar                                |                            |                           |                            | x                         | x              |
| <i>Prunella vulgaris</i> (Lamiaceae)                       | Late           | Yes/No                                   |                            |                           |                            |                           | x              |
| <i>Rubus fruticosus</i> (Rosaceae)                         | Late           | Yes/Yes                                  | x                          | x                         | x                          | x                         | x              |
| <i>Teucrium scorodonia</i> (Lamiaceae)                     | Late           | Yes/Yes                                  |                            |                           |                            | x                         | x              |

<sup>1</sup> Only at early flowering of *R. ponticum*. <sup>2</sup> ANOVA for this species used only data from late flowering season. <sup>3</sup> Only at late flowering of *R. ponticum*. <sup>4</sup> Only at peak flowering of *R. ponticum*.



Supplement 3. Table of analyses design of pollinator visitation rates of individual native species. Type of statistical analysis (symmetrical = sym; asymmetrical = asym; separate analyses for different factors = sep), type of transformation, main factors with numbers of levels and the number of replicates are displayed. Mean squares of factors were eliminated or pooled with residual mean squares in the case *F*-ratios of associated tests were not significant at  $\alpha = 0.25$  (Winer et al., 1991).

| Plant species                | Type of analysis | Trans-formation   | Number of levels of main factors |                  |            |                   |                | Replication |
|------------------------------|------------------|-------------------|----------------------------------|------------------|------------|-------------------|----------------|-------------|
|                              |                  |                   | Invasion (I)                     | Location (L)     | Season (S) | Site (Si)         | Day (D)        |             |
| <i>Cirsium vulgare</i>       | sym              | none              | 2                                | 2                | -          | -                 | 2<br>(pooled)  | 3           |
| <i>Crataegus monogyna</i>    | asym             | none              | 2                                | [2] <sup>a</sup> | -          | 2                 | 2              | 3           |
| <i>Digitalis purpurea</i>    | sep              | x <sup>0.5</sup>  | 2                                | 2                | 2          | -                 | 2<br>(pooled)  | 3           |
| <i>Ilex aquifolium</i>       | sep              | x <sup>0.5</sup>  | 2                                | 2                | 2          | 2                 | 2<br>(pooled)  | 3           |
| <i>Lonicera periclymenum</i> | asym             | none              | 2                                | [2] <sup>a</sup> | -          | 2                 | 2<br>(pooled)  | 3           |
| <i>Lotus corniculatus</i>    | asym             | none              | 2                                | [2] <sup>a</sup> | 2          | 2                 | 2<br>(pooled)  | 3           |
| <i>Rubus fruticosus</i>      | asym             | x <sup>0.25</sup> | 2                                | [2] <sup>a</sup> | -          | 2<br>(eliminated) | 2<br>(pooled)  | 3           |
| <i>Ulex europaeus</i>        | sym              | none              | 2                                | 2                | -          | -                 | - <sup>b</sup> | 4           |
| <i>Vaccinium myrtillus</i>   | sym              | x <sup>0.25</sup> | 2                                | -                | -          | 2                 | 2<br>(pooled)  | 3           |

<sup>a</sup> Number in squared brackets indicate indirect factor of asymmetrical analysis

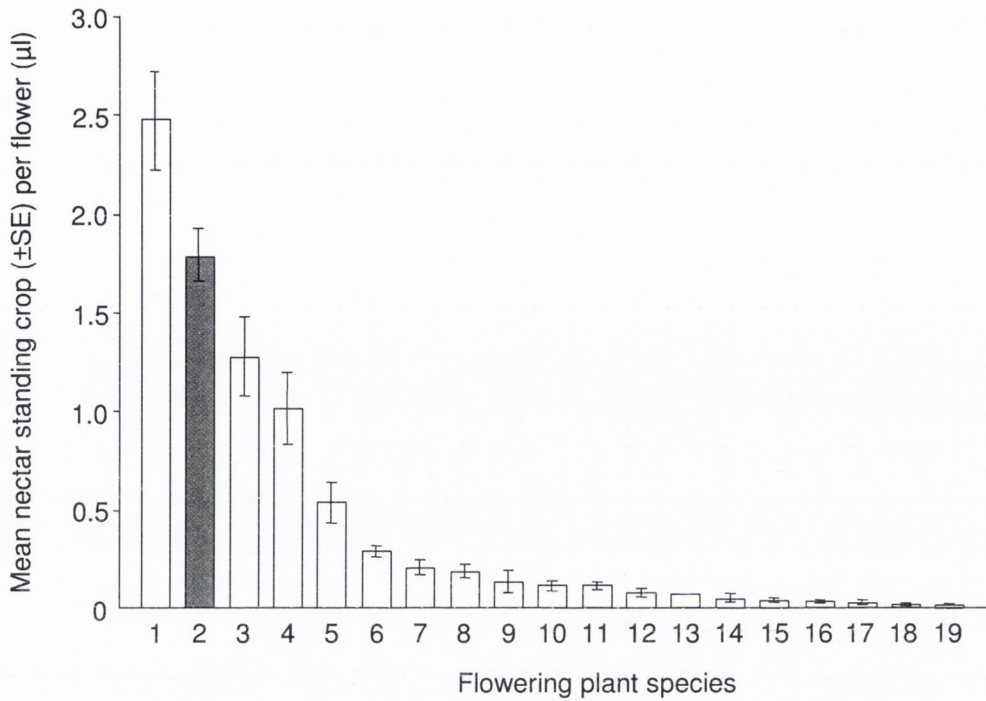
<sup>b</sup> At each site data only collected at a single day



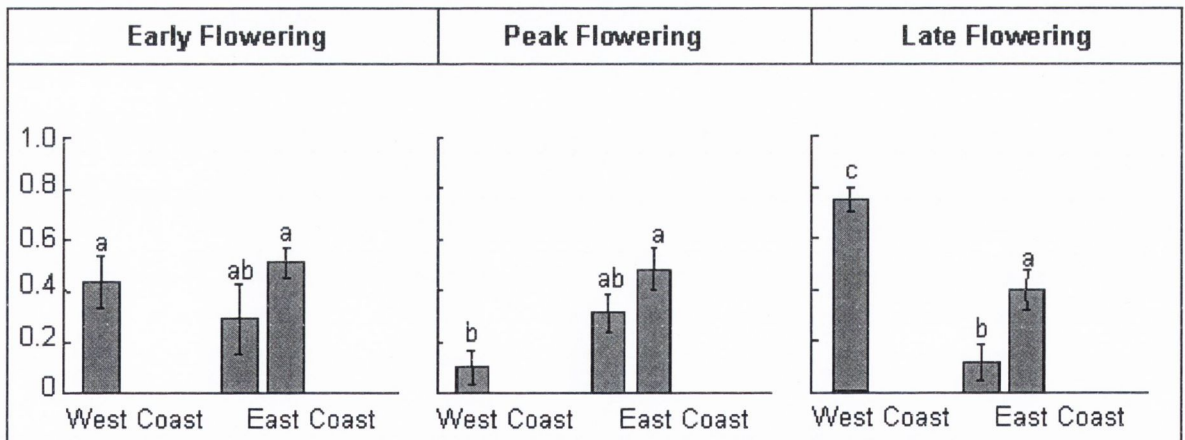


Supplement 4. Table of Fligner-Policello test statistic for pairwise comparisons between each individual native plant species and *Rhododendron ponticum*. I compared nectar standing crop (st.crop), nectar sugar secretion rate (sugar) and nectar volume secretion rate (volume). “df” refers to degrees of freedom of “native species, *R. ponticum*”. Fligner-Policello U was adjusted according to the Dunn-Sidák method (Day and Quinn, 1989). The statistic  $\hat{U}$  for large sample sizes approximates an asymptotic  $N(0,1)$  distribution (Hollander and Wolfe, 1999).  $P = 0.05^*$ ,  $P = 0.01^{**}$

| Native spp versus <i>R. ponticum</i> | df (st. crop) | <i>U</i> (st. crop) | df (secretion) | <i>U</i> (sugar) | <i>U</i> (volume) |
|--------------------------------------|---------------|---------------------|----------------|------------------|-------------------|
| <i>Cirsium palustre</i>              | 100,276       | -26.879 **          | 23,69          | -0.716           | -2.843 *          |
| <i>Chamaenerion angustifolium</i>    | 46,276        | -11.859 **          | 13,69          | -0.966           | -1.476            |
| <i>Corydalis claviculata</i>         | 30,276        | -13.158 **          | -              | -                | -                 |
| <i>Crataegus monogyna</i>            | 155,276       | -6.403 **           | 32,69          | -1.458           | -0.812            |
| <i>Digitalis purpurea</i>            | 176,276       | -5.268 **           | 38,69          | 1.182            | 0.627             |
| <i>Endymion non-scriptus</i>         | 64,276        | -13.274 **          | 16,69          | -2.479 *         | 0.216             |
| <i>Erica cinerea</i>                 | 80,276        | -32.782 **          | 11,69          | -13.199 **       | -13.199 **        |
| <i>Geranium robertianum</i>          | 36,276        | -11.205 **          | 14,69          | -0.435           | -0.911            |
| <i>Hypericum androsaemum</i>         | 15,276        | -46.290 **          | -              | -                | -                 |
| <i>Hypericum pulchrum</i>            | 38,276        | -48.123 **          | -              | -                | -                 |
| <i>Ilex aquifolium</i>               | 205,276       | -19.636 **          | 40,69          | 0.345            | 1.764             |
| <i>Lonicera periclymenum</i>         | 196,276       | 0.803               | 33,69          | -4.163 **        | -4.313 **         |
| <i>Lotus corniculatus</i>            | 255,276       | -13.546 **          | 59,69          | -3.379 **        | -3.657 **         |
| <i>Potentilla erecta</i>             | 63,276        | -28.877 **          | -              | -                | -                 |
| <i>Prunella vulgaris</i>             | 13,276        | -9.728 **           | -              | -                | -                 |
| <i>Rubus fruticosus</i>              | 163,276       | -11.668 **          | 37,69          | 3.177            | 2.026             |
| <i>Teucrium scorodonia</i>           | 57,276        | -17.461 **          | 12,69          | 0.035            | -0.256            |
| <i>Trifolium pratense</i>            | 103,276       | -25.311 **          | 6,69           | -1.122           | -2.927 *          |
| <i>Vaccinium myrtillus</i>           | 166,276       | -8,641 **           | 41,69          | -0.539           | -0.229            |
| <i>Vicia sepium</i>                  | 57,276        | -12.205 **          | 13,69          | -3.517 **        | -4.394 **         |



Supplement 5. Mean nectar standing crop of investigated flowering plant species. The bar in grey corresponds to alien invasive *R. ponticum*. 1 = *L. periclymenum*, 2 = *R. ponticum*, 3 = *D. purpurea*, 4 = *V. myrtillus*, 5 = *R. fruticosus*, 6 = *L. corniculatus*, 7 = *I. aquifolium*, 8 = *E. non-scriptus*, 9 = *P. vulgaris*, 10 = *T. scorodonia*, 11 = *V. sepium*, 12 = *C. angustifolium*, 13 = *G. robertianum*, 14 = *T. pratense*, 15 = *C. palustre*, 16 = *E. cinerea*, 17 = *C. claviculata*, 18 = *C. monogyna*, 19 = *P. erecta*.



Supplement 6. Pollinator visitation rates *Rhododendron ponticum*. Each bar represents a specific site at a specific flowering season. X-axes separate data into different locations. Y-axes show mean number (±SE) of pollinator visits per flower per 20 min. The three graphs display data from different flowering seasons. Bars without letters in common are significantly different from each other at  $P < 0.05$ . For comparison with native species see Fig. 2.1.





Supplement 7. Name and characteristics of study sites used for the manipulative experiment (Chapter 3, p. 57).

| Name of location                            | Altitude (m) | Latitude     | Longitude     | Treatment (no. aliens/no. natives) |
|---------------------------------------------|--------------|--------------|---------------|------------------------------------|
| Ballydoydowling, Glenealy                   | 115          | 6°11'04.1" W | 52°57'46.5" N | 12/3                               |
| Carrick Mountain, Sleanaglogh, Ashford      | 212          | 6°11'10.5" W | 52°58'45.4" N | 0/12                               |
| Ballinakill, Rathdrum                       | 137          | 6°12'45.6" W | 52°56'55.4" N | 3/12                               |
| Pollahoney, Arklow                          | 103          | 6°13'14.5" W | 52°48'15.3" N | 0/24                               |
| Ballinastraw, Stump of the Castle, Rathdrum | 173          | 6°13'40.8" W | 52°57'21.9" N | 0/15                               |
| Killeagh, Aughrim                           | 137          | 6°15'43.5" W | 52°51'56.1" N | 3/3                                |
| Knocknamohill, Woodenbridge                 | 51           | 6°15'44.3" W | 52°50'32.8" N | 0/3                                |
| Tinnakilly Upper, Aughrim                   | 158          | 6°18'28.7" W | 52°52'04.9" N | 0/6                                |
| Ow Valley, Craffield, Aughrim               | 98           | 6°21'26.7" W | 52°51'49.1" N | 12/12                              |
| Toberlownagh, Aughrim                       | 208          | 6°20'58.5" W | 52°48'53.5" N | 0/3                                |
| Ballymanus, Aughrim                         | 130          | 6°23'29.5" W | 52°52'46.6" N | 0/15                               |
| Ballymanus, Aughrim                         | 176          | 6°23'32.6" W | 52°52'26.3" N | 0/24                               |
| Ballymanus, Aughrim                         | 181          | 6°23'56.6" W | 52°52'40.2" N | 0/12                               |
| Coolalug Wood, Tinahely                     | 100          | 6°24'43.7" W | 52°48'35.1" N | 12/3                               |
| Askakeagh, Aughrim                          | 184          | 6°25'35.6" W | 52°51'12.7" N | 12/12                              |
| Ballinglen, Tinahely                        | 138          | 6°25'41.5" W | 52°49'41.8" N | 12/3                               |
| Slieveroe Mountain, Ballinglen, Tinahely    | 176          | 6°26'19.6" W | 52°50'22.1" N | 0/12                               |
| Ballycumber South, Ballinglen, Tinahely     | 155          | 6°26'35.1" W | 52°49'56.5" N | 0/15                               |
| Ballycumber North, Ballinglen, Tinahely     | 160          | 6°26'59.2" W | 52°50'38.5" N | 0/3                                |
| Ballycumber South, Ballinglen, Tinahely     | 197          | 6°26'59.8" W | 52°50'18.7" N | 3/12                               |
| Ballycumber North, Ballinglen, Tinahely     | 165          | 6°27'37.9" W | 52°51'09.4" N | 3/3                                |
| Kilcarney Cross, Knockananna, Hacketstown   | 203          | 6°29'20.0" W | 52°53'47.9" N | 3/12                               |
| Mullans North, Tinahely                     | 197          | 6°29'27.3" W | 52°50'35.9" N | 0/6                                |
| Mullin Cross, Knockananna, Hacketstown      | 209          | 6°29'48.7" W | 52°54'37.6" N | 12/12                              |
| Derreen Valley, Rathcoyle, Hacketstown      | 173          | 6°30'52.5" W | 52°53'58.2" N | 0/24                               |
| Laragh, Shillelagh                          | 169          | 6°32'59.9" W | 52°47'41.7" N | 3/3                                |
| Laragh, Shillelagh                          | 193          | 6°33'53.1" W | 52°47'34.9" N | 0/6                                |



Supplement 8. Analyses of bout length among different treatments. Values represent parameter estimates of a non-linear logistic regression model that was fitted to the observed mean numbers of flowers probed in a single inflorescence visit. Means  $\pm$  asymptotic SE and 95% asymptotic confidence intervals are displayed. Regression models did not significantly differ from each other in their parameters but showed much higher variability among large and small populations, respectively.

| Bout length                                 | <i>a</i>                           | <i>d</i>                           | <i>s</i>                        | df | <i>R</i> <sup>2</sup> |
|---------------------------------------------|------------------------------------|------------------------------------|---------------------------------|----|-----------------------|
| Overall                                     | 3.30 $\pm$ 1.00<br>1.14 - 5.45     | 2.20 $\pm$ 0.77<br>0.54 - 3.87     | 0.15 $\pm$ 0.09<br>-0.05 - 0.35 | 15 | 0.678                 |
| In mixed treatments with <i>R. ponticum</i> | 2.74 $\pm$ 1.91<br>-1.59 - 7.06    | 1.63 $\pm$ 1.32<br>-1.36 - 4.62    | 0.16 $\pm$ 0.24<br>-0.38 - 0.71 | 11 | 0.411                 |
| In pure <i>D. purpurea</i> treatments       | 3.01 $\pm$ 0.68<br>1.52 - 4.501    | 2.12 $\pm$ 0.60<br>0.80 - 3.44     | 0.16 $\pm$ 0.08<br>-0.03 - 0.35 | 13 | 0.755                 |
| In large populations                        | 4.83 $\pm$ 12.64<br>-23.76 - 33.42 | 3.52 $\pm$ 10.24<br>-19.65 - 26.69 | 0.11 $\pm$ 0.22<br>-0.38 - 0.60 | 11 | 0.539                 |
| In small populations                        | 3.85 $\pm$ 9.24<br>-16.11 - 23.81  | 1.77 $\pm$ 5.71<br>-10.56 - 14.11  | 0.07 $\pm$ 0.21<br>-0.38 - 0.51 | 15 | 0.186                 |

Logistic model:  $Y = \frac{a}{1 + d * e^{-s * X}}$

$a$  = upper asymptote;  $s$  = slope of graph;  
 $d$  = ratio of upper asymptote and  $y(x=0)$





Supplement 9. Taxonomic identity, absolute (*N*) and relative (%) frequency of pollinator species that visited *R. ponticum* and *D. purpurea* at invaded field sites and *D. purpurea* at uninvaded field sites in 2006. Frequency data was used to calculated Niche Overlap Indices (see methods p. 90).

| Insect species                | <i>R. ponticum</i> |      | <i>D. purpurea</i> <sup>1</sup> |      | <i>D. purpurea</i> <sup>2</sup> |      |
|-------------------------------|--------------------|------|---------------------------------|------|---------------------------------|------|
|                               | <i>N</i>           | %    | <i>N</i>                        | %    | <i>N</i>                        | %    |
| <b>Hymenoptera</b>            | 51 %               |      | 61 %                            |      | 100 %                           |      |
| Apidae                        |                    |      |                                 |      |                                 |      |
| <i>Bombus hortorum</i>        | 10                 | 12.0 | 7                               | 25.0 | 6                               | 31.6 |
| <i>Bombus lucorum</i>         | 5                  | 6.0  | 1                               | 3.6  |                                 |      |
| <i>Bombus pascuorum</i>       | 5                  | 6.0  | 8                               | 28.6 | 13                              | 68.4 |
| <i>Bombus pratorum</i>        | 18                 | 21.7 |                                 |      |                                 |      |
| <i>Bombus terrestris</i>      | 2                  | 2.4  | 1                               | 3.6  |                                 |      |
| Andrenidae                    |                    |      |                                 |      |                                 |      |
| <i>Andrena haemorrhoa</i>     | 1                  | 1.2  |                                 |      |                                 |      |
| <i>Andrena</i> sp             | 1                  | 1.2  |                                 |      |                                 |      |
| <b>Diptera</b>                | 48 %               |      | 36 %                            |      |                                 |      |
| Syrphidae                     |                    |      |                                 |      |                                 |      |
| <i>Eristalis horticola</i>    | 4                  | 4.8  | 1                               | 3.6  |                                 |      |
| <i>Platycheirus albimanus</i> | 1                  | 1.2  |                                 |      |                                 |      |
| <i>Sericomyia silentis</i>    | 23                 | 27.7 | 1                               | 3.6  |                                 |      |
| <i>Volucella pellucens</i>    | 1                  | 1.2  |                                 |      |                                 |      |
| Syrphidae sp 1                | 1                  | 1.2  |                                 |      |                                 |      |
| Syrphidae sp 2                |                    |      | 3                               | 10.7 |                                 |      |
| Syrphidae sp 3                |                    |      | 1                               | 3.6  |                                 |      |
| Calliphoridae                 |                    |      |                                 |      |                                 |      |
| Calliphoridae sp 1            | 1                  | 1.2  |                                 |      |                                 |      |
| Calliphoridae sp 2            | 1                  | 1.2  | 2                               | 7.1  |                                 |      |
| Calliphoridae sp 3            | 1                  | 1.2  |                                 |      |                                 |      |
| Diptera sp 1                  | 1                  | 1.2  |                                 |      |                                 |      |
| Diptera sp 2                  |                    |      | 1                               | 3.6  |                                 |      |
| Diptera sp 3                  | 2                  | 2.4  |                                 |      |                                 |      |
| Diptera sp 4                  | 4                  | 4.8  | 1                               | 3.6  |                                 |      |
| <b>Lepidoptera</b>            | 1 %                |      | 4 %                             |      |                                 |      |
| Unidentified                  | 1                  | 1.2  | 1                               | 3.6  |                                 |      |
| <b>Totals</b>                 | 83                 | 100  | 28                              | 100  | 19                              | 100  |
|                               |                    |      |                                 |      |                                 |      |
| Niche overlap index           |                    |      |                                 |      |                                 |      |
| 0.402                         |                    |      |                                 |      |                                 |      |
| 0.269                         |                    |      |                                 |      |                                 |      |
| 0.881                         |                    |      |                                 |      |                                 |      |

<sup>1</sup>*D. purpurea* at invaded field sites. <sup>2</sup>*D. purpurea* at uninvaded field sites.



Supplement 10. Number of collected DNA samples per bumblebee species at each investigated site for each year of sampling. Note that for three sites in 2006 sample sizes were smaller than the intended minimum sample size of 20 due to very low bee abundances.

| Location                 | 2005                |                   | 2006                |                   |
|--------------------------|---------------------|-------------------|---------------------|-------------------|
|                          | <i>B. pascuorum</i> | <i>B. lucorum</i> | <i>B. pascuorum</i> | <i>B. lucorum</i> |
| <b>Dublin</b>            |                     |                   |                     |                   |
| Invaded                  | 50                  | 45                | 71                  | 37                |
| Uninvaded                | 50                  | 25                | 40                  | 17                |
| <b>Wicklow Mountains</b> |                     |                   |                     |                   |
| Invaded                  | 45                  | 48                | 22                  | 50                |
| Uninvaded                | 30                  | 20                | 13                  | 13                |
| <b>Totals</b>            | 175                 | 138               | 146                 | 117               |

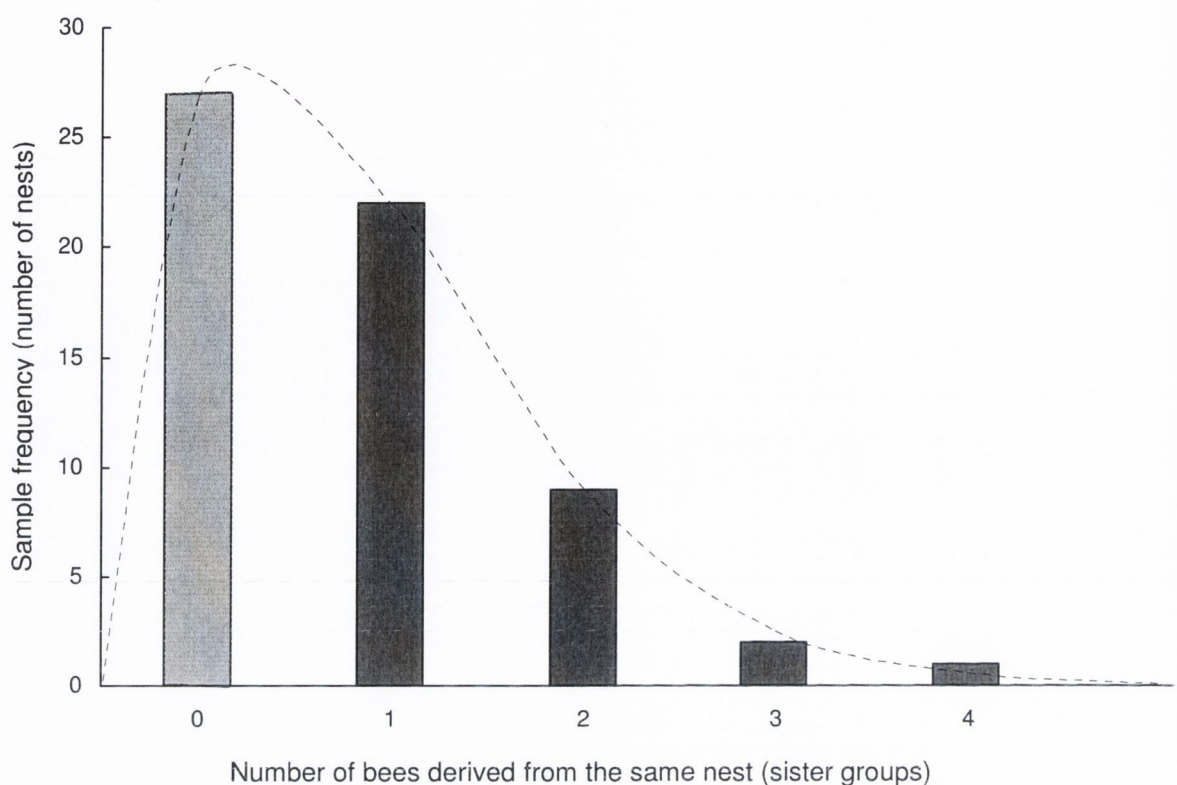
Supplement 11. Reaction cycle of performed polymerase chain reactions for different microsatellite primers.

| Step* |                  | Temperature (°C)                 | Time (sec) |
|-------|------------------|----------------------------------|------------|
| 1     | Heating          | 94                               | 180        |
| 2     | Denaturalisation | 94                               | 30         |
| 3     | Annealing        | 52 <sup>†</sup> /58 <sup>‡</sup> | 30         |
| 4     | Elongation       | 72                               | 30         |
| 5     | Extension        | 72                               | 600        |
| 6     | Pause            | 4                                | ∞          |

\* 34 cycles of steps 2 – 4

<sup>†</sup> B10, B11, B96, B119, B121    <sup>‡</sup> B100, B101, B116, B118, B124, B131, B132





Supplement 12. Example of a sister group sample distribution of *B. pascuorum* workers at the invaded Dublin site in 2005. The zero category (light grey bar) was not sampled but fitted by a best-fit Poisson distribution (dashed line) and accounts for colonies that could not have been sampled due to finite sample size.