An Ecological Assessment of *Impatiens glandulifera* in its Introduced and Native range and the Potential for its Classical Biological Control

By

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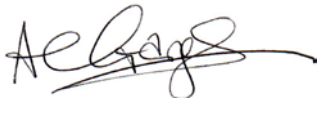
Declaration of Authorship

I, Robert Tanner, hereby declare that the research, experimental design and data analysis for this thesis was conducted by myself with the exception of the following:

- Sonal Varia (CABI) identified the 2007 ground Vortis samples presented in chapter 2 under my supervision and assisted in the 2008 sampling at Harmondsworth Moor.
- Alex Lee (Royal Holloway) conducted the experiment of plant performance in different soil types, under my supervision for chapter 4.
- Liang Jin (Royal Holloway) conducted the evaluation of the colonisation of arbuscular mycorrhizal fungi for the UK and Indian plants for chapter 5.
- Richard Weaver (Royal Holloway) assisted in collecting the Carabid samples for chapter 3.



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Abstract

The diversity of vegetation, the above and below-ground invertebrate community, and the soil mycobiota are intrinsically linked. However, few studies have assessed the impact of non-native invasive plant species at all levels. Here, dynamics of vegetation complexity, the invertebrate community, and abundance of arbuscular mycorrhizae (AM) were evaluated in relation to the abundance of *Impatiens glandulifera*.

The abundance of above-ground detritivores, herbivores, and predators was significantly lower in the invaded sites compared to the uninvaded sites. The below-ground community showed significant fluctuations within and between years, however, the overall abundance of the below-ground community showed no significant difference. At a species level, monocultures of *I. glandulifera* on exposed riverine sediments (ERS) impact on the ground beetle community by increasing the abundance of generalist ground beetles into the habitat. The presence of *I. glandulifera* may act to reduce the conservation potential of ERS by increasing competition between generalist and specialist Carabid species. In a third study, the impact of *I. glandulifera* was evaluated on AM fungi and native plant performance and the results revealed that below invaded stands, the levels of AM fungi are reduced and this has the potential to impact on native plant performance. These results suggest that invasion by *I. glandulifera* can lead to fragmented, destabilised ecosystems which require sensitive habitat restoration post-removal.

Since 2006, research has been conducted to evaluate the natural enemies on *I. glandulifera* in its centre of origin in the north-western Himalayas, where the autoecious, monocyclic rust pathogen *Puccinia komarovii* has been highlighted as having considerable potential as a biological control agent. When comparing the

ecology of *I. glandulifera* in the native and introduced range, the results suggest that *I. glandulifera* performs better in the introduced range and one of the major influencing factors is the release from its co-evolved natural enemies.

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1 General Introduction

1.1 Invasive species

Trade liberalisation and rapid globalisation are the main causes of increased spread of non-native invasive plant species across the globe over recent decades (Perrings *et al.*, 2002). Non-native or alien invasive species are recognised as the second greatest threat to biodiversity next to habitat loss, and can inflict irreversible damage to ecosystems with incurred costs measured in £-billions (Pimentel *et al.*, 2001; Pimentel *et al.*, 2005). Globally, non-native invasive plants are the major group of invasive species in terms of number of species and scale of impact (Sheppard *et al.*, 2006). In the UK, there are over 1,400 species of neophytes (plants introduced to Europe after AD 1500) of permanent residence, with an additional 2,000 recorded as having a sporadic occurrence over space and time (Preston *et al.*, 2002a). Only a small proportion of these species are truly invasive - approximately 40 plant species in the UK (Williamson, 1996) - but this minority has a disproportionate effect on natural ecosystems that can redefine the structure and composition of the countryside.

1.2 Traits of invasive plant species

In my opinion, almost any species, be it plant, animal or insect can become invasive given the appropriate environmental conditions and resources to allow the species to establish and spread. Pyšek and Richardson (2007) highlight that for a species to become established, the species in question will need to be equipped for a particular environment. Often, invasive plant species are successful when they possess one or more of the following characteristics: ability to tolerate a variety of habitat conditions (Hannah *et al.*, 1994), rapid growth (Grotkopp and Rejmánek, 2007), and reproduction

potential (including dispersal mechanisms) (Pyšek and Richardson, 2007; Pyšek *et al.*, 2009; Rejmánek and Richardson, 1996).

Understanding what traits may promote invasiveness in plant species is desirable to prevent the establishment of future invaders, either under the current environmental conditions or based on future estimations of climatic change (Bradley, et al., 2009). However, the plasticity to adapt life history characteristics has been shown to differ in native and non-native populations of the same species suggesting that invasive populations gain from being released from the regulation in the native range (ememy release hypothesis (Keane and Crawley,2002) and the evolution of increased competitive ability hypothesis (Blossey and Nötzold, 1995) are therefore able to adapt the traits that would favour a competitive advantage over native species (Chun *et al.*, 2007). Thus, being able to predict invasiveness in a species may be difficult as the invasiveness of the species may not be realised until the species is well established and already regarded as an ecological problem (Kolar and Lodge, 2001).

1.3 Definitions

In the field of invasion ecology, a number of terms are used to define and describe invasive species, often leading to confusion within the field of invasive species science (Ricciardii and Cohen, 2007) and even hampering the development of the science (Davis and Thompson, 2001). Aliens, invasive alien species (IAS), non-native invasive species, weeds, and pest species are commonly used interchangeably to describe non-native plant species which occur and impact on regions outside their native ranges (Colautti and MacIsaac, 2004; Richardson *et al.*, 2000).

Richardson *et al*. (2000) defines the following categories for invasive ecology:

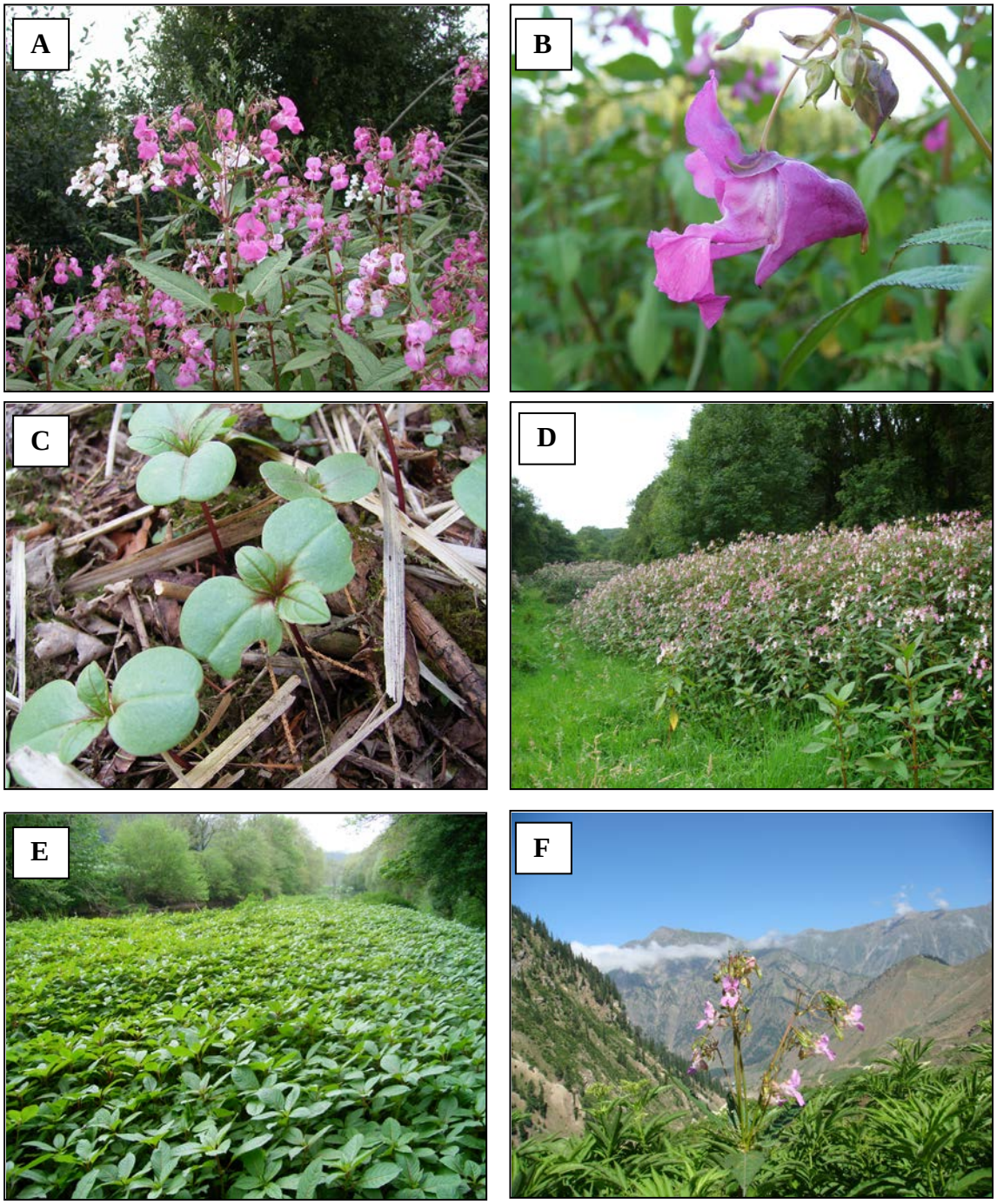
- **Alien plants** – Plant taxa whose presence in a given area is due to the intentional or accidental introduction as a result of human activity. Alien may be interchangeable with 'non-native' or 'introduced species'.
- **Naturalised plants** - Alien plants which sustain populations, year on year, without the influence of humans.
- **Invasive plants**- Naturalised plants which produce large numbers of offspring which may persist over large distances from the parent plant.
- **Weeds** – 'plants (not necessarily alien) that grow in sites where they are not wanted and which usually have detectable negative economic or environmental effects'.
- **Exotic range** and **introduced range** can justifiably be defined as the same. The exotic range is an area where the species has spread with the assistance of humans where it would otherwise be restricted from due to geographical barriers.
- **Native range** – A region where a species naturally occurs without direct or indirect human interference.

Ricciardi and Cohen (2007), highlighted that the term invasive has been consistently used wrongly in the field of invasive species science, and recommends the term 'invasive' should not be used to define a species that has a negative impact on the environment. This is due to the authors finding no correlation with the rate of establishment and spread, and the impact a species has on the environment. Throughout this thesis, I chose to use the terms 'non-native invasive species' or 'non-native invasive plant species' though following that of Ricciardi and Cohen (2007) it is not a given that

this definition implies *I. glandulifera* has an impact on the environment (unless otherwise quantified). Although it is probably the longest terminology, I consider it best describes what we are referring to a species which is not a naturally occurring resident of a region, and one that is expanding its population/area.

It is also important to highlight, that not all invasive species, or problematic plant species which have a negative effect on environments/habitats in the UK. Bracken (*Pteridum aquilinum* (L.) Kuhn), for example, is native to the British Isles but is regarded as an invasive species, with an ecological impact (Fowler, 2006). The species impacts on upland agricultural land and invades habitats of conservation importance. Another example would be ragwort (*Senecio jacobaea* Gaertn.), which is toxic to horses and is subject to a control act (Ragwort Control Act, 2003 (www.legislation.gov.uk/ accessed December 23rd 2011). Although it is not an offence to have the plant growing on your own land, under the 2003 act, it is an offense to allow the plant to spread to neighbouring land. Other native plant species regarded as injurious weeds in the UK, all with the potential of being invasive, include *Cirsium vulgare* (Savi) Ten., *Cirsium arvense* (L.) Scop., *Rumex obtusifolius* L. and *Rumex crispus*, where all are regarded as problematic weeds in agricultural systems (www.naturalengland.org.uk/ accessed December 23rd, 2011). The aforementioned species highlight that a problematic species/weed is one when it grows in an area where it is not wanted. In the right habitat, all of the previously mentioned species contribute to native biological diversity, in their own right and as being host to a plethora of native invertebrate and fungal pathogens.

Plate 1.1 *Impatiens glandulifera* in the introduced and native range



A. Colour variation in *Impatiens glandulifera* Royle flowers
B. Close up of *I. glandulifera* flower
C. Seedlings of *I. glandulifera*
D. Flowering monoculture of *I. glandulifera* within a floodplain of the river Camel, Cornwall, UK
E. *I. glandulifera* monoculture on the river Torridge, North Devon, UK
F. *I. glandulifera* in its native range, the foothills of the Himalayas, Pakistan

1.4 The study species: *Impatiens glandulifera*

Impatiens glandulifera Royle (Ericales: Balsaminaceae) (Plate 1), commonly known as Himalayan balsam, is one such non-native invasive plant species, which has spread rapidly throughout the UK and mainland Europe, since its introduction at the beginning of the 19th century (Beerling and Perrins, 1993; Pyšek and Prach, 1995). Native to the Western Himalayas, at altitudes of 2000-2500m, *I. glandulifera* is the tallest European annual, commonly attaining a height of 2m (Beerling and Perrins, 1993) and can even reach 3m at maturity in deciduous woodland (Andrews *et al.*, 2005). *Impatiens glandulifera* is an attractive plant with erect, hollow green stems with a reddish tinge. Leaves are arranged in whorls of 2-5, lanceolate and serrulate. Flowers are variable in colour from purple-pink and occasionally almost white (Blamey *et al.*, 2003) (Plate 1.1), and are produced from June through to October, long after most native annual plant species have senesced.

Seeds are large, black in colour at maturity, and are produced in capsules, which explosively dehisce when ripe; hence, the other common names for this plant are 'Touch-me-not' and 'Jumping Jacks'. There is a high level of variation of seed production between habitats and individuals (Willis and Hulme, 2004), an individual plant can produce up to 2500 seeds and propel the seeds up to 5 m from the parent plant. When *I. glandulifera* forms monocultures this can equate to a seed rain of 5000-6000 seeds m^{-2} (Beerling and Perrins, 1993).

1.5 Phenology

Beerling and Perrins (1993) state that the seeds germinate between February to March; though from personal observations, I consider February rather early in the season. However, the cotyledon stage can be seen as early as April, depending on a mild winter.

From May through to July rapid growth is seen as the plant attains its maximum height and leaf area. Flowering occurs as early as mid-June (Pers. obs.) and continues until late August. Seed-set occurs from late July and seed production is maintained until late September - early October until the first frosts kill the plants. Plants in the introduced range are thought to be more susceptible to frost than those in the native range and late spring frosts can kill early germinating seedlings (Beerling and Perrins 1993). However, quantitative data to substantiate this claim is currently lacking from the current literature. The seed bank is relatively short lived, persisting between 18 to 24 months (Beerling and Perrins, 1993), though seeds can remain viable for several years under artificial conditions with germination being achieved following a period of stratification at 4^{o}C (Mumford, 1990).

1.6 Phylogenetics of Balsaminaceae

The genus *Impatiens* contains approximately 1,200 species of annual and perennial herbs mainly distributed in the montane areas of tropical and subtropical Asia and Africa (Janssens *et al.*, 2009). The genus originated approximately 22 million years ago in Southwest China and the Himalayan basal clade, to which *I. glandulifera* belongs, radiated out of this region some 5.2 million years ago (Janssens *et al.*, 2009). The species *I. glandulifera* is estimated to be 3.7 million years old (Janssens *et al.*, 2009). *Impatiens* is one of two genera which make up the family Balsaminaceae. *Hydrocera*, the other genus in the family, is a monophyletic genus represented by *Hydrocera triflora* L., a semi-aquatic species endemic to the Indo-Malesian region (Janssens *et al.*, 2006).

Before recent advances in molecular phylogenetics, Balsaminaceae was included in a distinctly separate order- Balsaminales (Dahlgren, 1989) or more traditionally, as a

member of the order Geraniales in the sub-class Rosidae (Cronquist, 1981; Thorne, 2000). Geuten *et al.* (2004) and Anderberg *et al.* (2002) disputed such classifications, which were based mainly on morphological characteristics and as a result of their molecular phylogenetic studies, Balsaminaceae was reclassified as a family in the Ericales (an order of 26 families), sitting as a sister group to all other Ericales in the balsaminoid Ericales. The balsaminoid Ericales consist of the families Balsaminaceae, Marcgraviaceae, Pellicieraceae and Tetrameristaceae. Together this group comprises approximately 1,130 species. Palynological studies of the Balsaminoids showed the Balsaminaceae to be distinctly different from the other three families (Janssens *et al.*, 2005).

Since the reclassification at the family level and above, modern techniques have enabled infrageneric classification of *Impatiens* on a global perspective. Yuan Ming *et al.* (2004) and Janssens *et al.* (2006) used chloroplast *atp B-rccL* sequences from 86 species of Balsaminaceae to form a phylogenetic reconstruction of the family using parsimony and Bayesian approaches. It is evident from their research that the relationship between *Impatiens* species is strongly geographically structured, with distinct clades and closely related clades grouped within distinct geographical regions.

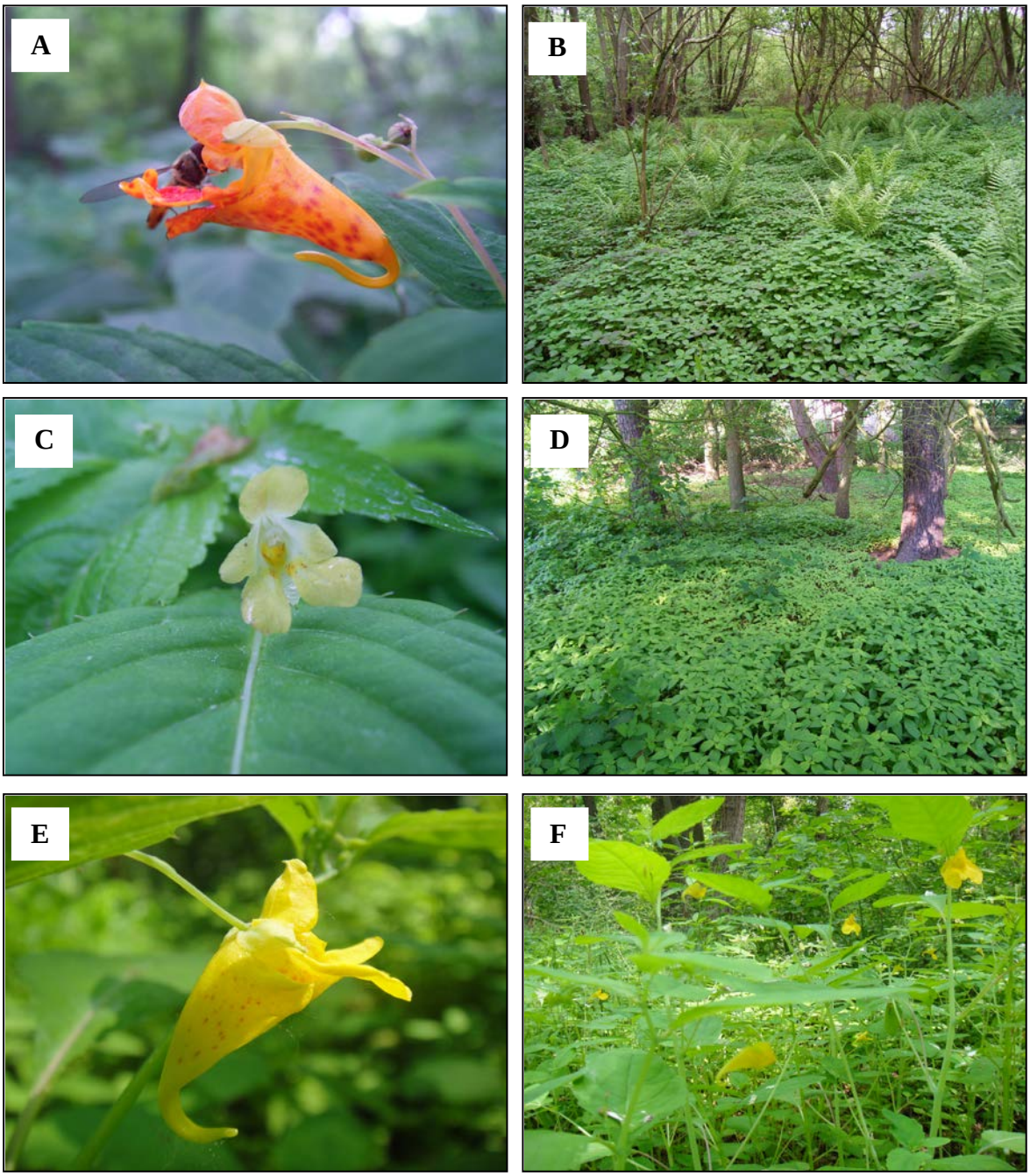
1.7 Other *Impatiens* in the UK countryside

Impatiens glandulifera is not the only invasive *Impatiens* in the UK, but it is the most widespread (Perrins *et al.*, 1993). *Impatiens capensis* Meerburgh (Plate 2 A/B), originally from North America, was introduced to the UK as early as 1822 (Perrins *et al.*, 1993) and is now present throughout the south east of the country. Although in the past this species was not considered as such a problem as *I. glandulifera*, in recent years, concern has grown over the spread of this weed in the south of England, where it

is reported as invasive in Kent, Thames region (north east) and Cornwall (Pers. comm., Trevor Renals, Environment Agency). *Impatiens parviflora* DC (Plate 1.2 C/D) is the second most invasive species of the genus in the UK.

Introduced to Europe from Central Asia as early as 1837, *I. parviflora* was first recorded in the British countryside in 1848 (Williamson, 1996). This, the smallest *Impatiens* present in the UK countryside, is found in lowland areas of the UK (Coombe, 1956) where it colonises bare ground and forms dense mats in shaded areas of woodlands and waste ground. Introduced in over 27 countries in Europe and widespread in nine (CABI, 2004), *I. parviflora* is not yet considered as much of an invasive problem as *I. glandulifera*, even though it is predicted to occupy approximately the same number of vice-counties (Perrins *et al.*, 1993). In the Lake District region of the UK, *I. parviflora* co-occurs with *Impatiens noli-tangere* L. where it may potentially compete for habitat requirements (Pers. comm., Paul Hatcher, Reading University). *Impatiens noli-tangere* (Plate 2 E/F) is the only native *Impatiens* to the UK (Hatcher, 2003) and it is the sole food plant for the endangered moth *Eustroma reticulatum* Denis & Schiffermüller (Hatcher *et al.*, 2004). This rare UK balsam species is only found in a few locations in the Lake District, and isolated populations in Wales. Occasionally, *I. balfourii* Hook (Adamowski, 2009; Perrins *et al.*, 1993) and *I. sultanii* Hook (National Biodiversity Network, available at: http://data.nbn.org.uk/, accessed 21st March, 2011) have been recorded in the UK countryside although their persistence over time fluctuates (Perrins *et al.*, 1993).

Plate 1.2 Other *Impatiens* species occurring in the UK countryside



A. *Impatiens capensis* Meerburgh flower
B. *I. capensis* spreading in Rush meadow, Silwood Park, Berkshire, UK
C. *Impatiens parviflora* DC flower
D. *I. parviflora* spreading at CABI-E-UK, Egham, Surrey, UK
E. *Impatiens noli-tangere* L. flower (picture taken in Hungary)
F. *I. noli-tangere* stand (picture taken in Hungary)

1.8 The introduction of *Impatiens glandulifera* into the UK

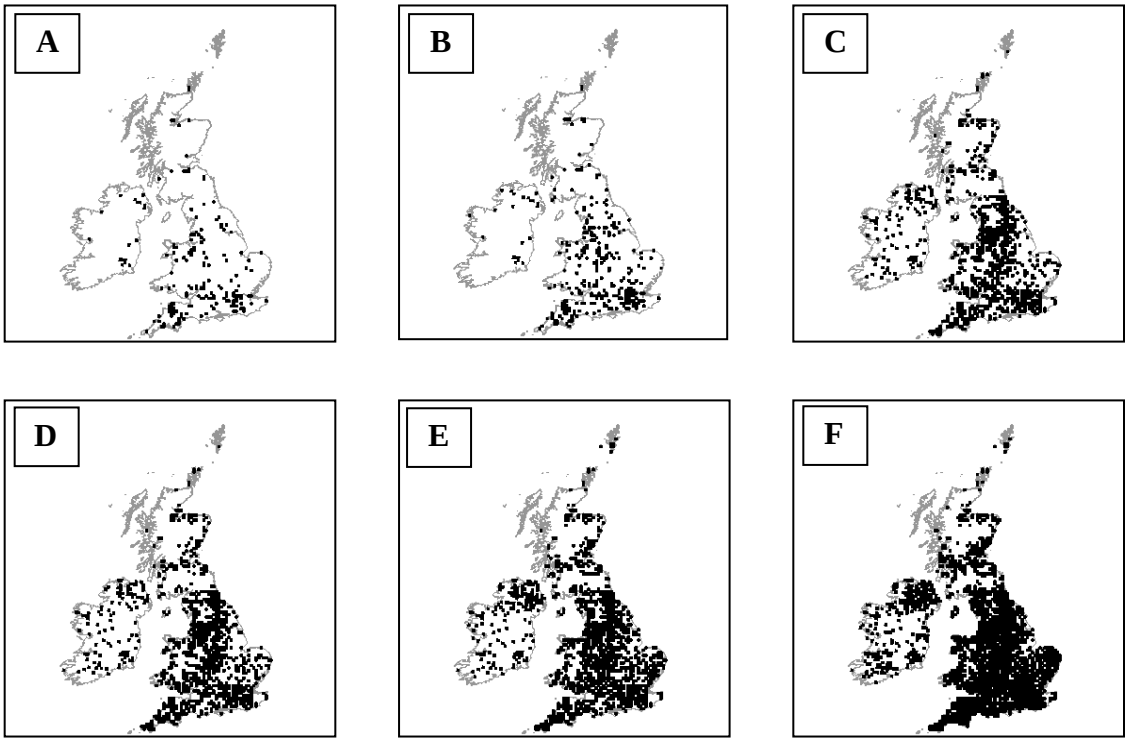
Little historic information is available on the introduction of *I. glandulifera* into the UK. It is commonly cited that John Forbes Royle, who was the curator of the East India Company's botanical garden in Saharanpur, northern India, introduced the species into the UK in 1839. However, this is probably incorrect, as Royle had already returned to the UK in 1837 after taking up a new appointment at King's College London (Pers. comm., Christopher Ashill). The *Gardeners' Chronicle* (1841) (available online www.archive.org) refers to the East India Company importing seeds of some Himalayan *Impatiens* species into England (although it does not specify *I. glandulifera*) and the Royal Horticultural Society (RHS) subsequently widely distributed the seeds.
There is also some disagreement on the year of introduction, with some botanical historians stating that *I. glandulifera* was introduced into the UK towards the end of the 19th century. However, Christopher Ashill (Pers. comm.) from the Royal Horticultural Society library, conducted some research on this subject and found the following on *I. glandulifera* in the Botanical register from 1840 (available online www.archive.org), 'this fine Balsam is the largest of four Indian species raised in the garden of the Royal Horticultural Society last year [1839], it having attained upwards of twelve feet in height by the end of August, although the seeds were not sown before the end of May.'

At that time, the garden of the Horticultural Society of London (later the Royal Horticultural Society) was in Chiswick so it is feasible to suggest that this plant was being grown in Chiswick in 1839. According to Dr. Brent Elliott from the RHS (Pers. comm.), 'the Horticultural Society made seeds available to its members (who at that time were called "Fellows"), although no records survive of such distribution. Thus, it is likely that the Horticultural Society of London itself was the first organisation to make the seed of *I. glandulifera* available to people in England'.

1.9 The spread of *Impatiens glandulifera* in the UK

In order for a plant species to become established and invasive in a region, a staged process is required. Theoharides and Dukes (2007) suggest a four-staged pattern needed for the successful widescale spread of an invasive plant species where firstly the species needs to be transported to a new region (section 1.8). Following on from the transportation of the species, the plant needs to be able to colonise and subsequently establish, persist in the environment. Lastly, if the environmental conditions and available habitats are available to the species the species will spread to become dominant in the environment. *Impatiens glandulifera* was first recorded from Europe from the UK in 1839 (Trewick and Wade, 1986). Soon after, the weed literally exploded out of the large Victorian gardens, where it was grown as a novel showpiece, to become naturalised in the British countryside by 1855 (Kent, 1975). Vigorous growth rates and prolific seed production have aided this species rapid expansion and its distribution is only limited by available high moisture habitat (Beerling and Perrins, 1993; Pyšek and Prach, 1993). Currently, there are few rivers in the UK that have not been colonised by *I. glandulifera* and as a result, British rivers have been referred to as 'balsam highways' (Perring, 1970). This prolific weed is now completely naturalized throughout riparian and open-wooded habitats occupying over 50% of the UK's 10 x 10km recording squares (Preston *et al.*, 2002b) earning it a listing in the top twenty invasive plants in the UK (Crawley, 1987) (Figure 1.1).

Figure 1.1 The spread of *Impatiens glandulifera* in the UK between 1950 and 2010. Where the occurrence is defined by the shaded area for (A) 1950 (B) 1970 (C) 1980 (D) 1990 (E) 2000 and (F) 2010. (Reproduced with permission from National Biodiversity Network, available at: http://data.nbn.org.uk/, accessed 21st March, 2011).



Similar rates of expansion have been seen in the Czech Republic. In 1995, *I. glandulifera* occupied approximately 56% of the total river length of that country and if the rate of spread remained constant as it was then Pyšek and Prach (1995) estimate all main rivers would be completely invaded by 2025. In Poland, *I. glandulifera* was introduced around 1890, in the southern region of the Sudety Mountains, where it has since spread to almost all regions of the country (Torarska-Guzik, 2005). Similar to the UK, (see section 1.9), *I. glandulifera* is regarded as one of the top 20 invasive alien plant species in Poland (Tokarska-Guzik, 2003). In Lithuania (Gudzinskas and Sinkeviciene, 1995) and Latvia (Helmisaari, 2010), *I. glandulifera* has spread rapidly, since its introduction to invade riverbanks of large, main rivers and their tributaries, Where *I. glandulifera* grows near riparian systems, the spread is aided by water currents, seeds are negatively buoyant, and are carried downstream. Germination takes place on the bottom of the water body and the seeds are carried downstream by the

current to the riverbank where they obtain a foothold in disturbed ground (Trewick and Wade, 1986). As well as natural processes of distribution, *I. glandulifera* has been spread by humans throughout the UK, either accidentally through the movement of topsoil, or by the deliberate introduction into the countryside as documented by Rotherham (2001).

1.10 Habitats invaded and tolerance to environmental differences

Impatiens glandulifera is predominantly a weed of riparian habitats in the UK, though the plant will flourish in damp woodlands and waste ground. In addition, *I. glandulifera* is often found by the side of roads and on railway embankments. *I. glandulifera* is occasionally found alongside canals, though often the high banks and low levels of fluctuation of the water body deem this habitat unsuitable for the plant to establish. Maule *et al.* (2000) showed that *I. glandulifera* is tolerant of a range environmental parameters including irradiance levels (0.3-100%) open ground photosynthetically active radiation (PAR), soil potassium (K) concentrations (46.1-267$\mu g\ g^{-1}$) and soil moisture content (6.5-85%). *I. glandulifera* is sensitive to frost where often the population is killed off by the first autumnal frosts (Beerling and Perrins, 1993).

1.11 What makes *Impatiens* successful invaders?

The physiological attributes of *I. glandulifera*, decribed in section 1.4 and 1.5, including producing a high abundance of propagules (that are projected from the parent plant), the tall stature of the species and the ability of the seeds to form dense seed banks all contribute towards *I. glandulifera* being a successful coloniser in the UK. However, to what extent are the attributes that enable *I. glandulifera* to be invasive, a common life strategy shared by the genus in general? Cockel (2011) highlights that many *Impatiens* species produce high propagules pressure and the seeds synchronously germinate in the

spring following a period of vernalisation. Primack and Miao (1992) showed that *I. capensis* seeds synchronously germinated in the spring months. For the northern hemisphere *Impatiens* species, this would be an evolutionary trait due to environmental adaptation.

The size of the seed bank and the ability of the seeds to survive overwinter is an important factor to the success of *I. glandulifera* in the UK (Grimes *et al.*, 1988). However, it is not just a characteristic of the one species in the genus. The size of *I. capensis* populations in North America, have shown to be directly related to the size of seed bank and the survival of the seeds over winter (Antlfinger, 1989). Many *Impatiens* species are tolerable of a wide variety of environmental. Temperature limits the invasion of *I. glandulifera* to the higher altitude regions in the UK (Willis and Hulme, 2002). Similarity, other *Impatiens* species limited by temperature in their natural habitats show invasive tendencies when transplanted to warmer regions. *Impatiens noli-tangere* shows invasive behaviour when grown in the south east of England, at a warmer temperature than that of its natural range, the Lake District region (personal communication, Paul Hatcher, Reading University).

1.12 Global distribution

Cockel and Tanner (2011) record the current global distribution of *I. glandulifera* to be:

- Present in 27 European countries (DAISIE, 2010), where it is widespread in 18 and invasive in 12 countries (CABI, 2004).
- Present in 11 states of the United States and invasive in 3 (USDA, 2010).
- Invasive in 8 states in Canada (Clements *et al.*, 2008).
- Present, though not invasive, in New Zealand (Sykes, 1982).
- Present, though not invasive, in the Russian Far East (Markov *et al.*, 1997).

- Present, though not invasive, in Japan (Drescher and Prots, 2003).

1.13 Associated plant species

Beerling and Perrins (1993) and Prowse (2001) have thoroughly reviewed the plant species associated with *I. glandulifera*, and their observations highlighted the relatively few species associated with stands of *I. glandulifera*. The species commonly associated with *I. glandulifera* include *Urtica dioica* L., *Galium aparine* L., *Rubus fruticosus* L., *Rumex obtusifolius* L., *Calystegia sepium* L. R.Br. and various grasses including species of *Fescue* and *Poa*.

1.14 Associated arthropod species

Relatively few arthropod species are associated with *I. glandulifera* in the UK. Beerling and Dawah (1993) list seven invertebrate families associated with *I. glandulifera* which include: Hemiptera: Aphididae, Cicadellidae, Nabiidae. Diptera: Agromyzdae and Lepidoptera: Sphingidae. Further, Beerling and Perrins (1993) list three species known to feed on *I. glandulifera*: *Aphis fabae* Scopoli (Hemiptera), *Impatientinum balsamines* Kallenbach (Hemiptera) and *Deilephila elpenor* L. (Lepidoptera). Prowse and Goodridge (2003) recorded slug (Gastropoda) damage to young seedlings in damp wooded habitat. In addition to the above, a number of pollinator groups visit *I. glandulifera* flowers, including *Bombus* species (Chittka and Schürkens, 2001; Lopezaraiza-Mikel *et al.*, 2007; Prowse and Goodridge, 2000) (see section 1.11 and 1.14) and more generalised groups of Diptera, Coleoptera and Hemiptera (Lopezaraiza-Mikel *et al.*, 2007). A similar impoverished invertebrate fauna is associated with the other non-native *Impatiens* species (*I. capensis* and *I. parviflora*) in the UK (pers. obs. Author).

1.15 Associated microbial species

Apart from *I. glandulifera* being recorded as forming sparse arbuscular mycorrhizal (AM) associations (Harley and Harley, 1987), there are no records of any other microbial associations in the UK. Prowse (2001) observed signs indicative of pathogen damage (leaf spots) within populations in the north of the UK, and similar observations have been seen throughout the UK (Pers. obs., Evans, Tanner and Shaw, CABI). However, attempts to isolate a fungal pathogen have failed and it is considered that the spotting is caused by invertebrate damage or abiotic stress.

1.16 Ecological impacts of *Impatiens glandulifera*

Some of the impacts associated with *I. glandulifera* have been documented (see table 1.1). However, more research is needed into the perceived effects that this non-native species could have on the ecosystems it invades which are often commonly cited on invasive species information websites (see as examples: http://www.naturalengland.org.uk, http://www.riverdee.org.uk and http://www.broads-authority.gov.uk (accessed 4th January, 2012)). Indeed, in the current literature there are clear gaps on the impact of invasive non-native plant species on ecosystem services (Vilá *et al*., 2009). Binimelis *et al*. (2007) classified four main categories of ecosystem services, and Vilá et al. (2009) suggest the impacts invasive species can have on them (in brackets); including (1) supporting (changing the composition of the habitat, species assemblages or soil properties), (2) provisioning (altering genetic resources, threatening endangered species), (3) regulating (changing pollinator services, altering erosion regimes) and (4) cultural (changing recreational uses, effecting ecotourism). Potentially, *I. glandulifera* can affect all of the aforementioned ecosystem services (see table 1.1). However, further research is needed to evaluate the effects on all.

The threat to biodiversity from this weed is significant especially in vulnerable habitats, including national parks and Sites of Special Scientific Interest (SSSI), as the plant is able to successfully compete directly with native species for space, light and nutrients. In a paired-plot removal experiment in a riparian system in the north-east of England, Hulme and Bremner (2006) found that the removal of *I. glandulifera* increased plant diversity and species richness, and when *I. glandulifera* forms monocultures, it may reduce species richness by up to 25%. However, Prowse (2001) and Hejda and Pyšek (2006) both showed that *I. glandulifera* has little effect on plant species richness in areas where the plant has invaded. Hejda and Pyšek (2006) showed that non-invaded plots only harboured 0.23 more plant species than invaded plots and removal of *I. glandulifera* allowed for natural restoration of the plant community.

Using multi-site removal and addition experiments, Prowse (2001) showed that no native plant species was excluded from an invaded stand, and plant species were not significantly affected by either removing or adding *I. glandulifera*. Maule *et al.* (2000) studied the factors influencing the growth and spread of *I. glandulifera* in mature woodland in the north of England and the effect the weed had on native woodland species. Using clearance trials, Maule *et al.* (2000) showed that in the UK *I. glandulifera* could successfully compete with native plants, including tree seedlings with the potential to inhibit the regeneration cycle of woodlands. However, when studying the potential impact of *I. glandulifera* on tree seedling growth in woodland dominated by silver birch (*Betula pendula* Roth.) and Norway spruce (*Picea abies* (L.) H.Karst.) in Germany, Ammer *et al.* (2011), found no impact of *I. glandulifera* on established tree seedlings. Tickner *et al.* (2001) showed *I. glandulifera* can successfully outcompete *U. dioica* in a riparian system though competition varied between the sites studied. In flooded sites, *Urtica dioica* L., had a negative impact on the germination of

I. glandulifera seeds though in mixed stands *U. dioica* was significantly affected by the presence of *I. glandulifera* with reduced growth and occurrence.

Table 1.1 The known and perceived impacts of *Impatiens glandulifera* with the potential impact on a particular ecosystem service (parentheses). Where (1) is supporting, (2) provisioning, (3) regulating and (4) cultural (see section 1.16).

Known effects	**Perceived effects**
Outcompetes native plant species:	Blocks drainage systems (3)
In riparian system (Tickner *et al.*, 2001) (1)	Increases flooding (3)
In woodland habitat (Maule *et al.*, 2000) (1)	Reduces invertebrate diversity (1)
Indirect competition with plant species:	Reduces soil mycobiota (1)
For pollinators (Chittka and Schürkens, 2001) (2)	Retards woodland regeneration (1)
Restricts access to rivers (Tanner, 2008) (4)	
Visually displeasing (Tanner, 2008) (4)	

In a study of indirect competition, Chittka and Schürkens (2001) showed that *I. glandulifera* is capable of reducing the fitness of native plant species through reduced seed-set, by luring pollinators away from native species due to its higher rate of sugar nectar production (0.47±0.12 mg per flower per hour), higher than any other known European species (Chittka and Schürkens, 2001). Prowse and Goodridge (2000) showed a similar preference for *I. glandulifera* by bees from the genus *Bombus* in a study of pollinator visitations to *I. glandulifera* and other plant species. Over time, such competition between plant species for pollinators could leave native species, which are unsuccessful at attracting pollinators genetically depauperate (Prowse and Goodridge, 2000).

Impatiens glandulifera can impede access to rivers for recreation and inspection, and when the plant dies back in the autumn it can leave riverbanks bare and liable to erosion thereby increasing sediment intake into the river system. This in turn has the potential

to smother habitat niches used by invertebrates and which are spawning grounds for fish. The invasion of *I. glandulifera* into an already stressed river network increases the risk of flooding which in turn may have detrimental effects on the ecological functioning of the habitat and thus impact on plant and invertebrate biodiversity. To-date, there has been no known research on the impact of *I. glandulifera* on invertebrate communities or diversity. As an aggressive coloniser, and an unpalatable species to the majority of UK invertebrate species, it is plausible to suggest that if *I. glandulifera* invades native communities and displaces native plant species, then their associated invertebrate species would also be displaced.

As mentioned in section 1.12, *I. glandulifera* is recorded as being sparsely mycorrhizal. It is known that mycorrhizal presence in soil can enhance plant species richness in a community by enhancing seedling establishment (Gange *et al.*, 1993). Therefore, in a weed monoculture, where no suitable host plants occur, the fungi would die, as mycorrhizae depend on plants for their carbon supply. It is feasible to suggest, that when *I. glandulifera* occupies an area over a long period, the soil would become deficient in these microorganisms and the health of the soil could be affected resulting in a reduced likelihood of recovery.

1.17 Economic impacts of *Impatiens glandulifera*

The Environment Agency has estimated it would cost between £150-300 million to eradicate *I. glandulifera* from the UK (Environment Agency, 2003). However, this figure is rather academic as the eradication of such a widespread invasive plant species would be near impossible. It is estimated that the total annual cost of invasive non-native species (plant pathogens, arthropods, mammals and plant species) to the British economy is £1.7 billion, of which £1-million is spent on controlling *I. glandulifera*

(Williams *et al.*, 2010). However, the actual annual cost of controlling *I. glandulifera* is likely to be considerably higher than the stated £1 million, if all sectors controlling and researching the species had been included, and the indirect costs related to biodiversity loss had been accounted for. Similar high costs related to the control of *I. glandulifera* have been recorded for mainland Europe, where Gelpke and Weber (2005) estimated it would cost between £923,133 and £5,839,691 to eliminate 95% of the current population in the Canton of Zürich, Switzerland.

1.18 Public perceptions of *Impatiens glandulifera*

Although difficult to quantify, it is worth noting the public perception of *I. glandulifera*. In a questionnaire, to conservationists and organisations with similar interests, Prowse (1997) asked for their perception of *I. glandulifera*. The majority, 89%, considered *I. glandulifera* to be a conservation problem. However, this is obviously not the case with certain members of the public, who actively promote the plant and spread the seed far and wide (Rotherham, 2001). Like *Buddleia davidii* Franchet and *Rhododendron ponticum* L., there is a conflict of interest between people who like the plant and those who do not, and those who recognise the issues associated with non-native plant species and those who do not.

1.19 Benefits of *Impatiens glandulifera*

The extended flowering time of *I. glandulifera*, compared to other UK natives (Prowse and Goodridge, 2000), coupled with high rates of sugar nectar production, means this plant is favoured by beekeepers (Showler, 1989; Starý and Tkalců, 1998). The recent decline in the populations of bees, in the UK (Blake *et al.*, 2011; Feltwell, 2010) has further highlighted the potential use this species may have in maintaining their populations (Showler, 1989). Apart from the benefits to *Bombus* populations, there are

no other benefits of this species to the UK countryside, apart, of course, to the few that consider monocultures of the plant in flower an attractive addition to the UK flora.

1.20 Legislation

Cockel and Tanner (2011) reviewed the current legislation with regard to *I. glandulifera* where they highlight that *I. glandulifera* was included within Schedule 9 of the 1981 Wildlife and Countryside Act in 2010, making it illegal to allow or cause the plant to grow in the British countryside. Defra and the Welsh Assembly are also considering banning the sale of *I. glandulifera* under Section 14ZA of the 1981 Wildlife and Countryside Act. On the European level, no additional laws govern *I. glandulifera*, though the EC Water Framework Directive (WFD) (2000) requires all member countries to bring their water bodies up to a 'good ecological status'. Although there is no specific mention of invasive non-native plant species management in the Directive, the impacts incurred by their occurrence are measurable within the guideline of what constitutes 'good ecological status'. Cockel and Tanner (2011) highlight, at an international level, that signatories of the 1992 Convention on Biological Diversity (CBD) (Secretariat of the Convention on Biological Diversity, 2005) are required to control invasive non-native species and restore habitats affected by them.

1.21 Current management strategies

Current control methods are labour intensive and difficult to implement, due to the often inaccessible habitats the weed grows in (Tanner, 2008). Often there are restrictions on the chemicals that can be used, if any, due to the sensitivity of the invaded habitat. Repeated control attempts over 2 or 3 seasons can exhaust the short-lived seed bank (Howell, 2002). However, cutting or spraying must be carefully timed, in June, to incorporate all plants at various growth stages and to prevent seed set (Prach, 1994).

Glyphosate® application is effective against *I. glandulifera* (Stensones & Garnett, 1994) but will also kill other plants in the near vicinity. 2,4-D amine will select only broad-leaved species and treatment should take place in early spring when the plant is at the rosette stage (Environment Agency, 2003). Cutting should sever the plant below the lowest node, preventing future seed set (Howell, 2002). Hand pulling is effective for eradicating small stands although it can leave banks bare and without root systems to hold soil in place, thereby adding to the potential for erosion. Total eradication from an area may be impossible if neighbouring habitats harbour populations (Wadsworth *et al.*, 1997; Wadsworth *et al.*, 2000). Thus, a catchment-scale approach to control is the only realistic method to manage this species. However, such a concerted approach is often difficult with traditional methods due to multiple land ownership along riparian systems and inaccessible habitats.

Since 2006, research has been conducted into the potential of classical biological control for the management of *I. glandulifera* in the UK. Classical biological control is defined as the utilisation of natural enemies (either plant pathogens or arthropod species) from the plant's native range in the regulation of host populations in the introduced/invasive range (DeBach, 1964). Classical biological control against non-native invasive plant species is receiving increased attention in the UK in recent years (Shaw and Tanner, 2008), due in part to the high costs associated with current control methods (Sheppard *et al.*, 2006). As a non-native species *I. glandulifera*, has no associated specific natural enemies in its introduced range, thus the species should be amenable to a classical biological control strategy (Shaw, 2003). Chapters 5 and 6 fully review the progress made in this field to-date.

1.22 Aims, objectives and hypotheses

The aim of this research was (also see table 1.2):

- To quantify the ecological impacts of *I. glandulifera* on invertebrate populations in the introduced range, both at a community and species level.
- Evaluate the impact of *I. glandulifera* on the arbuscular mycorrhizal community and subsequent native plant performance.
- To compare the ecology of *I. glandulifera* in the native and introduced ranges.
- To review the progress into the biological control of *I. glandulifera*.

The objectives of this research along with the associated hypotheses tested are detailed below:

Objective 1: The impact of *I. glandulifera* on the aboveground invertebrate community

In chapter 2, the main objective was to determine if *I. glandulifera* had an impact on the above-ground invertebrate community over time by comparing invaded habitats with uninvaded habitats. The specific hypotheses I aimed to test were:

- The presence of *I. glandulifera* has a negative impact on the above-ground invertebrate community by displacing functional groups within the invertebrate community structure.
- Seasonal fluctuations in the occurrence of *I. glandulifera* affect the abundance of invertebrate groups within the above-ground invertebrate community, either negatively or positively.

Objective 2: The impact of *I. glandulifera* on the carabid community of exposed riverine sediments (ERS)

In chapter 3, the main objective was to determine if colonisation by *I. glandulifera* affects the ground beetle community of ERS, thereby having an impact on the conservation status of these habitats. The hypotheses to be tested in this chapter 3 were:

- *I. glandulifera* has a negative impact on the ground beetle community on ERS.
- Specialist ground beetle species to ERS will be more negatively affected by the presence of *I. glandulifera* than generalist ground beetle species.

Objective 3: The impact of *I. glandulifera* on the below-ground invertebrate community

In chapter 4, the objectives were to evaluate the impact of *I. glandulifera* colonisation on the below-ground invertebrate community by comparing invaded and uninvaded sites in a multi-site method (as detailed in chapter 2) and to evaluate the performance of three native plant species grown in *I. glandulifera* invaded soil, and from soil under stands of native vegetation and measure the AM colonisation of each individual species to assess the levels of mycorrhizae in the invaded and uninvaded soils. The hypotheses to be tested in this chapter 4 were:

- The presence of *I. glandulifera* has a negative impact on the below-ground invertebrate community by displacing functional groups within the invertebrate community structure.
- Seasonal fluctuations in the occurrence of *I. glandulifera* affect the abundance of invertebrate groups within the below-ground invertebrate community, either negatively or positively.

- *I. glandulifera* will have a negative impact on the soil AM community and this will be expressed in a decreased percentage colonisation on the roots of plants grown in soil from under invaded stands.
- Native plants grown in the invaded soils will have reduced performance, expressed as plant biomass, when grown in soil from under invaded stands compared to uninvaded vegetation and this will be related to reduced AM colonisation on the roots.

Objective 4: Native and introduced range comparisons

In chapter 5, the objectives were to compare how environmental conditions, natural enemies, and AM influenced plant growth characteristics in the native range and evaluate if there were differences within the native range, and between the introduced and native range. The hypotheses to be tested in this chapter 5 were:

- Released from the pressure of host specific natural enemies, *I. glandulifera* populations in the introduced range will have increased growth (both height and total leaf area) compared to those in the native range.

Objective 5: The potential for biological control

The main objective of chapter 6 was to collate and review the potential for the biological control of *I. glandulifera* using the rust fungus *Puccinia komarovii* Tranz. There are no specific hypotheses associated with this chapter.

Table 1.2 Overview of the aims of the research, hypothesis tested and methods adopted

Areas of knowledge lacking	Aims of research	Hypothesis tested	Methods adopted
The effect of the invasion of *I. glandulifera* on native invertebrate populations in the UK.	To quantify the ecological impacts of *I. glandulifera* on invertebrate populations.	*I. glandulifera* has a negative impact on the above ground invertebrate community. *I. glandulifera* has a negative impact on the below ground invertebrate community. *I. glandulifera* has a negative impact on the ground beetle community of exposed riverine sediments Specialist ground beetle species on ERS will be more negatively affected by *I. glandulifera* than generalist ground beetles. Seasonal fluctuations in the occurrence of *I. glandulifera* affect the abundance of invertebrate.	Multi-site comparison study of invaded and uninvaded sites using an aerial and ground Vortis sampling (above ground invertebrates) (chapter 2), pitfall traps and hand searching methods (carabids) (chapter 3) and soil cores and Tullgren funnel light traps (below ground invertebrates) (chapter 4). Invertebrate community identified into taxonomic groups and to species (carabids only). Plant community sampled at all sites and percentage cover of each species estimated using randomly placed $1m^2$ quadrats. All invertebrate data related to plant percentage cover variables. Analysed using standard (repeated measure ANOVA, Peasons correlation, and regression) and multivariate methods (principal response curve (Van den Brink and ter Braak, 1999, and redundancy analysis (Crawley, 2007).
The effect of the invasion of *I. glandulifera* arbuscular mycorrhizal fungi and the implications of an invasion on native plant species.	To evaluate the impact of *I. glandulifera* on the arbuscular mycorrhizal community and subsequent plant performance.	*I. glandulifera* has a negative impact on the soil arbuscular mycorrhizal community. Native plant species will have a reduced performance when grown in soil from under invaded stands compared to uninvaded stands.	Chapter 4: three native plant species (*Plantago lanceolata* L., *Lotus corniculatus* L., *Trifolium pratense* L.) grown in invaded and uninvaded soils where performance (weight) and percentage colonisation of AM fungi (using root preparation method of Vierheilig *et al.* (1998) and cross eyepiece evaluation method of (McGonigle *et al.* (1990)). One way ANOVA used to compare difference between replicates from invaded and uninvaded soils. Regression analysis used to evaluate relationships between weight and AM colonisation.
The differences in the performance of native and introduced populations of *I. glandulifera*.	To compare the ecology of I. *glandulifera* in the native and introduced range.	Released from natural enemies, *I. glandulifera* will have increased growth in the introduced range.	Populations of *I. glandulifera* sampled in the UK, India and Pakistan for natural enemies and individuals were measured in the UK and India. Measurements included: height, total leaf area, above and below ground biomass, stem girth, percentage cover of natural enemies, and reproductive potential (seeds, pods and flowers). Two-way ANOVA used to compare differences between sites and regression analysis used to compare relationships between plant growth parameters.
The evaluation of novel control methods for *I. glandulifera*.	To review the progress into the biological control of *I. glandulifera.*		Literature review on the progress made on the biological control of *I. glandulifera.*

1.23 The Principal response curve

A prominent multivariate analysis conducted throughout this thesis is the use of the Principal response curve analysis (PRC) (Moser *et al.*, 2007; Van den Brink and ter Braak, 1999). The PRC is applied to community data to assess the structure of species communities and is based on redundancy analysis (RDA) (Moser *et al.*, 2007). Traditionally, the PRC has been adopted for analysis of mesocom experiments where a stressor (i.e. a pollutant) has been applied to a number of samples at varying concentrations and then measurements are taken over time to evaluate differences in the response of individual treatments (Van den Brink and ter Braak, 1999). In recent years, however, the PRC method has been used for ecological impact studies. Candolfi *et al.* (2004) applied the PRC to analyse an invertebrate abundance dataset where the authors were comparing invertebrates in genetically modified *Bt*-Corn and non-genetically modified corn. Devotto *et al.* (2008) applied the PRC to analyse the non-target effect of the biological control agent, *Beauveria bassiana* (Balsamo) and an insecticide for the control of *Dalaca pallens* Blanchard, on invertebrate populations.

The PRC method is particularly suited to evaluating the effect of the stressor, in this case the effect of *I. glandulifera*, on the response variable (in this case the invertebrate community) in a repeated measures design where measurements are taken over time (Moser *et al.*, 2007). Throughout this thesis I have used the PRC to evaluate the response of the invertebrate communities in sites invaded by *I. glandulifera* compared to uninvaded (native vegetation) sites. Where I have applied the PRC (chapter 2, 3 and 4) all of the data collected were from sites sampled repeatedly over time. The sum of three terms: mean abundance in the control, an error term, and week-specific treatment effect allows the model to evaluate the differences between the control and the

treatment in an easier to interpret graphic compared to the same analysis conducted with a RDA (Moser *et al.*, 2007).

The statistical model for the abundance analysis is:

$$y_{d(j)tk} = \bar{y}_{0tk} + b_k c_{dt} + \varepsilon_{d(j)tk}$$

Where $\bar{y}_{d(j)tk}$ is the abundance of species *k* in replicated treatment *j* of treatment *d* at time *t*. y_{0tk} is the mean abundance of species in week *t* in the control (d=0). b_k is the species weight and c_{dt} is the least square estimates of the coefficients which are estimated from the partial RDA. $\varepsilon_{d(j)tk}$ is an error term with mean zero and variance (taken from van den Brink and ter Braak, 1999). The PRC method plots a graphic of curves over time which represents c_{dt} plotted against *t* for each treatment which shows the deviation of the stressor, to that of the control at given sampling times, where the control is graphically represented as a horizontal line through zero.

The overall significance of the PRC is tested by Monte Carlo Permutations (van den Brink and ter Braak, 1999). The resulting output of the test allows the user to calculate the overall variance explained by the PRC and the part which is explained by time, treatment, and the interaction with treatment and time. Thus, allowing the user to evaluate if the community responds differently in the treatment to that of the control.

The advantage of the PRC is that it allows for an evaluation of the whole community at the species level, as in addition to the PRC graphic, the analysis produces a second vertical graphic of the taxon weight, in the form of a line-stack displayed on a third axis. This is a useful aspect of the PRC as we can evaluate the difference in abundance of

functional groups, or individual species that may be of particular interest. Using the taxon weight, it is possible to explain the response of each individual group/species to that of the treatment for any given time point. For example, in the case where a response curve has a positive deviation to that of the control, a negative taxon weight would indicate a decrease in abundance for that treatment compared to the control. Therefore, a positive taxon weight would indicate an increase in abundance for the treatment to that of the control, and the opposite would be shown if the response curve was negative to the control. The higher the taxon weight, be it positive or negative, the more the species is affected by the treatment whereas species with a taxon weight around zero are not regarded to be affected by the treatment.

Using the taxon weights, it is then possible to calculate the response of a species to a treatment at any given sampling time by comparing it to the geometric mean count in the control by:

$\exp(b_k \times c_{dt})$

Where the exponential of $(b_k \times c_{dt})$ is calculated for each of the species *k* at treatment *d* and sampling date *t* (taken from van den Brink and Ter Braak, 1999).

Thus, as an example, in an artificial scenario, where treatment A1 has a constant positive deviation of 1.5 from the control over five time points, and species B has positive taxon weight of 1.7; species C – 1.7, and species D – 0.01, it can be inferred that species B is affected by treatment showing an increase in abundance under treatment A1, to that of the control. Oppositely, species C is also affected by the treatment, but in this case it is negatively affected by the treatment showing a decrease in abundance compared to that of the control. Species D on the other hand is unaffected

by the treatment. Alternatively, as explained by van den Brink and Ter Braak, (1999), species D may behave differently to the pattern displayed by the graphic.

To evaluate the response of species B in more quantitative terms, i.e. the difference in the geometric mean count in the treatment to the control for a given time point or the difference between two time points, though in this artificial situation this would not be valid as treatment 1 remains constant at 1.5 over time. Calculating difference in abundance of species B would be exp(1.5*1.7) = 12.80. Thus, species B has increased approximately 13 fold in the treatment site to that of the control and species C has reduced in the treatment to approximately 7.8% of its geometric mean count in the control.

2 The temporal response of the above-ground invertebrate community to the fluctuating occurrence of the non-native species *Impatiens glandulifera*

2.1 Introduction

Chapter 1 highlights the main areas where research is lacking for the non-native species *Impatiens glandulifera* in the UK. This chapter covers an evaluation of the impact of *I. glandulifera* on the invertebrate community addressing objective 1 in section 1.20. For this chapter the focus is on the impact of *I. glandulifera* on the above-ground invertebrate community. In chapter 4, the impact on the below-ground invertebrate community is evaluated and both are discussed and compared in section 4.4 and in chapter 7.

Impact studies of non-native plant species on the community level are poorly represented in the current literature (Parker *et al.*, 1999). Even fewer studies have been conducted on the impact of non-native plants on invertebrate communities, apart from some recent examples (Gerber *et al.*, 2008; Litt and Steidl, 2010; Topp *et al.*, 2008). There are no known studies in the UK which evaluate the impact of non-native plant species on invertebrate communities, apart from the study of Beerling and Dawah (1993) where the authors compared the invertebrate assemblages found within stands of *I. glandulifera* to that of *Fallopia japonica* Houtt. (Ronse Decr.). Interestingly, Beerling and Dawah (1993) showed that *F. japonica* harboured a lower diversity of organisms to that of *I. glandulifera*, though unfortunately the authors failed to compare the invertebrate community to that of native vegetation.

As discussed in section 1.14, there is still some debate on the impact of *I. glandulifera* on native plant communities. However, all of the recent studies show *I. glandulifera* does displace native species, which therefore would have an impact on higher trophic levels (Litt and Steidl, 2010). Gerber *et al.* (2008) studied the impact of *Fallopia* species (*F. japonica*, *F. sachalinensis* (F. Scmidt ex Maxim) and *F. bohemica* Chrtek and Chrkova) on plant species richness and invertebrate populations in Switzerland, Germany and France. The authors showed that habitats invaded by *Fallopia* species supported a lower number of plant species and a lower abundance and species richness of invertebrates compared to uninvaded plots.

As a non-native invasive plant species which harbours few natural enemies in the introduced range we can assume *I. glandulifera* has a negative impact on habitat it invades and the species within. Since embarking on this research, I have discussed the results and experimental design with colleagues and sometimes I have had the question 'but isn't it a given fact that *I. glandulifera* will have an impact on the invertebrate community?' If this is, or is not the case, we still need to back this up with scientific evaluation, as the question will always remain hypothetical and unanswered.

Adair and Groves (1998) reviewed the methodologies available to evaluate the impact of invasive plants on biological diversity where they present four principal techniques: (1) multi-site comparisons, (2) time sequence studies, (3) weed removal experiments, and (4) weed addition experiments. Although their report focuses on the evaluation of biological diversity, all of the principal techniques are appropriate for evaluating other parameters like abundance patterns, biomass, and changes in community structure between orders, specialist and generalist species and feeding guilds; where all with the

exception of the latter can be related to plant and invertebrate species. Thus, from hereafter, I shall refer to these methods for evaluating the impact of non-native species.

Multi-site comparisons are the most widely used method for evaluating the impact of invasive weeds (Adair and Groves, 1998). The multi-site method involves selecting sites within a habitat where sites are divided by invaded and uninvaded stands. Sampling is conducted randomly within each site and often site means are used in the analysis. The advantage of this method is that a large amount of data can be collected relatively quickly and there is no need for the manipulation of the environment. The draw-backs of this method are that invaded sites are assumed to have had a similar composition to that of the uninvaded sites pre-invasion (Adair and Groves, 1998). Therefore, when conducting impact studies using multi-site comparisons it is important to (1) reduce variation between sites by comparing sites in similar habitats and (2) ensure that uninvaded sites have the potential to be invaded. The latter is important as an uninvadable site would suggest a composition different to that of an invaded site.

Prowse (2001)[2] conducted a comprehensive study of the impact of *I. glandulifera* on native plants species in the UK where the author used multi-site comparisons, removal, and addition experiments. Addition and removal experiments involve the manipulation of the occurrence of the non-native species, often in multiple sites and measuring the impact on recruitment or displacement of native species. Hulme and Bremner (2006)[2], studied the impact of *I. glandulifera* on native plant species, using removal experiments in a multi-site approach where paired plots consisted of one plot left as invaded and the second where *I. glandulifera* was removed. Again, Hejda and Pyšek (2006)[2] used removal experiments to evaluate the impact of *I. glandulifera* on the plant species

[2] See section 1.16 for a detailed evaluation of the findings of the studies

community. However, all studies were conducted over relatively short time periods of one or two seasons, and the recovery of the community can take considerable time to occur, especially if a species has been excluded from an area by the invader (Adair and Groves, 1998).

Time sequence studies monitor the spread of the non-native invasive plant species over time. Pre-invaded areas are monitored until eventual invasion (Adair and Groves, 1998). This method has the potential to provide an excellent data set if the study is able to predict the areas where the weed will spread. Such methods would be difficult to adopt for *I. glandulifera* in the UK, as management of the plant is a priority for most land managers and additional spread is undesirable. An alternative to this method would be to evaluate the impact of an invasive plant species along a gradient of invasion, from low to high percentage cover as demonstrated by Litt and Steidl (2010). The authors sampled the impact of the non-native grass *Eragrostis lehmanniana* Nees, on invertebrate populations over four years in the United Sates from a gradient of 0-91% total live biomass. Litt and Steidl (2010) showed that with every 100 g/m^2 increase in abundance of *E. lehmanniana* the number of invertebrate families decreased by 5%.

In this chapter, and chapter 4, I chose to evaluate the impact of *I. glandulifera* on invertebrate communities using the multi-site comparison method over two seasons. As reviewed in section 1.9, *I. glandulifera* is unusual in the fact that as a non-native widespread invasive plant species, the plant is an annual species and therefore prone to population fluctuations dictated by seasonal environment conditions. Thus, of interest would be how the invertebrate community responds to the fluctuating occurrence of *I. glandulifera*. Addition experiments were not considered an option due to the

conservation status of the habitats and by the request of the land-owners. In chapter 3, constrained by time, budget and traveling between sites, I again opted for the multi-site method but was restricted to sampling in just one season. Therefore, to enhance the study, I introduced an additional level, mixed vegetation, where the percentage cover of *I. glandulifera* was intermediate between the plant being absent and that of forming a monoculture (see section 3.2.1).

2.2 Methods

2.2.1 The study site

Harmondsworth Moor (Plate 2.1A) is a 135 ha public parkland situated in the county of Middlesex. Two rivers run through the park, the River Colne, running south of the eastern side of the park, and the River Wraysbury, running south of the western side of the park. The park, which is owned by British Airways, was a former gravel pit and landfill site. In the late 1990s, the land was restored and the park was opened in 2000. Harmondsworth Moor is the largest public parkland to be opened in the London catchment in the last 100 years. Although its former use may have led to the degradation of biological diversity, through sensitive conservation management the park has been restored to a more natural habitat and has been awarded the Green Flag Status every year since 2001 (Pers. comm., Paul Jarvis, British Airways).

The park is surrounded by a variety of different land usage, including industrial sites, urban sites, and transport networks (the M4 and M25). As a result, little can be done to reduce the influx of invasive plants from surrounding land into the parkland via the connecting river systems. Unfortunately this has led to the park being inundated with *I. glandulifera*, which has colonised the river banks and the grassland areas directly near the rivers flood plain (Plate 2.1B/C/D).

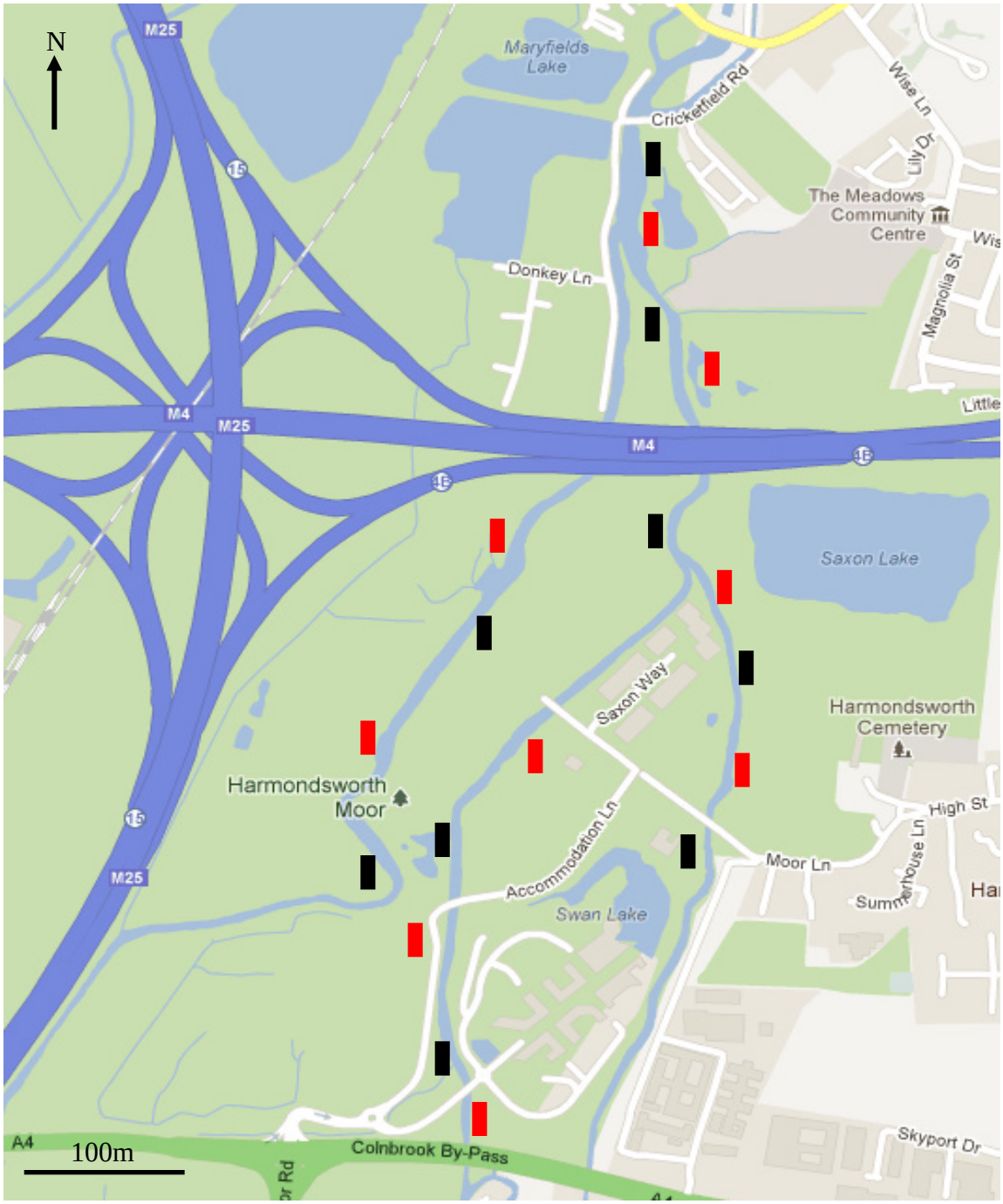
2.2.1.2 Sampling sites within the park

All sampling sites were selected within the park after a preliminary survey of the occurrence of *I. glandulifera* in late April 2007 (see figure 2.1 for site locations within Harmondsworth Moor).

Plate 2.1 Vegetation and equipment used at Harmondsworth Moor

A. An uninvaded site at Harmondsworth Moor dominated by grass species
B. An invaded site at Harmondsworth Moor
C. Impatiens glandulifera flowering at Harmondsworth Moor
D. Within the stand of an *I. glandulifera* monoculture
E. The aerial Vortis

Figure 2.1. A map of Harmondsworth Moor with the location of the sampled sites. Black rectangles indicate the positions of the invaded sites and red rectangles indicate the position of the uninvaded sites. All sites were 20m x 20m and at least 30m apart.



Site selection also followed the advice of the park managers. In total eighteen sites were selected, each 20m by 20m. These included nine sites where the percentage colonisation of *I. glandulifera* was no less than 60% of the total area, and therefore classed as invaded sites, and nine sites where the vegetation consisted of native stands, and therefore classed as uninvaded. All sites were at least 30m apart but otherwise similar in their position to the river and thus having the same potential to be invaded by *I. glandulifera*.

2.2.2 Invertebrate sampling

The invertebrate community was sampled monthly over two seasons including May through to, and including, September (2007) and including May and through to, and including, August (2008). All invertebrate sampling was conducted during a two-day period in each month. Invertebrates were sampled using two methods, a Vortis suction trap (to sample the ground/litter dwelling community), and an aerial Vortis suction trap (to sample the above ground/foliage community). Unfortunately, as the park is a public space, and following the request of the park managers, it was not deemed appropriate to sample invertebrates using permanent trapping methods such as pitfall traps due to potential disturbance.

2.2.2.1 Ground Vortis sampling

Ground Vortis suction traps (Burkard Manufacturing Co. Ltd,) have been widely used as sampling apparatus for ground dwelling invertebrate studies (Brook *et al.*, 2008). Vortis suction traps can be treated as a quantitative method for sampling invertebrates, in grassland systems, as the sampled area is equal to the aperture of the sampling tube, which provides an advantage over pitfall trap methods where the latter does not sample from a defined area (Brook *et al.*, 2008). The effectiveness of a Vortis suction trap has

been shown to be related to the timing of sampling (suction) for different invertebrate groups (Brook *et al.*, 2008). Less than 10 seconds sampling is adequate to capture 90% of species of planthoppers, spiders, and true bugs. The ground Vortis sampling was conducted each month throughout the summer months of 2007 and 2008. In total, each site was sampled nine times (5 in 2007 and 4 in 2008) throughout the course of the study. For each site, the ground Vortis sampling consisted of six ten-second vacuums per sample, where the sampler remained in the same location and turned in a 360^{o} position sampling 6 equally distant areas. This was then replicated six times throughout the site where the location of each sampled area was randomly selected. All samples were preserved in 70% alcohol prior to identification

2.2.2.2 Aerial Vortis sampling

The aerial Vortis (JCB Co. Ltd.) sampling follows the same principal of the ground Vortis where invertebrates are pulled into the apparatus using air movement and are subsequently captured (Stewart and Wright, 1995). The aerial Vortis was a reverse leaf blower with fine cloth secured to the end of the suction tube to capture invertebrates (Plate 2.1E). In a comparison study on the effectiveness of the reverse leaf blower compared to a standard ground Vortis suction trap, Stewart and Wright (1995) found that the reverse leaf blower was as efficient, for capturing species of Auchenorrhyncha, and performed better for capturing certain groups of beetles and spiders compared to the ground Vortis suction trap. The aerial Vortis sampling was conducted each month throughout the summer months of 2007 and 2008. In total, each site was sampled nine times (5 in 2007 and 4 in 2008) throughout the course of the study. For each site, the aerial Vortis sampling consisted of a sixty-second vacuum where the collector moved throughout the site moving the Vortis in a vertical and horizontal direction to encompass the structure of the vegetation. This was replicated six times throughout

each site and each sample period. Each sample was collected in a plastic bag, which was sealed and frozen prior to sorting and identification.

2.2.3 Vegetation sampling

Vegetation sampling was conducted in July of both years at each site. Six $1m^2$ quadrats were randomly placed within each site and individual species were identified, and their percentage cover per quadrat was visually estimated using a $10cm^2$ string grid within the $1m^2$ quadrat. Plant species were identified with the aid of keys and guides of Fitter *et al*. (1984), Phillips (1997) and Rose (1989).

2.2.4 Invertebrate identification

All invertebrates collected from the ground Vortis suction traps were identified to either order, sub-orders or distinct divisions within the orders[3] to allow for interpretation of the invertebrate community as a whole. As two people identified the invertebrates, checks were conducted on a subset of the samples in each sampled month to ensure consistency in identifications. For the aerial samples, the abundance of Coleoptera and Araneae were recorded, in addition to Auchenorrhyncha (Hemiptera: Homoptera), and Heteroptera (Hemiptera: Heteroptera). These groups were selected as the majority of species within these groups (with the exception of Araneae) are phytophagous and may potentially feed on *I. glandulifera* as oppose to just using the species as a resting point. Araneae were selected due to their value as an indicator species where their abundance suggests the presence of prey items (Neet, 1996).

[3] Sonal Varia (CABI) sorted the 2007 ground Vortis samples and assisted with the 2008 field sampling

2.2.5 Data analysis

2.2.5.1 The response of the invertebrate community to *Impatiens glandulifera*

All of the statistical analysis for the entire thesis was conducted using R version 2.12.2. (R Development Core Team, 2011). To analyse the effect of *I. glandulifera* invasion on the invertebrate community over time, multivariate analysis was considered the most appropriate method to evaluate the response of the communities to the effect of *I. glandulifera*. To evaluate the difference in invertebrate abundance between invaded and uninvaded sites the Principal Response Curve method (PRC) was used (van den Brink, *et al.*, 2009; van den Brink and ter Braak, 1999) (see section 1.23 for full details and worked examples).

For the ground Vortis samples, all replicates were pooled per site to give total abundance per site, per sampling date as a replicate. This was repeated for the aerial Vortis data-set. The native sites were the control sites and classified as uninvaded. The sites invaded by *I. glandulifera* sites were measured as deviations from the control and classified as invaded. A PRC analysis was performed on the ground Vortis data-set and the aerial Vortis data-set. Prior to the analysis, all of the invertebrate abundance data were log transformed with the value of 1 added to each data point (van den Brink and ter Braak, 1999).

2.2.5.2 Invertebrate abundance

The effect of *I. glandulifera* on invertebrate abundance, both total invertebrate abundance and selected individual sub-groups, was evaluated using a repeated measures ANOVA with treatment (invaded and uninvaded) and time as the main effects. As all

of the data were count data, all data sets were square root transformed, or log transformed with the value of 1 added (if the data sets contained zeros) to suit the assumptions of the test. The normality of the data and equality of variance was evaluated prior to the analysis.

2.2.5.3 The response of the invertebrate community to the percentage cover of vegetation

To evaluate the effect of the percentage cover of plant species on the invertebrate community, multivariate analysis was applied using the average percentage cover per quadrat, per season as the environmental variable. The vegetation data-set was classified into groups comprising of (1) *I. glandulifera* (2) trees and shrubs, (3) grasses and (4) forbs. Other non-native species were present including *I. capensis* and *Phalaris arundinacea* L., though their percentage cover was minimal in all sites and therefore did not warrant a separate non-native group. The invertebrate data-set from the ground Vortis and aerial Vortis sampling was pooled for each month and summed for each year, giving total invertebrate abundance for each group, per site, per year as a replicate. An indirect ordination analysis, Detrended Correspondence Analysis (DCA) was performed on the log transformed invertebrate data-set to evaluate whether to use linear or non-linear methods. If the gradient lengths from the resulting output were long (i.e. > 4) the data set was deemed as non-linear thus invoking the use of Canonical Correspondence Analysis (CCA). If the gradient lengths were < 4 this suggested the data-set was linear thus invoking the adoption of Redundancy Analysis (RDA) (ter Braak and Prentice, 1988; ter Braak and Smilauer, 1988). The invertebrate data-set had gradient lengths < 4, therefore RDA was performed on the log transformed invertebrate data-set, where the analysis incorporates environmental variables (percentage cover variables) into the multivariate analysis.

2.2.5.4 Vegetation

To evaluate any differences in plant species richness between invaded and uninvaded sites, and any differences between years, a two-way factorial ANOVA was performed on the total number of plant species per site. The data were square-root transformed prior to analysis to suit the assumptions of the test. All percentage cover data was averaged over the six sampled quadrats per site to give a site mean per year as the replicate. To evaluate any difference in the percentage cover of *I. glandulifera*, forbs and grasses between years, a one-way analysis of variance was conducted where the percentage cover variables were arc-sine transformed prior to analysis.

2.3 Results

2.3.1 The ground dwelling community

In total, 128,337 invertebrates were collected and identified into taxonomic groups from the ground Vortis during this study. There was a significant shift in the ground-dwelling invertebrate community composition between the invaded and uninvaded habitats for 2007 ($F_{1,80} = 11.27$, $P < 0.05$) and 2008 ($F_{1,64} = 9.879$, $P < 0.05$) (Figure 2.2). For 2007, the first canonical axis of the PRC explained 82.2% of the total variation where 18.51% was explained by time, and 13% by treatment (including the interaction with time). For 2008, the first canonical axis of the PRC explained 78.1% of the total variation where 41% was explained by time, and 6.4% by treatment (including the interaction with time). The groups Formicoidea, Coleoptera, Acari, Isopoda, Thysanoptera, Sternorrhyncha, Vespoidea, Araneae, Collembola, Heteroptera, and Auchenorrhyncha were all reduced in abundance in 2007 compared to the uninvaded sites, though in 2008 only Araneae, Auchenorrhyncha, Collembola, Heteroptera Vespoidea, and additional Stylommatophora were reduced in the invaded sites (Table 2.1). None of the invertebrates groups studied showed a positive association with the invaded stands. Table 2.2 shows the mean abundance per site for 2007 and 2008 of all invertebrate groups captured in the ground dwelling community.

The PRC graphic portrays the seasonal fluctuations of the invertebrate community in response to the growth of *I. glandulifera* in the invaded sites (Figure 2.2). Throughout 2007, the invertebrate community in the invaded sites deviates from the uninvaded habitats reaching a peak in September, driven by the invertebrate groups mentioned above. In 2008, there was a higher variation between the sampling months compared to 2007, and this may be a result of the seasonal spatial fluctuations in the percentage

cover of *I. glandulifera* between years and the recolonisation of native species (see section 2.3.3 and Appendix 1).

The total invertebrate abundance was consistently higher in the uninvaded habitats ($F_{1,16}$ = 14.52, P < 0.05) (Figure 2.3A). The total invertebrate abundance was also significantly influenced by time where the abundance was lower in 2008 compared to 2007 ($F_{8,128}$ = 6.018, P < 0.01) (uninvaded 2007: 1288.667 ± 121.346, invaded 2007: 646.422 ± 70.386; uninvaded 2008: 725.194 ± 96.427, invaded 2008: 420.861 ± 57.552).

The invertebrate group Auchenorrhyncha had the greatest decrease in abundance in the invaded sites compared to the uninvaded sites. Significantly more Auchenorrhyncha were present in the uninvaded stands compared to the invaded ($F_{1,16}$ = 47.52, P < 0.001) (Figure 2.3B). The Auchenorrhyncha community was significantly influenced by time where the abundance was lower in 2008 compared to 2007 ($F_{8,128}$ = 7.635, P = 0.001) (uninvaded 2007: 124.84 ± 11.903, invaded 2007: 30.444 ± 3.028; uninvaded 2008: 48.527 ± 8.456, invaded 2008: 20.166 ± 3.061). The invaded sites had the highest deviations from the uninvaded sites in September 2007 and July 2008 (Table 2.3), where Auchenorrhyncha was reduced to approximately 15.7 % (September 2007) and 23.3 % (July 2008) of the geometric mean count in the uninvaded sites.

Figure 2.2 Principal Response Curve of the invertebrate groups from the ground Vortis. Where (A) is 2007 and (B) is 2008. The control (uninvaded) is expressed as a horizontal line through zero and the black line is the response of the invertebrate community in the invaded sites over time (compared to the control). The invertebrates groups to the right of the graphic are ordered in their taxon weight corresponding to the y-axis. Invertebrate groups with a taxon weight of higher than 0.5 or lower than -0.5 are significantly influenced by the invaded sites.

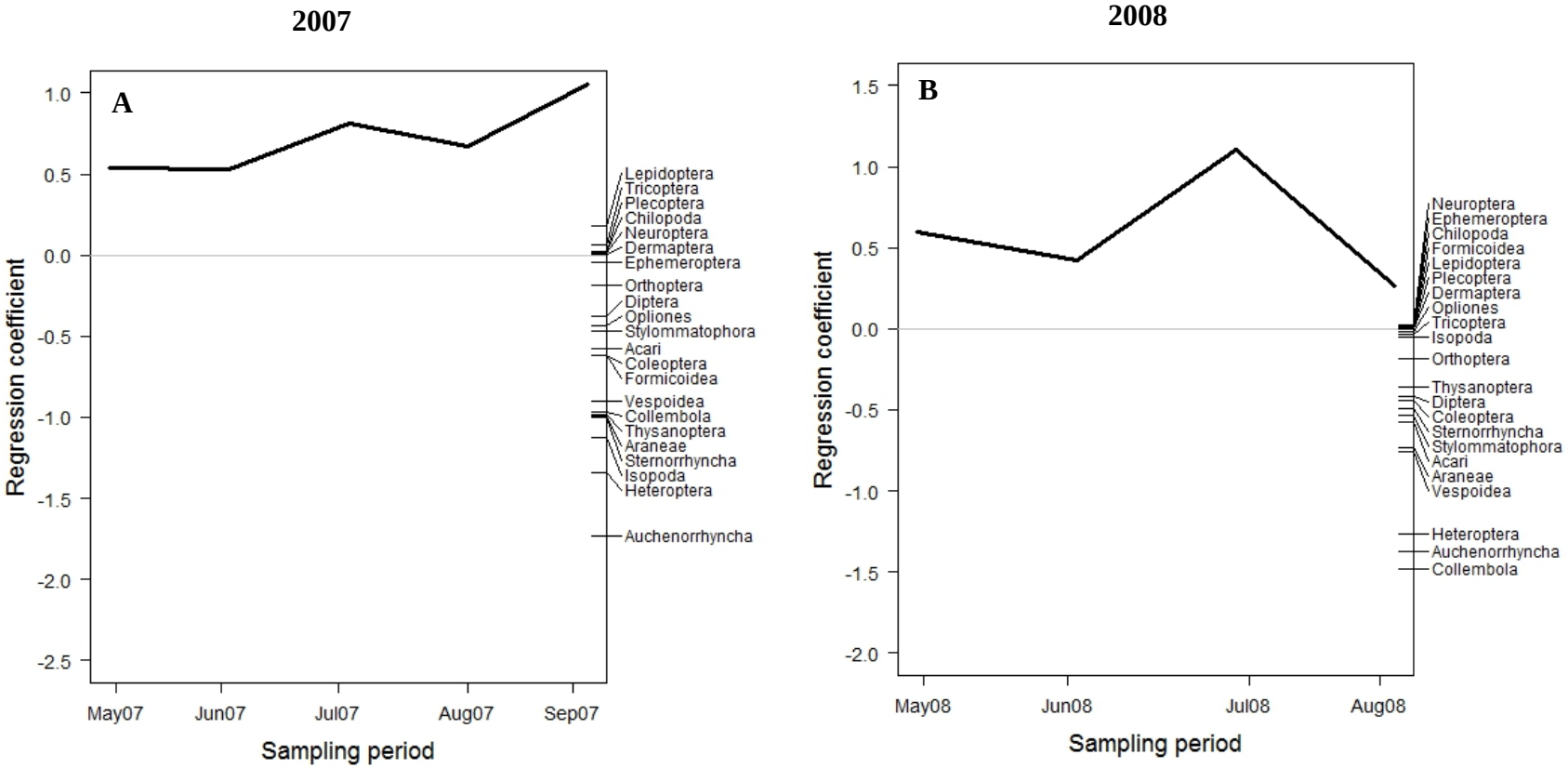


Table 2.1 The feeding guilds and taxon weights of the invertebrate groups from both the ground Vortis and aerial Vortis. Taxon weights in bold indicate a significant response to the invaded sites for both the aerial and ground Vortis samples, where a positive weight indicates an increase in abundance in the invaded sites and a negative weight indicates a decrease in abundance compared to the control. The feeding guild lists the dominant feeding guild within the group.

Group	Feeding guild	Taxon weight (b_k)			
		Ground Vortis		Aerial Vortis	
		2007	2008	2007	2008
Acari	Omnivores	**-0.579**	-0.574		
Araneae	Predators	**-0.989**	**-0.733**	**-1.613**	**-1.414**
Auchenorrhyncha	Phytophagous	**-1.732**	**-1.37**	**-2.287**	**-1.811**
Chilopoda	Predators	0.018	0.0178		
Coleoptera	Omnivores	**-0.614**	-0.443	**-1.541**	**-1.683**
Collembola	Detritivores	**-0.969**	**-1.479**		
Dermaptera	Omnivores	0.008	0.001		
Diptera	Omnivores	-0.381	-0.414		
Ephemeroptera	Omnivores	-0.048	-0.025		
Formicoidea	Omnivores	**-0.622**	-0.009		
Heteroptera	Phytophagous	**-1.343**	**-1.267**	**-2.1**	**-1.641**
Isopoda	Detritivores	**-1.122**	-0.0516		
Lepidoptera	Phytophagous	0.182	-0.009		
Neuroptera	Predators	0.015	0.025		
Opliones	Omnivores	-0.436	-0.021		
Orthoptera	Phytophagous	-0.188	-0.185		
Plecoptera	Predators	0.022	0.001		
Sternorrhyncha	Phytophagous	**-1.002**	-0.492		
Stylommatophora	Phytophagous	-0.461	**-0.537**		
Thysanoptera	Omnivores	**-0.987**	-0.362		
Tricoptera	Omnivores	0.067	-0.038		
Vespoidea	Omnivores	**-0.902**	**-0.755**		

Table 2.2. Mean invertebrate abundance per site from the ground Vortis samples for the uninvaded and invaded sites for 2007 and 2008

Year	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
site (Uninvaded)	1		2		3		4		5		6		7		8		9	
Group																		
Acari	1140	116	1333	180	288	75	581	92	178	133	580	91	1066	125	103	56	143	66
Araneae	207	141	264	243	320	177	757	66	258	180	225	90	208	53	90	47	128	172
Auchenorrhyncha	555	361	664	368	974	344	489	76	827	101	682	122	538	187	237	64	652	124
Chilopoda					1													
Coleoptera	137	58	265	36	166	122	167	29	149	33	176	55	139	21	37	36	90	83
Collembola	4086	4233	3222	2923	3752	2316	7642	2281	3217	787	3314	1627	2185	1599	2251	534	2673	1335
Dermaptera							2											
Diptera	536	334	511	252	471	150	380	197	519	188	305	140	301	141	147	193	271	206
Ephemeroptera	2						1				1							
Formicoidea	111	10	73	3	39		1221	4	3		55	5	30	13	4	45		4
Heteroptera	71	33	67	30	82	24	120	26	34	27	75	52	115	23	26	21	63	47
Isopoda	98	162	166	126	104	109	1092	22	159	132	108	37	50	17	38	36	30	53
Lepidoptera		21	1	18		1	4	104	0	9		24		22	1	7	2	15
Neuroptera																		
Opliones	11	3	10	5	10	5	7	1	14	11	6		6		3	3	6	10
Orthoptera	5	6	4	4	13	3	1	2	6	10	2		2	2		13	6	31
Plecoptera																1		
Sternorrhyncha	52	20	104	83	87	21	311	24	44	22	292	10	97	10	33	4	42	24
Stylommatophora	48	21	60	11	18	5	88	3	91	25	25	3	32	5	16	4	18	23
Thysanoptera	57	8	31	8	13	5	52	12	24	2	67	4	34	16	1	3	3	3
Tricoptera				3		2		5										1
Vespoidea	275	115	258	84	183	86	151	49	199	39	142	57	142	48	48	46	95	42

Table 2.2 (continued)

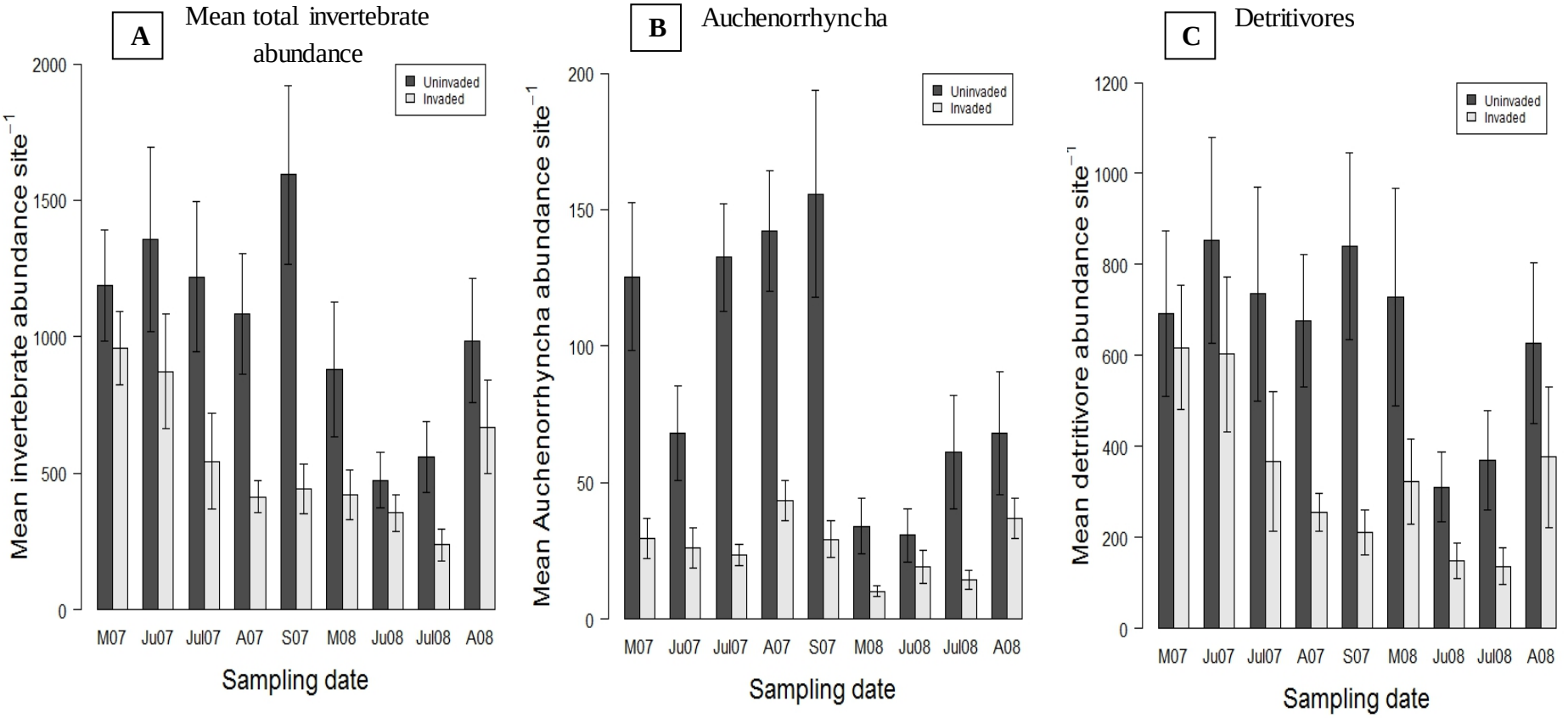
Year	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
site (Invaded)	1		2		3		4		5		6		7		8		9	
Group																		
Acari	163	37	124	51	118	115	187	76	168	118	328	192	301	65	239	65	173	163
Araneae	130	79	107	71	185	105	390	84	149	115	62	87	100	31	68	52	36	140
Auchenorrhyncha	107	82	118	116	207	70	203	54	201	71	194	113	125	63	125	57	90	100
Chilopoda						1	1						1					
Coleoptera	122	64	101	39	143	63	77	31	117	68	104	70	81	4	57	45	37	87
Collembola	1919	447	2112	774	3031	738	2926	1873	2651	1323	845	880	1240	477	1850	959	1444	940
Dermaptera					2		1											
Diptera	499	147	683	251	403	181	547	251	361	271	172	126	257	65	183	276	129	600
Ephemeroptera		1	1										1					
Formicoidea	23	15	9	13	18	10	144	2	10	1	29	245	12	4	4		2	1
Heteroptera	13	4	10	15	38	14	28	9	26	9	13	14	15	14	9	14	34	23
Isopoda	48	107	48	21	68	31	147	15	86	52	20	59	17	11	10	50	13	111
Lepidoptera	2	52	1	19	8	16	6	6	1	12	23	5	1	32		5		14
Neuroptera	1					1												
Opliones	3	1	5		6	6	7	7	6			3	5		2	4	1	2
Orthoptera	5	3		1	22	2		1	1	2			2		1	7		15
Plecoptera							1			1								
Sternorrhyncha	93	8	32	72	39	8	74	16	55	8	95	11	33	13	38	6	57	17
Stylommatophora	35	4	36		31	8	87	7	21	2	7	2	19		7	5	5	13
Thysanoptera	1	5	5	7	6	3	9		7	1	18	8	2	14	1	3	4	6
Tricoptera	4					1		1		1				1		1		
Vespoidea	88	45	75	54	120	22	131	45	122	53	62	56	83	37	47	27	40	55

Table 2.3 The resulting Principal Response Curve coefficients for the ground Vortis and aerial Vortis samples for the invaded sites + sampling date which are contrasts to the control (uninvaded).

Sampling method	Sampling date								
	May	June	July	Aug.	Sept.	May	June	July	Aug.
	2007					2008			
Ground Vortis	0.53	0.527	0.808	0.673	1.05	0.594	0.421	1.104	0.263
Aerial Vortis	0.762	0.578	0.976	0.882	0.774	0.736	0.651	0.891	0.8

Detritivore abundance (Isopoda and Collembola) was significantly decreased in the invaded sites compared to the uninvaded sites ($F_{1,6} = 17.765$, $P < 0.001$) (Figure 2.3C). Time was a significant factor where fewer individuals were collected during 2008 compared to 2007 ($F_{1,128} = 3.078$, $P < 0.001$) (uninvaded 2007: 759.711 ± 86.572, invaded 2007: 410.556 ± 58.18; uninvaded 2008: 509.134 ± 83.182, invaded 2008: 246.333 ± 49.297).

Figure 2.3 Differences in invertebrate abundance for the ground Vortis samples between invaded and uninvaded sites. Where (A) mean total ground-dwelling invertebrate abundance ± S.E, (B) mean Auchenorrhyncha abundance ± S.E and (C) mean detritivore abundance ± S.E for the ground Vortis sampling in the invaded and uninvaded sites for each month sampled. Total invertebrate abundance, Auchenorrhyncha, and detritivores abundance was all higher in the uninvaded sites compared to the invaded sites. (M: May, Ju: June, Jul: July, A: August and S: September).



2.3.2 Aerial Vortis

In total 8,709 invertebrates were identified into taxonomic groups from the aerial Vortis over the two season study. Similar to the ground Vortis samples, the aerial Vortis samples showed a significant shift in the invertebrate community when comparing the invaded and uninvaded sites for 2007 ($F_{1,80} = 88.467$, $P < 0.05$) and 2008 ($F_{1,64} = 30.015$, $P < 0.05$) (Figure 2.4A/B). For 2007, the first canonical axis of the PRC explained 95.1% of the total variation where 10.32% was explained by time and 48.19% by treatment (including the interaction with time). For 2008, the first canonical axis of the PRC explained 97.7% of the total variation where 7.06% was explained by time and 30.13% by treatment (including the interaction with time). All of the groups studied (Auchenorrhyncha, Heteroptera, Coleoptera and Araneae) showed a decrease in abundance in the invaded sites compared to the uninvaded sites for each sampling date. It is interesting to note that all four taxonomic groups show a similar position on the aerial and ground Vortis PRC graphic indicating that these invertebrate groups show a similar response to the presence of *I. glandulifera* in both micro-habitats. The aerial PRC graphic shows some evidence of seasonal fluctuations though this is not as pronounced as that seen in the ground Vortis.

The total number of invertebrates was significantly higher in the uninvaded sites compared to the invaded sites for each sampling date ($F_{1,16} = 74.434$, $P < 0.001$) (Figure 2.5A). There was a significant effect of time ($F_{8,128} = 3.34$, $P < 0.05$) (uninvaded 2007: 92.222 ± 7.574, invaded 2007: 23.177 ± 3.803; uninvaded 2008: 77.416 ± 9.482, invaded 2008: 15.25 ± 2.053) where less invertebrates were collected in 2008 compared to 2007 (see table 2.4). Similar to the ground Vortis, Auchenorrhyncha were the most affected by *I. glandulifera* with significantly fewer individuals found in the invaded sites ($F_{1,16} = 49.449$, $P < 0.001$).

Figure 2.4 Principal Response Curve of the invertebrate groups from the aerial Vortis. Where (A) is 2007 and (B) is 2008. The control (uninvaded) is expressed as a horizontal line through zero and the black line is the response of the invertebrate community in the invaded sites over time (compared to the control).

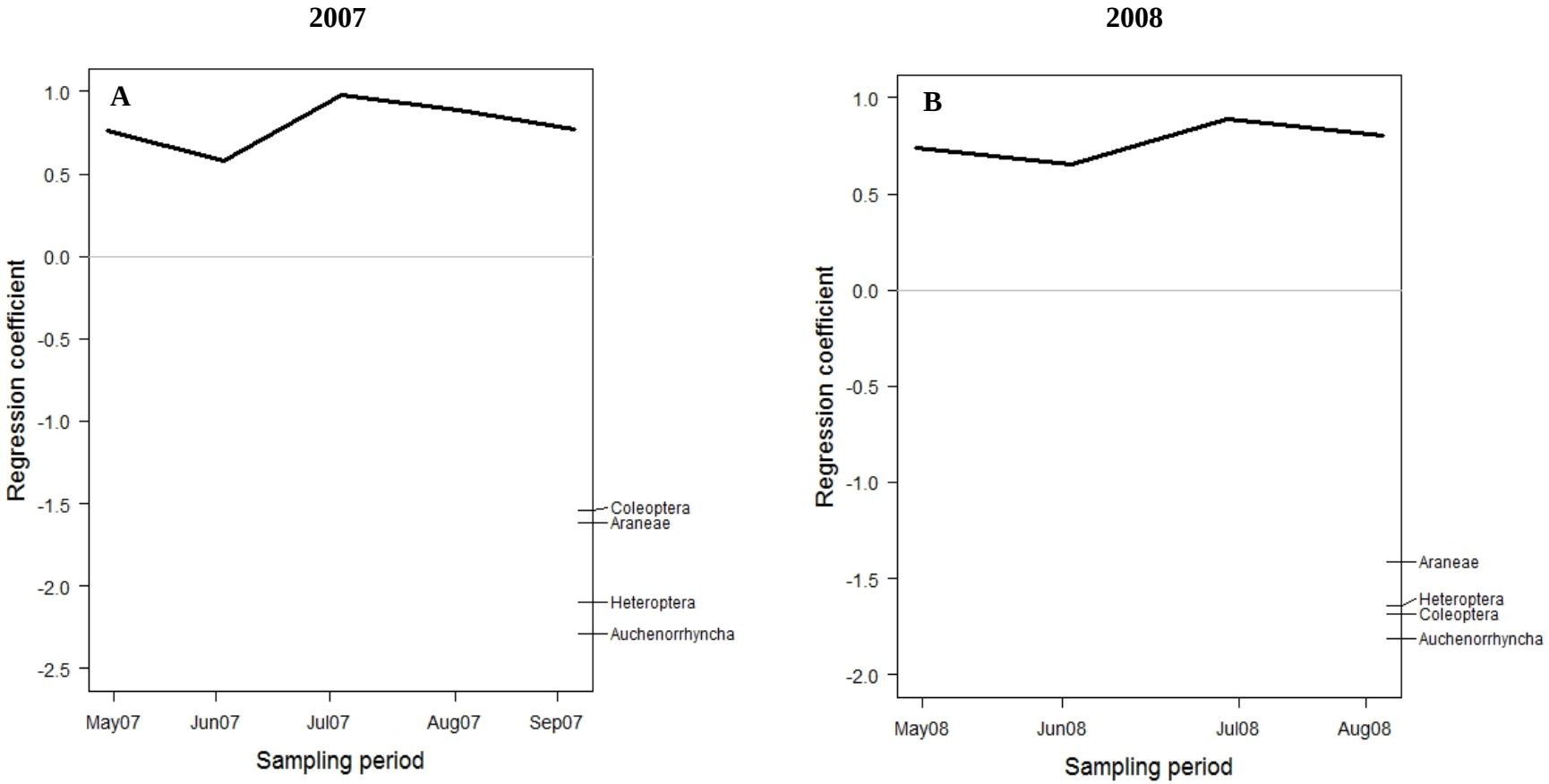


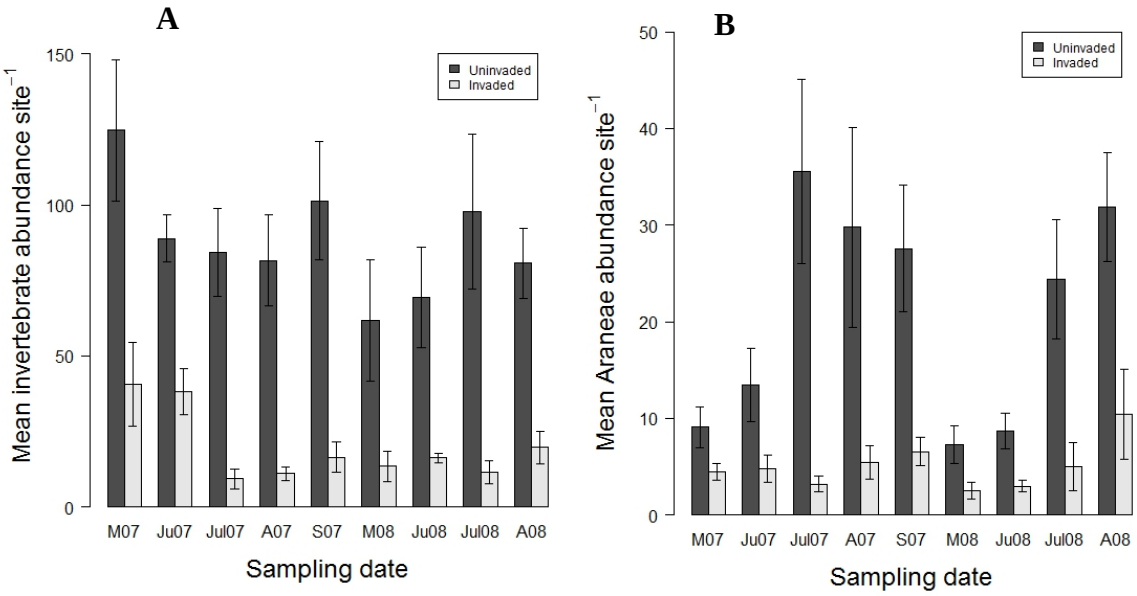
Table 2.4. Mean invertebrate abundance per site from the aerial Vortis samples for the uninvaded and invaded sites for 2007 and 2008

Year	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
site (Uninvaded)	1		2		3		4		5		6		7		8		9	
Group																		
Araneae	106	96	144	86	118	64	297	94	129	111	83	63	82	57	34	43	46	37
Auchenorrhyncha	370	287	266	267	354	201	177	35	287	143	285	204	128	45	166	66	163	74
Coleoptera	95	31	57	48	38	18	102	78	66	55	41	37	52	20	8	38	11	81
Heteroptera	85	32	100	54	53	53	62	21	48	51	76	93	59	58	87	19	55	27

Year	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
site (Invaded)	1		2		3		4		5		6		7		8		9	
Group																		
Coleoptera	11	5	11	5	9	3	4	35	22	1	3	5	4	3	9	4	23	7
Auchenorrhyncha	7	22	34	25	28	13	14	20	50	35	113	29	128	27	153	15	121	23
Heteroptera	2	5	4	9	10	8	5	12	13	15	11	2	4	17	12	9	18	6
Araneae	15	10	26	13	29	6	37	75	34	30	24	25	25	8	16	7	14	15

Again, there was a significant effect of time ($F_{8,128} = 1.294$, $P < 0001$) (uninvaded 2007: 48.8 ± 6.311, invaded 2007: 14.4 ± 3.491; uninvaded 2008: 36.722 ± 6.789, invaded 2008: 5.805 ± 0.883).

Figure 2.5 Differences in invertebrate abundance for the aerial Vortis samples between invaded and uninvaded sites. Where (A) is the mean total invertebrate abundance ± S.E and (B) is the mean Araneae abundance ± S.E for the aerial Vortis sampling in the invaded and uninvaded sites for each month sampled. (M: May, Ju: June, Jul: July, A: August and S: September).



The abundance of Araneae was significantly lower in the invaded sites ($F_{1,16} = 32.93$, $P = < 0.001$) (Figure 2.4B), and there was a significant effect of time ($F_{8,128} = 5.454$, $P < 0.001$) (uninvaded 2007: 23.088 ± 3.425, invaded 2007: 4.888 ± 0.572; uninvaded 2008: 18.083 ± 2.737, invaded 2008: 5.25 ± 1.389). There was an indication of an interaction between treatment and time ($F_{8,128} = 1.952$, $P = 0.0576$), suggesting that this group followed different temporal patterns in the invaded and uninvaded sites.

2.3.3 Vegetation changes between years

There was a significant shift in the percentage cover of *I. glandulifera* in the invaded sites between 2007 and 2008 where *I. glandulifera* was significantly reduced in 2008 ($F_{1,16} = 110.09$, $P < 0.001$) (Figure 2.6A, also see Appendix 1 which details the differences in the plant species community percentage cover between 2007 and 2008). Seasonal fluctuations in the occurrence of *I. glandulifera* have been observed throughout the study period, in a variety of habitats (Pers. obs., Author), often as a result of a late frost which has a high mortality on the emerging seedlings, rather than direct competition with native plant species. With the decrease in cover of *I. glandulifera*, grasses (Figure 2.6B), and trees and shrubs showed no significant increase in percentage cover between years ($F_{1,16} = 2.701$, $P = 0.119$). Forbs increased in 2008, colonising the void left by a reduced percentage cover of *I. glandulifera* ($F_{1,16} = 35.836$, $P < 0.001$) (Figure 2.6C). Plant species richness between the invaded and uninvaded sites ($F_{1,32} = 0.438$, $P = 0.512$) between years remained constant ($F_{1,32} = 1.685$, $P = 0.203$) suggesting there was no recruitment of new species into the invaded areas as a result of the reduced percentage cover of *I. glandulifera*.

2.3.4 The relationship between vegetation cover variables and invertebrate groups

The invertebrate group – percentage cover bi-plot explains 13.06% of the total variation for 2007 ($F_{4,13} = 2.708$, $P < 0.05$) and 10.27% of the total variation for 2008 ($F_{4,13} = 1.617$, $P < 0.05$) (Figure 2.7). For the 2007 bi-plot, axis 1 is correlated with the percentage cover of *I. glandulifera* and explains 34.77% of the total variation whereas axis 2 is correlated with the percentage cover of forbs and explains 6.39% of the total variation. For 2008, axis 1 is correlated with *I. glandulifera* and explains 17.01% of the total variation and axis 2 is correlated with grasses and explains 9.12% of the total

variation. Almost all invertebrate groups are negatively associated with the percentage cover of *I. glandulifera* with the exception of Lepidoptera in 2007 and Formicoidea in 2008. For both years, trees and shrubs appear to be unimportant in determining the structure of the invertebrate community.

Figure 2.6 The seasonal change in the percentage cover of vegetation in the invaded sites between years. (A) *Impatiens glandulifera*, (B) forbs and (C) grasses between 2007 and 2008 in the invaded sites. The box and whisker plots show the median percentage cover expressed as the solid horizontal line within the box. The top and bottom of the box show the 75th and 25th percentiles, respectively. The vertical dashed line shows the interquartile range and the points show the outliers of the data (Crawley, 2007). Where *Impatiens glandulifera* showed a significant reduction in percentage cover in the invaded sites in 2008 ($P < 0.001$), the percentage cover of forbs was significantly increased ($P < 0.001$). The percentage cover of grasses remained constant between seasons ($P = 0.119$).

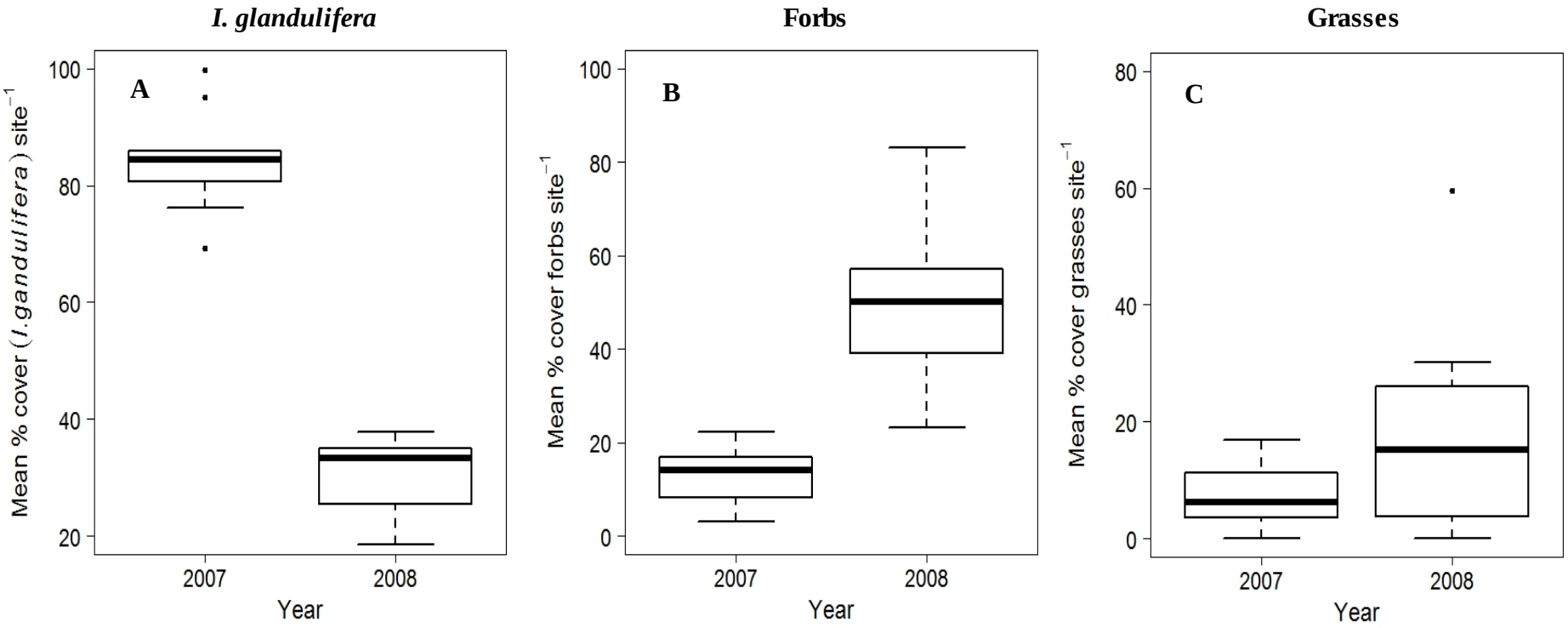
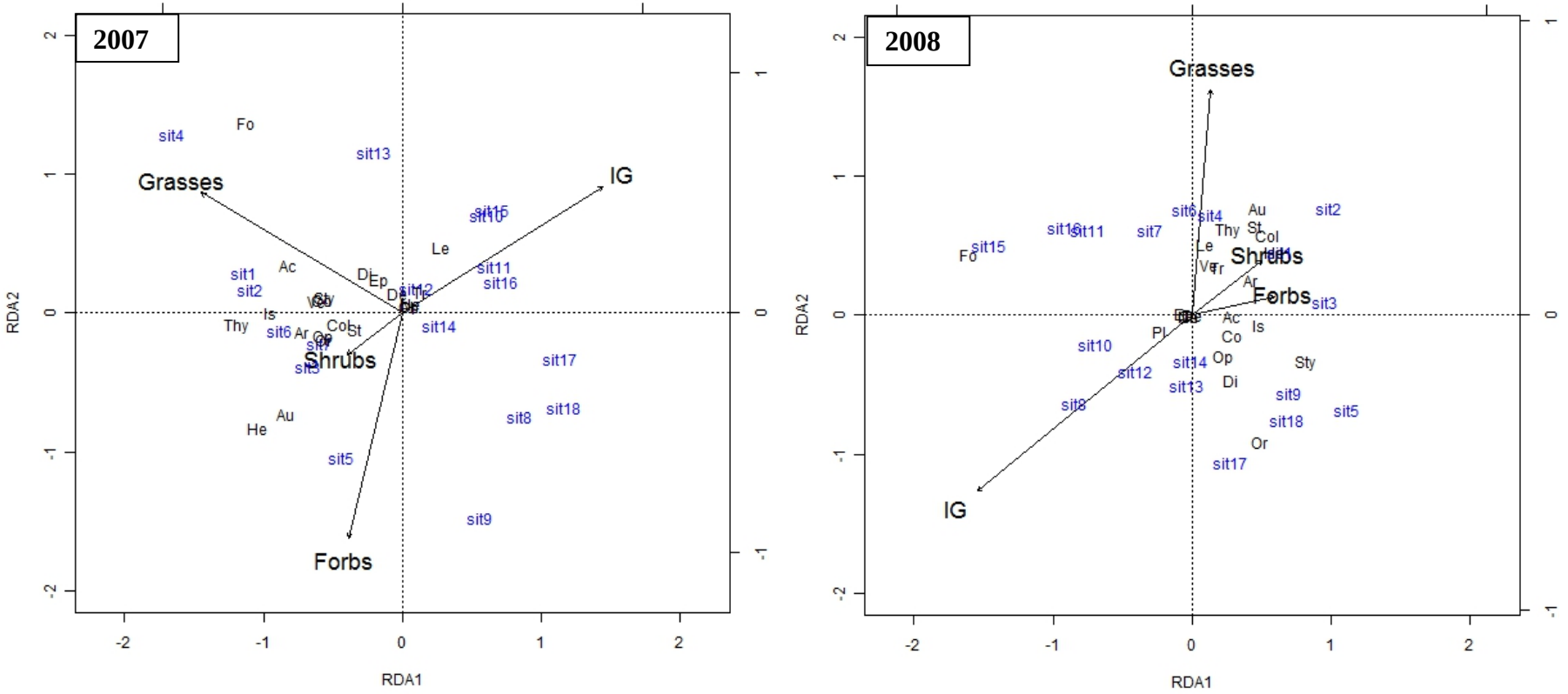


Figure 2.7 Biplot of the percentage cover of vegetation (grasses, forbs, shrubs (including trees) and *Impatiens glandulifera*) and invertebrate abundance for both the ground Vortis and aerial Vortis data sets combined for 2007 and 2008. Plot labels are: Ac: Acari, Ar: Araneae, Au: Auchenorrhyncha, Ch: Chilopoda, Co: Coleoptera, Col: Collembola, De: Dermaptera, Di: Diptera, Ep: Ephemeroptera, Fo: Formicoidea, He: Heteroptera, Is: Isopoda, Le: Lepidoptera, Ne: Neuroptera, Op: Opliones, Or: Orthoptera, Pl: Plecoptera, St: Sternorrhyncha, Sty: Stylommatophora, Thy: Thysanoptera, Tr: Tricoptera, Ve: Vespoidea. Sit 1-18 correspond to individual sites where sites 1-9 are the uninvaded sites and sites 10-18 are the invaded sites. The environmental label IG: *Impatiens glandulifera*.



2.4 Discussion

This study shows that the above-ground invertebrate community is affected by the presence of *I. glandulifera*. Both the ground-dwelling and foliage-inhabiting invertebrates showed seasonal shifts in the composition of groups in invaded sites compared to uninvaded. However, the shift of the invertebrate community away from the invaded sites was more pronounced for the foliage dwelling invertebrates compared to the ground dwelling invertebrates and this may potentially be due to the availability of food in the canopy of the invaded stands compared to within and below the monoculture of *I. glandulifera*.

The vast majority of invertebrate groups had a negative correlation with the percentage cover of *I. glandulifera* as opposed to the positive relation to native plant species indicating that the invertebrate community comprises of species that are dependent on native vegetation as their food source and/or oviposition or are not utilizing a community which is more heterogeneous in structure than native vegetation (Kappes *et al.*, 2007). These results are consistent with other studies that show an impact of non-native plant species on invertebrate populations (Gerber *et al.*, 2008; Kappes *et al.*, 2007; Litt and Steidl, 2010). Few invertebrate species were observed feeding directly on *I. glandulifera* during the duration of the study with the exception of the elephant hawk moth, *Deilephila elpenor*. Knight (2010) shows that invertebrate species, that do feed on non-native plant species in the UK, generally have a reduced performance on the non-native plant species compared to their preferred host. In an experiment where the author reared larvae of the elephant hawk moth on its native host plant (*Chamerion angustifolium* L.) and *I. glandulifera* (a non-native host), the elephant hawk moth performed better (expressed as biomass and survival) on the native host. Tallamy

(2004) suggests that ornamental plants are initially selected for wholesale because they are regarded as pest-free and unpalatable to invertebrates in the introduced range, which may go some to explain the lack of natural enemies found feeding on *I. glandulifera* in the UK.

The within season and between season fluctuations of the invertebrate community in response to the invaded sites is elegantly shown by the PRC graphic and resulting output. When relating the PRC to the growth and phenology of *I. glandulifera,* an association is clearly seen in the ground micro-habitat though less defined in the aerial micro-habitat. From May to June 2007, the response remains parallel to that of the uninvaded habitats until just after June when a deviation was seen away from the uninvaded sites until September (the last sampling period of 2007). This would coincide with the rapid growth and subsequent monopoly of *I. glandulifera* within the invaded habitats that leads to shading of the invaded habitat and reduced amount of sunlight availability within the invaded stands. The dense monoculture would remain until the first frost in late September or October. A similar pattern was observed in 2008, though the variability of the line is more erratic. However, the important point to note is that in both micro-habitats the PRC never falls below zero, therefore the structure of the invertebrate community, as expressed by the taxon weights, remains constant throughout the sampling period.

The similarity in plant species richness between the invaded and uninvaded sites reflect the results of other studies including those of Prowse (2001) and Hejda and Pyšek (2006), in the fact that plant species richness seemed unaffected by *I. glandulifera*. Uninvaded sites had an average of 11.8 plant species per site in 2007 compared to 12.3 plant species per site in invaded plots while in 2008, 8.1 plant species per site were

identified in the uninvaded sites compared to 10.2 plant species in invaded sites. The results conflict with those of Hulme and Bremner (2006) who showed species recruitment was significantly increased when *I. glandulifera* was removed from an area. The results of this study show only the percentage cover of native species increased rather than species richness. Hulme and Bremner (2006) physically cleared *I. glandulifera* from plots during their experimental design and this may potentially enhance native plant colonisation, compared to natural fluctuations of the population, by providing an empty niche.

The significant reduction in the percentage cover of *I. glandulifera* between the two seasons allowed for an evaluation of the recovery of the invertebrate population in response to the fluctuating changes in plant species occurrence. As discussed in section 1.5, *I. glandulifera* seedlings are vulnerable to late spring frosts and this was the case in late April and early May 2008, where the early germinating seedlings were killed reducing the overall abundance of the population. Interestingly, with the reduced percentage cover of *I. glandulifera* in the invaded sites, the invertebrate community failed to recover significantly between years. However, future research would benefit from a longer timescale study to evaluate how the invertebrate community responds to fluctuating plant species composition and native plant species recovery. The 2008 redundancy analysis still showed a negative association with almost all of the invertebrate groups and the total invertebrate abundance for both the aerial and ground dwelling micro-habitats remained consistently higher in the uninvaded habitats compared to the invaded habitats.

The lack of a significant recovery of the invertebrate community in the invaded sites may potentially be due to the quality and quantity of native vegetation in the invaded

sites compared to the uninvaded sites (Stiling and Moon, 2005). Although plant species richness remained relatively constant in both the invaded and uninvaded sites between years, the sheer abundance of *I. glandulifera* and over shadowing of native species, which was observed after the 2008 vegetation survey, may lead to reduced niche availability for the invertebrate community. In addition, indirect effects as a result of the occurrence of *I. glandulifera* may influence the performance and quality of native plant species in the invaded sites (Vilá *et al.*, 2011) (see section 4.3.2).

Whereas the foliage-dwelling invertebrate community showed a consistently negative association with the invaded sites in the ground-dwelling community, 10 invertebrate groups showed no response to the invaded sites (Table 2.1). Of these 10 invertebrate groups, 60% were winged mobile groups like Diptera and Ephemeroptera. It is plausible that these groups may use *I. glandulifera* stands as resting sites within the community as a whole. Other invertebrate groups included conspicuous species like grasshoppers (Orthoptera) which could be using the invaded habitats as refuges from predator species. As highlighted by van den Brink and ter Braak (1999), care should be taken when interpreting PRC results. Groups with a taxon weight of between - 0.5 and 0.5 are not regarded to react significantly to the effect of *I. glandulifera*. A response may be present but this may be reflected as a different pattern to that of the PRC.

In certain cases, non-native invasive plants have been shown to benefit certain groups of invertebrate species (as discussed within Gerber *et al.*, 2008). Some of the more vigorous invaders, for example non-native plant species from the genus *Fallopia*, almost permanently simplify an invaded habitat, in the terms of structural diversity, year on year leading to beneficial niches for exploitation by more mobile predatory invertebrate groups (Kappes *et al.*, 2007). However, as the results from this study show,

the impact of *I. glandulifera* on native plants is more seasonal and varies from year to year. Often plant species present in uninvaded plots are present in invaded plots, though in lower abundance, cover and vigour. Therefore, the carrying capacity of native plant species for the invertebrate community within invaded stands may be significantly reduced.

The majority of phytophagous invertebrates within the invaded stands are unlikely to be utilising *I. glandulifera* directly as a food source (Beerling and Perrins, 1993). Most invertebrate species would be utilising the invaded habitat as part of the wider environment, potentially as a resting area or a corridor for movement between stands of native vegetation. When *I. glandulifera* grows in scattered monocultures, dividing native vegetation, the invaded community may have an indirect impact on fragmented areas of native vegetation, and the vegetation within the invaded stands by increasing the pressure exerted on native species by natural enemies.

Total invertebrate abundance was significantly decreased in the invaded sites compared to the uninvaded sites for both the ground dwelling and foliage dwelling community. There was a general decreasing trend in total invertebrate abundance in the invaded stands for both micro-habitats in 2007, though in 2008 this trend was less obvious. Indeed, in general, the overall abundance of invertebrates was lower in 2008 compared to 2007 and this could potentially be due to the increased rainfall observed during the sampling times in 2008 compared to 2007. The two groups with the strongest negative response to *I. glandulifera*, in both the foliage and the ground micro-habitats were Auchenorrhyncha and Heteroptera, both of which comprise solely of herbivores. Gerber *et al.* (2008) discuss how herbivorous insects are groups within the invertebrate community which should show the strongest negative response to non-native species, as

with the lack of specialist natural enemies the non-native is an unpalatable alternative to their preferred host plant. Although the order Coleoptera comprises omnivorous species, few ground beetles (carabid) were collected within the samples and the majority of individuals belonged to the family Chrysomelidae, entirely a family of herbivorous species.

As an indicator group, Araneae (spiders), provide an indication of the health of the community (Neet, 1996) and it is interesting to note that in both micro-habitats, during both years, Araneae were consistently negatively associated with invaded sites. This indicates that as a predatory group, dependent on prey species, there is an overall negative response in the whole community and therefore an overall shift in the food web dynamics of the invertebrate community to the invaded sites, where herbivore and predator species respond together. Although a slight recovery of the spider community was shown in the invaded sites in 2008, after the percentage cover of *I. glandulifera* was significantly reduced, the group did not significantly recover (taxon weight – 0.989: 2007; - 0.733: 2008). A similar lag phase of recovery was shown in a study comparing spider diversity in invaded and uninvaded stands of *Chromolaena odorata* (L.) R.M. King and H. Robinson, in South Africa (Mgobozi *et al.*, 2008). Here, the authors showed that even after physical removal of the invasive plant, two Araneae families (Cyrtaucheniidae and Uloboridae) did not return to the managed stands. Although the habitat structure is simplified in invaded stands, compared to uninvaded, which may increase the capture rate of prey species, the abundance of prey species was significantly reduced in this study. As a predatory group, spiders may be potentially more sensitive to the natural fluctuations of an invasion than more mobile prey species. Indeed, the peak activity of spiders is later in the season when *I. glandulifera* has

achieved its maximum height and aerial monopoly of the invaded habitat, even at a lower percentage cover.

The total abundance of detritivores was significantly lower in the invaded habitats and both Collembola and Isopods showed a strong negative response to the invaded sites compared to the uninvaded sites. Decomposition of vegetation by these groups is an important process in the cycling of nutrients within the ecosystem. Dangles *et al.* (2002) showed differences in the ability of three species of detritivores to process leaves of the non-native species *F. japonica* indicating that reduced detritivore diversity in invaded stands might have ecosystem consequences. As *I. glandulifera* is now our tallest annual species in the UK and can form extensive branching, the above ground biomass can be significantly greater than native forb species, thereby more material is incorporated into the ecosystem. However, in a study comparing the decomposition rates of native and introduced plant species, Bottollier-Curtet *et al.* (2011) showed *I. glandulifera* and *U. dioica* had similar rates of decomposition and were among the highest rates of the ten plant species studied. This study was conducted within the water body and decomposition rates may differ to that on land and/or the species composition within an aquatic environment compared to a terrestrial environment are different (Nowlin *et al.*, 2008). Indeed, Dangles *et al.* (2002 showed that species composition of detritivores influences the decomposition rates of *F. japonica*. Dried stems of *I. glandulifera*, from the previous season were found throughout invaded stands the following season, and beyond which may have implications of the recycling of nutrients and the productivity of the invaded sites.

At an ecosystem level, the reduced abundance and invertebrate groups found in invaded stands dominated by *I. glandulifera*, compared to uninvaded stands, has consequences

at higher levels in the food chain. Birds, mammals, and amphibians feed on the abundance of invertebrates found within native stands of vegetation and if *I. glandulifera* is significantly reducing the invertebrate abundance on a national scale this has serious impacts which would potentially feed through the system impacting on higher trophic levels. Research into the impact of *Solidago* species on grassland birds in eastern Europe showed the invasion significantly reduced bird species richness by reducing the habitats available for nesting and potentially food availability (Skórka *et al.*, 2010). When studying the impact of *Salix x repens* L., an invasive riparian tree species in eastern Australia, Holland-Clift *et al.* (2011) showed sites dominated by this species has reduced bird diversity. In the same region, Greenwood *et al.* (2004) showed *Salix x repens* reduced invertebrate abundance and composition of the community.

Further research is needed into the potential impacts of invasive non-native plant species at an ecosystem level, including multi-trophic levels. Understanding the impact at a species level is essential for conservation objectives. For example if *I. glandulifera* is affecting rare species, endemic to invaded habitats conservation/control measures should be adopted. Further studies would benefit from recording species composition, whether actual species or morphospecies. This study has highlighted the impact of just one non-native invasive plant species but further studies, on other non-native plant species, would be beneficial to focus management efforts. Indeed, these studies are challenging to achieve, due to the multitude of interactions within the community and the array of expertise required. Expertise would be required from all disciplines of biology including ornithologists, entomologists, botanists, and community ecologists. In addition, multi-habitat comparisons would provide tangible insights into how plant invasions affect species within varying complexities of floral composition and faunal interactions.

3 The impact of *Impatiens glandulifera* on carabid assemblages of exposed riverine sediments in the south west of England

3.1 Introduction

River systems are undoubtedly one of the most diverse habitats found within the British Isles (Boon *et al.*, 1992). Their benefits include natural amenities for relaxation and recreation, they harbour high levels of biological diversity, act as natural flood management, provide water for consumption and irrigation, and act as corridors for the movement of nutrients and species in an otherwise fragmented landscape (Boon *et al.*, 1992; Norris and Thoms, 1999). All of the aforementioned benefits contribute to river systems providing a high level of ecosystem services (Loomis, *et al.*, 2000). Intensive habitat modification throughout the UK, through agriculture, transportation and urbanisation, has lead to many river systems becoming polluted and modified (Brown *et al.*, 2010; Larsen and Ormerod, 2010; Reaney *et al.*, 2011).

Channel and bankside management and the extraction of water for human benefit have led to increased pressure on these vulnerable habitats at an alarming and unsustainable rate. Increased bank erosion which results in increased sediment intake into the water body (Collins *et al.*, 2010; Environment Agency, 2010), pollutants (Batty *et al.*, 2010; Millier *et al.*, 2010), climate change (Whitehead *et al.*, 2009) and altered water flow has lead to a change in the species composition in discrete areas and species extinction at local and national levels (Environment Agency, 2010). In addition to anthropogenic

pressures on riparian systems, abiotic influences such as seasonal variations in hydrological processes, which in turn can alter geomorphic factors within the catchment, in space and time, render riparian habitats prone to high levels of disturbance (Sadler and Bell, 2000; Bell and Sadler, 2003a).

One type of habitat within riparian systems which is prone to disturbance due to its position, are the Exposed Riverine Sediments (ERS) (Bates *et al.*, 2007; Sadler *et al.*, 2004). The distribution of ERS throughout the catchment is influenced by geomorphological and hydrological influences, thus their occurrence is patchy in both time and space (Sadler *et al.*, 2004). Eyre and Lott (1997) describe the most common locations of ERS being downstream on the inside of a river bend where the water flows slower compared to the outside of the bend. ERS are formed when the river is in full flow; sediment is carried downstream and deposited at a slow flowing part of the river. When the water levels decrease in the spring and summer months the ERS are left exposed, often until the autumn months when the rivers increase in capacity because of autumnal rains. Throughout the spring and summer months ERS may become submerged sporadically by flooding events. ERS form the interface between the aquatic and terrestrial environment and thus harbour a unique habitat, which contain a diversity of invertebrate groups (Sadler *et al.*, 2004).

ERS have long been known to harbour high levels of invertebrate diversity including ground beetles (Coleoptera: carabidae) (Eyre *et al.*, 2002), flies (Diptera), and spiders (Araneae), some of which are endemic to ERS habitats (Eyre *et al.*, 2001a; Eyre *et al.*, 2002; Sadler and Bell, 2000; Sadler and Bell, 2002). Additionally, rare and endangered species have long been associated with ERS habitats. Three rare ground beetle species *Perileptus areolatus* Creutz, *Bembidion testaceum* Duft. and *Lionychus quadrillum*

Duft., all with a high fidelity to ERS, are on the UK's Biodiversity Action Plan (BAP) list (Eyre and Luff, 2002).

Chapter 2 showed that *I. glandulifera* impacts on the invertebrate community by changing the composition of invertebrate groups within invaded sites compared to native vegetation. In addition, the abundance of invertebrates was shown to decrease within invaded sites compared to the uninvaded sites. This in itself is of scientific interest. But if *I. glandulifera* is impacting on the species composition of an invaded habitat where the invasion displaces specialist species and increases generalist species, then this has further conservation impacts and thus needs evaluating. Thus, evaluation is needed at a species level.

To evaluate the impact of *I. glandulifera* on the invertebrates on ERS, ground beetle species (carabidae) were chosen as the focal group for this study. Carabids were chosen, firstly, due to the conservation importance of some species within this group on ERS, and secondly because they have long been used as bio-indicators for ecosystem health (Allegro and Sciaky, 2003; Rainio and Niemelä, 2003). In particular, ground beetle communities have been sampled to evaluated ecosystem alterations because of human impacts (Avain and Luff, 2010) and habitat change (Taboada, 2008**)**. Ground beetles are regarded as good bio-indicators for a number of reasons. As a group, they are sensitive to environmental change and any impacts can be evaluated at a species level by either a decrease or increase in abundance and diversity. In addition, community level shifts can be evaluated by classifying individuals in feeding guilds, specialists or generalists, and rare or common species. Rainio and Niemelä (2003) describe a generalised pattern in ground beetle communities as a response to disturbance

where the immobile specialist species decrease in abundance to the stressor and mobile generalist species increase.

Few studies have evaluated the effect of non-native invasive plant species colonisation on ground beetle populations in Europe. Topp *et al.* (2008) studied the impact of *Fallopia sachalinensis* F. Schmidt invasion on ground beetle assemblages in semi-natural woodland and rivers in Germany and found colonisation affected the community by reducing the numbers of predators and increasing the number of detritivores. Hyman (1992) highlighted the threat to *Bembidion semipunctatum* Donovan, an ERS specialist and a species with conservation status (Notable A) by the colonisation of *I. glandulifera*, where he suggested that invasion may decrease the available niches required by the species. However, to-date no studies have quantified this claim.

As *I. glandulifera* is predominantly a weed of riparian systems in the UK, and is found growing on and near ERS the threat is conceived as likely. A study evaluating any potential impacts is further justified due to the increased occurrence of *I. glandulifera* along UK river systems. Using data collected from the 2006-2008 River Habitat Survey, the UK Environment Agency (2010) estimate that *I. glandulifera* is now the most commonly occurring non-native invasive plant species present in our riparian habitats, occurring in over 13% of river lengths across England and Wales; a significant increase from the baseline data of 1995-1997 (Raven *et al.*, 2000).

3.2 Methods

3.2.1 The sites

All sample sites selected for this study were situated along riparian systems in the south west of England in the counties of Cornwall and Devon (Figure 3.1). Sites were selected during a preliminary vegetation census in April 2006, which involved recording the presence, absence, and percentage cover of *I. glandulifera* for each ERS site as a whole. A number of environmental factors have been shown to affect the quality of the ERS and therefore the abundance and diversity of the carabid faunas. Variations in substrate, habitat heterogeneity (within the ERS), available hibernation habitats for beetles (on or around the ERS), percentage of shade, livestock trampling, and native vegetation cover can all affect ground beetle populations (Sadler & Drake, 2004; Bates *et al.*, 2007). Therefore, environmental variables that could have an impact on ground beetle species assemblages, and which were not being directly measured as variables within this study, were kept as similar as possible through site selection. Sites were selected where disturbance of any kind (i.e. human activity and livestock) was minimal. Furthermore, advice was sought from the Environment Agency on the quality of ERS sites in the region and from previous studies of ground beetles on ERS by Bell and Sadler (2001), Bell and Sadler (2003a), Bell and Sadler (2003b), and Sadler and Drake (2004). Following this information, it was decided to include ERS sites from Devon where the quality of the sites was regarded as higher than those in Cornwall (see Table 3.1 for site details).

In total, nine sites were selected based on the presence and absence of *I. glandulifera* on the ERS (see Appendix 2 for ERS site photographs and local maps). Three different vegetation types were distinguished: (1) ERS without *I. glandulifera* (uninvaded), (2)

ERS with *I. glandulifera* monoculture (invaded), where at least 50% of the total area of the ERS was covered with *I. glandulifera* with only a few other plants visible among *I. glandulifera* seedlings and (3) ERS with a scattered occurrence of *I. glandulifera* where the percentage cover was no more than 40% of the total vegetation cover (mixed) (Table 3.1).

Sites without *I. glandulifera* were evaluated for their potential of being invaded through visual inspection of their location within the catchment. If an ERS was free from *I. glandulifera* and did not have the potential to be invaded, for example, if it was situated on a nearside bend of the river then it was excluded from the survey and others which had the potential to be invaded, but were currently uninvaded were selected.

Figure 3.1 The location of sampled exposed riverine sediment sites in Devon and Cornwall

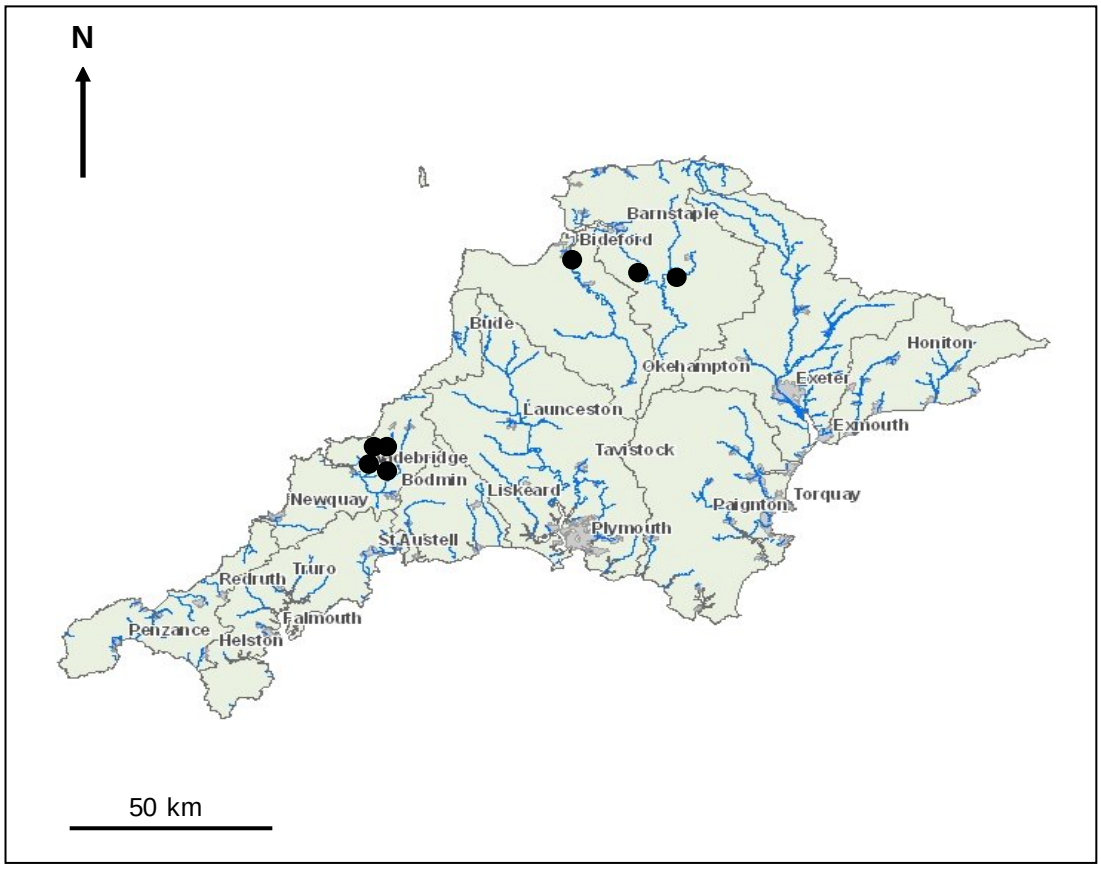


Table 3.1 Site details of the nine exposed riverine sediments (ERS). Three different vegetation types were distinguished: (1) ERS without *I. glandulifera* (uninvaded), (2) ERS with *I. glandulifera* monoculture (invaded), where at least 50% of the total area of the ERS was covered with *I. glandulifera* with only a few other plants visible among *I. glandulifera* seedlings and (3) ERS with a scattered occurrence of *I. glandulifera* where the percentage cover was no more than 40% of the total vegetation cover (mixed). ERS type: Mid = situated within the river and Side = forming an interface with the river and the riverbank.

ERS label	Location	River	Geographical coordinates		ERS type	Vegetation type	Mean % cover per quadrant (n= 6), per site		
			Latitude (N)	Longitude (W)			Native vegetation	*I. glandulifera*	Bare ground
1	Devon	Taw	50 54 770	003 54 168	Mid	Mixed	71	25	9
2	Devon	Mole	50 59 405	003 53 077	Side	Invaded	27	50	29
3	Devon	Torridge	50 28 451	004 47 968	Side	Uninvaded	21	0	79
4	Cornwall	Camel	50 28 388	004 45 917	Side	Mixed	52	32	8
5	Cornwall	Ruthern	50 28 451	004 47 968	Side	Uninvaded	78	0	26
6	Cornwall	Ruthern	50 28 475	004 47 959	Side	Uninvaded	57	0	50
7	Cornwall	Camel	50 20 920	004 47 871	Side	Invaded	6	93	6
8	Cornwall	Camel	50 20 930	004 47 954	Mid	Mixed	51	44	1.
9	Cornwall	Camel	50 28 938	004 47 914	Mid	Invaded	40	50	8

3.2.2 Vegetation sampling

Previous studies by Bell and Sadler (2003a), Bell and Sadler (2003b), Bates *et al.* (2007) and Sadler and Drake (2004) have shown that the percentage cover of vegetation on an ERS can have an impact on the carabid assemblages within. Therefore, just categorising the absence and occurrence of *I. glandulifera* for each site would be inadequate to evaluate any potential effects of the species on the carabid community. It is important to evaluate the percentage cover and species richness of vegetation, along with the percentage cover of bare ground within each ERS in order to measure the variation between individual sites and then relate these environmental variables to the abundance of the carabid community. In addition, the percentage cover may change during the course of the season as plant species grow and shade out others. After the preliminary vegetation sampling in April, each ERS was sampled again during the month of June using six $1m^2$ quadrats randomly placed within each ERS habitat. The percentage cover of vegetation was estimated for each species within each quadrat (see Table 3.2 for mean percentage cover of plant species per ERS). In addition, the percentage cover of bare ground was recorded within each quadrat. Plant species were identified with the aid of field guides Fitter *et al.* (1984), Phillips (1997), Rose (1989).

3.2.3 Carabid sampling

Carabid species were sampled during a period to coincide with maximum ground beetle activity on ERS and the growth of *I. glandulifera*, therefore sampling was conducted between May and August 2006. Studies by Bates *et al.* (2007), Bell and Sadler (2003a), Bell and Sadler (2003b), Sadler and Drake (2004) show using a combined sampling approach, including pitfall trapping, timed hand searching and excavation of the substrate maximises the likelihood of collecting both mobile and immobile species on ERS. The combined sampling method approach also enables the user to overcome

some of the bias incurred by pitfall trapping (as detailed in section 3.2.3.1) in particular their over representation of more mobile species. However, for this study, keeping the vegetation structure intact was important from a scientific and conservation perspective and therefore excavations were not considered appropriate, as they would disturb the surface structure, and vegetation on the ERS. Therefore, ground beetles were sampled using pitfall traps and timed hand searching throughout the duration of the study.

3.2.3.1 Pitfall trapping

Pitfall trapping is the most frequently used method for estimating ground beetle populations though if the study is not designed to take into consideration the potential bias associated with pitfall traps the sampled community may be unrepresentative of the population (Cheli and Corley, 2010; Woodcock, 2005). The type of trap (Morrill *et al.*, 1990), number of traps, position (in relation to other traps (Woodcock, 2005), and within the substrate (Digweed *et al.*, 1995)), preservative used within the trap (Lemieux and Lindgren, 1999) and the structure of surrounding vegetation (Melbourne, 1999; Woodcock, 2005) have all been shown to influence the species caught and capture success. All of the aforementioned factors were taken into account when designing the study with the pitfall traps.

Six pitfall traps were used at each site to sample the carabid community. The number of traps were chosen to ensure each trap could be placed at equal distances to one another at the ERS site with the smallest area (Woodcock, 2005). The traps were placed in each ERS in a 2 x 3 grid with the distance between each pitfall trap modified to the size and shape of the ERS. The grid approach to setting out the traps was adopted upon the recommendation of Woodcock (2005) as it provides an even coverage of the area sampled. The pitfall traps were plastic beakers 8cm in diameter and 10cm deep. Each

pitfall trap was placed into the sediment with the rim flush with the surface of the ERS. Increased vegetation diversity and thus structural heterogeneity around the trap can increase the surface area, both horizontally and vertically for the ground beetles to utilize, thus reducing the effectiveness of the trap (Woodcock, 2005). Therefore, the vegetation structure was removed around each trap in a 5cm diameter as recommended by Woodcock (2005). Approximately 100ml of concentrated sodium chloride solution was placed into each pitfall to act as a preservative. Ethylene glycol is often used as the preferred preservative for pitfall trapping though as the traps were on a riparin system, with the risk of flooding, this method was deemed inappropriate. All pitfall traps were emptied and replenished every 14 days. It is acknowledged that 14 days sampling time is a relatively long period to leave traps before replenishment, however, time and funding restrictions dictated this timeframe. As some of the sites were some distance from one-another, all sites in Cornwall were changed on one day and those sites in Devon were changed the following day. All specimens were preserved in 70% alcohol prior to identification.

3.2.3.2 Timed hand searching

Timed hand searching was conducted at each site where the sampler moved throughout the ERS turning over stones and collecting ground beetles either by hand or with an aspirator. The sampling was standardised for each site a period of 20 minutes. Hand searching was timed in order to standardise across space and time. Hand searching was conducted on the same day as the pitfall traps were collected and replenished. All specimens were preserved in 70% alcohol prior to identification.

3.2.4 Sampling effort

Unfortunately, due to high rainfall during May and June 2006, many of the pitfall traps were lost. In addition, the increased rainfall resulted in the rivers flooding and six of the nine ERS were submerged for much of the month of May making both sampling methods impossible to conduct. Therefore, complete sampling was achieved over an eight week period with four sampling times between June and August 2006.

3.2.5 Carabid identification and classification

All ground beetles collected were identified to species using the identification keys of Forsythe (2000) and Lindroth (1996). Species were further classified by their conservation status using field guides and key references of Forsythe (2000), Hyman (1992) and Lindroth (1996). Lastly, species were ranked due to their association/fidelity to ERS following a combination of rankings from Bell and Sadler (2003a), Bell and Sadler (2003b), Bates *et al.* (2006), Eyre *et al.* (2001b) and Sadler and Drake (2004). Those species, which have a high association to ERS habitats, i.e. those species that are dependent on ERS for all or part of their life cycle, were graded 1. Species that were highly associated with ERS, but were often found in other habitat types were graded 2. Species, which were commonly found on a variety of habitats and regarded as having little association with ERS, were graded 3. Species graded 1 and 2 were regarded as specialists to an ERS, and species graded 3 were regarded as generalist species (Bell and Sadler, 2003a; Sadler and Drake, 2004).

3.2.5 Data analysis

Ground beetle abundance was estimated for each site and each sampling date by pooling the pitfall traps and hand searching datasets with the exception of the constrained Redundancy Analysis where sampling dates were also pooled. The percentage cover of

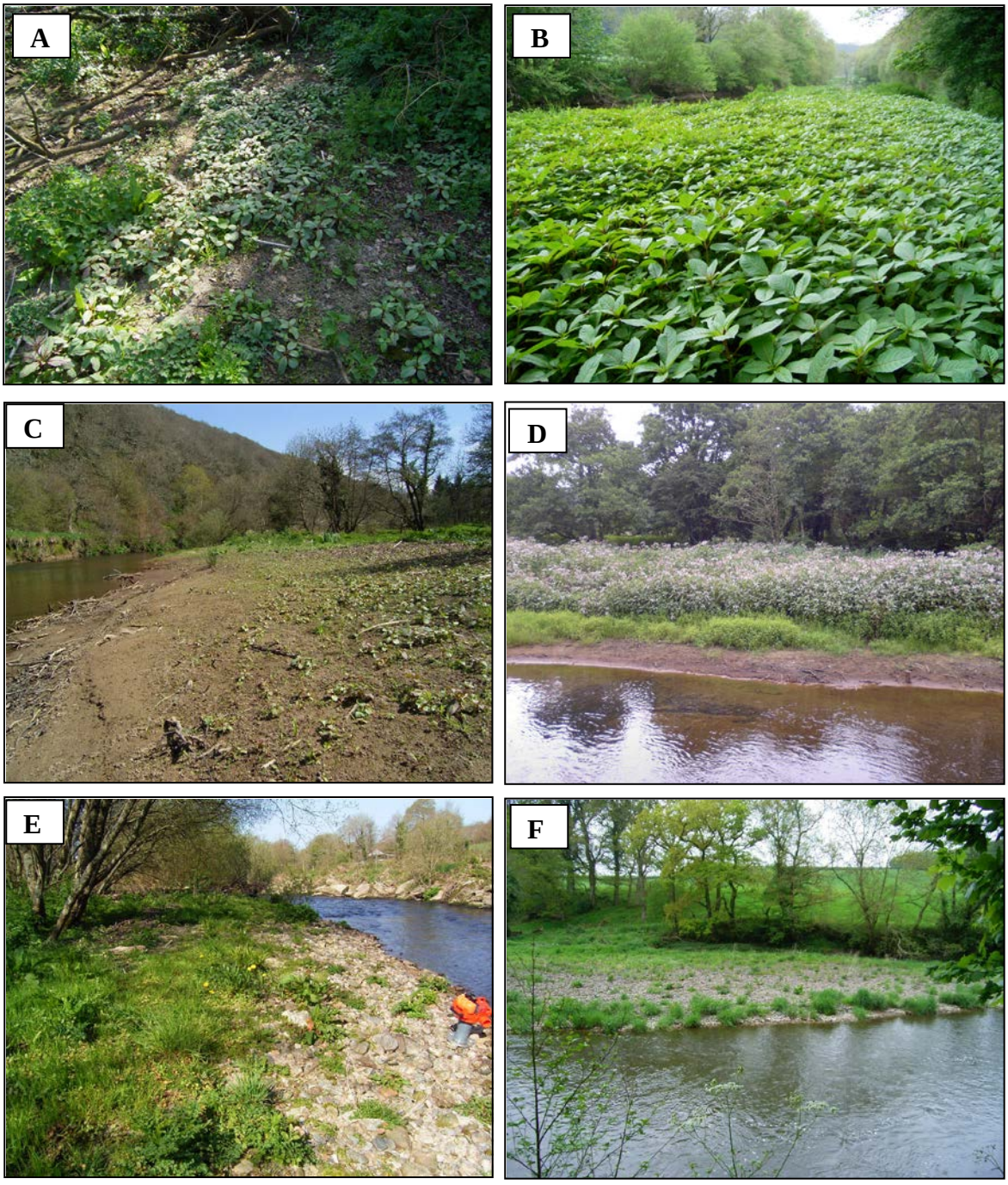
I. glandulifera, native vegetation, and bare ground of all six quadrats was averaged to give site means as a replicate. Carabid diversity was measured using the Shannon-Wiener diversity index (*H*) (Morin, 1999).

The effect of vegetation type on the ground beetle community was evaluated using the multivariate Principal Response Curve (PRC) method where the uninvaded ERS vegetation type was treated as the control (see section 1.23 for a full description and justification). As discussed in section 1.23, the PRC allows interpretation at the species level. All data on carabid abundance was log transformed prior to analysis with the value of 1 added to each data point before transformation (van den Brink and ter Braak, 1999). The significance of the PRC was tested using a Monte-Carlo permutation test. At the species level, further evaluation is possible by comparing the species weight between treatments and their deviation from the control, and time.

The effect of *I. glandulifera* percentage cover, native plant species percentage cover and bare ground percentage cover on ground beetles was evaluated using a constrained Redundancy Analysis (RDA). Model selection followed the same method as detailed in section 2.2.6.3. The detrended correspondence analysis (DCA) produced gradient lengths less than 4 thus invoking the adoption of the linear method (RDA). In addition to exploring patterns of the percentage cover variables on total generalist abundance and total specialist ground beetle abundance (summed over all sample dates), a Pearson product-moment correlation analysis was performed where the percentage cover data was arc-sine transformed and the count data was square-root transformed to suit the assumption of the test.

The difference in plant species richness between the ERS habitats was evaluated using a one-way Analysis of Variance where the species richness data was square root transformed prior to analysis. The effect of ERS vegetation type on total carabid diversity and the diversity of specialist ground beetles was analysed using a repeated measures ANOVA with treatment and time as the main effects. The normality of the data and equality of variance was evaluated prior to the analysis. If the repeated measures ANOVA showed a significant difference for either treatment or time a pairwise t-test was performed on the sample means to evaluate which treatment/times differed. To evaluate the differences in percentage cover of native species and bare ground, between ERS habitats, a one-way analysis of variance was used where the percentage cover variables were arc-sine transformed prior to analysis.

Plate 3.1 Exposed riverine sediments in the south west of England



A. *Impatiens glandulifera* seedlings on exposed riverine sediment in Cornwall
B. *I. glandulifera* invading the river Torridge, North Devon
C. An invaded exposed river sediment in April 2006 and (D) the same exposed riverine sediment in August 2006
E. A mixed vegetation exposed riverine sediment in Cornwall
F. An uninvaded exposed riverine sediment in North Devon

3.3 Results

3.3.1 Vegetation

Plant species richness was significantly lower in the invaded habitats compared to the mixed and uninvaded ERS ($F_{2,51} = 8.038$, $P < 0.001$) (Figure 3.2). The mixed ERS habitats and uninvaded ERS habitats had a significantly higher percentage cover of native plant species compared to the invaded habitat ($F_{2,51} = 6.973$, $P<0.05$) (mixed 58.38 ± 7.47; invaded 24.5 ± 6.254; uninvaded 52.27 ± 7.357), whereas all three ERS habitats significantly differed with regard to percentage cover of bare ground ($F_{2,51} = 5.958$, $P < 0.001$) (mixed 6.22 ± 2.255; invaded 14.72 ± 5.308; uninvaded 52 ± 6.549) (see Table 3.2 for plant species composition for each ERS).

3.3.2 The Carabid community

In total, 1,924 ground beetles were collected and identified over the course of the study (1,577 from the pitfall traps and 347 from hand searching). The sampled community consisted of 45 species from 14 genera (Table 3.3) (see table 3.4 for the mean total abundance of carabid species caught over the duration of the study for each sampling method and each ERS site). Of these, 20% were categorised as specialist species to ERS. Only three species (6.8%) collected are recognised as having conservation status, namely *B. semipunctatum* and *Tachys scutellaris* Stephens (both Notable A), and *Pterostichus cristatus* Duf. (Notable B).

There was an overall significant shift in the carabid community when comparing the invaded and mixed ERS vegetation type to the control ($F_{1,24} = 6.5435$, $P < 0.05$). The first canonical axis of the PRC explained 54.14% of the total variation and of this 13% was explained by time and 29% by treatment (including the interaction with treatment

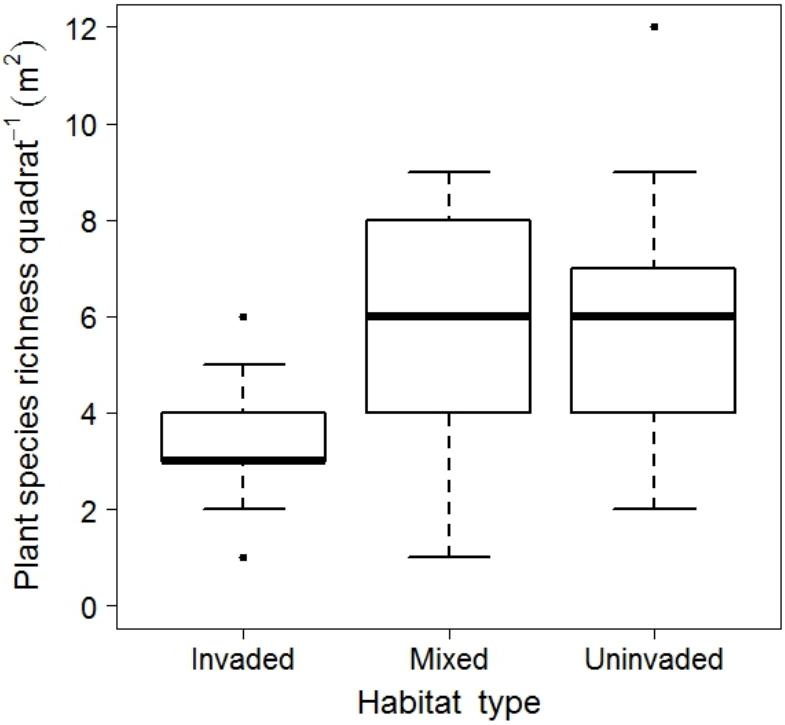
Table 3.2 Plant species composition for each exposed river sediment sampled. Figures given in the table are mean percentage cover per quadrat (n=6) per ERS. Three different vegetation types were distinguished: (1) ERS without *I. glandulifera* (uninvaded), (2) ERS with *I. glandulifera* monoculture (invaded), where at least 50% of the total area of the ERS was covered with *I. glandulifera* with only a few other plants visible among *I. glandulifera* seedlings and (3) ERS with a scattered occurrence of *I. glandulifera* where the percentage cover was no more than 40% of the total vegetation cover (mixed).

Site type	Mixed	Invaded	Uninvaded	Mixed	Uninvaded	Uninvaded	Invaded	Mixed	Invaded
Site label	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Species									
Ajuga reptans L.			0.3		0.8				
Allium ursinum L.	1.5				3.8				
Anthoxanthum odoratum L.	6.7				1.2				
Anthriscus sylvestris Hoffm.					21.7			45.0	
Arum maculatum L.				0.3					
Buddleja davidii Franch.	2.0								
Calystegia sepium (L.) R.Br.	1.7	1.7	0.8	0.3					
Cardamine flexuosa With.	0.7		0.7	1.3		1.2			8.3
Carex echinata Murray					1.7	0.3			
Carex remorta L.					5.8				
Carex pendula Huds.					1.7				
Carex viridula Michaux.	2.5								
Centaurea scabiosa L.					1.3				
Cerastium fontanum Baumg.			1.0						
Cerastium glomeratum Thuill.			0.8			0.8			
Equisetum arvense L.	3.3								
Festuca rubra L.	0.8				1.7				
Galium aparine L.	0.8	7.8		10.7	4.2	3.3	0.5		20.0
Heracleum sphondylium L.	1.7		1.7	0.8	0.3				
Holcus lanatus L.	6.7		0.5						
Impatiens glandulifera Royle	25.0	50.8	0.2	32.5			93.3	44.2	50.0
Juncus effusus L.									
Montia sibirica L.			1.8	1.3	4.3	16.7		1.5	
Oenanthe crocata L.				0.3		4.2			

Table 3.2 (continued).

Site type	Mixed	Invaded	Uninvaded	Mixed	Uninvaded	Uninvaded	Invaded	Mixed	Invaded
Site label	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Species									
Percicaria hydropiper L.		3.3	4.0		0.8		0.2		1.7
Percicaria lapthafolium L.	2.5	5.8							
Plantago major L.					0.8	0.3			
Poa trivialis L.	9.3			9.2					
Primula veris L.	0.7				5.0	0.5			
Ranunculus ficaria L.				0.3	1.7	1.7			
Ranunculus repens L.				5.0	0.8	0.8			
Rubus fruticosus L.		0.7	1.3	0.8	3.3	0.8			
Rumex obtusifolius L.	7.5	0.5			5.0			2.0	
Rumex sanguineus L.			0.8						
Rumex acetosa L.		1.3	2.7	1.7					
Rumex crispus L.	0.8	2.5	1.0	2.5	1.5	5.3			
Salix alba L.	16.7								
Silene dioica (L.) Clairv.	0.7		1.2	0.3	8.3	1.7		2.5	
Stellaria holostea L.	2.5				0.8				
Urtica dioica L.	2.3	3.3	2.2	12.5		11.7	0.4	0.8	18.3
Veronica hederifolia L.				4.5	0.7	0.8			
Veronica montana L.					0.8	7.5			

Figure 3.2 Average plant species richness in the three ERS vegetation types. The box and whiskers plot show the median percentage cover expressed as the solid horizontal line within the box. The top and bottom of the box show the 75th and 25th percentiles, respectively. The vertical dashed line shows the interquartile range and the points show the outliers of the data (Crawley, 2007). Plant species richness was lower in the invaded ERS when compared to the mixed and uninvaded ERS.



and time) (Figure 3.3). The response of the ground beetle community was more pronounced in the invaded ERS compared to the mixed vegetated ERS (Figure 3.3) when both are compared to the uninvaded ERS. Whereas the mixed ERS habitats remained relatively constant over time, the invaded ERS habitats showed a higher deviation at the beginning of the study moving closer to the control in weeks 4 and 6 and deviating away from the control in August (Figure 3.3 and Table 3.5). Within the PRC analysis, only those species with a high taxon weight (either positive or negative) are considered to be significantly affected by the habitat types.

Figure 3.3 Principal response curves with species weight for ground beetles indicating the effect of ERS habitat over the course of the study. The control (uninvaded) is expressed as a horizontal line through zero and the black solid line is the response of the carabid community in the mixed ERS sites over time. The dotted line response of the carabid community in the invaded ERS sites. The carabid species to the right of the graphic are ordered in their taxon weight corresponding to the y-axis. Invertebrate groups with a taxon weight of higher than 0.5 or lower than -0.5 are significantly influenced by the invaded sites.

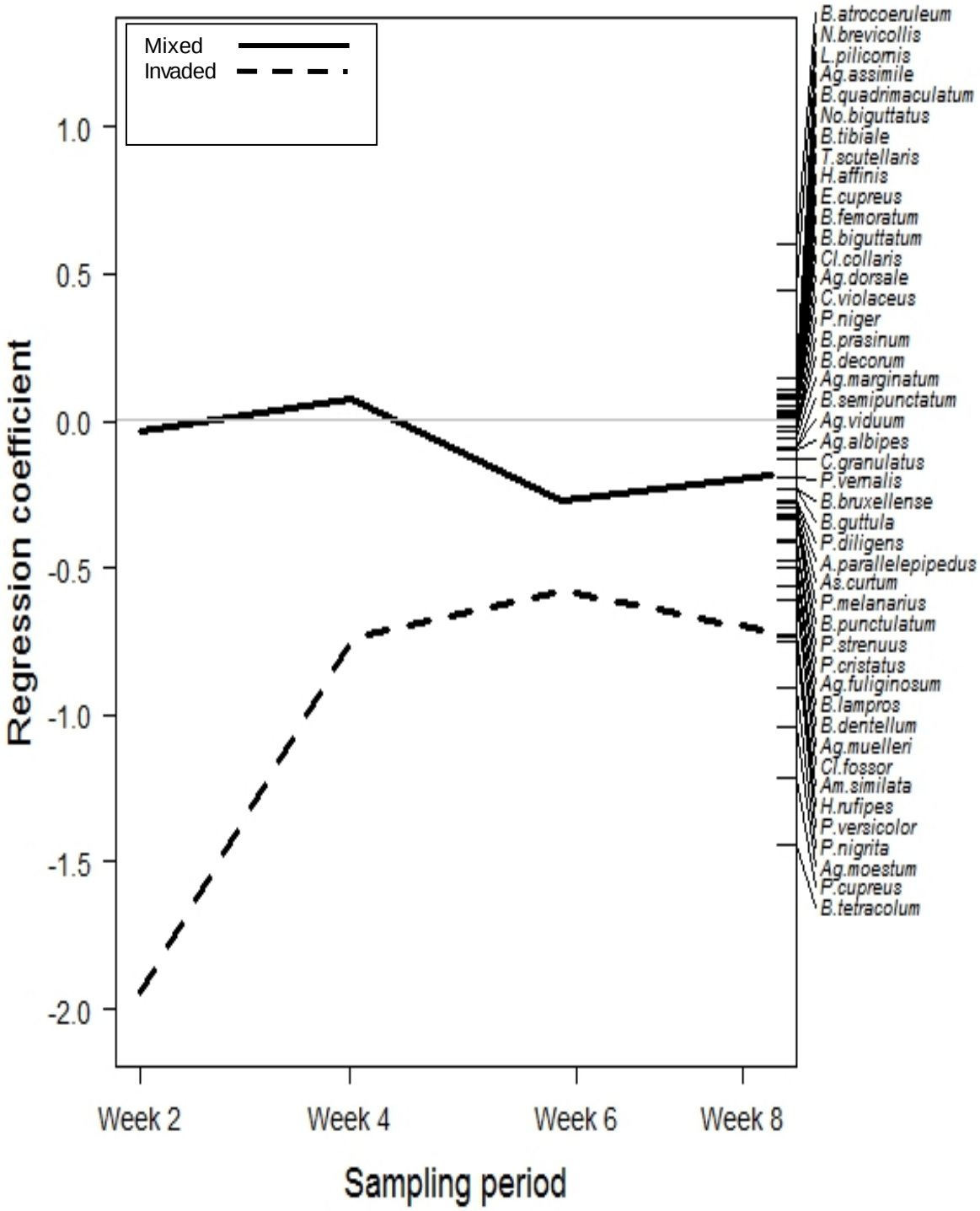


Table 3.3 Carabid species collected during the course of the study. The table provides their conservation status (Not. A- Notable A, Not. B- Notable B), fidelity ERS (1 and 2= specialist, 3= generalist), feeding class and taxon weight (taken from the Principal response curve analysis). Those species highlighted in bold are significantly affected by the treatments (invaded and mixed ERS).

Species	Status	Fidelity	Feeding class	Taxon weight (b_k)	Species	Status	Fidelity	Feeding class	Taxon weight (b_k)
Abax parallelepipedus Pil	None	3	Predatory	-0.276	***Bembidion tetracolum* Say**	**None**	**3**	**Predatory**	**-1.399**
Agonum albipes (Payk.)	None	3	Predatory	-0.096	*Bembidion tibiale* (Duft.)	None	1	Predatory	0.068
Agonum assimile (Payk.)	None	3	Predatory	0.099	*Carabus granulatus* (L.)	None	3	Predatory	-0.13
Agonum dorsale (Panz.)	None	3	Predatory	0.016	*Carabus violaceus* (L.)	None	3	Predatory	0.012
Agonum fuliginosum (Panz.)	None	3	Predatory	-0.392	*Clivina collaris* (Hbst.)	None	2	Predatory	0.016
Agonum marginatum (L.)	None	3	Predatory	-0.06	***Clivina fossor* (L.)**	**None**	**3**	**Predatory**	**-0.594**
***Agonum moestum* (Duft.)**	**None**	**3**	**Predatory**	**-1.011**	*Elaphrus cupreus* Duft.	None	3	Predatory	0.045
***Agonum muelleri* (Hbst.)**	**None**	**3**	**Predatory**	**-0.544**	*Harpalus affinis* (Schr.)	None	3	Phytophagous	0.047
***Agonum viduum* (Panz.)**	**None**	**3**	**Predatory**	**-0.92**	***Harpalus rufipes* Deg.**	**None**	**3**	**Phytophagous**	**-0.713**
***Amara similata* (Gyll.)**	**None**	**3**	**Phytophagous**	**-0.703**	*Loricera pilicornis* (F.)	None	3	Predatory	0.142
Asaphidion curtum (Heyden)	None	3	Predatory	-0.289	*Nebria brevicollis* (F.)	None	3	Predatory	0.43
***Bembidion atrocoeruleum* Stephens**	**None**	**1**	**Predatory**	**0.576**	*Notiophilus biguttatus* (F.)	None	3	Predatory	0.077
Bembidion biguttatum (Fabricius)	None	3	Predatory	0.021	*Pterostichus cristatus* Duf.	Not. B	3	Predatory	-0.396
Bembidion bruxellense (Wesm.)	None	3	Predatory	-0.224	***Pterostichus cupreus* (L.)**	**None**	**3**	**Predatory**	**-1.175**
Bembidion decorum (Zenk.)	None	1	Predatory	-0.038	*Pterostichus diligens* Sturm	None	3	Predatory	-0.261
Bembidion dentellum (Thun.)	None	2	Predatory	-0.483	*Pterostichus melanarius* (Ill.)	None	3	Predatory	-0.313
Bembidion femoratum Sturm	None	2	Predatory	0.034	***Pterostichus niger* (Schall.)**	**None**	**3**	**Predatory**	**-0.878**
Bembidion guttula (F.)	None	3	Predatory	-0.224	*Pterostichus nigrita* (Pank.)	None	3	Predatory	-0.022
Bembidion lampros (Hbst.)	None	3	Predatory	-0.459	*Pterostichus strenuus* (Panz.)	None	3	Predatory	-0.328
Bembidion punctulatum Drap.	None	1	Predatory	-0.316	***Pterostichus vernalis* Panz.**	**None**	**3**	**Predatory**	**-0.728**
Bembidion prasinum (Duft.)	None	1	Predatory	-0.037	*Pterostichus versicolor* (Strm.)	None	3	Predatory	-0.191
Bembidion quadrimaculatum (L.)	None	2	Predatory	0.084	*Tachys scutellaris* Stephens	Not. A	3	Predatory	0.047
Bembidion semipunctatum (Donovan)	Not. A	1	Predatory	-0.092					

Table 3.4 The total abundance of invertebrate identified for each exposed riverine sediment from the pitfall traps (Pitfall) and handsearching methods (Hand) over the course of the study. Three different vegetation types were distinguished: (1) ERS without *I. glandulifera* (uninvaded), (2) ERS with *I. glandulifera* monoculture (invaded), where at least 50% of the total area of the ERS was covered with *I. glandulifera* with only a few other plants visible among *I. glandulifera* seedlings and (3) ERS with a scattered occurrence of *I. glandulifera* where the percentage cover was no more than 40% of the total vegetation cover (mixed).

Site number		1		2		3		4		5		6		7		8		9	
ERS type		Mixed		Invaded		Uninvaded		Mixed		Uninvaded		Uninvaded		Invaded		Mixed		Invaded	
Sampling method		Pitfall	Hand	Pitfall	Hand	Pitfall	Hand	Pitfall	Hand	Pitfall	Hand	Pitfall	Hand	Pitfall	Hand	Pitfall	Hand	Pitfall	Hand
	Species																		
Abax	*parallelepipedus* Pil			7		1				3				4		1			
Agonum	*albipes* (Payk.)			6				1				1		1		4		3	
Agonum	*assimile* (Payk.)		1		1	1	1	2	10	8		1		8	1	8		1	1
Agonum	*dorsale* (Pont.)											1							
Agonum	*fuliginosum* (Panz.)							1		2		1		3				3	
Agonum	*marginatum* (L.)			1		3								1					
Agonum	*moestum* (Duft.)			10		6		5				1		53		7		6	
Agonum	*muelleri* (Hbst.)				1									11				4	
Agonum	*viduum* (Panz.)									1		4		2		1			
Amara	*similata* (Gyll.)			8				3		1				5				8	
Asaphidion	*curtum* (Heyden)			1						1						1		9	
Bembidion	*atrocoeruleum* Stephens	1		1		11				1		6				2			
Bembidion	*biguttatum* (Fabricius)				2		16		17		4		2		4		2		
Bembidion	*bruxellense* (Wesm.)	2		2				1						8			1	1	
Bembidion	*decorum* (Zenk.)																		
Bembidion	*dentellum* (Thun.)		11	8	7	1	1	2				2	2	8		1		1	
Bembidion	*femoratum* Sturm		2		1				3		2				5		3		
Bembidion	*guttula* (F.)					1			2	2				14		10		4	
Bembidion	*lampros* (Hbst.)	1		16		1		9		13		15		3				2	
Bembidion	*prasinum* (Duft.)						1												

Table 3.4 (continued)

Site number		1		2		3		4		5		6		7		8		9	
ERS type		Mixed		Invaded		Uninvaded		Mixed		Uninvaded		Uninvaded		Invaded		Mixed		Invaded	
Sampling method		Pitfall	Hand	Pitfall	Hand	Pitfall	Hand	Pitfall	Hand	Pitfall	Hand	Pitfall	Hand	Pitfall	Hand	Pitfall	Hand	Pitfall	Hand
Bembidion	*punctulatum* Drap.	4		12	1	3		7		2		1		1					
Bembidion	*quadrimaculatum* (L.)		1	2			1			1					6	5	8		21
Bembidion	*semipunctatum* (Donovan)											1							
Bembidion	*tetracolum* Say	11		39		7		12		72				104		27		81	
Bembidion	*tibiale* (Duft.)	14	8	39	2	74	8	3	4	2	1	2		50	13	4	4	3	11
Carabus	*granulatus* (L.)		13	2	20	2	13	1	14		25		3	2	10		25		19
Carabus	*violaceus* (L.)	1		1		2								5					
Clivina	*collaris* (Hbst.)			1		7						3						1	
Clivina	*fossor* (L.)					1								13		1		6	
Elaphrus	*cupreus* Duft.																		
Harpalus	*affinis* (Schr.)	1		1						2				3		1	1		
Harpalus	*rufipes* Deg.			10				3		4		3		34		1		3	
Loricera	*pilicornis* (F.)			0						3		1		3		2			
Nebria	*brevicollis* (F.)	3		1		2		10	3	13				2		3			
Notiophilus	*biguttatus* (F.)							2		2				19					
Pterostichus	*cristatus* Duf.			1												2		7	
Pterostichus	*cupreus* (L.)	1		25		22				1				27		4		21	
Pterostichus	*diligens* Sturm			24		10				2		1		11		6		3	
Pterostichus	*melanarius* (III.)			7		13		2				1		8				5	
Pterostichus	*niger* (Schall.)			4	1	1		1	2		1			2		0			
Pterostichus	*nigrita* (Payk.)			10			1	2		20		1		41		8		11	
Pterostichus	*strenuus* (Panz.)			18	1	1		13		5	2			4				1	
Pterostichus	*vernalis* Panz.	1																4	
Pterostichus	*versicolor* (Strm.)	1		44		3		11		1		6		3		7		9	
Tachys	*scutellaris* Stephens					1													

Table 3.5 The resulting Principal Response Curve coefficients the mixed and invaded ERS + sampling date which are contrasts to the control (uninvaded).

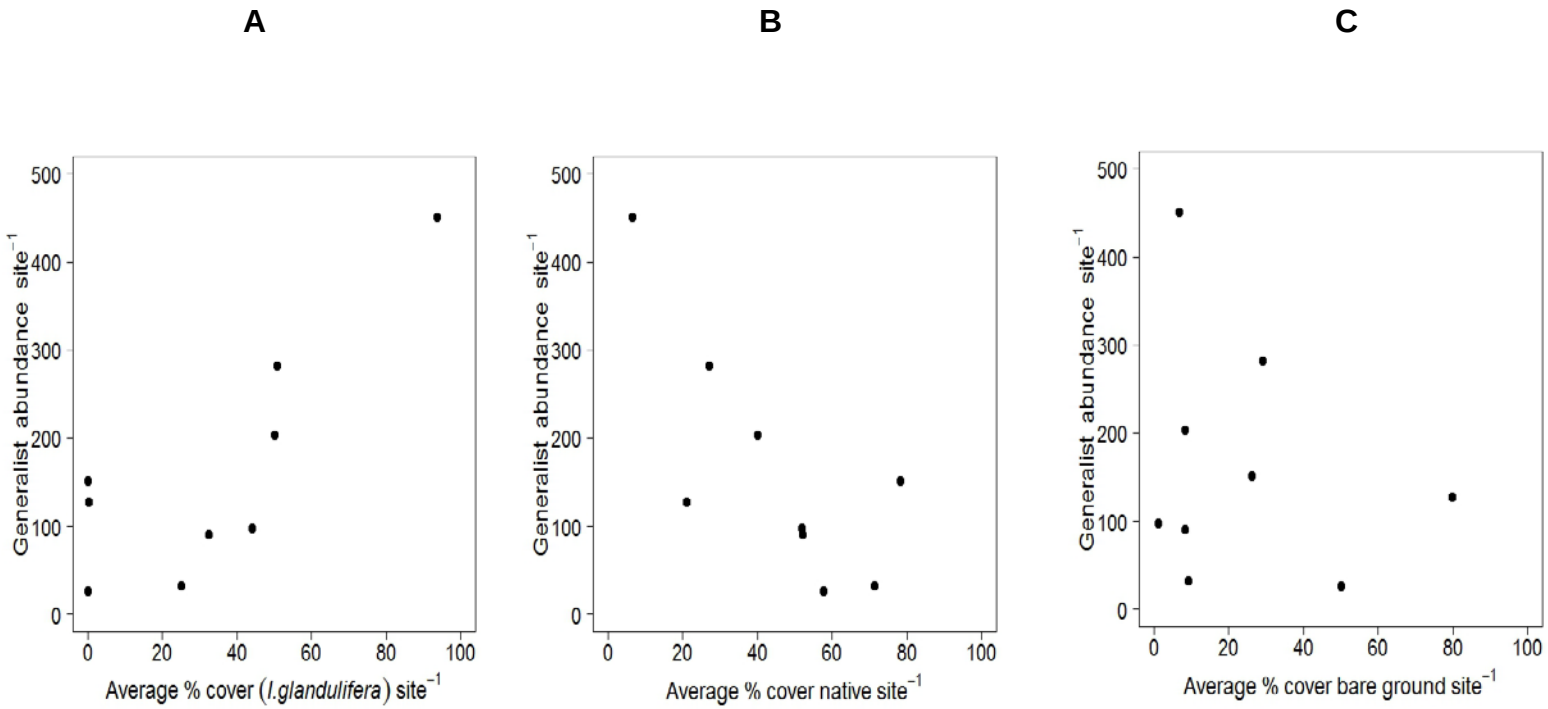
ERS type	Sampling period (week)			
	2	4	6	8
Mixed	-0.035	0.07	-0.261	-0.177
Invaded	-1.883	-0.718	-0.557	-0.698

Therefore, those species with a species taxon weight between 0.5 and -0.5 are considered to show a weak response to the treatment though all species are shown in Figure 3.3 to represent the community as a whole. All ten species which showed a taxon weight lower than - 0.5 (Table 3.3), and were therefore positively associated with the invaded, and to a lesser extent the mixed ERS, were generalist species. There was no relationship between the percentage cover of *I. glandulifera*, percentage cover of native plant species and percentage cover of bare ground and the ground beetle community ($F_{3,5} = 2.59$, $P = 0.13$). However, the invaded ERS had a higher overall abundance of generalists compared to the mixed and uninvaded ERS ($F_{2,6} = 5.911$, $P = < 0.05$) (invaded, 312 ± 72.5; mixed 73.6 ± 20.9; uninvaded 101.3 ± 38.3 per site). There was a positive correlation between the abundance of generalist ground beetles and the percentage cover of *I. glandulifera* per site ($r = 0.721$, $N = 9$, $P < 0.05$) (Figure 3.4A) and a negative correlation between the percentage cover of native vegetation per site ($r = -0.773$, $N = 9$, $P < 0.05$) (Figure 3.4B). There was no correlation between generalist abundance and the average percentage cover of bare ground ($r = -0.377$, $N = 9$, $P = 0.317$) (Figure 3.4C).

The overall abundance of specialist ground beetles was similar in all three habitats ($F_{2,6} = 0.008$, $P = 0.992$) (invaded, 48.6 ± 3.6; mixed 50.3 ± 2.7; uninvaded 55.3 ± 17.7 per site). Only one species, *Bembidion atrocoeruleum* Stephens, a specialist ground beetle

of ERS, showed a taxon weight greater than 0.5, indicating a negative association with the invaded and mixed ERS. *B. atrocoeruleum* was reduced in the invaded ERS to between 34 and 73% of its geometric mean count in the control over the course of the study.

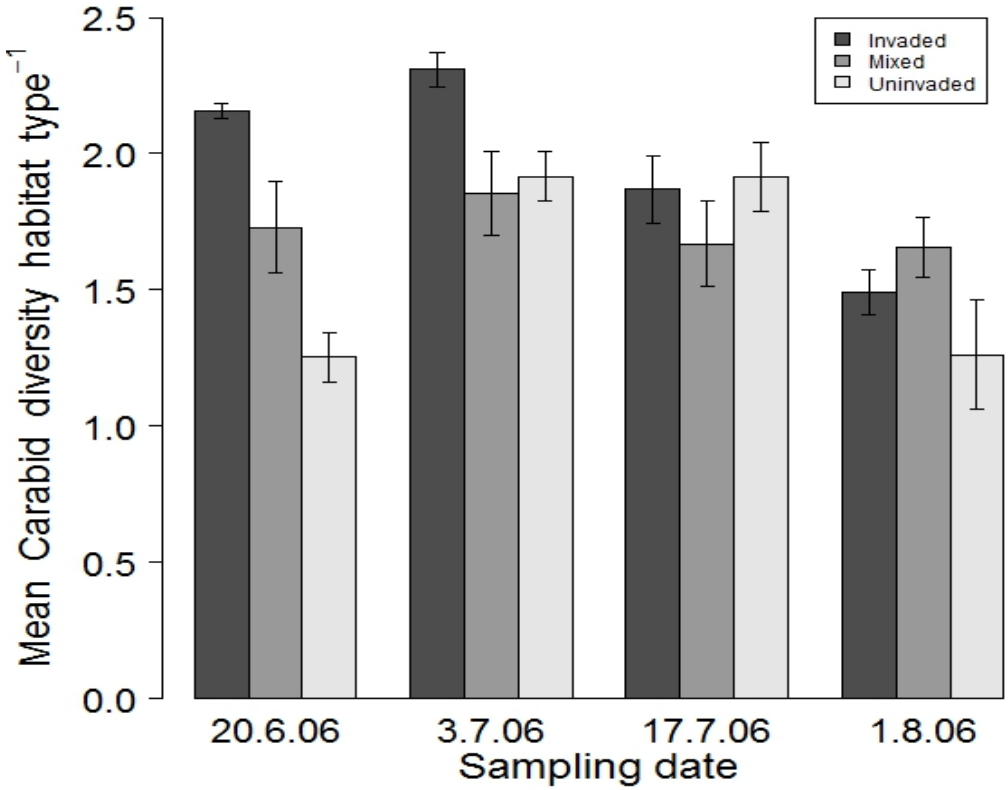
Figure 3.4 The relationship between generalist carabid abundance and percentage cover environmental variables. Where (A) shows a positive correlation for *Impatiens glandulifera* (B) shows a negative correlation for native vegetation, and (C) shows no relation with bare ground.



3.3.3 Carabid diversity

There was no evidence that carabid diversity was affected by the ERS type ($F_{2,6}$ = 2.097, P = 0.204). However, the diversity of ground beetles in the invaded ERS was affected by time ($F_{3,18}$=3.9807, P= 0.024) (Figure 3.5). There was no evidence that specialist diversity was affected by ERS vegetation type ($F_{2,6}$ = 0.117, P = 0.819) or time ($F_{3,18}$ = 0.996, P = 0.417). There was no difference in generalist species richness between sites (invaded habitats (23.6 ± 1.66), mixed (14.6 ± 2.02), uninvaded (15.66 ± 2.84) ($F_{2,6}$ = 0.055, P = 0.946). The total specialist species richness for each habitat type was similar (invaded 4.33 ± 0.66, mixed 4.33 ± 0.33, uninvaded 3.66 ± 1.21) ($F_{2,6}$ = 0.655, P = 0.621).

Figure 3.5 Mean carabid diversity (Shannon-Wiener diversity index *H*) per habitat type over time ± S.E. All exposed riverine habitat types had a similar diversity over time.



3.4 Discussion

The results of the study of the impact of *I. glandulifera* on ground beetles on ERS show that when *I. glandulifera* invades an ERS, the invasion can alter the composition of the ground beetle community and, potentially, the conservation potential of ERS by attracting a higher abundance of generalist ground beetles to the invaded habitats. The influx of generalist ground beetle species may lead to increased competition for scarce resources with those species endemic to ERS. There is a concern in conservation ecology that fragmented habitats, and habitats that are disturbed by natural and anthropogenic influences, have an increase in generalist species which are better able to exploit disturbance than specialist species (Christian *et al.*, 2009; Marvier *et al.*, 2004). Specialist carabid species are particularly vulnerable to habitat disturbance that reduce their niche requirements (Gandhi *et al.*, 2008; Niemlä, 2001).

In contrast to the results presented in this chapter, Hansen *et al.* (2009) showed that invasion by *Centaurea maculosa* Lam., in the USA supports a lower abundance of generalist ground beetle species compared to native vegetation. The authors also showed that specialist ground beetles were more abundant in the invaded vegetation than in the native vegetation. The presence and invasion of *I. glandulifera* may act to fragment the native vegetation, and niche availability within riparian systems, resulting in 'pockets' of native and invaded vegetation along riparian systems. Being the tallest annual species in the UK with a tendency to form dense monocultures (Beerling and Perrins, 1993), *I. glandulifera* may act as a physical barrier for the movement of specialist ground beetles to and from the ERS throughout the summer months. Specialist ground beetles are generally more immobile than generalist ground beetle species found within ERS habitats (Bates *et al.*, 2006). Carabid species composition

can be affected by the vegetation structure and resulting changes in microclimate conditions as a result of plant invasions. When studying the impact of *Campylopus introflexus* (Hedw.) invasion on carabid species in costal dune systems in Germany, Schirmel et al. (2011) showed that carabid species richness was higher in the uninvaded areas of the protected dune systems. The structural diversity within ERS dominated by native vegetation would be higher than that of an invaded ERS. Species like *Veronica hederifolia* L, *Galium aparine* and *Stellaria holostea* L, are all commonly found on uninvaded ERS, are reasonably short growing and bushy compared to the tall stemmed *I. glandulifera* populations where branching may form up to 1m above the ground (Pers. obs., Author).

The response of the ground beetle community to the ERS dominated by *I. glandulifera* was more profound, in relation to the uninvaded ERS, than the response seen in the mixed vegetation ERS. The significant deviation in the invaded ERS at the beginning of the study, to that of the uninvaded and mixed ERS suggests that the impact of *I. glandulifera* on the carabid community structure is more significant during June and early July (coinciding with the peak activity of carabid species on ERS) compared to the later in the season (late July and early August). The high levels of natural disturbance which are characteristic of ERS habitats may act to regulate the carabid populations found within ERS to a higher degree than in other more stable environments and the influx of another stressor (a non-native plant species) into the system could lead to further compound the changes in species composition. When studying the impact of garlic mustard (*Alliaria petiolata* Bieb.) on carabid assemblages in a more stable system, forest interiors in the USA, Dávalos and Blossey (2004) found no difference in the species composition in invaded and uninvaded sites.

Although generalist abundance was significantly higher in invaded ERS compared to mixed and uninvaded ERS ($P = < 0.05$), the diversity of both specialist and generalist ground beetles was similar between all ERS over time. The overall diversity of ground beetle species decreased over time throughout the duration of the study. The PRC shows that the deviation of the invaded community moved closer to that of the uninvaded and the mixed vegetation in July, whereas the mixed vegetation deviates slightly from the uninvaded ERS. These results are different to those of Topp *et al.* (2008) who showed the abundance of carabid species, and species richness, decreased in stands of *F. sachalinensi*s compared to native vegetative communities. In contrast, the results presented in this chapter support the findings of WeiBin *et al.* (2008) who found that carabid communities altered in the presence of the invasive weed *Ageratina adenophora* (Spreng.) R.M.King & H.Rob. when compared to native vegetation in China, but the diversity of species remained unchanged. It should be noted that the results presented in this chapter may be explained by the peak activity period of the ground beetles declining over time though vegetation growth and the shading of the habitat by *I. glandulifera* may also influence carabid diversity.

An important aspect of plant invasion ecology is the time the invaded population has been established in the community. With time, the effects of the invasion are confounded, or potentially irreversible, as the population expands competing with native plants and displacing their associated invertebrate species (Pawson *et al.*, 2010). The time *I. glandulifera* has been established on the mixed and dominated ERS unfortunately is unknown. However, if the mixed vegetative ERS are more recently colonised by *I. glandulifera*, it is plausible to suggest a similar reduction in native plant species may be seen, similar to the ERS dominated with *I. glandulifera*.

This study provides an indication that *I. glandulifera* outcompetes native plant species in ERS when the invasive population forms monocultures. This is in contrast to the results in section 2.3.4 where plant species richness was similar in the invaded and uninvaded sites. Studies of non-native invasive plant invasions within riparian systems show the invader often has the competitive advantage over native plant species (Fierke and Kauffman, 2006; Maskell *et al*., 2006). Potentially, plant species colonising ERS may be weaker competitors, or the pressure exerted by such high disturbance, coupled with competition from *I. glandulifera* may be a cause of the reduced species richness seen on ERS inundated with *I. glandulifera*. Both the uninvaded and mixed vegetation ERS had a similar average species richness and percentage cover of native plants compared to the lower percentage cover and species richness found in the invaded ERS.

Poa trivialis L. and *Holcus lanatus* L. were more dominant on the mixed vegetated ERS as were biannual species from the genera *Rumex*. These species persist longer throughout the season than *I. glandulifera* and their above-ground biomass and overwintering root system may maintain the surface of the ERS by trapping sediment during the winter and spring months. However, increased sediment may lead to smothering available niches for prey species such as Collembola and Diptera larvae, thereby lowering their abundance. The majority of ground species captured during the study were predatory species (95%) and prey abundance would be regarded as a factor influencing the occurrence of ground beetle species within and between ERS (Sadler *et al*., 2004). In addition, sediment accumulation can lead to successional changes in the composition of vegetation on the ERS, which may alter the hibernation potential of the habitats (Eyre and Lott, 1997). Throughout the autumn and winter months, *I. glandulifera* roots would rot beneath the surface of ERS whereas grasses and other perennial species would persist from season to season. Although rotting vegetation is a

potential food source for detritivores, it would not provide a suitable niche for hibernation as rotting material would attract prey, predators, and opportunistic fungi.

The fact that *I. glandulifera* is shown to outcompete native plant species on ERS suggests that *I. glandulifera* should be managed in both highly invaded ERS, and equally ERS where the species is interspersed with native vegetation. Not managing a population of *I. glandulifera* interspersed with native vegetation, may potentially lead to the build-up and dominance of the species as it outcompetes native plant species. Complications obviously arise when managing *I. glandulifera* in areas of high conservation importance. Manual removal through work parties, commonly volunteers at the weekend (known as 'balsam bashing'), may do little to improve the situation as removing plants from the ERS would severely disrupt the surface of the ERS leading to increased erosion. Again, chemical control although selective, would be undesirable within ERS.

The lack of a correlation between the percentage cover of bare ground and the abundance of generalist ground beetle species is to be expected as bare ground is not a niche requirement for generalist species on ERS (Bates *et al.*, 2006). However, it is surprising that the abundance of specialist ground beetles showed no correlation to any of the percentage cover variables (vegetation and bare ground). Sadler *et al.* (2004) highlight that many specialist carabid species are dependent on bare ground habitats for parts of their life cycle and the colonisation of vegetation is negatively correlated with specialist abundance and diversity. The lack of any correlation may be a reflection of the lack of success of trapping at the beginning of the study when traps were washed away during the peak period of ground beetle activity. In addition, the high water levels during May and June may have influenced specialist populations.

An interesting result from this study is the negative correlation found between the percentage cover of native vegetation and generalist abundance compared to the positive correlation with *I. glandulifera* percentage cover (Figure 3.4). All ground beetle species associated with the invaded ERS habitats were hydrophilic species, and *Pterostichus nigrita* (Pank.), *P. cupreus* (L.), *P. versicolor* (Strm.), in particular are shade preferring species which may indicate why they are attracted to the tall stands of *I. glandulifera*. However, this fails to explain why a negative pattern is seen with native vegetation. Potentially, these large carnivorous carabid species may prefer the invaded habitats due to the reduced structural diversity of the vegetation making it easier to navigate the ERS surface and find and catch prey species (Hyman, 1992). Only two species, *Harpalus affinis* (Schr.) and *H. rufipes* Deg. can be described as predominantly seed-feeders and only the latter was associated with the invaded ERS. Although *I. glandulifera* is a prolific seed producer, there is no evidence in the literature that these comparably large seeds would comprise the diet of either species. Therefore, as a direct food source *I. glandulifera* would be of low value to any of the carabid species recorded in this study.

The high levels of disturbance in riparian systems, coupled with the associated difficulties of sampling on ERS habitats, highlights the necessity for multiple season intensive sampling when studying invertebrates within this system. The current research provides an indication that *I. glandulifera* affects the carabid community of ERS by increasing the generalist carabid abundance. However, the low abundance of specialist carabid species collected during the course of the study, in particular the absence of *P. areolatus*, *B. testaceum* and *L. quadrillum* (all BAP species) in any of the collections, does not allow us to infer any direct effect of *I. glandulifera* on species with high conservation importance. Pitfall traps, as discussed in section 3.2.3.1, are biased to

fast moving mobile species (often generalist species) and under-represent more immobile species (often specialist species). There were clear difference in the species assemblages caught between the two sampling methods (Table 3.4) where larger fast moving species like *Carabus granulatus* (L.), *C. violaceus* (L.) and *Pterostichus cristatus* Duf. were only identified from the pitfall trap method.

Including timed hand searches, as recommended by Bates *et al.* (2007), and Bell and Sadler (2003a), can incorporate more cryptic species into the dataset, and this is shown in the dataset where 13 species of *Bembidion* were collected in the hand searching method compared to 9 species of *Bembidion* captured in the pitfall traps (Table 3.4). However, the overall capture rate was higher for the pitfall trapping method (88% of the total) compared to the timed hand searches (22%). The difference in species composition between the two sampling methods supports the use of multi-sampling methods for sampling carabid communities (Bates *et al.*, 2007).

Understanding and addressing the impacts of invasive non-native plant species in riparian systems is essential for conserving the unique flora and fauna within these highly fragmented and managed ecosystems. It is alarming to consider the sheer abundance of non-native plant species within our riparian systems and the threat they pose on the dynamics of riparian systems (Environment Agency, 2010). Indeed, it is questionable if such a highly disturbed habitat can ever be resilient to invasion by introduced plant species. However, combined control efforts at a catchment scale coupled with habitat restoration, incorporating the knowledge of scientists and land managers, coupled with adequate funding from government and NGO's is the only way forward.

4 Below-ground impacts of *Impatiens glandulifera*: implications for management practices and habitat restoration

4.1 Introduction

Soil provides native plant species with essential resources needed which in turn promote the biological diversity of invertebrate organisms and fungi, which provide ecosystem services such as conservation, fertility- that increases the productivity of agricultural systems and increases the abundance of beneficial organisms to naturally control pest species. Intensive agriculture and pollution (Tilman, 2002), urbanisation (Chen, 2007), climate change (Defra, 2009), erosion due to biotic (Hooke, 2006) and abiotic pressures (Kauffman *et al.*, 1983), and the colonisation of non-native plants (Broz *et al.*, 2007; Maurel *et al.*, 2010) are all known to impact on the quality of soils and diversity of the below-ground community, including both arthropod and fungal biota.

The Environment Agency (2004) estimate that 2.2 million tonnes of top soil is eroded in the UK each year. *Impatiens glandulifera* has often been blamed for increased soil erosion in riparian habitats (Burkhart and Nentwig, 2008; Cockel and Tanner, 2011). However, to date, this claim is unsubstantiated and lacks scientific evaluation. In addition, there is currently no quantifiable scientific research to evaluate the impact of *I. glandulifera* on soil organisms. The research conducted in chapters 2 and 3 has focused on the above-ground impacts of *I. glandulifera* on invertebrate communities. However,

for a concise evaluation of the impact of *I. glandulifera* at an ecosystem level, below-ground potential impacts are an important aspect and thus warrant evaluation.

There are now a number of scientific studies that show how below-ground herbivores affect and maintain the structural composition and fitness of above-ground vegetation communities (Brose, 2008; Carson and Root, 2000; Scherber *et al.*, 2010). The interactions between below-ground and above-ground invertebrates, and the plant community are complex and are in a constant state of perturbation both on temporal and spatial scales (Eisenhauer *et al.*, 2011a). Soil herbivores enhance the diversity and stability of plant communities by promoting some plant species over others (Bardgett *et al.*, 2005; Deyn *et al.*, 2003). Invasion by non-native invasive plant species can potentially lead to a decrease in plant community diversity by replacing native plant species (Gerber *et al.*, 2008). In turn, the reduction in plant species diversity may cause the replacement and local extinction of below-ground invertebrate species, as seen in other systems (Hooper *et al.*, 2000). As discussed in section 2.3.3, there was no difference in plant species richness between the invaded and uninvaded sites at Harmondsworth Moor, though the percentage cover of forbs and grasses was significantly reduced in invaded plots. Reduced cover of native plants, as an effect of invasion by *I. glandulifera*, may potentially have indirect consequences on the fitness of natives as below-ground herbivores may exert increased pressure on the diminished population.

Currently, few studies have evaluated the impacts of non-native plants on the soil invertebrate community. Van der Putten *et al.* (2009) highlight that most of the research conducted to-date on plant invasions has been conducted in an above-ground context. Where below-ground studies have been researched, the results are often

conflicting when studying different systems and different species. For example, Rudd (2009) studied the potential impact of *Lonicera* x *bella* Zabel, an invasive honeysuckle species in the United States, on soil invertebrate diversity using pitfall traps and soil cores, and showed there was no effect of the non-native plant species on soil invertebrate diversity or abundance. When studying the relationship between the abundance of earthworms in Congolese Eucalyptus plantations, which are invaded by the non-native *Chromolaena odorata* L., Mboukou-Kimbata *et al.* (2007) showed earthworm densities were higher in the invaded stands. Belnap and Phillips (2005) showed an increase in invertebrate species richness in areas invaded by *Bromus tectorum* L. in south-eastern Utah, USA. As highlighted by Wolfe and Klironomos (2005), the lack of additional studies does not allow for further comparisons, and the authors go on to suggest that it is difficult to make generalisations about the impact of non-native plant invasions on the soil biota due to the paucity of research in this field.

When studying the below-ground impacts of a non-native plant species, in order to obtain a holistic view one cannot ignore the potential impacts on the soil fungal biota. Indeed, plants, invertebrates, and soil fungi are intrinsically linked and most native plants species reply on soil fungi for their colonisation, establishment, growth, and nutrient acquisition (Gange *et al.*, 1990; Gange *et al.*, 1993). Over 80% of all plant families are mycorrhizal dependent (Jeffries *et al.*, 2003). Arbuscular mycorrhizal fungi (AM fungi) form a symbiotic relationship and interface between the plant's root structure and the soil substrate (Brundrett, 2002). The extensive hyphal structure increases the surface available to the plant to absorb water and nutrients, mostly phosphorous, from the soil. The AM fungi benefit through the acquisition of carbon from the process of plant photosynthesis, which is then incorporated into the below-ground system and cycled through the ecosystem. It has been shown that AM fungi

play a vital role in the structuring of the plant community of natural ecosystems and the health of the soil and associated native species are dependent on a permeated network of AM fungal hyphae (Gange and Ayres, 1999). The symbiotic association of land plants and AM fungi dates back to the early evolution of primitive plants colonising land and is fully reviewed in Brundrett (2002).

Impatiens glandulifera, unlike the majority native plant species in the UK, is not dependent, or only weakly dependent on AM fungi for its successful colonisation of new habitats (Beerling and Perrins, 1993; Harley and Harley, 1987). Indeed, this holds true for many non-native plant species in the UK (Harley and Harley, 1987). The lack of any mycorrhizal association in some of our more prolific non-native plant species enables these species to invade habitats where the mycorrhizal network has been degraded, either by natural disturbance or a previous invasion by another non-mycorrhizal dependent non-native plant species (Reinhart and Callaway, 2006).

AM fungi do not have high levels of host specificity (Klironomos, 2000) though the symbiotic relationship between the fungus and the host is more pronounced and beneficial for some plant species interactions compared to others (Johnson *et al.*, 1997; Klironomos, 2000). Even non-native plant species that are weakly dependent on AM fungi may utilise the AM fungal network in the process of an invasion, as the AM fungal network forms a symbiosis with numerous plant species within the community (Reinhart and Callaway, 2006). An invading non-native invasive plant species may potentially exploit the benefits of the plant-microbial association without contributing as much as the native plants to the association. Thus, as the non-native species dominates the community over time, the AM fungal network is left depauperate at a cost to the

native species and the fungal network, while the non-native species benefits due to the lack of competition (Reinhart and Callaway, 2006).

In contrast to the lack of research on the impacts of plant invasions on soil invertebrates, in recent years there has been a considerable amount of research conducted on the effects of plant invasions on the microbial community (see Belnap and Phillips, 2001; Kourtev *et al.*, 2003; Roberts and Anderson, 2001). Studies conducted on non-native plant species that are associated with AM fungi show that the non-native species benefit from this association when invading new habitats and competing with native plant species. For example, Harner *et al.* (2010) showed that AM fungi directly benefit the colonisation, establishment, and growth of the non-native spotted knapweed (*Centaurea stoebe* L. subsp. *micranthos* (Gugler) Hayek) in riparian systems in the United States. Marler *et al.* (1999) tested the effect of AM fungi on interspecific and intraspecific competition between a native species (*Festuca idahoensis* Elmer) with a lower colonisation of AM fungi than that of the non-native invader (*Centaurea maculosa* Lam.) in rangeland in Western North America. In greenhouse experiments, the authors showed that although AM fungi had no direct effect on either species when grown together, however, with an AM fungal inoculum in the soil, *C. maculosa* showed increased competition. In comparison, where studies have been conducted with non-native plant species that have a low dependency on AM fungi, these studies have shown that the non-native species can deplete the AM network within the invaded areas compared to uninvaded areas (Vogelsang *et al.*, 2005).

In the UK, the management of non-native invasive plant species has focused on the control and eradication of invaded populations. However, to-date, there have been no known studies on the impact of non-native invasive plants on the soil community, and,

therefore, restoration attempts are potentially futile if impacts occur and they are not addressed. If an invasion is having a detrimental impact on the soil, remediation coupled with restoration to support the recolonisation of native plants species would be needed.

4.2 Methods

4.2.1 The site

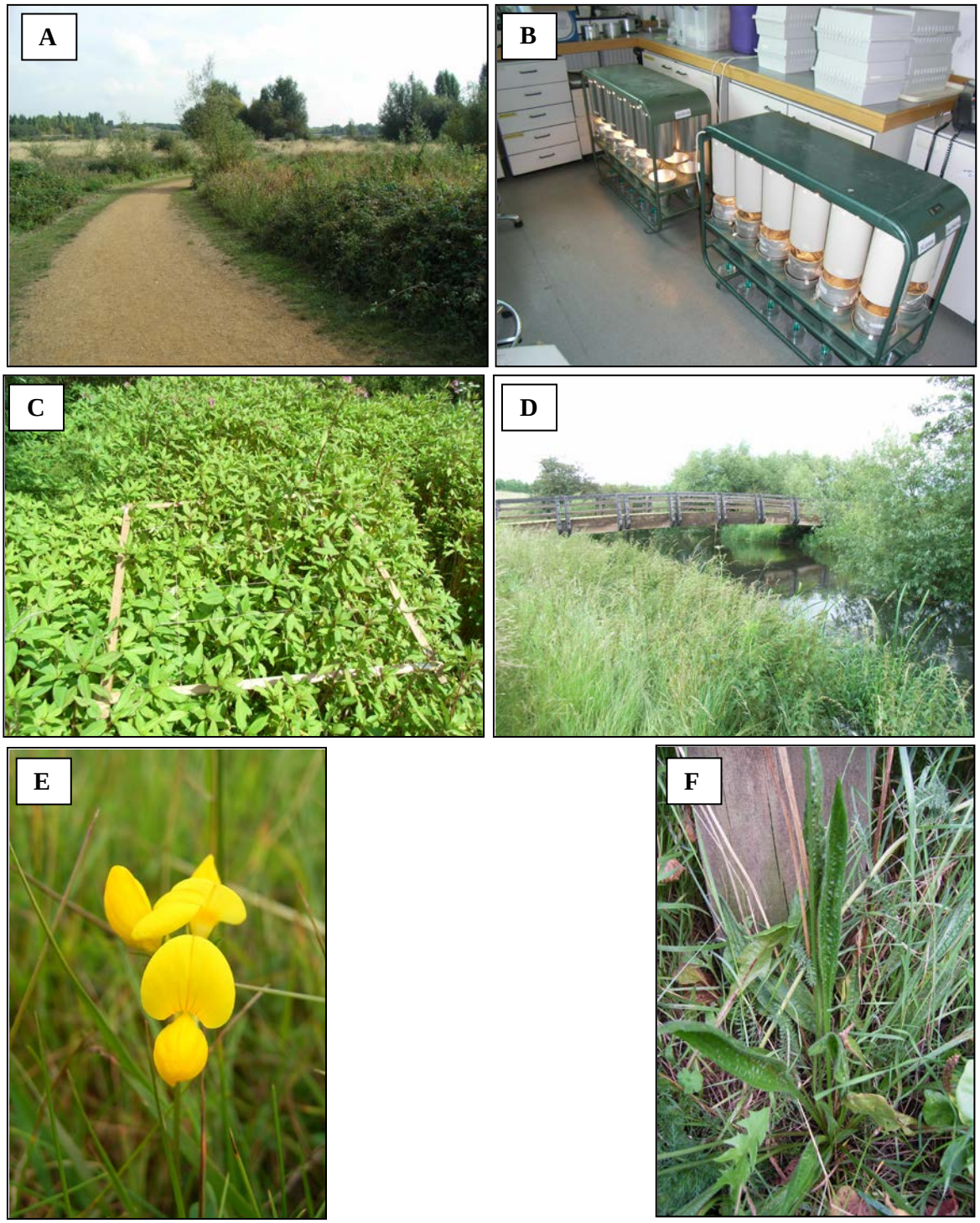
All samples for this study and experiment were collected from Harmondsworth Moor (Plate 4.1 A/B) (see section 2.2.1 for a full site description) during the summer months of 2007 and 2008 (see section 4.2.2.1 for sampling effort).

4.2.2 Evaluating the response of below-ground invertebrates to the presence of *Impatiens glandulifera*

4.2.2.1 Invertebrate sampling

To evaluate any impacts of *I. glandulifera* on the below-ground invertebrate community, soil cores (10cm diameter and 25cm in depth) were taken from beneath stands of *I. glandulifera* and native vegetation. The soil invertebrate community was sampled monthly over two seasons between May and September 2007 and May and August 2008. The sampled sites were the same as in section 2.2.1.2, therefore eighteen sites were sampled in total (nine invaded sites and uninvaded sites). One soil core was taken from each site during each sampling period. The position of the sampled soil was randomly selected from within the sampled site. Ground-dwelling invertebrates were extracted from the soil cores using a Berlese Tullgren Funnel (Burkard Manufacturing Co. Ltd.). Each soil sample was placed inside a separate funnel within the trap and covered with Clingfilm to prevent invertebrates escaping from above. A light source was initiated from above thereby influencing the movement of the invertebrates away from the source.

Plate 4.1 Vegetation and equipment used for below-ground sampling



A. Footpath and cycle track through the moor
B. Berlese Tullgren Funnel set up with soil core samples
C. Vegetation quadrat
D. Footbridge over the river Wraysbury
E. *Lotus corniculatus* L. Harmondsworth Moor
F. *Plantago lanceolata* L. Harmondsworth Moor

All invertebrates were preserved in 70% alcohol prior to identification. Below the funnel, a test tube was attached with approximately 10ml of ethylene glycol to capture and preserve any invertebrates emerging from the soil (Plate 4.1C). All invertebrates were identified to Order following the guide of Chinery (1993).

4.2.3 Vegetation sampling

The vegetation data used in this chapter is the same as that of chapter 2 (see section 2.2.3 for sampling method). Thus, in brief, the percentage cover of *I. glandulifera* was estimated for each invaded site on a monthly basis for the duration of the study by a visual assessment of each site. In addition, during July of both years, six $1m^2$ quadrats (Plate 4.1D) were randomly placed within each site and individual species were identified and their percentage cover per quadrat was estimated.

4.2.4 Measuring invertebrate biomass

Following identification, all species were dried to obtain a measurement of dry weight for total invertebrate abundance per soil core. The majority of alcohol was pipetted from the samples and the test tubes were placed in a drying cabinet for a 72 hour period at 60^oC. Specimens were then weighed using Mettler AT200 scale (precision to 0.0001g).

4.2.5 Evaluating plant performance from invaded and uninvaded soils

To determine if *I. glandulifera* impacts on the below-ground AM network and plant performance three native plant species which were found to be present at Harmondsworth Moor, namely *Plantago lanceolata* L. (Plate 4.1E), *Lotus corniculatus* L. (Plate 4.1F), and *Trifolium pratense* L. were grown in soil from beneath stands of *I.*

glandulifera and from beneath stands of native vegetation[4]. Seeds of each plant species were germinated on damp filter paper within a 9 cm diameter Petri dish. Approximately 100 seeds were germinated for each species within a controlled temperature room (20^{o}C) with a light regime of 16 hours light: 8 hours dark.

4.2.5.1 Soil samples

Soil was collected from five invaded and five uninvaded sites within Harmondsworth Moor during the month of July 2008. The sites were different to those used in section 2.2.1.2 and 4.2.2.1. Soil samples were extracted from the ground using a soil core (see section 4.2.2.1 for diameters). All soil samples from beneath invaded sites were combined, mixed, and potted into thirty 10cm diameter plant pots where each pot contained approximately 250 gm of soil. This was then repeated for the uninvaded soil samples and all pots were labelled with their corresponding soil type.

4.2.5.2 Plant propagation and maintenance

Upon germination, 10 seedlings of each species were sown into pots containing each soil type. One seed was placed in each pot. The developing plants were then maintained under the same controlled conditions for a period of four months where the plants were watered from above every 48 hours using approximately 50ml rainwater. After four months, each plant was harvested from the soil and the above-ground vegetation was cut from the below-ground roots. For each replicate, the above-ground wet and dry biomass was measured. Dry mass was measured after placing the plants in a drying cabinet for a period of 72 hours at 60^{o}C.

[4] Alex Lee (Royal Holloway) conducted the experiment on plant performance under my supervision

4.2.5.3 Root preparation and staining

The method of root preparation and staining followed that of Vierheilig *et al.* (1998). Roots were gently extracted from the soil of each replicate where above ground biomass was evaluated. Each root sample was washed under tap water for a period of 60 seconds until all soil particles were removed. Following a period of drying to remove excess water the roots were cut into 1cm lengths and immersed in a 10% potassium hydroxide solution (KOH) (10% w/v: 10g KOH in 100ml aqueous solution) and placed in a water bath at 80^{o}C for 25 minutes. Upon removal from the KOH the root pieces were washed under running water for a period of 5 minutes where the surplus water was removed with standard blotting paper. The root pieces were then added to clean vials where approximately 20ml of staining solution (84.4 : 15 : 0.6, dH2O : 1% HCI: Quink blue pen ink) was added, enough to cover the roots. The vials were then placed back into the water bath for a period of 15 minutes. The vials were then removed and stored in the staining solution until they were analysed under a microscope.

4.2.5.4 Evaluation of roots for mycorrhizal colonisation

To evaluate the percentage AM colonisation of each root sample three 1cm lengths of root were selected from each replicate. Following the cross-hair eyepiece method of McGonigle *et al.* (1990), the percentage AM colonisation was evaluated where 100 root encounters with the cross-hair was recorded for the presence and absence of AM fungi. Thus, if the cross-hair encounters roots 100 times and in only 20 of those encounters AM structures were observed the percentage colonisation for that sample was 20%. This process was then repeated for all root samples.

4.2.6 Data analysis

4.2.6.1 The principal response curve

To evaluate the effect of *I. glandulifera* on the soil invertebrate community a multivariate approach was adopted using the principal response curve (see section 1.23 for a full description). As in chapter 2, the uninvaded sites were taken as the control. Each soil core was an individual replicate and prior to analysis abundance was log transformed with the value of 1 added.

4.2.6.2 Invertebrate abundance

The effect of *I. glandulifera* on invertebrate abundance, both total invertebrate abundance and individual groups, was evaluated using a repeated measures ANOVA with treatment (invaded and uninvaded) and time as the main effects. The normality of the data and equality of variance was evaluated prior to the analysis and if the data deviated from the assumptions of the test, the data were log transformed or square root transformed.

4.2.6.3 Vegetation

To evaluate any differences in plant species richness between invaded and uninvaded sites, and any differences between years, a two-way factorial ANOVA was performed on the total number of plant species per site. The data were square-root transformed prior to analysis to suit the assumptions of the test. All percentage cover data was averaged over the six sampled quadrats, per site, to give a site mean per year as the replicate. To evaluate any difference in the percentage cover of *I. glandulifera*, forbs, and grasses between years a one-way analysis of variance was conducted where the percentage cover variables were arc-sine transformed prior to analysis. If the data

deviated from the assumptions of the test, the data were transformed by log transformation or square root transformation.

4.2.6.4 Invertebrate biomass

The effect of *I. glandulifera* on invertebrate biomass was evaluated using a repeated measures ANOVA with treatment (invaded and uninvaded) and time as the main effects. Each soil core sample represents one replicate. The normality of the data and equality of variance was evaluated prior to the analysis and if the data deviated from the assumptions of the test, the data were transformed by log transformation or square root transformation.

4.2.6.5 Relationship between invertebrate abundances and percentage cover of vegetation

To assess the relationship between below-ground invertebrate abundance and the percentage cover of vegetation a multivariate approach was adopted. An indirect ordination analysis, Detrended Correspondence Analysis (DCA) was performed on the log transformed invertebrate dataset to evaluate whether to use linear or non-linear methods (see section 2.2.6.3 for a full description). The gradient lengths were less than 4 thus a Redundancy analysis (RDA) was performed on the invertebrate data set and the environmental variables (percentage cover of vegetation) (see section 2.2.6.3 for a full description).

4.2.6.6 Evaluating differences in above-ground plant biomass between soil types

To evaluate differences in plant performance of the three plant species grown in the invaded and uninvaded soils, both the fresh weight, and dry weight of the above ground

biomass were analysed using a one-way analysis of variance, with individual plants as replicates. The normality of the data and equality of variance was evaluated prior to the analysis and if the data deviated from the assumptions of the test, the data were transformed by log transformation or square root transformation.

4.2.6.7 Evaluating the difference in percentage mycorrhizal colonisation between soil types

A one-way analysis of variance was performed on the percentage AM colonisation where the average colonisation was calculated for the three 1cm root pieces per plant and the average was taken as the replicate. The percentage colonisation data was arc-sine transformed prior to analysis.

4.2.6.8 Relationship between percentage colonisation of mycorrhizal and dry weight

To evaluate any relation between dry weight and percentage colonisation of AM, a regression analysis was performed for each plant species. The data were checked for normality and the percentage mycorrhizal colonisation data was arc-sine transformed prior to analysis.

4.3 Results

4.3.1 The invertebrate community

There was a significant shift in the below-ground invertebrate community when comparing the invaded sites to the control (uninvaded) for 2007 ($F_{1,80} = 8.54$, $P < 0.05$) (Figure 4.1A). However, the 2008 data showed no difference between the invaded and uninvaded sites ($F_{1,64} = 1.39$, $P = 0.9$) (Figure 4.1B). The first canonical axis of the PRC explained 59.91% of the total variation where 12.82% was explained by time and 7.56% by treatment (including the interaction with time). The groups Haplotaxida, Coleoptera, Acari, Collembola and invertebrate larvae all showed a significant response to *I. glandulifera* (Table 4.1). All of the aforementioned groups showed a negative response to the invaded sites at the beginning of both seasons and as the season progressed the response moved to a positive association with the invaded habitats compared to the uninvaded. In contrast to the above-ground invertebrate datasets (both ground Vortis (section 2.3.1) and aerial Vortis (section 2.3.2)), the PRC fluctuates above and below the control (uninvaded sites) indicating that the invertebrate community is affected both positively and negatively within and between seasons. Collembola showed the highest response to the invaded sites where the population at the beginning of the study (May) was reduced in the invaded sites to 87% of its geometric mean count of the uninvaded sites and increased in the invaded sites (see table 4.1 and 4.2). In August 2007, Collembola increased in the invaded sites to 430% of the geometric mean count of the uninvaded sites. The groups Formicoidea, Isopoda, Myriapods, and Thysanoptera all showed no response to *I. glandulifera*.

Figure 4.1 Principal Response Curve of the invertebrate groups from the 2007 (A) and 2008 (B) soil cores. The control (uninvaded) is expressed as a horizontal line through zero and the black line is the response of the invertebrate community in the invaded sites, compared to the control, over time. The invertebrates groups on the third axis are ordered in their taxon weight corresponding to the y-axis. The 2007 data set shows a significant shift in the invertebrate community between the invaded and invaded sites ($P < 0.05$) though for 2008 there was no significance ($P = 0.9$).

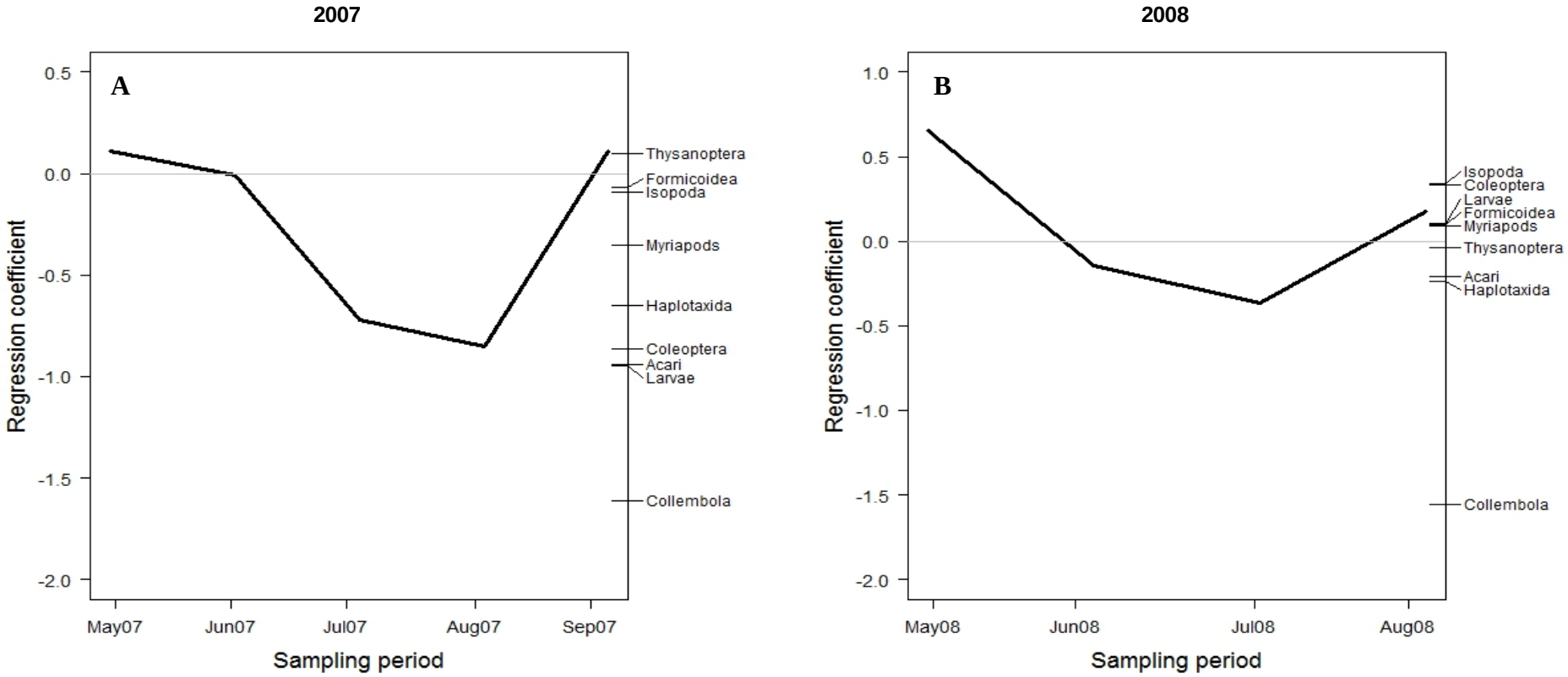


Table 4.1 Invertebrate groups including their main feeding guild and resulting Principal Response Curve taxon weight (b_k) identified for the soil core sampling. Groups in bold indicate a significant response to *Impatiens glandulifera*. The feeding guild lists the dominant feeding guild within the group.

Group	Feeding Guild	Taxon weight (b_k)	
		Soil core 2007	Soil core 2008
Acari	Omnivores	**-0.93986**	-0.20989
Coleoptera	Omnivores	**-0.86142**	0.33083
Collembola	Detritivores	**-1.61155**	**-1.55814**
Formicoidea	Omnivores	-0.06792	0.09623
Haplotaxida	Detritivores	**-0.64849**	-0.2356
Isopoda	Detritivores	-0.0922	0.33718
Larvae	Omnivores	**-0.9455**	0.10661
Myriapods	Detritivores/Predators	-0.35587	0.09377
Thysanoptera	Phytophagous	0.09559	-0.0902

Table 4.2 The resulting Principal Response Curve coefficients for the soil core sampling methods for invaded sites + sampling date which are contrasts to the control (uninvaded).

Sampling method	Sampling date								
	May-07	Jun-07	Jul-07	Aug-07	Sep-07	May-08	Jun-08	Jul-08	Aug-08
Soil core	0.1127	-0.0066	-0.7189	-0.8494	0.112	0.6568	-0.1475	-0.3674	-0.1730

Table 4.3 The total number of soil dwelling invertebrates identified for each site (uninvaded and invaded) for 2007 and 2008.

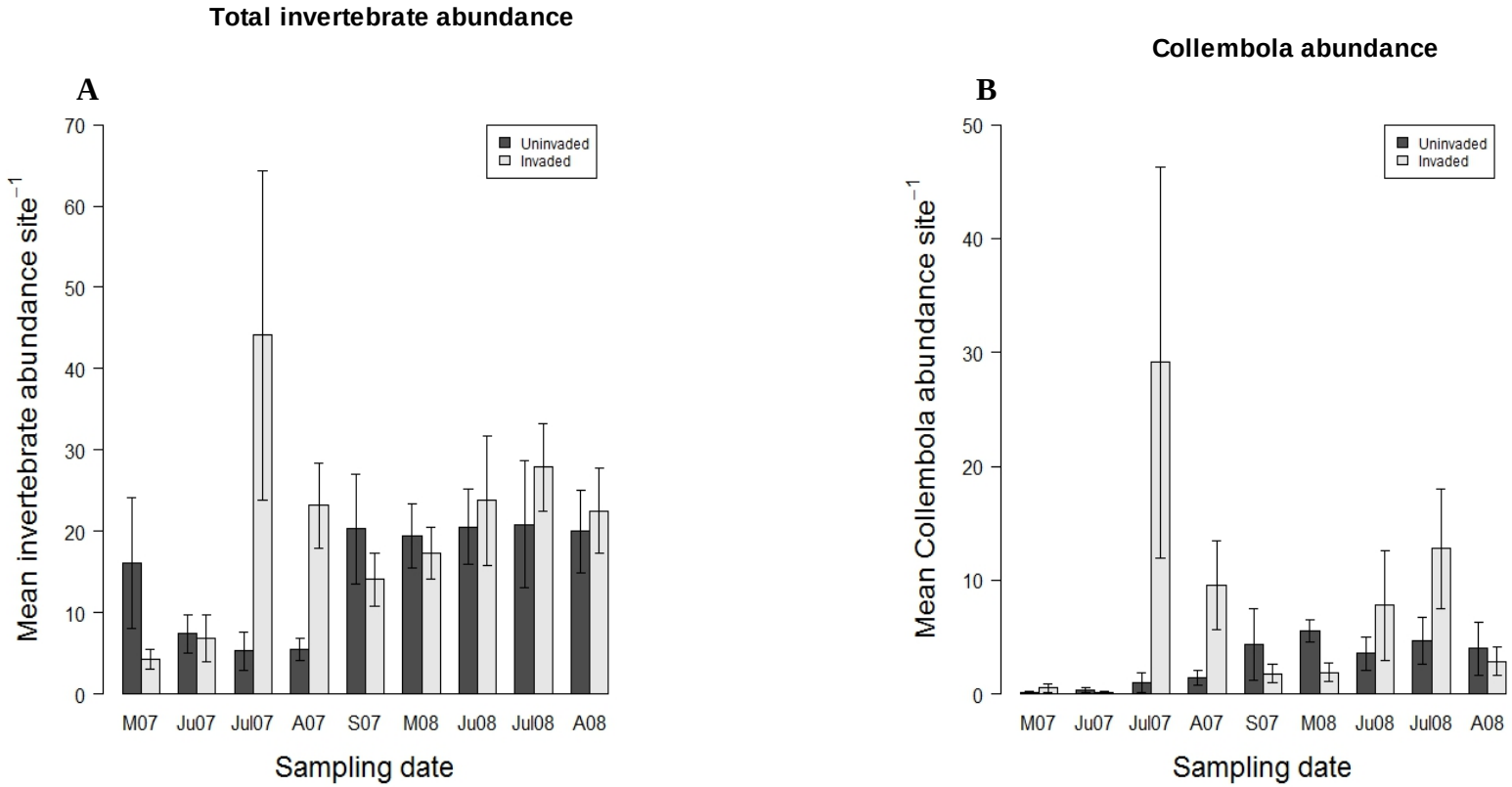
Year	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
site (Uninvaded)	1		2		3		4		5		6		7		8		9	
Group																		
Acari	34	40	4	7	12	18	2	32	9	21	17	20	12	16	26	23	19	21
Coleoptera	13	5	3	2	7	3	1	5			5	3	3	1	11	4	6	
Collembola	38	13		17	2	8	4	18	6	36	4	15	2	21	8	20	1	12
Formicoidea	6	2		2		1			4	5	1	6	60	9			1	
Haplotaxida	1	7	2	3	4	14	2	8	3	6	8	4	6	4	8	16	3	10
Isopoda	5	1	13	2	1	2	4	13		41	3	1	7	2	1	12	1	12
Larvae	4	4	9	3	5	5	7	7	5	16	12	1	7	12	32	19	5	12
Myriapods	2	11	3	5	2	2	2	6		30		5		19	2	2		4
Thysanoptera	1						1									1		

Year	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
site (Invaded)	1		2		3		4		5		6		7		8		9	
Group																		
Acari	23	3	6	7	5	28	1	14	38	27	9	26	19	72	17	7	26	31
Coleoptera	15	2	6	2	9	10	5	6	11	3	9	2	5	7	6	2	8	2
Collembola	139	48	1	34	8	8	9	6	122	7	8	17	63	57	7		13	51
Formicoidea				1		6	8	1										1
Haplotaxida	6	5	9	7	4	4	1	7	9	17	5	10	7	6	7	4	12	12
Isopoda	4			6		20	1	1	6	7		12	1	25	1	5	2	29
Larvae	16	8	11	14	7	8	11	19	15	12	15	11	17	13	24	12	21	10
Myriapods	6				3	5	5	2	3	4	3	11	5	8		3		14
Thysanoptera												3				2		

Overall, the total abundance of invertebrates was similar between the two habitat types ($F_{1,16} = 2.347$, $P = 0.145$) (Figure 4.2A) (see table 4.3 for the total number of soil dwelling invertebrates identified for each site (uninvaded and invaded) for 2007 and 2008). For total abundance there was a significant influence of time when comparing the invaded and uninvaded sites ($F_{8,128} = 3.025$, $P < 0.05$), and a significant influence of treatment and time ($F_{8,128} = 2.772$, $P < 0.05$), indicating the invertebrate communities responded differently over time between the invaded and uninvaded sites. For both the invaded and uninvaded sites a higher abundance was seen in 2008 compared to 2007 (uninvaded 2007: 11.088 ± 2.328, invaded 2007: 18.711 ± 4.627; uninvaded 2008: 20.194 ± 2.656, invaded 2008: 22.888 ± 2.831). Each habitat type produced different general trends over the course of the study. In 2007, the uninvaded sites showed a general decrease in total below-ground invertebrate abundance throughout the growing season reaching the highest abundance in September, whereas in 2008 invertebrate abundance remained relatively constant. In contrast, in the invaded sites the total invertebrate abundance showed a general increase reaching its highest levels in June 2007 and July 2008.

Overall, Collembola abundance was similar between the two habitat types ($F_{1,16} = 1.669$, $P = 0.212$) though there was a significant influence of time ($F_{1,16} = 4.118$, $P < 0.001$) and a significant influence of treatment and time ($F_{8,128} = 2.581$, $P < 0.05$) (Figure 4.2B). Again, this indicates that the Collembola community responded differently in the invaded and uninvaded sites. A higher abundance was seen in the uninvaded sites in 2007 compared to 2008 (uninvaded 2007: 1.444 ± 0.671, invaded 2007: 8.222 ± 3.742; uninvaded 2008: 4.444 ± 0.864, invaded 2008: 6.333 ± 1.88).

Figure 4.2 Mean total invertebrate abundance ± S.E (A) and mean Collembola abundance ± S.E (B) for the soil core samples from the invaded and uninvaded habitats for each month sampled. There was no difference in abundance of total invertebrates ($P = 0.14$) and Collembola ($P = 0.21$) between the invaded and uninvaded habitats, though there was a significant influence of time (Total: $P < 0.05$; Collembola: $P < 0.001$), and an interaction between time and treatment (Total and Collembola: $P < 0.05$). M: May, Ju: June, Jul: July, A: August and S: September).



There was no difference between the soil invertebrate abundance and the environmental variables (percentage cover) in the RDA for 2007 ($F_{4,13} = 1.363$, $P = 0.13$) or 2008 ($F_{4,13} = 0.911$, $P = 0.64$) and hence the graphic is not shown.

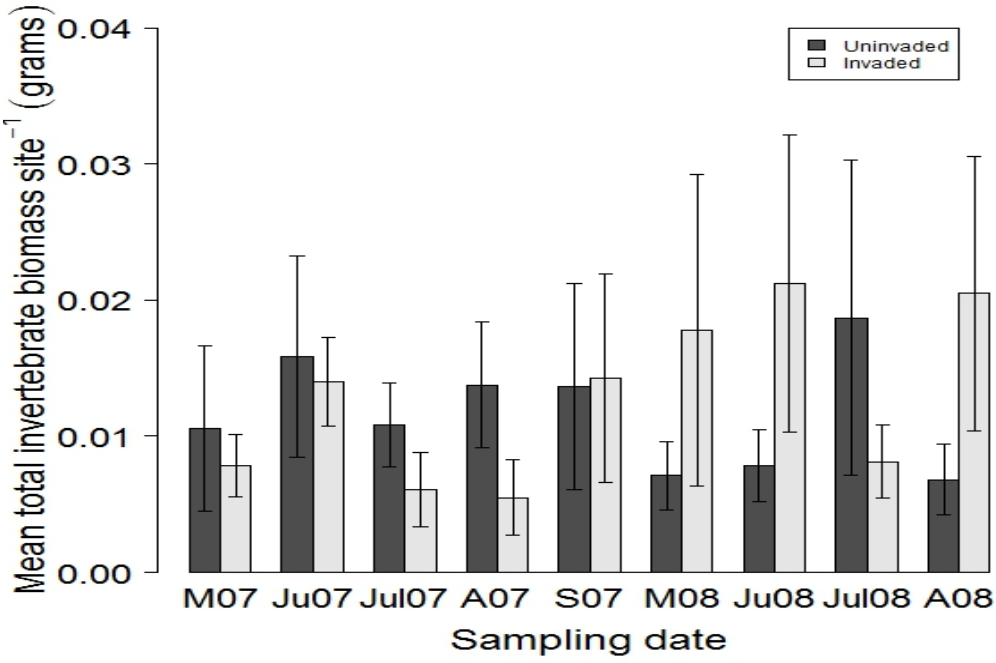
Total invertebrate biomass was similar between the two habitat types ($F_{1,16} = 0.064$, $P = 0.804$) (Figure 4.3), though there was a significant influence of time ($F_{8,128} = 1.063$, $P < 0.001$) (uninvaded 2007: 0.016 ± 0.004, invaded 2007: 0.014 ± 0.002; uninvaded 2008: 0.013 ± 0.003, invaded 2008: 0.01 ± 0.002).

4.3.2 Plant performance in the different soil types

All three plant species showed a higher percentage of colonisation of AM fungi when grown in the uninvaded soils compared to the invaded soils: *P. lanceolata* ($F_{1,18} = 17.707$, $P < 0.001$) (invaded 12.4 ± 2.44; uninvaded 29.7 ± 2.6) (Figure 4.4A), *L. corniculatus* ($F_{1,18} = 36.406$, $P < 0.001$) (invaded 12.4 ± 1.6; uninvaded 24.4 ± 1.2) (Figure 4.4B), *T. pratense* ($F_{1,18} = 26.306$, $P < 0.001$) (invaded 12.8 ± 1.6; uninvaded 23.7 ± 1.1) (Figure 4.4C).

P. lanceolata and *L. corniculatus* both had a higher biomass (both dry (Figure 4.5A/B) and fresh weight (Figure 4.6A/B)) when grown in the uninvaded soils compared to the invaded soils (*P. lanceolata* dry weight: $F_{1,18} = 10.134$, $P < 0.001$, (invaded 0.114 ± 0.024; uninvaded 0.536 ± 0.13), fresh weight: $F_{1,18} = 40.554$, $P < 0.001$ (invaded 0.733 ± 0.117; uninvaded 3.334 ± 0.367)) (*L. corniculatus* dry weight: $F_{1,18} = 21.572$, $P < 0.001$, (invaded 0.024 ± 0.004; uninvaded 0.061 ± 0.006), fresh weight: $F_{1,18} = 22.514$, $P < 0.001$ (invaded 0.268 ± 0.069; uninvaded 0.714 ± 0.064)).

Figure 4.3 Mean total invertebrate biomass ± S.E for the invaded and uninvaded habitats for each month sampled. There was no difference between the invaded and uninvaded habitats (P = 0.8), though there was a significant influence of time (P < 0.001). M: May, Ju: June, Jul: July, A: August and S: September).



T. pratense showed no difference in either dry (Figure 4.5C) or fresh weight (Figure 4.6C) between the two soil types (dry weight: $F_{1,18} = 1.183$, P = 0.291, (invaded 0.044 ± 0.006; uninvaded 0.035 ± 0.005), fresh weight: $F_{1,18} = 0.016$, P = 0.899 (invaded 0.533 ± 0.093; uninvaded 0.515 ± 0.107).

There was a positive relationship between dry weight and percentage AM colonisation for *P. lanceolata* and *L. corniculatus* ($R^2 = 0.206$, F1,18 = 5.927, P < 0.05; $R^2 = 0.32$, F = 9.752, P < 0.05), respectively) (Figure 4.7A/B). However, there was no relationship between dry weight and percentage AM colonisation for *T. pratense* ($F_{1,18} = 0.652$, P = 0.429) (Figure 4.7C).

Figure 4.4 The difference in the percentage of Arbuscular mycorrhizal fungi (AM fungi) colonisation of the three plant species grown in invaded and uninvaded soil types. (A: *Plantago lanceolata*, **B:** *Lotus corniculatus* , **C:** *Trifolium pratense*). All three species had a significantly higher AM colonisation ($P < 0.001$) when grown in uninvaded soil compared to invaded soil. The box and whiskers plot show the median percentage cover expressed as the solid horizontal line within the box. The top and bottom of the box show the 75^{th} and 25^{th} percentiles, respectively. The vertical dashed line shows the interquartile range and the points show the outliers of the data (Crawley, 2007).

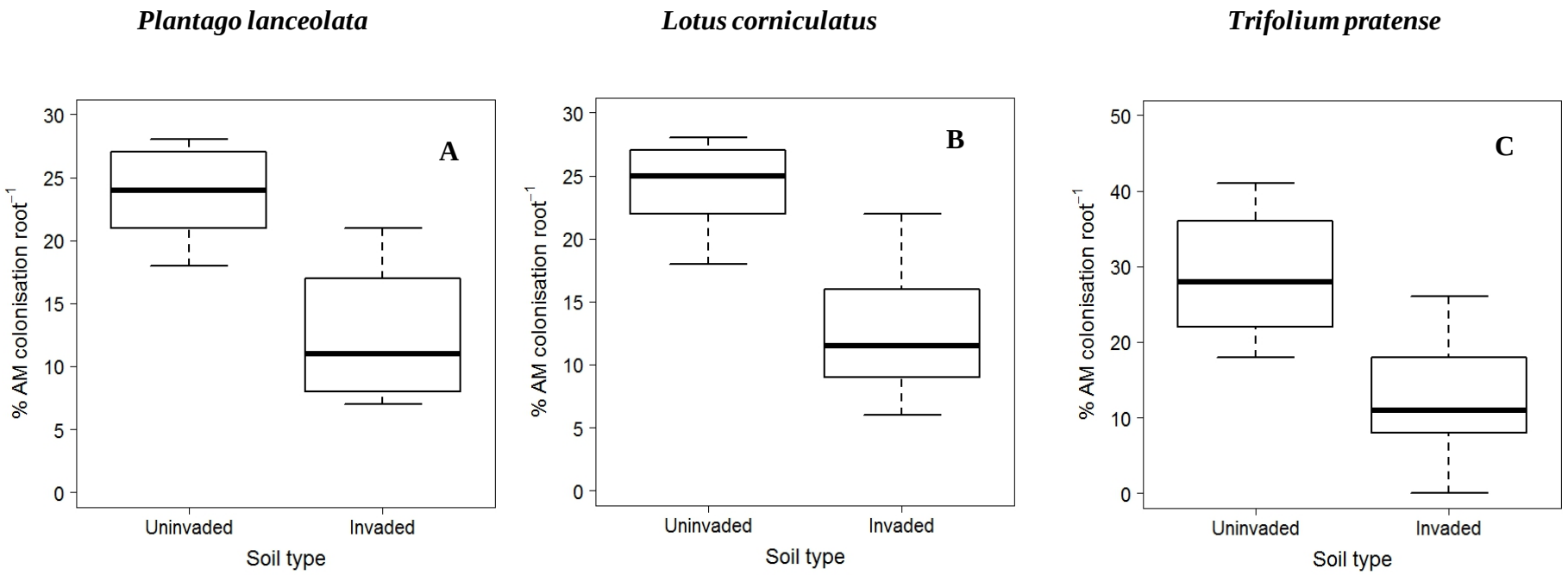


Figure 4.5 The difference in above-ground dry weight (grams) of the three studied plant species grown in invaded and uninvaded soil types. (A: *Plantago lanceolata*, **B:** *Lotus corniculatus*, **C:** *Trifolium pratense*). *Plantago lanceolata* and *Lotus corniculatus* both showed a significant reduction in dry weight when grown in the invaded soil type ($P < 0.001$). There was no difference in the dry weight of *Trifolium pratense* between the two soil types ($P = 0.29$). The box and whiskers plot show the median percentage cover expressed as the solid horizontal line within the box. The top and bottom of the box show the 75th and 25th percentiles, respectively. The vertical dashed line shows the interquartile range and the points show the outliers of the data (Crawley, 2007).

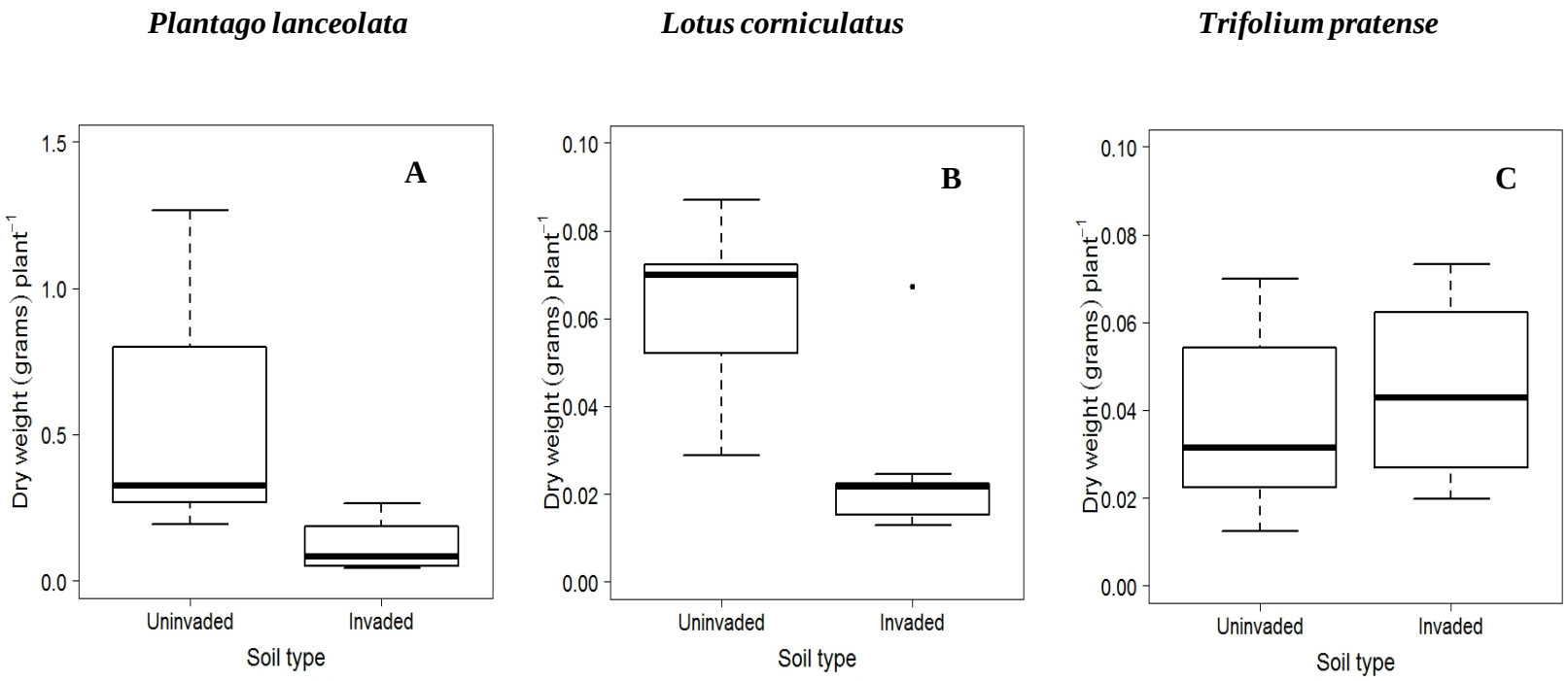


Figure 4.6 The difference in above-ground fresh weight (grams) of the three studied plant species grown in invaded and uninvaded soil types. (A: *Plantago lanceolata*, **B:** *Lotus corniculatus*, **C:** *Trifolium pratense*). *Plantago lanceolata* and *Lotus corniculatus* both showed a significant reduction in fresh weight when grown in the invaded soil type ($P < 0.001$). There was no difference in the fresh weight of *Trifolium pratense* between the two soil types ($P = 0.89$). The box and whiskers plot show the median percentage cover expressed as the solid horizontal line within the box. The top and bottom of the box show the 75^{th} and 25^{th} percentiles, respectively. The vertical dashed line shows the interquartile range and the points show the outliers of the data (Crawley, 2007).

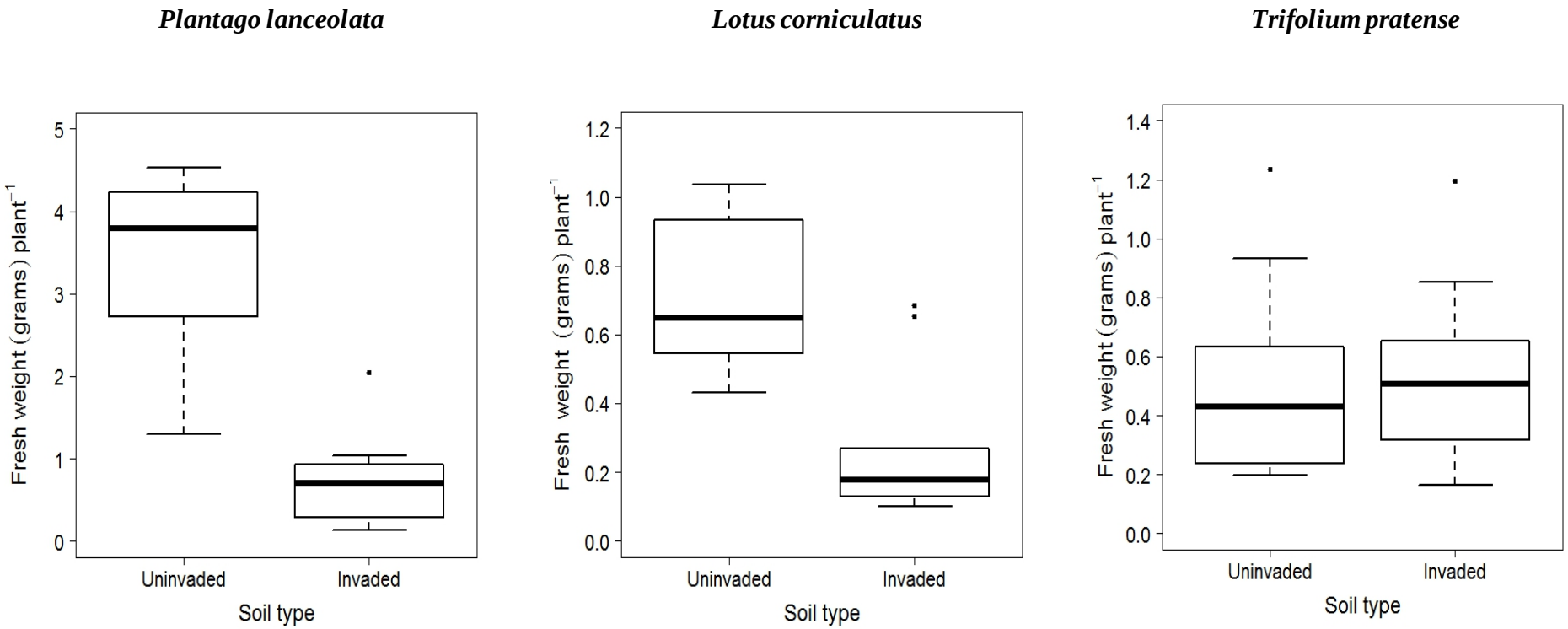
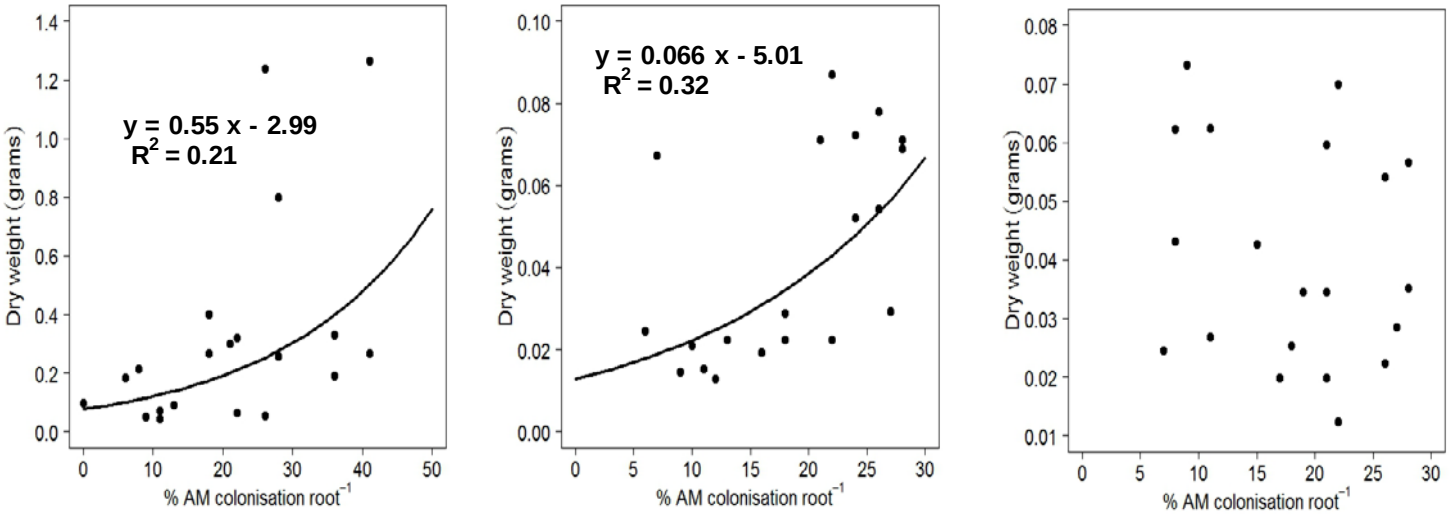


Figure 4.7 The relationship between dry weight and percentage colonisation of Arbuscular mycorrhizal fungi (AM fungi) for all three study plant species. (A: *Plantago lanceolata*, **B:** *Lotus corniculatus*, **C:** *Trifolium pratense*). Both *Plantago lanceolata* ($P < 0.05$) and *Lotus corniculatus* ($P < 0.05$) showed a positive relationship between the percentage colonisation of AM fungi and above-ground dry weight. There was no relationship between the dry weight of *Trifolium pratense* and percentage colonisation of AM fungi ($P = 0.43$).

(A) ***Plantago lanceolata*** **(B)** ***Lotus corniculatus*** **(C)** ***Trifolium pratense***



4.4 Discussion

The results of this study show that the impact of *I. glandulifera* on the below-ground invertebrate community does not follow the same patterns as that of the impact on the above-ground invertebrate community. Whereas in section 2.3.1, Collembola and Coleoptera responded in a negative response consistently throughout the season to the presence of *I. glandulifera*, in the below-ground system the same two groups showed positive associations with the invaded sites compared to the uninvaded during the peak summer months for both seasons sampled.

The fact that the total invertebrate abundance, and the abundance of the group most strongly affected by the invaded sites (Collembola), as shown in the PRC, indicates that below-ground invertebrates may be more buffered to the occurrence of *I. glandulifera* than the above-ground community. The PRC analysis was only significant in 2007 ($P < 0.05$) and in contrast to the PRC in section 2.3.1 (for the ground-dwelling invertebrates) and section 2.3.2 (for the foliage dwelling invertebrates), the significance was predominantly driven by a positive association with one invertebrate group. The invasion by *I. glandulifera* may have limited direct negative impacts on the below-ground invertebrate community. The roots of *I. glandulifera* are comparably large to those of native forbs and grasses and are augmented by adventitious roots produced from the lower nodes of the stem (Beerling and Perrins, 1993). The increased root mass in an invaded site may act to increase the amount of food available to detritivores throughout the growing season, and the rest of the year as the roots break down. This may go some way to explaining the increased abundance of Collembola in invaded sites.

Brown and Gange (1990) suggest that root herbivores are more affected by root quantity rather than quality, and this may suggest why Collembola appear to be associated with the invaded sites compared to the uninvaded sites. The invasion of *I. glandulifera* may have indirect effects on the plant species composition of the above-ground community by attracting detritivore species into the invaded sites, which feed on the lower quantity of native roots, lowering the fitness of native species within the invaded site. In addition, Brown and Gange (1989) showed that herbivory by soil invertebrates suppresses plant species richness, which in turn may have indirect effects on the below-ground invertebrate community (Wolfe and Klironomos, 2005). The groups that showed the most negative response to the invaded sites in section 2.3.1 and 2.3.2, were herbivores and these groups would be heavily dependent on native plant species as a food source and for oviposition and development.

In a typical grassland habitat, Collembola are one of the most abundant invertebrate groups (Eisenhauer *et al.*, 2011b). The abundance of Collembola show seasonal shifts where generally their abundance would be lower in the drier summer months (Hopkin, 1997). The presence of *I. glandulifera* may act to alter the water content within the soil under an invaded site as the roots of *I. glandulifera* have been shown to contain a high percentage of water content, up to 88% (Beerling and Perrins, 1993). It is also a plausible that *I. glandulifera* does not alter the water content of the soil but rather persists as an invasive population in moister soil environments and this may account for the increased abundance of Collembola found within the invaded sites.

Impatiens glandulifera may act to increase or alter the productivity of invaded sites by increased leaf fall as suggested for *F. japonica* invasions (Maurel *et al.*, 2010). Temporal impacts on ecosystem functioning may be caused by the slower breakdown of

organic material compared to native species. *I. glandulifera* has a similar chemical concentration of nitrogen, phosphorus, and potassium to that of *F. japonica* (Beerling and Perrins, 1993) which has been shown to have lower decomposition rates to that of native species. Indeed, for *I. glandulifera*, the previous seasons stems are clearly present as dried material in the summer months of the following season, and in its native range, the previous season's leaves are present in the leaf litter the following June (Pers. obs., Author). Changes in the rate and amount of decomposition of organic matter in the invaded sites may lead to food material becoming available at different times of the year compared to the native, uninvaded sites.

The majority of invertebrate groups (Formicoidea, Isopoda, Myriapods and Thysanoptera) in the below-ground community were unaffected by the presence of *I. glandulifera* in 2007, when the percentage cover of the species was at its highest. The groups Acari, Coleoptera, Haplotaxida, and invertebrate larvae significantly recovered in 2008, when the percentage cover of *I. glandulifera* naturally reduced. Coupled with the fact that for both years there was no significant relation between the percentage cover of *I. glandulifera* and the abundance of individual groups (2007: $P = 0.13$; 2008 $P = 0.64$), further suggests that the presence and abundance of *I. glandulifera* does not affect the below-ground invertebrate community as significantly as the above-ground invertebrate community. Indeed, abundance and total biomass were similar within the invaded and uninvaded sites. The large size of the error bars for total invertebrate biomass (see section 4.3) indicates a large size range for below-ground invertebrate species. Indeed, while sorting through the samples, the morphology, and size of Collembola varied considerably within sites and suggests a high diversity of species were captured in both the invaded and uninvaded sites.

Non-native invasive plant species have been shown to impact on ecosystem functioning and processes by displacing plant species and functional invertebrate groups in the community (Kettenring and Adams, 2011; Koutika *et al.*, 2011). Understanding the effects of the occurrence of *I. glandulifera* at an ecosystem level (both above and below-ground) is essential to understand the impacts in the context of ecosystem functioning and processes, and the resulting ecosystem services ascertained from an intact community. Scientists have realised the importance, in terms of habitat conservation, of valuing natural ecosystem services, from the value of pollinators (Mayer *et al.*, 2011), rivers which act as corridors for nutrient transportation (Tomlinson *et al.*, 2011), and indeed soil to maintain life on earth (Price, 2011). Within the invaded sites, differences in the response of the invertebrate community varied within each micro-habitat (foliage-dwelling, ground-dwelling and below-ground), where a general decrease in the negative response was observed as one moved from the canopy to the below-ground community. The influx of organisms into a community as a result of *I. glandulifera* invasion and increased Collembola abundance below-ground, or the reduction of foliage dwelling predators (spiders) and below-ground mycobiota, can potentially lead to disrupted, fragmented communities as it is known that all are intrinsically linked (Masters, 2004; Wolfe and Klironomos, 2005).

Research conducted by Hulme and Bremner (2006) suggests that once *I. glandulifera* is eradicated from an area, the void left is quickly colonised by other non-native plant species and not by native species. All three plant species showed a significant difference in the percentage colonisation of AM fungi where the percentage colonisation was lower in the plants grown in invaded soils. Even though *I. glandulifera* may utilise the AM fungal network, this relationship may be far from symbiotic and *I. glandulifera* may be exploiting the benefits from the AM fungal

network without passing nutrients back to the fungi. Over time, and with the decrease in native plant abundance, this may act to deplete the AM fungal network under stands of *I. glandulifera*. It is interesting to note, that *I. glandulifera* did not outcompete native plants at Harmondsworth Moor (see chapter 2.3.4), therefore the AM fungal network may potentially always be available for the species to exploit.

The decreased levels of AM colonisation on the three native plant species studied may act to decrease their fitness and subsequent competitive ability within invaded stands. This could result in reduced species richness in the invaded stands, especially if native species are not aggressive competitors, or are already stressed through a highly disturbed environment (see section 3.3.1). It is interesting to note that *T. pratense* showed no difference in fresh or dry weight between the two soil types and there was no correlation between the dry weight and percentage colonisation of AM fungi. This suggests that *T. pratense* is less dependent on AM fungi symbiosis for its growth and acquisition of nutrients, or that the wrong AM species were present within the invaded stands, or both.

Essentially establishing a habitat resistant to subsequent alien plant invasion, post-removal of *I. glandulifera*, is fundamental for long-term habitat restoration. Harris (2003) discusses how measuring the microbial community can indicate the levels of degradation and thus plan for remedial actions to restore the habitat. The strong indication that *I. glandulifera* reduces levels of AM fungi beneath invaded stands is demonstrated in this chapter by the significant reduction in the percentage colonisation of AM on the roots of both *P. lanceolata* and *L. corniculatus* grown in soil from under invaded stands. Coupled with the positive correlation found with the two

aforementioned species between dry weight and the percentage colonisation of AM fungi if is feasible to conclude that these two species rely on AM fungi for their acquisition of nutrients and subsequent growth and fitness.

If just the presence of *I. glandulifera* in a plant community - being weakly dependent on AM fungi - and its competitive ability with native plant species, which act to reduce the abundance of native plant species, is enough to reduce and degrade the AM fungal network over time, is currently not clear. Further studies would be needed where precise evaluations of the microbial communities were conducted with molecular techniques to evaluate the abundance of AM fungi in the soil pre-and post-invasion. Methods, which involve the use of phospholipid fatty acid analysis (PLFA), could be adopted to detect the shifts in the microbial community. To-date, there have been no known studies on the allelopathic effects of *I. glandulifera*, although Lobstein *et al.* (2001) and Clements *et al.* (2008) both identified levels of naphthaquinone, a secondary chemical with known allelopathic properties, in the leaves and stem tissue of *I. glandulifera*. Research conducted by Maurel *et al.* (2011) shows that *F. bohemica* significantly supresses native plant colonisation and establishment due to allelopathic effects. If the same is true for *I. glandulifera*, this adds yet another stressor into the invaded environment.

To-date there have been no studies on the effect of manual management on non-native invasive species to the soil biota. However, in other systems, such as agricultural, manual disturbance of the soil has been shown to change the quality and quantity of AM fungi (Curaqueo *et al.*, 2011; Jansa *et al.*, 2003; Mirás-Avalos *et al.*, 2011). Manual, mechanical, and chemical control of *I. glandulifera* may potentially add to the disturbance of the microbial community by further disrupting and depleting the AM

network. It is feasible to suggest that current traditional management techniques for the control of *I. glandulifera*, such as hand-pulling and mechanical control, coupled with the plant's weak association with AM, are doing little to improve habitat restoration post-removal. Indeed, biological control may be the only feasible, cost effective, method of controlling *I. glandulifera* in the UK. With biological control, a quick-fix solution is not expected. Any biological control agent would not aim to eradicate the invasive population (Shaw, 2003). Instead, the biological control agent would weaken the competitive advantage of *I. glandulifera* enabling native species to successfully compete with the non-native plant species. This in turn would allow for the natural recolonisation of the native plant community and the subsequent AM network and invertebrate populations.

5 An ecological comparison of *Impatiens glandulifera* in the native and introduced range

5.1 Introduction

It is widely accepted that only a small proportion of introduced plant species actually become invasive (Williamson, 1996). Williamson and Fitter's (1996) 'ten rule' suggests only one in ten imported plants appear in the wild, and one in ten of these are able to sustain self-perpetuating populations. Of the latter, only one in ten become invasive and have detrimental economic impacts. However, it is this small minority, which incur high economic (Williams *et al.,* 2010) and ecological impacts (Gaertner *et al.*, 2009; Vilà *et al.*, 2011). Studying the whole-range ecology (native and introduced populations) of plant species is an essential component to understanding plant invasions in introduced regions (Hierro *et al.*, 2005; Zuppinger-Dingley *et al.*, 2011). Increasing our knowledge on the ecology and interactions of established invasive non-native plant species in their native range and understanding the regulations, which control their spread, can aid control in the introduced range (Harris *et al.*, 2011; St. Quinton *et al.*, 2011). In addition, this understanding can be applied to future plant imports where plants with weedy traits or which have high reproductive potential, coupled with the ability to adapt to favourable introduced conditions, and are highly regulated by biotic and abiotic variables in the native range, are regarded as undesirable horticultural additions (Kleunen *et al.*, 2010; Lambdon *et al.*, 2008).

Invasive non-native plant species are often regarded as being more successful; showing increased biomass (Kleunen *et al.*, 2011; Prati and Bossdorf, 2004), fecundity (Caňo *et*

al., 2008; Erfmeier and Bruelheide, 2004; Ebeling *et al.*, 2008), density (Jakobs *et al.*, 2004) and a wider geographical distribution than their native congeners (Crawley, 1987; Jakobs *et al.*, 2004). A number of hypotheses have been proposed to explain why introduced populations show increased performance where the common denominator for most is the escape from regulation coupled with the ability to adapt and exploit decreased regulation in the introduced range.

The Enemy Release Hypothesis (ERH) states that when a plant species is introduced into an introduced region it is released from the regulation of natural enemies (both arthropods and plant pathogens) which regulate the population in the native range. Thus, introduced populations are able to grow with increased growth and range expansion than native plant species (Keane and Crawley, 2002). The release from specialist natural enemy pressure affords non-native plant species an advantage over native plant species, which are suppressed by their array of natural enemies (both specialist and generalist species). In addition to the release from specialist natural enemies, the ERH predicts that specialist natural enemies on closely related species, if present in the introduced range, will not adapt to feed on the introduced plant species, and generalists within the introduced range will have a greater impact on native species over introduced species (Keane and Crawley, 2002).

Even though generalist predators may attack non-native invasive plant species, the pressure exerted by these generalist species is generally lower than on their native counterparts (Olckers and Hulley, 1991). Recent research on invertebrate – non-native plant species associations, suggests that introduced species are more resistant to generalist attack than their native congeners (Krebs *et al.*, 2011). Most invertebrate species regarded as generalist species are confined to a relatively small number of plant

species. Faced with a monoculture of a non-native, these invertebrate herbivores may feed on non-natives even though the shift has been shown to affect their fitness (Tallamy, 2004).

The ERH is the underlying scientific basis to classical biological control in the fact that this process aims to readdress the imbalance between native and introduced natural enemy associations by re-associating introduced invasive populations with host-specific natural enemies, which affect the introduced population by reducing the abundance and/or fecundity of those populations. However, although one of the most widely referenced hypotheses for plant invasions, experimentation to test the ERH shows conflicting results. For example, Cappuccino and Carpenter (2005) evaluated herbivory on nine non-native invasive plant species and nine native plant species in the US and concluded that the invasive populations had a significantly lower herbivory levels compared to the native species. Hartley *et al.* (2010) showed similar results when comparing the abundance, species richness and herbivory of native and introduced tree species in the US. Of equal interest, Schaffner *et al.* (2011) showed generalist invertebrates had a greater impact against spotted knapweed (*Centaurea stoebe*) in the native range compared to that of the introduced range.

Studies opposing the ERH include that of Kleunen and Fischer (2009), where the authors evaluated the data from 140 North American plant species that are naturalised in Europe. Searching fungus-host databases, van Kleunen and Fischer (2009) showed that the geographical spread of these introduced plant species in Europe was negatively associated with their release from co-evolved fungal pathogens in their native range. Colautti *et al.* (2004) conducted a review on studies that had tested the ERH and found only 60% of studies supported the predictions made. In another experiment testing the

ERH, Agrawal and Kotanen (2003) showed that native invertebrate herbivores had a higher impact (leaf area feed on) on introduced compared to native species.

Along the same lines as the ERH, the Endophyte Enemy Release Hypothesis (E-ERH) (Evans, 2008), again has implications for classical biological control. The E-ERH proposes that when plants are introduced into exotic regions they lose their co-evolved endophytes, which afford the plant protection from natural-enemy damage and tolerance to abiotic factors in the native range. The E-ERH predicts that there is a cost to the plant species in the native range for this increased level of protection, which may be expressed as decreased growth and fecundity (Rudgers *et al.*, 2004; Schulz and Boyle, 2005). Thus, when released from co-evolved endophytes, plants grow with increased vigour but with reduced defences, therefore, re-associating natural enemies with endophyte-free introduced populations may explain some of the success in classical biological control (Evans, 2008). Similarly, the Evolution of Increased Competitive Ability (EICA) Hypothesis (Blossey and Nötzold, 1995) proposes that when non-native plants are released from natural-enemy pressure, these plants will invest less in the secondary chemicals used to deter natural enemies in the native range, which will in turn promote the adaption of the species to invest more into growth and fecundity. Again, under the EICA Hypothesis the introduced population has reduced defences if re-associated with co-evolved natural enemies from the plant's native range.

The Novel Weapon Hypothesis (Muller, 1969) highlights that some non-native plant species may arrive in introduced regions with competitive traits such as allelopathic and biochemical defences. These defences promote competition with native species by altering the habitat (as in the case of allelopathy) or by reducing attack from generalist natural enemies (as in the case of novel biochemical defences) (Lind and Parker, 2010;

Müller, 2009). The study of Krebs *et al.* (2011) supports the Novel Weapon Hypothesis with regard to biochemical defences where the authors compared the fitness, expressed as biomass, of invertebrate species fed on native and non-native species. The invertebrates fed on the non-native species *F. japonica* had a decreased fitness compared to those fed on native plant species. Murrell *et al.* (2010) found support for allelopathic defences where the authors showed that *F.* x *bohemica* Chrtek & Chrtková restricted the growth of native forbs when grown together and the effect was dissipated with the addition of activated carbon.

Other hypotheses, including the Empty Niche Hypothesis (Elton, 1958), suggest that the introduced species may be able to exploit niches which are unfavourable to native species and go some way to explaining how habitat availability in the introduced range may also determine the success of an invasive species. A similarly related idea is the Disturbance Hypothesis, which suggests that introduced species are better adapted to exploit disturbed habitats than their native counter-parts. Indeed, invasive non-native plant species like *I. glandulifera* and *F. japonica*, thrive in disturbed habitats, however, equally so do some native species like *Urtica dioica*.

A combined holistic ecological view and evaluation of the ecology, phenology, and population constraints of the species in question in its native range, where the researcher evaluates both natural and unnatural populations, and compares these to introduced populations, may provide tangible insights which can advance our understanding of why a particular species is invasive in the introduced range. The ultimate goal of the surveys in the native range, which are presented in this chapter, was to collect and identify natural enemies from *I. glandulifera,* which potentially can be used as classical

biological control agents in the plant's introduced range. Although not specified in detail here, considerable research was conducted evaluating the life cycle and host range of the majority of species detailed within this chapter, up until the point of non-target impact, after which those species were rejected. Tanner *et al.* (2008) detail the research conducted on a selection of these species prior to 2008 and Tanner (2008) reviews the potential for the biological control of *I. glandulifera* prior to initial exploratory surveys in the native range.

5.2 Methods

5.2.1 The sites

Between 2006 and 2010, surveys were untaken to evaluate the natural enemies on *I. glandulifera* in the plant's native range (Pakistan and India). In total, in the native range, 30 distinct sites were sampled for natural enemies (Table 5.1). In addition, during 2010, plant measurements were taken from individual plants within the native (n = 135 individuals) and introduced range (n = 111 individuals). In total, five sites were selected for comparison: three native sites which included (1) a high altitude population, hereafter referred to as Rohtang (Plate 5.1A) (2) a riparian population, here after referred to as Solang (Plate 5.1B) and (3) a population growing within close proximity to a settlement and within an orchard, hereafter referred to as Chandrkhani (Plate 5.1C), and two introduced sites (1) a riparian/grassland population (Harmondsworth Moor, Middlesex N 51 29 582 E 000 29 023), hereafter referred to as Harmondsworth and (2) a wooded habitat (Sunningdale, Berkshire N 51 23 619 E 000 29 023), hereafter referred to as Sunningdale.

5.2.2 Fungal pathogen surveys

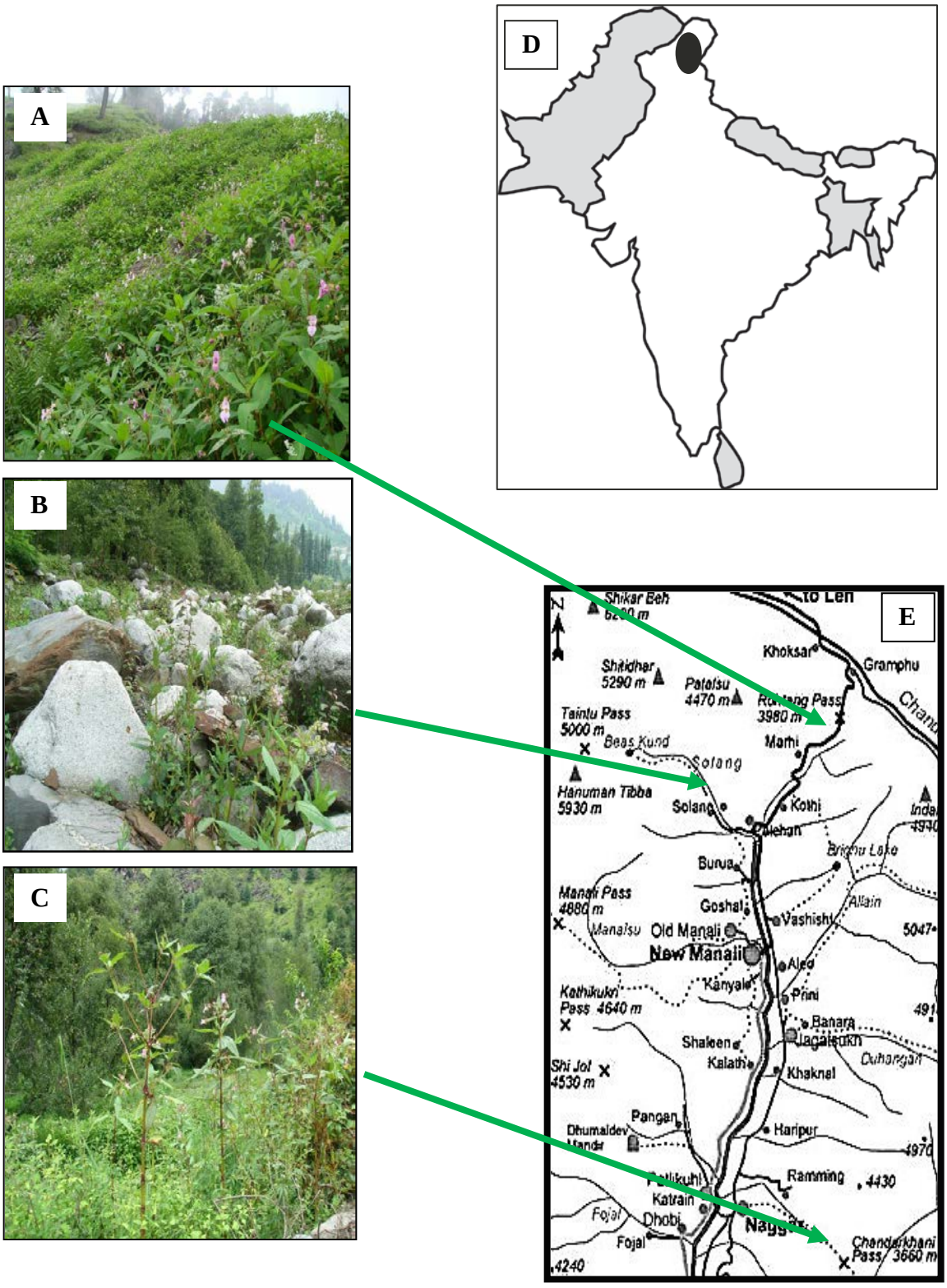
At each site, plants were randomly selected along a W-shaped sampling transect, through the whole population, for evidence of fungal pathogens (Holderness, 2002). The samples were collected, stored in a plant press and either directly exported to the CABI-Europe UK quarantine facility under a Defra plant health licence, or held with collaborators until export permissions were obtained. Leaf material infected with biotrophic fungi, and cultured samples of facultative fungi were either identified in the country of collection or sent to the CABI Microbial Identification Service, Egham, UK.

Table 5.1 Sites surveyed for natural enemies of *Impatiens glandulifera* in the native range (India and Pakistan)

Site ID	Country	Landmark	County/District	Habitat	Geographical coordinates		Altitude	Year sampled
					Latitude (N)	Longitude (E)		
1	Pakistan	Ayubia Nathia Gali	Murree Hills	urban	34 04 104	073 23 903	2438	06
2	Pakistan	Changa Gali	Murree Hills	urban	33 59 696	073 23 148	2569	06
3	Pakistan	Changa Gali	Murree Hills	road	33 59 930	073 22 952	2483	06
4	Pakistan	Ayubia National Park	Ayubia	meadow	34 01 768	073 24 485	2523	06
5	Pakistan	Doonga Gali	Ayubia	meadow	34 03 289	073 24 782	2472	06
6	Pakistan	Naran	Khagan Valley	road	34 52 102	073 36 563	2362	06,08
7	Pakistan	South Naran	Khagan Valley	riparian	34 53 025	073 38 043	2377	06,08
8	Pakistan	Malooq National Park	Khagan Valley	meadow	34 53 780	073 41 489	3022	06,08
9	Pakistan	North Naran	Khagan Valley	meadow	34 56 022	073 48 439	2651	06,08
10	Pakistan	North Naran	Khagan Valley	meadow	34 54 436	073 40 684	2829	08
11	Pakistan	North Naran	Khagan Valley	meadow	34 54 919	073 47 885	2815	08
12	Pakistan	North Naran	Khagan Valley	meadow	34 56 022	073 45 439	2651	08
13	Pakistan	Balukdi Town	Khagan Valley	meadow	34 56 087	073 50 257	2830	08
14	Pakistan	Balukdi Town	Khagan Valley	meadow	34 58 365	073 55 771	3098	08
15	Pakistan	Laloza Lake	Khagan Valley	meadow	34 55 012	073 45 843	3130	08
16	Pakistan	Jalckuda	Khagan Valley	meadow	34 58 477	073 55 765	3186	08
17	Pakistan	Besal	Khagan Valley	meadow	34 56 598	073 52 352	2935	08
18	Pakistan	Besal	Khagan Valley	meadow	34 56 380	073 51 562	2938	08
19	Pakistan	Burwai	Khagan Valley	meadow	34 57 541	073 55 067	3042	08
20	India	Raksham Village	Sangla Valley	semi-agri	31 24 354	078 19 863	2983	08
21	India	Raksham Village	Sangla Valley	semi-agri	31 24 364	078 19 871	3130	08
22	India	Jalori Pass	Sangla Valley	riparian	31 34 581	077 21 718	1938	08
23	India	Chandrkhani Pass	Kullu Valley	settlement	32 06 482	077 11 532	2159	08,09,10*
24	India	Bhahang Town	Kullu Valley	road	32 16 736	077 10 852	2049	08,09
25	India	Bhrugha lake	Kullu Valley	meadow	32 19 142	077 12 357	3102	08,09
26	India	Solang	Solang Valley	riparian	32 19 129	077 09 359	2450	08,09,10
27	India	Solang	Solang Valley	riparian	32 19 132	077 09 371	2459	08,09,10*
28	India	Rohtang	Kullu Valley	meadow	32 19 778	077 12 707	3067	08,09,10*
29	India	Dhundi	Kullu Valley	meadow	32 21 215	077 07 526	2837	09,10
30	India	Nagar	Kullu Valley	riparian	32 08 687	077 10 536	1725	09,10

* Denotes plant measurements taken

Plate 5.1 Main focal survey sites in India for invertebrates, fungal pathogens, and plant measurements. Where (A) = Rohtang; (B) =Solang and (C) = Chandrkhani. The black dot on (D) indicates the location of (E) in the context of the country as a whole.



5.2.3 Arthropod surveys

At each site, signs of damage on *I. glandulifera* indicative of damage by arthropod species were inspected and live specimens were collected from all aerial parts of the plant and stored in ventilated plastic boxes with feeding material to sustain the culture. Thirty plants were selected at each site along the same sampling transect as detailed in section 5.2.2. Stem and root damage was assessed by uprooting individuals plants and dissecting the stems and roots. Arthropod specimens were collected and preserved by pinning and/or preserving in 70% alcohol prior to identification. Similar to the fungal pathogens, species of perceived importance, from a biological control perspective (damaging to the host plant), were either identified in-country or exported to the UK and identified by the Natural History Museum, London.

5.2.4 Plant measurements

Plant growth parameters were measured twice during 2010 once in the month of June and again in August at each site in the native and introduced range. Plants were randomly selected from within each population. For each individual, measurements included total height (from the soil surface to the maximum height), girth of stem (taken below the lowest node), the total number of leaves, the length and width of each leaf (taken at the widest point), and an estimate of the percentage damage (of the whole plant) from natural enemies. In addition, the same individuals were uprooted and the above and below-ground biomass was recorded. Biomass was taken as wet weight due to limited resources in the Himalayas. Roots were cleaned of any attached soil before they were weighed. A section of each root was dried using a plant press where the paper was changed every 24 hours until all of the moisture had been extracted from the root sample. The dried roots were then analysed for the percentage AM fungal colonisation[5]

[5] Ling Jin (Royal Holloway) measured the AM fungi colonisation for the Indian and UK samples

following the method described section 4.2.2.7. In June, the total number of plants was counted in 6 or 8 randomly positioned $1m^2$ quadrats at each site. In August, the number of flowers, number of immature pods, and number of mature seeds were counted on each plant in the introduced range and at Rohtang and Solang in the native range. The Chandrkhani population was not in flower, thus this site was not recorded. Seeds were collected where available for a comparison of UK and Indian biomass.

5.2.3 Leaf area estimation

In August 2010, approximately 100 leaves were randomly selected from individual plants within each site. For each leaf, the length and width (at the widest point) was measured and the exact area was estimated using ImageJ (version 1.44) (Abramoff *et al.*, 2004). A photograph was taken of each leaf next to a 30cm ruler in order to calibrate each photograph. The image was then loaded into the ImageJ software and adjusted to a binary image before leaf area was estimated. The accuracy of the software was confirmed by measuring an object of known area and comparing the output. A linear regression analysis was conducted to determine which of the leaf measurements (length or width or length x width) best represented the actual area calculated by ImageJ and the resulting model was applied to each leaf measurement taken.

5.2.6 Data analysis

For each site, individual plants were treated as replicates. All data was appropriately transformed (arc-sine transformation in the case of percentage data; square root transformation for count data and log transformation for biomass, ratio and height data) to suit the assumptions of the test. A two-way analysis of variance was conducted on the response variable with site and season as fixed effects. The number of flowers, immature pods, and mature seeds were summed to give a measure of the reproductive

potential per plant at the time of sampling. To evaluate if seed weight differed between each country, a one-way analysis of variance was performed using individual seeds as replicates. To assess if there were relationships between the plant growth parameters a regression analysis was conducted for each data set using individual plants as replicates. Where both countries' data sets showed a significant relationship, the regression slopes were compared to evaluate if they were significantly different from the other using an analysis of covariance.

5.3 Results

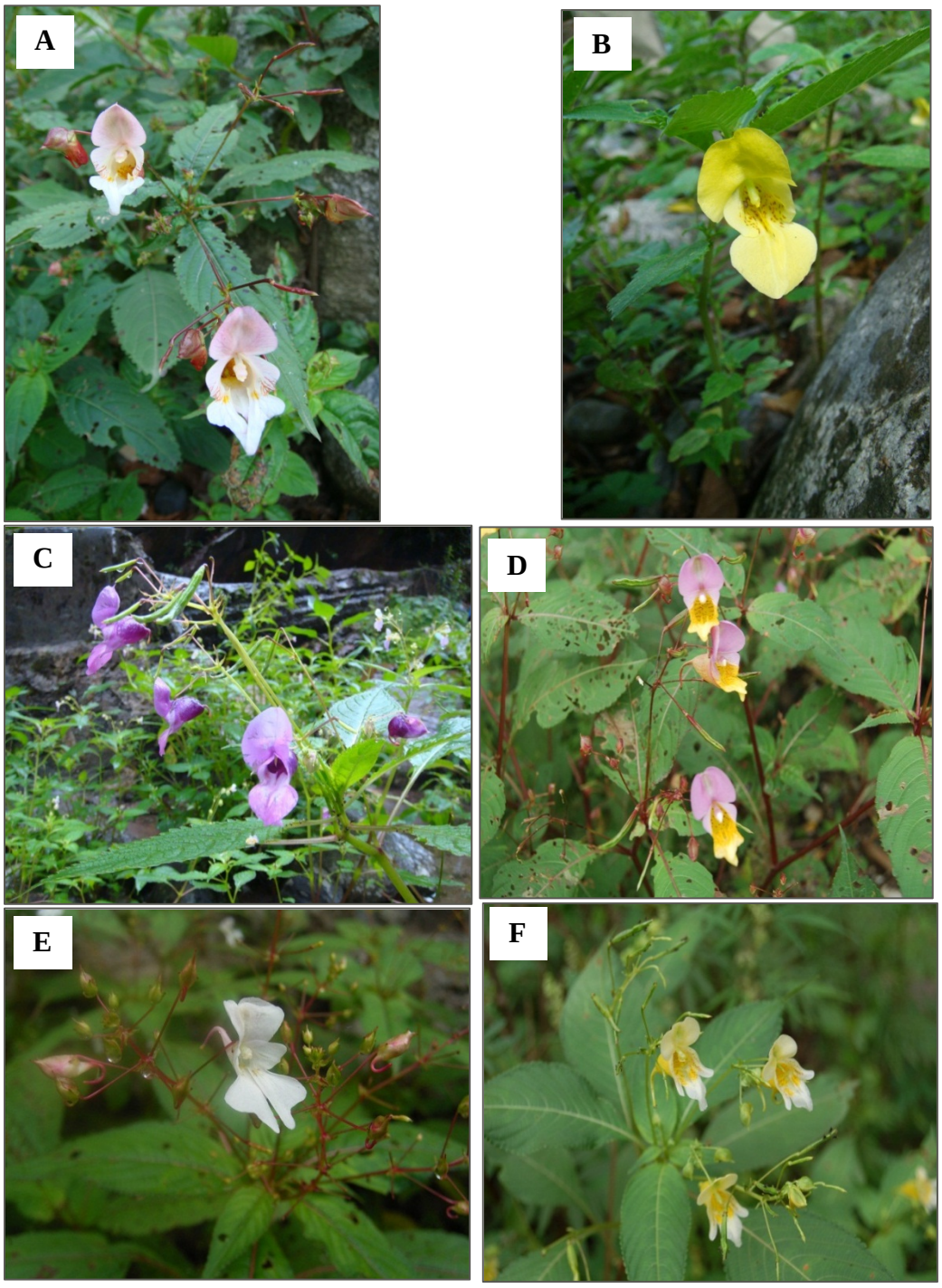
5.3.1 The occurrence of *Impatiens glandulifera* in the Himalayas

In the plant's native range, *I. glandulifera* was recorded between 2,377m and 3,102m.a.s.l. Populations were observed to grow in a number of habitats including waste-ground, along roadsides and ditches, in semi-agricultural and urban areas, deciduous wooded habitats, riparian systems and high altitude meadow habitats (Table 5.1).

5.3.2 Associated plant species

In the native range, *I. glandulifera* is associated with a relatively low number of plant species including representatives from Polygonaceae (*Rumex* and *Polygonum*), Cannabaceae, Balsaminaceae (Plate 5.2), Ranunculaceae, Fabaceae and Asteraceae. At Rohtang, the species most commonly associated with stands of *I. glandulifera* was *Polygonum polystachum* Wall. Ex Meisn (Himalayan knotweed). At Solang, three *Impatiens* species (*I. radiata* Hook. f., (Plate 5.2A), *Impatiens scabrida* DC. (Plate 5.2B) and *Impatiens sulcata* Wall. (Plate 5.2C)) grew in close proximity to *I. glandulifera* often interspersed within mixed populations. At Chandrkhani, *Cannabis* species, grasses, and *Malus* species dominated the associated flora.

Plate 5.2 Other *Impatiens* species encountered in the Himalayas



A. *Impatiens radiata* Hook. f. (Kullu Valley India)
B. *Impatiens scabrida* DC. (Kullu Valley, India)
C. *Impatiens sulcata* Wall. (Kullu Valley, India)
D. *Impatiens bicolor* Royle (Khagan Valley, Pakistan)
E. *Impatiens brachycentra* Kar. & Kir. (Khagan Valley, Pakistan)
F. *Impatiens edgeworthii* Hook. F. (Khagan Valley, Pakistan)

5.3.3 Natural enemy associations

In the current literature, there is little information about the natural enemy complexes on *I. glandulifera* in the plant's native range. In every population surveyed, almost all parts of the above-ground structure showed symptoms of attack by both fungal pathogens and arthropod species. The natural enemy associations varied between sites but were consistent at each site over time. The following section details all of the fungal pathogens found on *I. glandulifera* in the native range and those arthropods that were identified to species level.

5.3.3.1 Plant pathogens

The leaf-spot, *Phoma exigua* var. Desm. [Ascomycota: Pleosporales: Incertae sedis] (Herb. IMI no. 394868) (Plate 5.3A) was widespread, in the fact that it was found at sites in both India and Pakistan (Table 5.2), though symptoms were localised within sites and between sites. The pathogen causes discrete lesions, sometimes coalescing, on the leaves of the plant. The lesions were typically composed of concentric rings of brown necrotic leaf tissue, surrounded by a purple ring. Upon maturity, the lesions fall out of the leaf leaving the typical shot-hole symptoms indicative of many leaf-spot pathogens (Plate 5.3B).

A *Septoria* leaf-spot [Ascomycota: Capnodiales: Mycosphaerellaceae] (Herb. IMI no. 396825 (India) IMI no. 396826 (Pakistan)) (Plate 5.3C) was found throughout the plant's native range (Table 5.2) and inflicted considerable damaged to infected individuals. Similar to the *Phoma* leaf-spot, the pathogen causes distinct shot-holes on the leaves upon maturity. The infected spots were smaller than those of the *Phoma* leaf-spot, though when infection was high, as the season progressed then infection coalesced across the whole leaf surface area (Plate 5.3D).

Two mildew species, *Plasmopara obducens* J. Schröt. [Oomycota: Peronosporales: Peronosporaceae] (Herb. IMI no. 395161) and *Sphaerotheca balsaminae* Wallr [Ascomycota: Erysiphales: Erysiphaceae] (Herb. IMI no. 395162) (Plate 5.2E), were observed on the leaves and stems of *I. glandulifera* in Pakistan and India (Table 5.2). Due to the wide host range of other closely related mildew species, both of these species were regarded as having low priority as biological agents (Pers. comm., Paul Cannon, CABI).

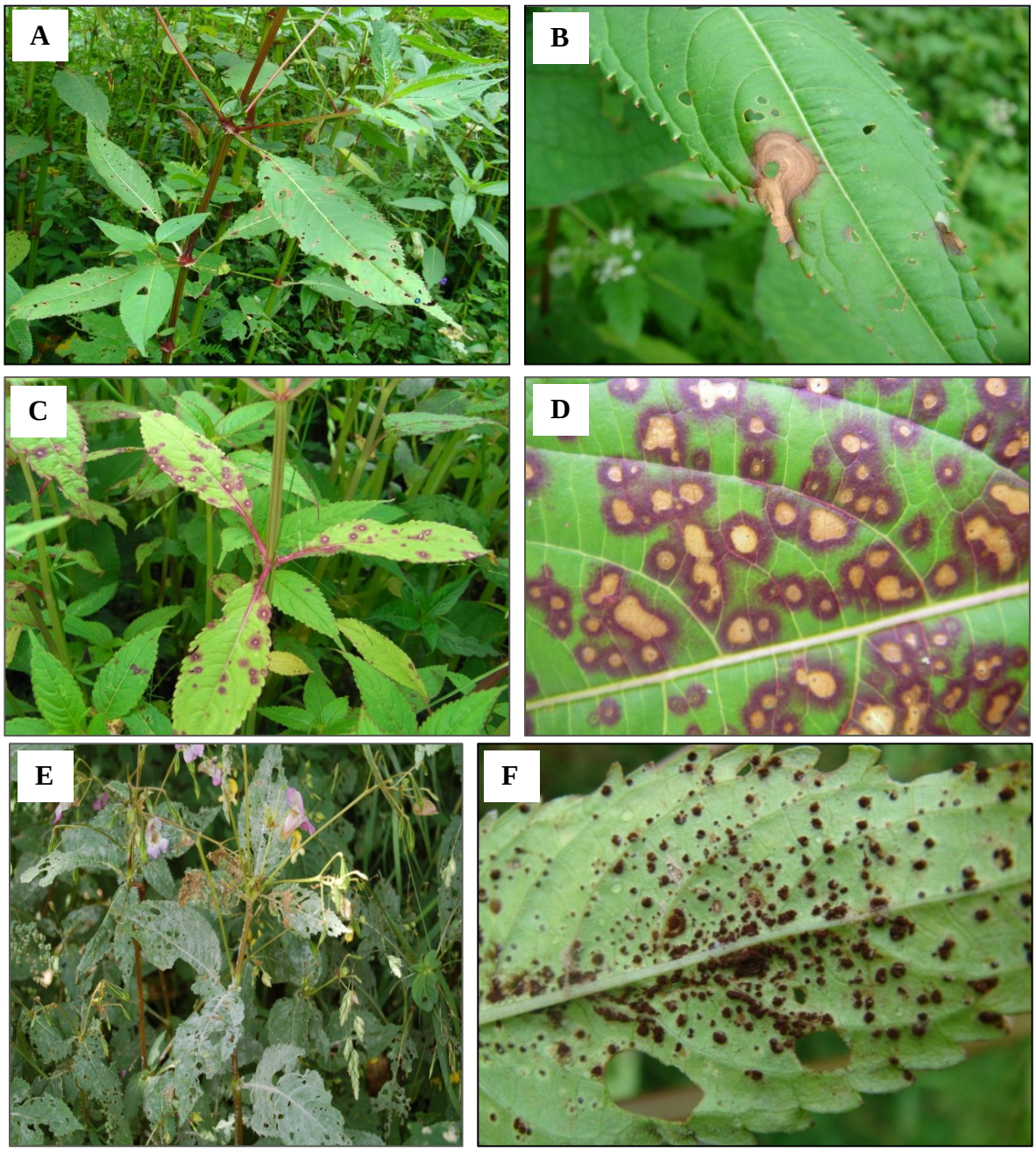
Table 5.2 Plant pathogens collected from *Impatiens glandulifera* throughout the Himalayas. Where In. = India and Pa. = Pakistan. Site numbers correspond to those in Table 5.1.

Species	plant part attacked	Country/sites recorded
Puccinia komarovii Tranz.	leaves/stems	In.26;27;28;29;30 Pa.9;10;11;12;17;18
Puccinia cf. *impatiensis*	leaves	Pa.10
Phoma exigua var. Desm	leaves	In.*Pa.*
Septoria sp.	leaves	In.*Pa.*
Sphaerotheca balsaminae Wallr.	leaves/stems	In.*Pa.*
Plasmopara obducens J. Schoröt.	leaves/stems	In.*Pa.*

* Denotes the organism was recorded throughout the area surveyed

Two rust fungi [Basidomycota: Pucciniales: Pucciniaceae] were collected on *I. glandulifera* within the native range. *Puccinia* cf. *impatientis* (Schwein.) Arthur (Herb. IMI no. 394851) was found infecting the leaves of individual *I. glandulifera* plants within the population at one site in the Khagan valley north of Naran, Pakistan, in a natural meadow habitat at 2,651m.a.s.l. The 'cf' (close to) denotes that an exact identification of the rust was not possible and that *P. impatiensis* is the described species it most closely resembles. In addtion, *P. komarovii* Tranz. (Herb. IMI no. 403542), was found infecting the stem (aecial stage) and leaves (uredinal and telia stage) of *I. glandulifera* in Pakistan and India (Plate 5.2F).

Plate 5.3 Plant pathogen damage on *Impatiens glandulifera* in the Himalayas



A. *Phoma exigua* var. Desm. damage on *Impatiens glandulifera* (India)
B. *Phoma exigua* var. with typical shot-hole symptoms (India)
C. *Septoria* leaf-spot symptoms on *I. glandulifera* (India)
D. *Septoria* leaf-spot close up on leaf of *I. glandulifera* (India)
E. *I. glandulifera* infected with *Plasmopara obducens* J. Schröt. and *Sphaerotheca balsaminae* Wallr. (Pakistan) (H.C. Evans, CABI)
F. Urediniospores of *Puccinia komarovii* Tranz. on leaf of *I. glandulifera* (India) (H. C. Evans, CABI)

5.3.3.2 Arthropods

Damage indicative of arthropods was observed on all aerial parts of *I. glandulifera* throughout the plant's native range, though feeding damage on the leaves was the most conspicuous. After reviewing all of the photographic documentation of the arthropods collected and observed on *I. glandulifera* in the native range, it is estimated that 37 distinct species were observed, though this may be an underestimate as not all larvae were reared to adults and identified.

Metialma suturella Marshall [Coleoptera: Curculionidae] (Plate 5.4A) was found at two riparian sites in India where it was found in abundance in the Solang valley (Table 5.3) (Tanner *et al.*, 2010). From a biological control perspective this species was highlighted as having potential, however, subsequent host range testing determined this species had a host range wider than desired (Tanner *et al.*, 2008). Although initially thought to be host specific to *I. glandulifera* in 2008, subsequent surveys to the same locations in 2009 and 2010 identified individuals developing within *I. scabrida*, *I. radiata* and *I. sulcata* populations.

Alcidodes fasciatus (Redtenbacher) [Coleoptera: Curculionidae] (Plate 5.4B) was found feeding and ovipositing on the stem of *I. glandulifera* in three localised sites in India (Table 5.3). Larvae were found developing inside the stem, however, larvae were also found developing within stems of *P. polystachum* thus rendering this species unsuitable as a potential classical biological control agent.

Table 5.3 Arthropod species collected on *Impatiens glandulifera* in the Himalayas. Where In. = India and Pa. = Pakistan

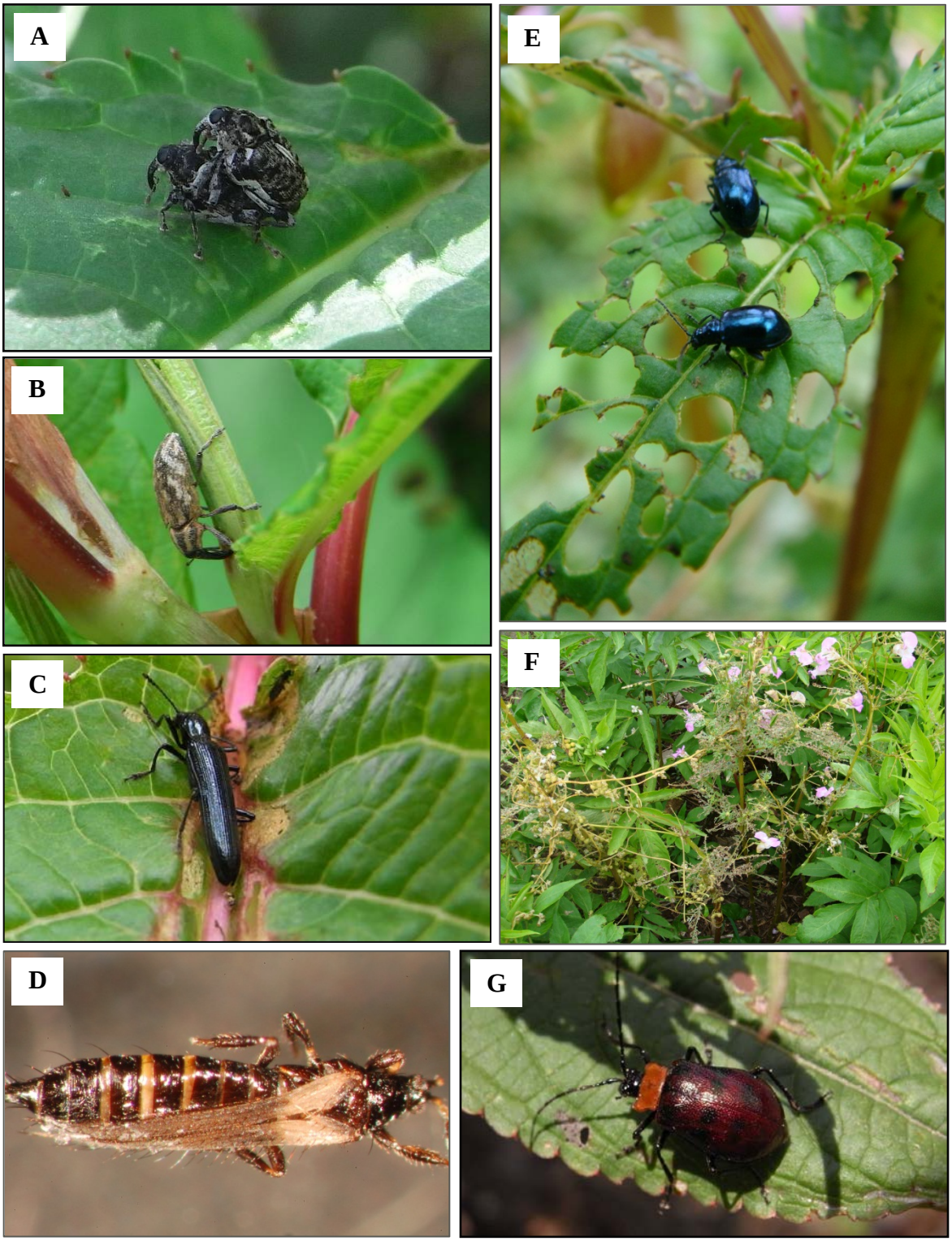
Species/taxonomic group	Plant part attacked	Country/sites recorded
Altica himensis Shukla	leaves/stem/pods	In.*Pa*
Languriophasma cyanea (Hope)	leaves/stems	In. 26;27
Metialma suturella (Marshall)	leaves/stems/roots	In. 26;27
Alcidodes fasciatus(Redtenbacher)	leaves/stems	In.26;27;29
Meristata spilota (Hope)	leaves	In.26;27;29
Evacanthus repexus (Distant)	leaves	In.26;27;29 Pa.7
Deilephila rivularis (Boisduval)	leaves	In.21;30
Chilocrates patulus (Walker)	unknown	In.23;26
Taeniothrips major Bagnall.	flowers/leaves	In.*Pa.*
Helicidae spp.**	leaves/stems/pods	In.Pa.
Hemiptera: Aphrophora spp.**	unknown	In.Pa.
Coleoptera: Chrysomelidae spp.**	leaves	In.Pa.
Coleoptera: Curculionidae	leaves	In.Pa.
Diptera	unknown	In
Lepidoptera spp.**	leaves/stems	In.Pa.
Hemiptera spp.* *	leaves	In.Pa.

*Denotes the organism was recorded throughout the area surveyed
**Denotes where more than one unidentified species recorded

Languriophasma cyanea (Hope) [Coleoptera: Languriidae) (Plate 5.4C) was collected within the Solang valley at two sites (Table 5.3). The adults were found feeding on the leaves of *I. glandulifera* and other *Impatiens* within the vicinity. Larvae were found developing within the stems of *I. glandulifera*.

Meristata spilota (Hope) det. Shute (Chrysomelidae: Galerucinae) was found feeding on leaves of *I. glandulifera* at three sites in in the Kullu Valley, India. This relatively large chrysomelid beetle was not considered a priority for evaluation as a biological control agent, due mainly to the discovery of other plant pathogens and arthropod species that showed greater levels of damage to *I. glandulifera*.

Plate 5.4 Arthropod natural enemies collected from *Impatiens glandulifera* in the Himalayas



A. *Metialma suturella* Marshall (R.H. Shaw, CABI)
B. *Alcidodes fasciatus* (Redtenbacher)
C. *Languriophasma cyanea* (Hope) (R. H. Shaw, CABI)
D. *Taeniothrips major* Bagnall (x 200 magnification)
E. *Altica himensis* Shukla
F. Damage caused by *A. himensis*
G. Unidentified Chrysomelidae beetle (R. H. Shaw, CABI)

Taeniothrips major Bagnall [Thysanoptera: Thripidae] (Plate 5.4D) belongs to the genus which includes around 20 species, all of which are associated with flowers (Natural History Museum report 2006-735). Adults were observed feeding on the flowers of *I. glandulifera* while nymphs were more commonly observed feeding on the leaves of the plant. *T. major* often occurred in high numbers in the field and was observed causing considerable damage to *I. glandulifera* plants.

Altica himensis Shukla [Coleoptera: Chrysomelidae] (Plate 5.4E) was abundant throughout the area surveyed and evidence of its characteristic feeding damage was found in every population sampled. The beetle has a known distribution ranging from India, Kumaun Hills, Kashmir and Bhutan (Natural History Museum report 2006-736). The female, which is slightly larger than the male, lays a cluster of eggs on the underside of the leaf and upon hatching the 1st instars feed on the leaves of *I. glandulifera* and once the larvae are mobile they feed on the seed pods, flowers and stems of the plant. When high in abundance, *A. himensis* can skeletonise all aerial parts of the plants (Plate 5.4F). From a biological control perspective, this species was highlighted as having potential due to the severe damage caused by this species. However subsequent host range testing determined this species had a host range wider than desired (Tanner *et al.*, 2008) which is similar to the results of Jyala (2002) and Shah and Jyala (1998).

Evacanthus repexus (Distant) [Hemiptera: Cicadellidae] was found on *I. glandulifera* plants in three sites in India and one site in Pakistan (Table 5.3). The damage associated with this leafhopper was minimal and the abundance of this species was low within populations of the plant.

Deilephila rivularis (Boisduval) [Lepidoptera: Sphingidae] was collected at two sites within India (Table 5.3) feeding on leaves of *I. glandulifera*. Little is known about the biology of this hawk-moth apart from it tends to occur between 2000 and 4000m asl. (Pers. comm., Tony Pittaway CABI).

Chilocrates patulus (Walker) [Hemiptera: Miridae] was collected from aerial foliage of *I. glandulifera* in India (Table 5.3). Little damage was associated with the presence of this species and only adult specimens were collected. A number of miscellaneous arthropods were collected throughout the surveys (Table 5.3), including representatives from invertebrate Orders and Suborders Lepidoptera, Hemiptera, Coleoptera (Plate 5.4G), Aphrophora and Helicidae.

5.3.3 Plant performance comparisons

The linear regression model for calculated leaf area (length x width) to actual area was the best fit for both the Indian ($R^2 = 0.986$, $F_{1,98} = 6712$, $P < 0.001$) and UK ($R^2 = 0.989$, $F_{1,100} = 9452$, $P < 0.001$) data sets. Therefore, the equation $y = 0.63 x – 0.2807$ was applied to all length x width leaf measurements for India and the equation $y = 0.6621 x + 0.8816$ was applied to the length and width leaf measurements for the UK. In the two equations y is the estimated area calculated from the actual area x (length x width).

There was a significant difference in height between sites ($F_{4,237} = 95.124$, $P < 0.001$) (Figure 5.1A). Within the native range, *I. glandulifera* was significantly smaller at Solang and Rohtang compared to those plants at Chandrkhani ($P < 0.001$). Within the introduced range, the plants at Sunningdale were significantly taller than those at Harmondsworth ($P < 0.05$). Between countries, plants at Harmondsworth and Sunningdale were significantly taller than those at Solang and Rohtang ($P < 0.001$),

though similar to Chandrkhani. For height there was a significant interaction between site and season ($F_{4,237} = 40.863$, $P < 0.001$), where Solang and Chandrkhani showed an increased growth rate from June to August compared to Rohtang, Sunningdale and Harmondsworth.

There was a significant difference in the total leaf area per plant between sites ($F_{4,237} = 52.48$, $P < 0.001$) (Figure 5.1B). Within the native range, Rohtang had a significantly lower total leaf area per plant compared to Chandrkhani and Solang ($P < 0.001$). Within the introduced range, Sunningdale had a significantly higher total leaf area compared to Harmondsworth ($P < 0.001$). Between countries, Harmondsworth had a significantly higher total leaf area compared to Rohtang ($P < 0.001$) and Sunningdale had a significantly higher total leaf area per plant compared to all native sites ($P < 0.001$). There was a significant interaction between site and season ($F_{4,237} = 3.63$, $P < 0.05$) where Rohtang and Chandrkhani showed a greater increase in total leaf area from June to August compared to Harmondsworth, Solang and Sunningdale.

There was a significant difference in the below-above-ground biomass ratio between sites ($F_{4,237} = 47.812$, $P < 0.001$) where: within the native range Solang had a significantly higher below-above-ground ratio compared to Chandrkhani ($P < 0.05$) (Figure 5.1C). Within the introduced range, both sites were significantly different in the below-above-ground biomass ratio ($P < 0.05$). All of the native sites had a significantly higher below-above-ground biomass ratio compared to the introduced sites ($P < 0.05$) . There was a significant difference interaction between site and season ($F_{4,237} = 21.532$, $P < 0.001$). Solang and Rohtang displayed a mean decrease in the below-above-ground ratio between seasons while the Sunningdale, Chandrkhani and Harmondsworth increased.

There was a significant difference in the density of plants per m^2 between the sites ($F_{4,33}$ = 24.94, P < 0.001) where within countries, Sunningdale (104.37 ± 8.14) had a higher density of plants per m^2 compared to Harmondsworth (44 ± 8.18) (P < 0.001). Within the native range, all three sites had a similar density of plants per m^2 (Chandrkhani: 17.62 ± 5.33; Solang: 21.37 ± 5.2; Rohtang: 30.37 ± 4.29). Between countries, Sunningdale had a significantly higher density of plants per m^2 compared to all native sites (P < 0.001) and Harmondsworth had a significantly higher density compared to Rohtang and Chandrkhani (P < 0.001).

There was a significant difference between the percentage colonisation of AM fungi between sites ($F_{4,231}$ = 17.14, P < 0.001) (Figure 5.2A). Within the native range, Solang had a higher percentage colonisation of AM fungi than Rohtang (P < 0.05). Within the introduced range, both sites had a similar percentage colonisation of AM fungi.

Figure 5.1Variation in plant growth parameters between the three sites in India and the two sites in the UK. Where (A) shows the difference in height ($P < 0.001$), (B) total leaf area per plant ($P < 0.001$) and (C) the below - above ground biomass ratio ($P < 0.001$). The box and whiskers plot show the median corresponding dependent variable expressed as the solid horizontal line within the box. The top and bottom of the box show the 75th and 25th percentiles, respectively. The vertical dashed line shows the interquartile range and the points show the outliers of the data (Crawley, 2007). X axis labels = In1= Chandrkhani; In2= Rohtang; In3= Solang; UK1= Harmondsworth and UK2= Sunningdale. Means with different letters are significantly different. Where appropriate letters underlined depict sites between countries that are significantly different.

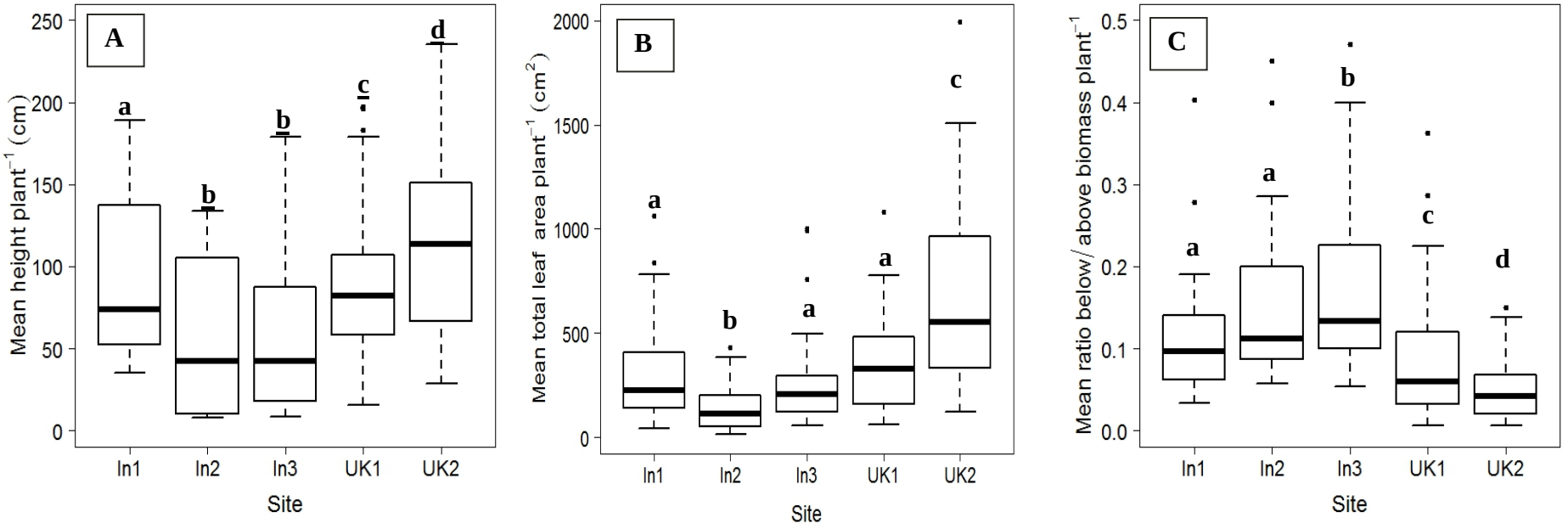
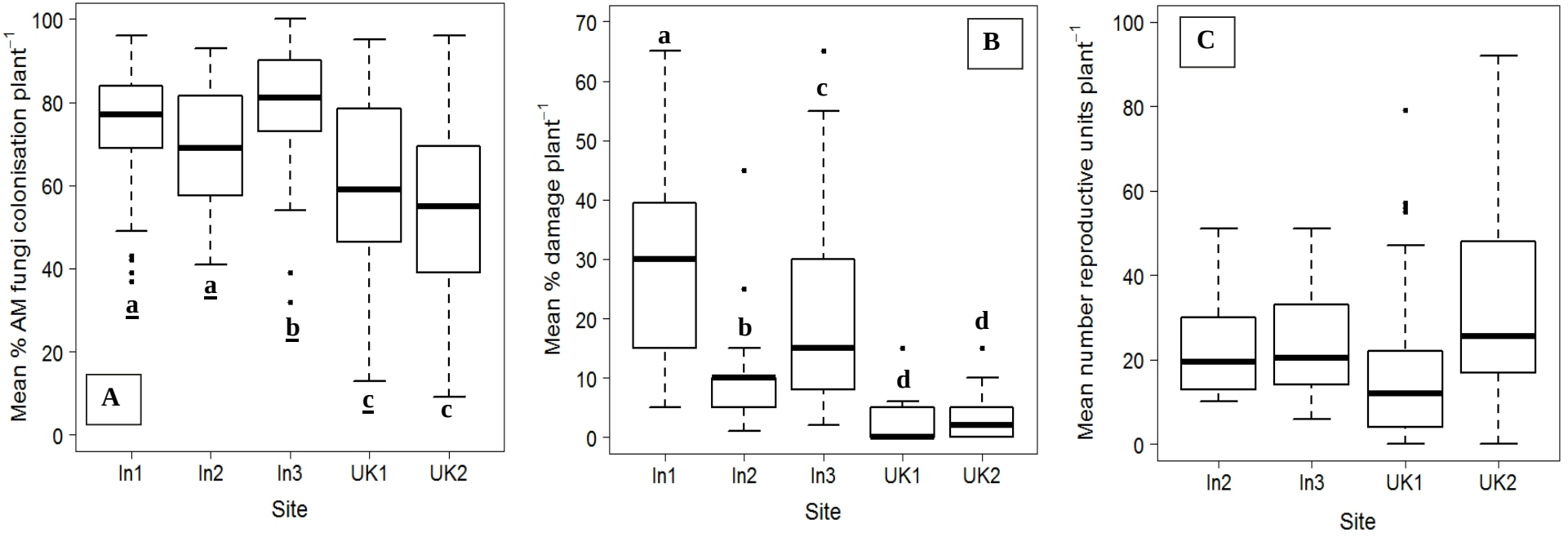


Figure 5.2 Variation in the percentage colonisation of AM fungi, percentage damage of natural enemies and number of reproductive units (flowers, seed pods and seeds) between the sites in India and the two sites in the UK. Where (A) shows the difference in AM percentage colonisation ($P < 0.001$), (B) percentage damage from natural enemies ($P < 0.001$) and (C) number of reproductive units ($P = 0.071$). The box and whiskers plot show the median corresponding dependent variable expressed as the solid horizontal line within the box. The top and bottom of the box show the 75th and 25th percentiles, respectively. The vertical dashed line shows the interquartile range and the points show the outliers of the data (Crawley, 2007). X axis labels = In1= Chandrkhani; In2= Rohtang; In3= Solang; UK1= Harmondsworth and UK2= Sunningdale. Means with different letters are significantly different. Where appropriate letters underlined depict sites between countries that are significantly different..



Between the native and introduced range, Rohtang had a significantly higher percentage colonisation of AM fungi compared to Sunningdale ($P < 0.05$) whereas Solang and Chandrkhani both had a higher percentage colonisation of AM fungi compared to both introduced sites ($P < 0.001$). There was a significant interaction between site and season ($F_{4,231} = 4.34$, $P < 0.05$) where the two introduced sites showed a decrease in the AM fungal colonisation, Rohtang and Solang remained constant, and Chandrkhani decreased.

There was a significant difference in the percentage damage from natural enemies between sites ($F_{4,237} = 92.035$, $P < 0.001$) (Figure 5.2B). Within the native range all sites were significantly different in the percentage damage from natural enemies ($P < 0.001$) . Within the introduced range, both sites had a similar percentage damage from natural enemies ($P > 0.05$) where both sites had significantly less percentage damage per plant than those at the native sites ($P < 0.001$).

There was an indication that Sunningdale had a higher number of reproductive units (seeds, pods and flowers) per plant compared to all other sites ($F_{3,91} = 2.427$, $P = 0.071$) (Figure 5.2C). Seed weight was greater in the native range (n = 105, mean 0.019 ± 0.0006) compared to the introduced range (n = 102, mean 0.015 ± 0.00049) ($F_{1,203} = 17.745$, $P < 0.001$).

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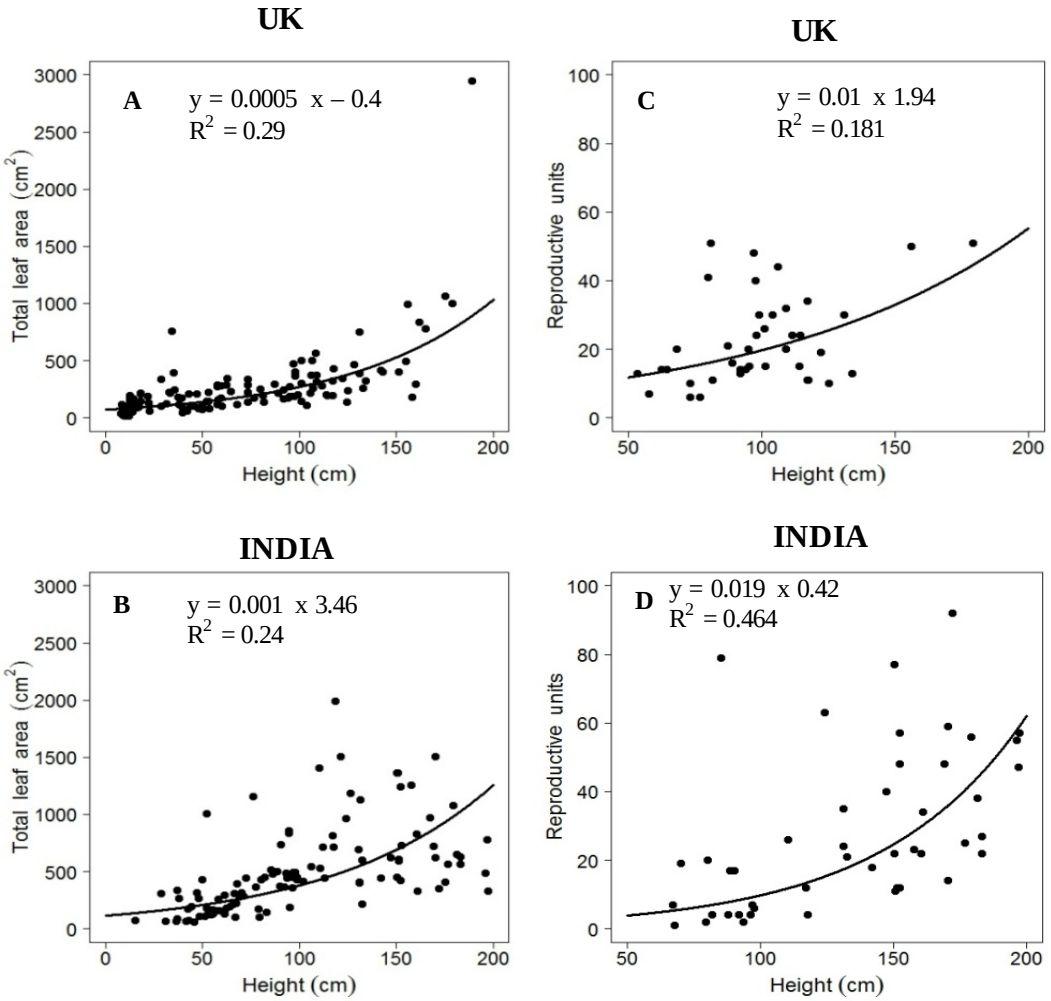
Figure 5.3 The relationship between plant growth parameters for UK and India. Figure (A and B) shows a significant relation between height and total leaf area for both the UK and India, respectably ($P < 0.001$). Figure (C and D) shows a significant relationship between height and reproductive units (flowers, immature pods and seeds) for both the UK ($P < 0.05$) and India ($P < 0.001$).



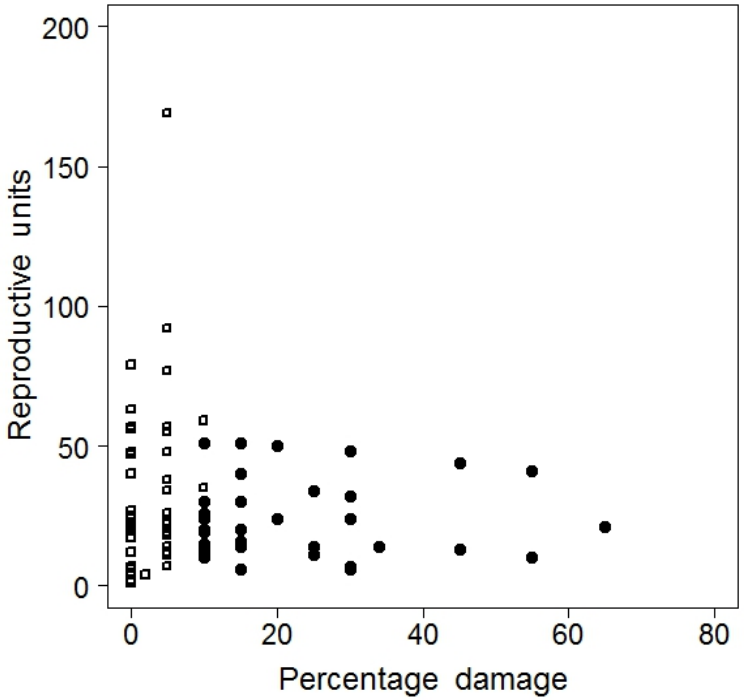
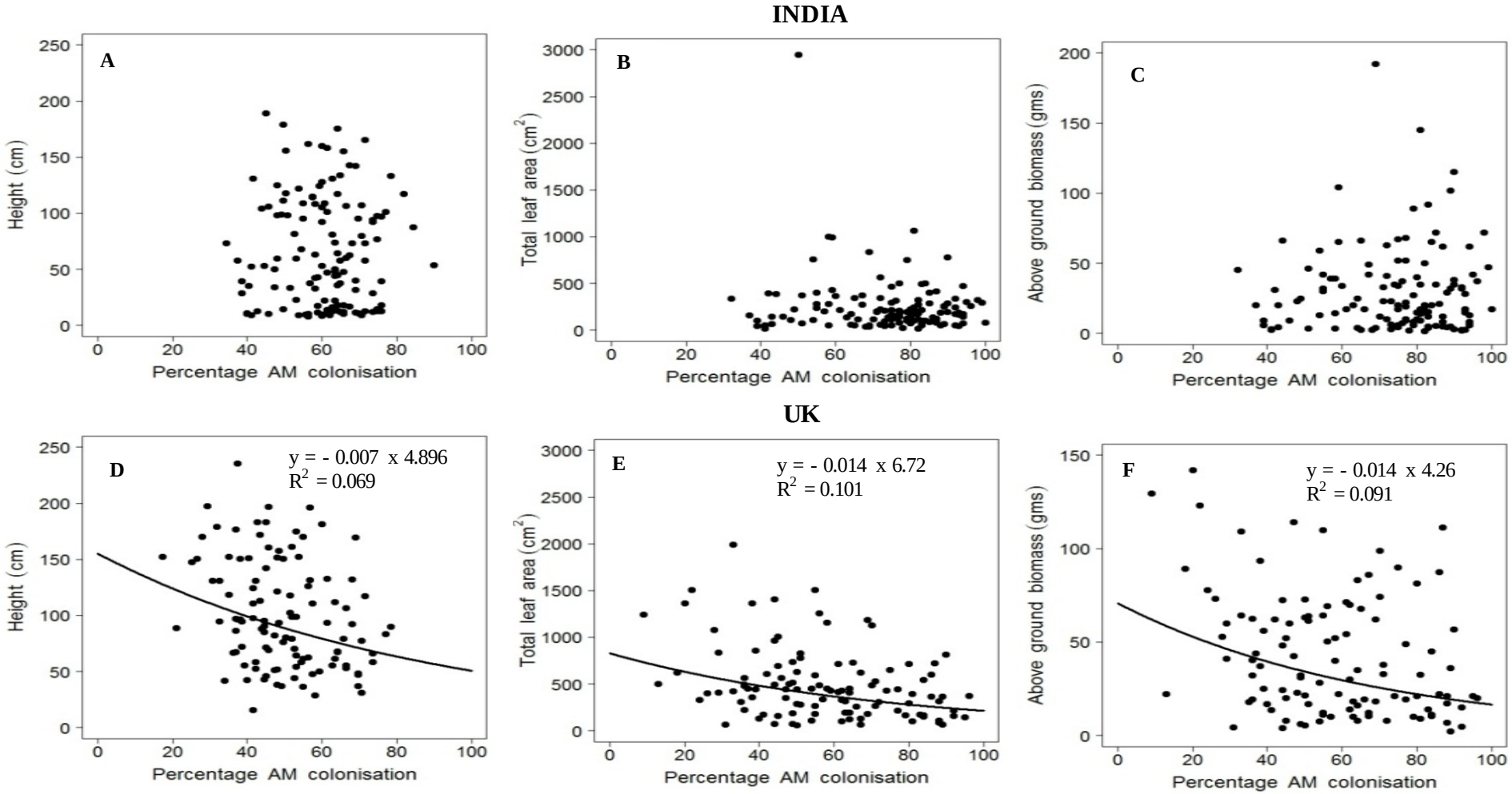


Figure 5.4 The relationship between percentage damage of natural enemies and the number of reproductive units for UK and India. Where the dots are replicates from India and the squares are replicates from the UK.

Figure 5.6 The relationship between AM fungal colonisation and plant growth parameters for India and the UK. For India, there was no relationship between AM fungal colonisation and the three plant parameters (A) height (B) total leaf area and (C) above ground biomass. For the UK there was a significant negative relationship between AM fungal colonisation and all three plant growth parameters (D) height (E) total leaf area and (F) above ground biomass.



There was a significant positive relationship between plant height and total leaf area for both the introduced ($R^2 = 0.295$, $F_{1,109} = 47.1$, $P < 0.001$) and native range ($R^2 = 0.235$, $F_{1,134} = 42.54$, $P < 0.001$). In addition, there was a significant relationship between height of individual plants and the number of reproductive units for the introduced range ($R^2 = 0.181$, $F_{1,142} = 10.47$, $P < 0.05$) and the native range ($R^2 = 0.464$, $F_{1,45} = 40.74$, $P < 0.001$). There was no relationship between percentage damage from natural enemies, per plant and the number of reproductive units in the introduced range ($F_{1,45} = 0.173$, $P = 0.678$). There was an indication of a negative relationship between percentage damage of natural enemies and the number of reproductive units in the native range ($F_{1,45} = 3.577$, $P = 0.065$).

There was a significant negative relationship between the percentage AM colonisation and plant height for the introduced range ($R^2 = 0.069$, $F_{1,108} = 9.097$, $P < 0.05$). However, there was no significant relationship between AM colonisation and plant height in the native range ($F_{1,129} = 11.96$, $P = 0.997$). There was a significant negative relationship between AM colonisation and total leaf area for plants in the introduced range ($R^2 = 0.101$, $F_{1,108} = 13.29$, $P < 0.001$), though again there was no relationship in the native range ($F_{1,129} = 0.009$, $P = 0.922$). There was a significant negative relationship between the AM colonisation and the above-ground biomass of plants in the introduced range ($R^2 = 0.091$, $F_{1,108} = 11.96$, $P < 0.001$). In the native range, there was no significant relationship between the AM colonisation and the above-ground biomass ($F_{1,129} = 0.007$, $P = 0.932$).

5.4 Discussion

Before the initiation of the biological control programme against *I. glandulifera* in 2006, there was little information available on the natural enemies associated with the plant in the native range. Fowler and Holden (1994) suggest this is due to *I. glandulifera* having little economic or medicinal value in the native range. Indeed, throughout the numerous surveys, when talking with local people there seem to be few uses for the plant, except the occasional reference to the seeds being edible, though even then it seems they are of low value when compared to the fruits produced by *Rubus* species (Eriksson *et al.*, 2003). The only published ecological and distribution data was from Himalayan floral field guides (Blatter, 1927; Polunin and Stainton, 1997; Sharma and Jamwal, 1988).

Impatiens glandulifera is regarded as a species that is endemic to high altitude meadows, and fringes of woodlands (Polunin and Stainton, 1997). However, because of human resource requirements, small and large settlements have changed the natural state of some areas in the Himalayas allowing for connectability between habitats. Gullies dug into the land, which enable water to flow from higher altitudes to irrigate farmland and provide water for human requirements, has resulted in connecting areas, which were previously divided by ecological barriers. Seeds expelled from the pods of *I. glandulifera* are thus able to become incorporated into man-made streams and are able to establish at lower altitudes, which would have been impossible without this anthropogenic disturbance. Natural Himalayan rivers are fast moving at high altitudes with few suitable areas along the riverbank where *I. glandulifera* can obtain a foothold, unless the river expands out into a flood-plain. Human disturbance along river systems, including bank side settlements and farmland, allows for *I. glandulifera* to colonise such

areas. Thus, one can argue that even in the native range of *I. glandulifera* there are natural and unnaturally occurring populations.

Within the native range, the occurrence of natural enemies showed distinct differences between the sites surveyed. Fewer invertebrate species were found in Pakistan compared to the Indian region of the Himalayas though all of the plant pathogens were found in both countries. The populations surveyed in Pakistan are on the edge of the plant's western native range. Potentially, invertebrate populations would be more restricted by natural barriers than wind-blown spores of a rust species. However, this does not explain the widespread occurrence of the two leaf-spot species, which are spread mainly by rain splash. A more plausible explanation is that both leaf spots are not host specific to *I. glandulifera* and are hosts to other *Impatiens* species that occur throughout the Himalayas. Indeed, the host range of both leaf-spot species has been proved wider than *I. glandulifera* under controlled conditions (Tanner *et al.*, 2008).

The topological structure of the Himalayas, with its high mountainous regions and deep valleys, may account for irregular natural enemy occurrence between populations within the native range. Although Solang, Rohtang and Chandrkhani are in relatively close proximity to each other, each site has distinct natural enemy assemblages. The genus *Impatiens* colonised the Himalayan relatively recently, in evolutionary terms, and the natural enemies would have co-evolved with the species. It is feasible that populations became isolated from one another as a result of Plio-Pleistocene climate fluctuations (Pers. comm., Janssens, Katholieke University), and some of the natural enemies co-evolved with discrete populations.

It is also plausible to suggest that the low numbers of arthropod natural enemies observed during the surveys in the highest site in India (Rohtang) and the high altitude sites in Pakistan, may simply be due to climatic conditions rather than any geographical barriers. Arthropod species are dependent on the number of day degrees available in a given region for their development (Bale, 2002). If the higher altitude areas do not satisfy the day degrees requirements of the arthropod species found at lower altitudes, arthropod species would be more restricted or even excluded from these regions.

Of the invertebrate species, *M. suturella* and *L. cyanea* could be regarded as *Impatiens* specialists based on their life cycles being synchronised with the phenology of *I. glandulifera*. Both species utilise the growth and structure of the plant where the adult female lays eggs in the stem early in the season (June-July). The stem provides food and a safe haven for the developing larvae. Both species pupate in the root mass of the plant during the winter months and emerge as adults the next spring (Pers. obs., Author and Shaw CABI). However, it is interesting to note that the occurrence of these two Coleoptera species was confined to one area, the Solang valley, where the diversity of *Impatiens* species was highest and both species were found developing and feeding on other *Impatiens* species.

Both rust species, *P. impatiensis* and *P. komarovii,* can be regarded as highly host specific to the genus *Impatiens* due to the nature of infection which is synchronised with the phenology of the plant's life cycle. Although both species of rust have other known hosts, *I. noli-tangere* and *I. parviflora*, respectiveley, it is likely that these are pathotypes (*formae speciales*) of the species which are host specific at the species level (see section 6.4). It is not suprising that both species were found in more natural areas within the Himalayas, and were absent in unnatural habitats such as at Chandrkhani.

The two species of rust are different in the fact that *P. impatiensis* is a heteroecious species, where only the uredina and telia are expressed on *I. glandulifera*. In mainland Europe, the aecial spore stage is found on *Adoxa moschatellina* L. Converseley, *P. komarovii* is an autoecieous monocyclic rust pathogen which completes its life cycle on *I. glandulifera* (Tanner *et al.*, 2010). The pycnial and aecial spore stages are found infecting the stem and causing distortion and hypertrophy of young seedlings early in the season and the uredina and telia are found on the lower surface of the leaves later in the year.

It is interesting to note the relatively low number of natural enemies observed attacking *I. glandulifera* in the native range (37 arthropod species and 6 plant pathogens) compared to other weeds where explorations have evaluated the natural enemy complexes in the native range. For example, Shaw (2007) conducted a review on the phytophagous arthropods recorded from 24 plant species in their native range which have been targets for biological control programmes and found, on average, each plant species supported 110 arthropod species, of which 5.81% (n=6 arthropod species) would be monophagous. In addition, Shaw (2007) presents the number of natural enemies recorded on *F. japonica* in the plant's native range, from surveys conducted by the author and from literature reviews where 186 arthropod species and 50 fungal pathogens have been recorded.

By 2008, almost all of the natural enemies detailed within this chapter were recorded with the exception of one or two species, which were of little interest from a biological control perspective. This may suggest bias from a biocontrol scientist's perspective, as a heightened consideration is given to visible damage associated with the plant species and any natural enemy association. Thus, a biocontrol scientist may miss those species

which may be regarded as broadly generalists and/or show little signs of associated damage. Although further species could potentially be discovered on *I. glandulifera*, if the geographical range of the surveys had been increased, bi-seasonal intensive surveys were conducted in 2008, 2009 and 2010, which have encompassed the limits of the native range of *I. glandulifera* thus it is fair to suggest that the sampling effort was adequate.

Isolation, by geographical barriers, extreme environmental conditions, and the dominance of natural enemies like *P. komarovii, A. himensis,* and the *Septoria* leaf-spot may all suggest why *I. glandulifera* has a low diversity of natural enemies. An additional, plausible explanation may be that the native range of *I. glandulifera* is relatively restricted in area (approximately 800km in length and 50 km wide) compared to that of *F. japonica* (Williamson, 1996). In addition, one should consider the life forms of the species, where *I. glandulifera* is an annual species and would be expected to have a considerably lower above and below-ground biomass, total leaf area and occurrence to that of the perennial species, *F. japonica*.

The plant measurement data show that, in general, *I. glandulifera* is taller, has a higher total leaf area, and has less natural enemy damage in the introduced range compared to the native range. These results are consistent with other native – introduced range comparisons (Ebeling *et al.*, 2008; Erfmeier and Bruelheide, 2004; Widmer *et al.*, 2007). In both the introduced and native range, height was significantly associated with total leaf area and reproductive units (the sum of seeds, pods and flowers), suggesting that across the current distribution of *I. glandulifera*, the above-ground morphological ratios between the growth parameters measured are similar.

The density of *I. glandulifera* per m^2 was consistent within the native sites though the UK populations showed significant differences to each other and differences were seen between the ranges with the expectation of Harmondsworth and Solang. The height of individual populations showed differences between the sites surveyed, where in general, in the native range, the more naturally occurring populations showed a smaller height compared to the semi-natural site Chandrkhani, and the both of the introduced sites. Sunningdale, the only wooded habitat, showed the highest average height, which is consistent with the results of Maule *et al.* (2000), where the authors observed plants over 3m tall in wooded habitats.

We cannot rule out that the increased performance seen in the introduced range is due to environmental factors, where the UK climate is more benign than that seen in the Himalayas. Indeed, in this comparative study, it was not possible to control for altitude, which has been shown to have an influence on plant performance (Balasuriya, 1999; Kofidis *et al.*, 2007; Pan *et al.*, 2009). Mean height and mean total leaf area did generally decrease within the native range with increased elevation. However, the seasons in both ranges are similar in the fact that they follow the same patterns of winter, spring and summer and autumn; though the Himalayas, of course, have more extreme variations to that of the UK.

In general, the increased performance of *I. glandulifera* in the introduced range, coupled with the diminished associated natural enemies is consistent with the predictions of the ERH. However, further research would be required to fully substantiate differences in plant growth parameters between countries, including increasing the sample size and number of sites sampled in each country. It is also interesting to note that the Chandrkhani populations were taller and had a higher total leaf area than the two more

natural native populations, though natural enemy damage was significantly higher. The natural enemies at Chandrkhani were more generalist than those found at the two other native sites. It is possible that generalist natural enemies inflict less of a cost to *I. glandulifera* than specialist natural enemies, which have adapted to attack the plant at all stages of its life history thus incurring an accumulative cost on the plant.

Even though the percentage damage by natural enemies was significantly higher in the native range compared to the introduced range, there was only an indication of a negative relationship between percentage damage and reproductive units (flowers, pods and seeds) in the native range. From a biological control perspective, the damage inflicted on the plant and any subsequent reduction in reproduction, is desirable especially in the case of an annual that spreads by seed propagules. However, the data on reproductive units should be taken with caution, as they provide only a snap shot in time. In the UK, and in India, *I. glandulifera* flowers and sets seed for a prolonged period thus any effect on seed set may be more or less pronounced than that shown. However, the complete lack of any association between percentage damage and reproductive units in the introduced range suggests that those generalist arthropod species that do feed on *I. glandulifera* have no effect.

The results show that *I. glandulifera* produces heavier seeds in the native range compared to the introduced range. These results conflict with studies of other native-introduced range comparisons (Buckley *et al*., 2003; Ebeling *et al*., 2008). However, both of the aforementioned studies compared seed size of perennial shrub species and in this study, we are comparing an annual species. The difference in seed weight between the native and introduced populations may be put down to environmental factors between the countries. It is plausible to suggest that in the Himalayas, the severity of the winter would favour individuals to produce smaller quantities of seeds with a higher

biomass. A higher number of seeds, with lower biomass may be an adaptation of the introduced populations to a more tolerable climate.

One cannot ignore the fact that the smaller stature seen in the native range may also be due to direct competition with other associated plant species, either above-ground, competing for light, space requirements, or below-ground where species compete for space, nutrients, and beneficial microbes. The plant species associated with *I. glandulifera* are relatively few in the Himalayas, similar to the low diversity seen within stands in the introduced range (Beerling and Perrins, 1993). However, the most dominant species are perennials such as representatives from *Ranunculus* and the aggressive invader *P. polystachum* that has an extensive root system, which would easily compete for underground resources. Indeed, the native sites had a higher below-ground to above-ground ratio than the introduced sites, which suggests native populations may put down more root biomass to compensate for increased competition in the native range.

The lack of any association between height, total leaf area and above-ground biomass, and the percentage colonisation of AM fungi, for the Indian samples compared to the negative relationship seen for the above in the introduced range, is interesting and suggests that the introduced populations associate with an incompatible, newly encountered AM fungal species. Indeed, the negative relationship shown within the introduced populations implies there is a cost associated with this relationship that may simply be due to the level of specificity between the plant and the AM fungi (Sanders, 2002). In the introduced range, *I. glandulifera* may potentially be associating with the wrong AM fungi. No relationship between the measured plant growth parameters and AM fungal colonisation in the native populations may imply the AM fungi and the plant is in a symbiotic relationship where the two are mutually beneficial to each other.

Indeed, other factors may influence the growth parameters more than the colonisation of AM fungi; including photoperiod, altitude, and nutrient acquisition. Ayres *et al.* (2006) highlight that when plants are competing with other plant species, the effects of AM fungi are often dissipated. Indeed, in the native range of *I. glandulifera*, competition with native plant species would be higher than in the introduced range where the plant has a competitive advantage over native plant species. However, it may be the case that any relationship is easier to elucidate in introduced populations due to the reduced interactions with natural enemies and reduced competition with associated plant species as assumed by the enemy release hypothesis (Keane and Crawley, 2002).

This study highlights that a multitude of factors, and the release from them, can influence morphological differences of a plant species in the native and introduced range. No one factor can be taken in isloation when considering why a non-native plant species performs differently in the introduced range compared to its perfomance in the native range. Indeed, plants that are heavily regulated in the native range and have medium to high reproduction potential may be considered undesirable additions to the horticultural market but it is still difficult to predict any adaptations which may occur in the introduced range.

6 A summary of the progress made with the biological control of *Impatiens glandulifera* using the rust fungus *Puccinia komarovii*

6.1 Introduction

As a management tool for controlling weed species, classical biological control has been practiced worldwide for over 100 years where there have been approximately 7,100 introductions (this figure includes repeated introductions in one country) of classical biological control agents, using 2,677 species (Cock *et al.*, 2010). However, although European countries have been the source for over 380 releases of classical weed biological control agents, there has been only one released against a non-native invasive plant species in the European Union, which was the psyllid, *Aphalara itadori* Shinji against *Fallopia japonica* in the UK (Djeddour and Shaw, 2010; Shaw *et al.*, 2009; Shaw *et al.*, 2011). Although research into classical biological control of non-native invasive plant species is in its infancy in Europe, compared with other geographical regions, such as South Africa, Australia and North America (Cock *et al.*, 2010), the interest from UK Government departments (Shaw, 2003; Sheppard *et al.*, 2006) and European funding bodies (Cock and Seier, 2007) has increased in the last decade (Cortat *et al.*, 2010).

This surge in interest stems from the fact that for most of our established problematic non-native plant species, eradication, or even control on a nationwide scale, is no longer a feasible option (Djeddour *et al.*, 2008; Shaw and Tanner, 2008). High costs of implementing more traditional control methods, such as chemical application and

manual removal, coupled with the reduced number of chemical products available to control non-native plant species, mean that classical biological control may be the only sustainable method of control for some of the UK's most prevalent widespread plant species. The widespread distribution of species like *F. japonica*, *I. glandulifera* and *Heracleum mantegazzianum* Sommier and Levier implies that any realistic attempt to control these species on a catchment, regional, or national scale would require a self-perpetuating control method, which may feasibly only be achieved with classical biological control.

A possible reason why there has been so little research into classical biological control in Europe is that non-native invasive plants rarely affect agricultural systems in this geographical region and, therefore, until recently, the economic and ecological impacts of environmental weeds have received little attention (Sheppard *et al.*, 2006). Previously, the focus on invasive species in Europe has been on the ecology and life history of invasive species with applied management and control coming second best (Sheppard *et al.*, 2006). The release of *A. itadori* for the control of *F. japonica* was initiated in 2011 at a number of locations throughout England and Wales and was the result of 10 years of research (Shaw *et al.*, 2009). This was the first release of an exotic classical biological control agent against a non-native invasive weed in the European Union, and has potentially set a precedent for future releases (Shaw *et al.*, 2009, Shaw *et al.*, 2011).

The majority of classical biological control releases worldwide have involved arthropod biological control agents (Shaw, 2003). In comparison, the history of classical biological control using fungal pathogens against non-native invasive plant species is relatively recent, beginning in the 1970s (Evans *et al.*, 2001). This appears somewhat

surprising, with the benefit of hindsight, as fungal control agents, and in particular rusts, have proven to be highly host specific and when applied to control invasive weed populations successes have been spectacular (Evans and Tomley, 1994; Tomley and Evans, 2004), with few non-target impacts recorded (Barton, 2004; Waipara *et al.*, 2009). Evans *et al.* (2001) suggest that concerns over the safety of moving plant pathogens between countries is one reason why classical biological control using fungal pathogens is slow on the uptake. In addition, many nations, including Europe, lack a clear regulatory pathway for licencing fungal biological control agents (Seier, 2005; Sheppard *et al.*, 2006), which may still lead to biological control researchers and sponsors alike, favouring biological control research using arthropod biological control agents.

The rust pathogen, *Puccinia komarovii* was first observed infecting *I. glandulifera* in the native range (the Indian region of the Himalayas) in 2008. This chapter reviews the research and scientific advances that have led to this rust pathogen becoming the most suitable classical biological control agent for the control of *I. glandulifera* in its introduced range. In addition, this chapter reviews the future research and considerations needed, in order to release a microorganism, as a biological control agent in Europe.

6.2 Pre-survey biogeographical research

An understanding of the geographical range of the target species, along with prior information on its life cycle and seasonality, is essential to plan and implement successful surveys for natural enemies in the plant's native range. Pre-survey research, including reviewing herbarium samples, can lead to an indication of site localities and

landmarks where the target species has been recorded in the past. Interestingly, this may also provide information on natural enemies. In 2005, an evaluation of herbarium records of *I. glandulifera* in its native range was conducted at Kew Herbarium, London. In addition to obtaining detailed site localities, there was clear evidence of a high level of natural-enemy activity on the target species in its native range (*fide* Herb-K records, Nov., 2005). There were clear visual indications of pathogen damage and rust pustules, indicative of the genus *Puccinia* (Pers. com., Evans).

6.3 In-country collaboration

In-country collaboration, in the plant's native range, is essential for the successful delivery of a biological control programme. In-country support is essential to provide local knowledge of the occurrence of the plant, both from botanical experts and in-country reference collections (library and herbarium). It also helps with understanding local language, customs and assists planning and implementing surveys. In fact, in-country collaboration is now a prerequisite for foreign scientists, wishing to conduct biological surveys in India. Thus, for the biological control of *I. glandulifera*, CABI collaborated with the National Bureau of Plant Genetic Resources, New Delhi
Under a MoU titled 'The study of biological control of invasive plant species and Indian natural enemies'.

6.4 Identification

In order to gain export permission, prior species identification is desirable, and in some cases essential, to satisfy the legislative procedures of the country of origin. This often necessitates local official identification, and the rust infecting *I. glandulifera* was identified by the Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi, who named it *Puccinia komarovii* Tranz. Specimens were deposited within

their herbarium (Accession number HCIO 49555). It is interesting to note that this is the first time *I. glandulifera* has been recorded as a host of *P. komarovii.* A subsequent search of national and international fungal databases (www.indexfungorum.org/names/names.asp and nt.ars.grin.gov/fungaldatabases/, accessed 1st September, 2011) resulted in 33 records of *P. komarovii* infecting nine species of *Impatiens* (see table 6.1 for *Impatiens* and *Puccinia* associations).

Puccinia komarovii was first recorded from *I. parviflora* in central Asia in 1904 (Coombe, 1956; Sydow and Sydow, 1904). Gaumann (1959) also indicates that *I. amphorata* Edgeworth (syn. *I bicolor*) is a host of *P. komarovii,* and also names *I balsamina* L., *I. capensis, I. firmla, I scabrida* and *I thomsoni* Hook. as hosts. In an inoculation experiment, conducted by Blumer (1937) using *P. komarovii* collected from *I. parviflora* in Switzerland, the author states that the rust infects *I. balsamina, I. capensis, I. formula* and *I. scabrida*, but not *I. amphorata, I. holstii* Engl. & Warb, *I. roylei* Walp. = *I. glandulifera* and *I. sulatani* Hook. When reviewing this, Gaumann (1959) stated that the lack of infection on *I. amphorata* in Blumer's experiment indicates that several different varieties of *P. komarovii* may exist. Indeed, there is still some confusion to whether Blumer (1937) actually achieved sporulation or a hypersensitive reaction in his experiments, as he only records *I. balsamina* as producing 'severe infection in the form of circular, pale, later brown lesions bearing uredosori on the underside of the leaves'.

In 2011, *P. komarovii* was collected from *I. parviflora* in Hungary, by CABI scientists, and inoculated onto *I. parviflora, I. glandulifera, I. scabrida* and *I. balsamina* in an experiment which was replicated (3 replicates per species) and repeated twice in the quarantine unit at CABI E-UK. Infection, in the form of the expression of rust pustules

on the underside of the inoculated plants was only achieved on *I. parviflora* and *I. balsamina* (Tanner, Ellison and Varia, unpublished). In addition, *P. komarovii* ex. *I. glandulifera* (Indian Himalayas) has been shown not to infect *I. parviflora* in a series of host range tests. Thus, it is plausible to concur with the suggestion of Gaumann (1959), that there are a number of different varieties of *P. komarovii* that are species specific within the genus of *Impatiens*. Interestingly, research conducted by the Hungarian Academy of Sciences, confirms that the two pathotypes of *P. komarovii* (ex. *I.parviflora* and ex. *I. glandulifera*), are indistinguishable molecularly based on ITS sequencing (Kiss *et al.*, unpublished). Based on the host range of *P. komarovii* ex. *I. glandulifera*, it will therefore be feasible to rename this pathotype as a *forma specialis* of the type *P. komarovii*.

Table 6.1 *Puccinia* species associated with *Impatiens*. Data obtained from www.indexfungorum.org/names/names.asp and nt.ars.grin.gov/fungaldatabases/, accessed 1st September, 2011

***Impatiens* species**	***Puccinia* species**	**Region**
Impatiens capensis Meerb.	*Puccinia impatientis* (Schwein.) Arthur (1903)	USA, Canada, Europe
	Puccinia rubigo-vera impatientis Arthur	USA
	Puccinia recondita Rob. Ex Desm.	USA
Impatiens glandulifera Royle	*Puccinia komarovii* Tranz.	India,Pakistan
Impatiens parviflora DC.	*Puccinia komarovii*	Europe, Russia,China
Impatiens amplexicaulis Edgeworth	*Puccinia komarovii*	China,Nepal
Impatiens brachycentra Kar. & Kir.	*Puccinia komarovii*	China
Impatiens edgeworthii Hook. f.	*Puccinia komarovii*	Pakistan
Impatiens racemosa DC.	*Puccinia komarovii*	Pakistan
Impatiens radiata Hook. f	*Puccinia komarovii*	Pakistan
Impatiens thomsoni Hook.f.	*Puccinia komarovii*	India
Impatiens urticifolia Wall.	*Puccinia komarovii*	China
Impatiens noli-tangere L.	*Puccinia impatientis*	Europe, Russia, Pakistan

6.5 Exporting *Puccinia komarovii* from the country of origin

Gaining official approval to export *P. komarovii* proved to be a time consuming process due to difficulties interpreting newly enforced legislation surrounding acquisition and

export of biological material from India. Indeed, the export of biological control agents has become more difficult in recent years worldwide as countries get to grips with the United Nations Convention on Biological Diversity (1992) and the rights to the biodiversity within their borders (Cock *et al.*, 2010). In India, the Biodiversity Act 2002 imposed restrictions on exporting biological material, and other nations have imposed similar legislations to protect their bio-resources (Tanner and Djeddour, 2010).

Cock *et al.* (2010) argues that biological control agents should be exempt from these legislative processes as the exchange of biological control agents is mutually beneficial to all countries concerned. Nations that impose restrictions are, or have been in the past, recipients of biological control organisms. Even with the implementation of the Indian Biodiversity Act 2002, there are however clear provisions and guidelines set out to facilitate collaborative research and sharing of bio-resources for scientific purposes (National Biodiversity Authority of India, available at http://nbaindia.org/ accessed 25th October, 2009).

There are two possible approaches; the first requires in-country collaboration made official by a signed MoU and endorsed by the appropriate governmental department. If the above criteria are met, the collaborating organisations can complete a Material Transfer Agreement (MTA), which is then submitted to an expert committee, including both pathologists and quarantine experts, for consideration. If the MTA is approved, it is then sent to the appropriate central governmental department for endorsement (in this case the Department of Agriculture Research and Education (DARE)), and material can be exported for research purposes. The alternative is to apply directly to the National Biodiversity Authority (NBA) of India. However, if an application to NBA was unsuccessful this could mark the end of the biological control programme in India. For

the *I. glandulifera* biological control programme, securing in-country collaboration to facilitate exploratory surveys and scientific research in-country was an essential component, hence, the former pathway was used.

The aecial stage of the rust pathogen was imported into the UK, from India in June 2010 on live *I. glandulifera* plant material under Defra licence (no. PHL199C/6334) and the Germplasm Exchange Reference (FS No. 11/2010). Plants were cleaned of any soil and were contained in sealed zip-lock bags, which were then sealed within a cool box for the duration of the journey. Plants were only removed from this containment upon arrival at CABI's Defra licenced quarantine facility.

6.6 Field observations and life cycle determination

Puccinia komarovii was first observed infecting *I. glandulifera* populations in India during July 2008 (Tanner *et al.*, 2009). The aecial stage was found expressed on only two or three plants within the population at the Rohtang site. Further intensive surveys were conducted in this region between 2009- 2010, where during June and July surveys the aecial stage was found in abundance infecting *I. glandulifera* seedlings between 6cm and 18cm tall. Infection was patchy at the sites where it was found, but under suitably damp microclimatic conditions, more than 50% of the plants were found to be infected (Pers. obs., Ellison and Tanner). Piskorz and Klimko (2006) recorded a higher level of infection on *I. parviflora* in Wielkopolski National Park, Poland, where over 90% of the population was infected with *P. komarovii* when the density of the host was higher than 90 individuals/m^2. The June survey was the earliest period we had sampled the plant in the native range, and then the infection was observed at an early stage with fresh aecia still being produced on the swollen stem below the cotyledons.

Hypertrophy and possibly hyperplasy of the hypocotyls induced by the rust was evident (Plate 6.1A/B/E). Plants infected with the stem rust are significantly taller, due to longer hypocotyls, which often also have a wider girth, than uninfected plants. This would aid aeciospore dispersion into wind currents, as infected plants would be higher in the canopy. Although there is only one generation of aeciospores in each season, they were still observed to have a significantly detrimental impact on the plants they infected; if the distribution was somewhat patchy.

Table 6.2 The proposed life cycle of *Puccinia komarovii* on *Impatiens glandulifera* in the Himalayas based on field observations

Period	Life cycle of *Puccinia komarovii* on *Impatiens glandulifera* in the Himalayas
Jan-March	Both teliospores and *I. glandulifera* seeds are dormant (potentially under snow).
April- May	Temperature increase and snow melt (providing water for seedling germination) trigger germination of Himalayan balsam seedlings. Seedlings emerge through previous season's leaf litter infected with teliospores. Teliospores germinate (induced by environmental triggers, including possibly plant volatiles) and produce basidiospores which are released and infect the hypocotyl of young seedlings.
June- July	Infection develops within the plant stem and aecial cups erupt onto the surface, containing aeciospores. The aecia induce the hypocotyl, to extend and become significantly longer than those of uninfected plants, carrying the aecia above the canopy. Aeciospores are dispersed by wind and rain splash, which infect the leaves, and after a currently unknown biotrophic incubation period within the leaves (possibly weeks) urediniospores are produced on the underside of the leaves. Urediniospores are released and disperse locally in small eddies to infect leaves of other individuals in the population; and more widely in wind currents to spread between populations. This is the cycling stage of the rust – with more than one generation within the season. It can also be very damaging to the plants, if rust levels reach epidemic proportions.
Aug-Sept	The cooling environmental temperature, and potentially a chemical reaction in the aging leaves, trigger the formation of teliospores, which either are released from the telia into the soil or fall attached to the leaf as the plant begins to die and sheds it leaves. Seeds produced in the capsules are released and dispersed.
Oct-Dec	Both teliospores and *I. glandulifera* seeds are dormant on or just below the soil surface (potentially under snow).

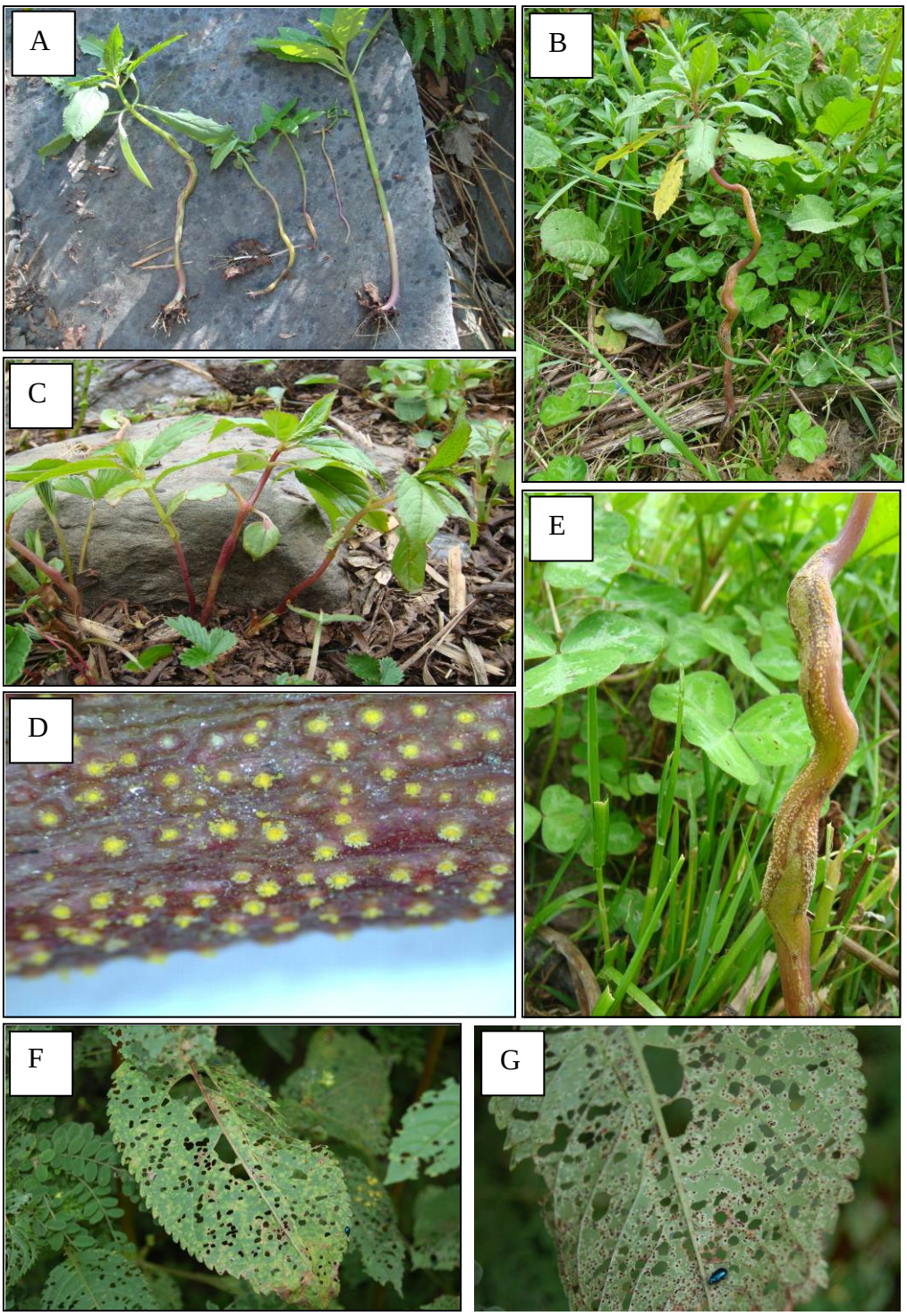
It is likely that the aecial stage of the rust causes premature death of infected individuals, and hence no seed set. This is consistent with the observations of Bacigalowa *et al*. (1998) and Eliáš (1995) who recorded up to 100% mortality on seedlings of *I. parviflora* infected with *P. komarovii* in mainland Europe. In other rust-host systems, similar high levels of mortality have been shown, for example the impact of *Puccinia lagenophorae* Cooke on *Senecio vulgaris* L. (Paul and Aryes, 1986). Individuals of *Impatiens glandulifera*, infected with the aecial stage of the rust soon lose their early season height advantage, looking significantly smaller and less healthy than the surrounding uninfected plants, a few weeks after seed germination. The aecia-infected hypocotyls often split open (Plate 6.1E) and secondary infections are clearly visible, leading to plant collapse. Similar secondary infection, because of infection by *P. lagenophorae* on *S. vulgaris*, has been shown to increase mortality of individuals (Hallett and Ayres, 1992). Certainly, in late July and August, plants with aecia on the base of the stems are hard to find.

The aeciospores erupt from the aecial cups (Plate 6.1D) to infect young leaves of *I. glandulifera*, to form the urediniospores (the cycling stage) some 2 weeks after initial infection. In July and August, at the same sites where the aecia were found, uredinia and telia were observed on the older plant leaves. The urediniospores are spread through the populations by wind currents and go on to infect the wider population. This spore stage may cycle through two or more generations until later in the season when, potentially triggered by biochemical changes within the host and a cooling of the ambient temperature, the infection is expressed as teliospores (the over wintering stage) on the underside of the leaves. Often there is an overlap in the production of uredinia and telia, potentially an evolutionarily adaptation to maximise the persistence and occurrence of the pathogen. When the plant begins to senesce, the telia fall to the

ground either still attached to the leaves or become incorporated into the soil. The following spring, the snow melt and increased temperature induce the germination of the *I. glandulifera* seeds and the telia germinate to form the basidiospores, which infect the hypocotyl of the plants.

Evans (1987) highlights the importance of understanding and evaluating the life cycle of biological control agents prior to any application for release. Obviously, the life cycle detailed above was initially based on field observations, as well as on the life cycle of *P. komarovii* on *I. parviflora*. However, molecular comparisons of all three spore stages (aecial, uredinia and telia) proved that they all belong to the same species, confirming *P. komarovii* is an autoecious rust pathogen on *I. glandulifera* (Kiss *et al.*, unpublished). Additionally, experimental infection under quarantine conditions, where the aecia were inoculated onto the leaves of *I. glandulifera*, resulted in the formation of uredinia and teliospores. The last link in the experimental confirmation of the life cycle, the infection of the basidiospores onto the hypocotyl is yet to be confirmed. However, telia and basidiospores have successfully been germinated under controlled conditions, where germination was triggered by priming the spores at 4^{o}C for a two-month period.

Plate 6.1 *Puccinia komarovii* infecting *Impatiens glandulifera* in the Himalayas



A. *Impatiens glandulifera* infected (and uninfected- right) with the aecial stage (C.Ellison, CABI)
B. *I. glandulifera* showing severe twisting of the stem due to infection
C. Field evidence of host specificity in the native range- the plant in the middle is *I. glandulifera* infected with the stem rust and either side is *Impatiens sulcata*, both uninfected
D. Close up of the aecia cups on the stem of *I. glandulifera*
E. Close up of an infected stem where the epidermis has broken due to infection
F. Chlorosis on the upper side of an infected *I. glandulifera* leaf (H. C. Evans, CABI)
G. Uredinia infection on the underside of *I. glandulifera* leaf

6.7 Distribution and persistence

Chapter 5 describes the distribution of *P. komarovii* in the areas surveyed in India and Pakistan. At all locations in India, *P. komarovii* was found year on year at the same locations both in wooded habitats and riparian systems. The latter is interesting, since during 2009, the aecial stage of a rust (hypocotyl stem infection) was observed for the first time at a river site in the Solang valley. This is encouraging from a biological control perspective, as before this discovery it was unclear if the rust was able to persist in a riparian habitat. As *I. glandulifera* is predominantly a weed of riparian habitats in the UK, the rust pathogen would be required to persist and be self-perpetuating throughout the catchment region. In Europe, *P. komarovii* is predominantly found infecting *I. parviflora* in wooded habitats, though often these border riparian catchments (Pers. obs., Author). Thus, it may be the case that the wooded habitats would remain the source of the inoculum throughout the winter months. The natural survival of the rust throughout the winter, and the subsequent germination of the teliospores the following spring (induced by a prolonged cold period) are vital for the persistence of the rust within *I. glandulifera* populations. The survival of *P. lagenophorae* during the winter months in Switzerland is critical to start the spring infection on *S. vulgaris* (Frantzen and Müller-Schärer, 1999; Frantzen and Müller-Schärer, 2006).

6.8 Inoculation and infection parameters

Prior knowledge, based on ecological data and climatic measurements is often needed to successfully infect introduced biotypes upon returning to UK quarantine. Whilst surveying *I. glandulifera* in the native range, temperature data loggers (LogTag® HAXO-8) which recorded both temperature and humidity, every 5 minutes, were placed within infected populations for up seven days. These provided baseline data for the initial inoculation experiments.

In general, a number of inoculation methods are assessed to determine the most appropriate for infection in the lab to best recreate that seen in the plant's native range. Pathogens are inoculated onto the target with various brush and spore suspension techniques and, preferably, maintained in a dew chamber for a pre-determined time (dew period) before being transferred to a temperature-controlled greenhouse or CT-room, and monitored daily for symptoms (Agrios, 2005; Evans and Ellison, 2005; Evans and Tomley, 1994). Spore suspension in Tween80, spores brushed dry onto the plant, or in a talc/spore mix (ratio 3:1), are all useful methods for such assessments. For this particular plant-host association, a talc /spore mix proved to be the most suitable, as it provided infection similar to that seen on infected plants in the native range. After inoculation, plants were placed in a dew chamber for 48 hours (15^{o}C inner chamber, 13.5^{o}C outer chamber- the temperature indicative of the morning dew period in the Himalayas) to aid sporulation. After this period, plants were placed in cages and observed for developing symptoms (Evans and Ellison, 2005; Evans and Tomley, 1994). The controlled temperature room was set at 23^{o}C day and 16^{o}C night with a 15L/9D light regime.

6.9 The test plant list

Apart from actually finding a potential biological control agent to test, the compilation of the test plant list is one of the most fundamental components of a classical biological control programme. The species within the list are used to evaluate and confirm the host specificity of the biological control agent. Plants were selected for host range testing using the Centrifugal Phylogenetic Method (Wapshere, 1974). Following this method, closely related plant species are selected from the same genus and/or family of the target species. The list is then expanded to more distantly related species in other families within the same order to the target species. In addition, Wapshere (1974)

advocates the inclusion of species with a similar morphology, and biochemical composition to that of the target species, though often these species are already included, as they constitute the closely related species. However, the latter may include species that grow in a similar ecological niche to that of the target species. Finally, plants with are attacked by similar agents to the agent being tested are incorporated into the list. Briese (2005) highlights that recent advances in plant phylogenetics should be incorporated into the formulation of modern-day test plant lists. Previous plant relationship classifications before molecular advances were based largely on morphological relationships and modern molecular methods have served to reclassify the relationships of many plant species (Briese, 2005).

The global market in horticultural sales of *Impatiens* is worth £-millions per year where most of the business comes from the sale of just two species and their varieties, *I. walleriana* (Busy Lizzie) and *I. hawkeri* (the New Guinea hybrids) (Morgan, 2007). Lagging far behind these two species in the sheer numbers of varieties available are *I. balsamina* (the first *Impatiens* species to be cultivated as early as 500 years ago (Morgan, 2007). In 2011, the Royal Horticultural Society (RHS) listed 30 species and an additional 38 varieties on its plant finder website (http://apps.rhs.org.uk/rhsplantfinder/ accessed 11 September, 2011). All of the species detailed on the web site, with the exception of three species, were included in the proposed test plant list. The exclusion of the three species was made on the advice of the owner of the National *Impatiens* Collection, Ray Morgan due to the named species being either very difficult to propagate or uncommon in horticultural circles.

In addition, there are numerous suppliers of *Impatiens* seeds in the UK and abroad, though again most concentrate on the Busy Lizzie and New Guinea hybrids. Chiltern

seeds supply seeds from 4 species (*I. balsamina, I. textori, I. glandulifera* and *I. walleriana*) and nine varieties of the latter. Thompson and Morgan supply three species of *Impatiens* and 12 varieties of *I. walleriana*, whereas Nicky's seeds supplies one species of *Impatiens* and 34 varieties of *I. walleriana* and 2 varieties of *I. hawkeri*. The four varieties of both *I. hawkeri* and *I. walleriana* included in the test plant list reflect the high number of varieties available for both these species.

In my opinion, a test plant list should evolve throughout the course of a biological control programme, as the natural enemy-plant interactions become better understood and/or additional closely related plant species become available or unavailable. In total, the test plant list for the biological control of *I. glandulifera* includes 60 plant species and 8 varieties (Appendix 1). Of these, 39 are ornamental species, 14 are native, 3 are introduced (into the UK countryside) and 4 are closely related economically important plants (though many of the ornamental species may be considered as economically important species). The list includes representatives from all families within the order Ericales which are either native or available through the horticulture trade.

Where a plant order contains a substantial number of species, for example *Erica*, more than one species was included in the test plant list to fairly represent that order (see Appendix 4). As *Impatiens* was previously classified in the order Geraniales, one representative of this group was also included in the test plant list, namely *Geranium robertianum* L. Three safeguard species are included in the test plant list from similar habitats to where *I. glandulifera* grows. These species were chosen from the author's field knowledge when surveying *I. glandulifera* in the UK and from Beerlings and Perrins (1993).

6.10 Host specificity testing

Determining the host range of *P. komarovii* is a critical step in the development of the biological control programme as the safety of non-target species is paramount. Host specificity testing is focused on evaluating the potential risk to plant species within the proposed area of release though it is not assumed absolute (Ghosheh, 2005). Species in the test plant list would be assessed for their susceptibility to a biological control agent in a series of replicated tests under controlled quarantine conditions. Based on the evaluation of the infection parameters, three replicates of the target species, and multiples of 3 of a selection of non-target species would be evaluated simultaneously. If any test produced a negative result on the target species, i.e. no or reduced sporulation, these tests would be treated as void and repeated. The current research into the host range testing of *P. komarovii,* is on-going, where to-date the rust shows a high level of host specificity to *I. glandulifera*.

6.11 Symptom evaluation

In combination with the host range testing, any symptoms which are abnormal on the inoculated non-target species are evaluated both macro and microscopically through the course of the host range tests. In the case of *P. komarovii,* chlorotic spotting appears on the lower and upper leaves of the host approximately 10 days after inoculation with pustule development and sporulation appearing on the underside of the leaves approximately 14 days post inoculation. Microscopic evaluation determined that the urediniospores germinate and enter the host through the stomata. The germ-tube forms an appressorium over the stomata and the infective hyphae develop within the host. All non-target species tested were maintained for a period of six weeks post inoculation, three times the length required for sporulation on the host species. Where symptoms develop on the non-target species, the leaf was cleared and stained and examined for

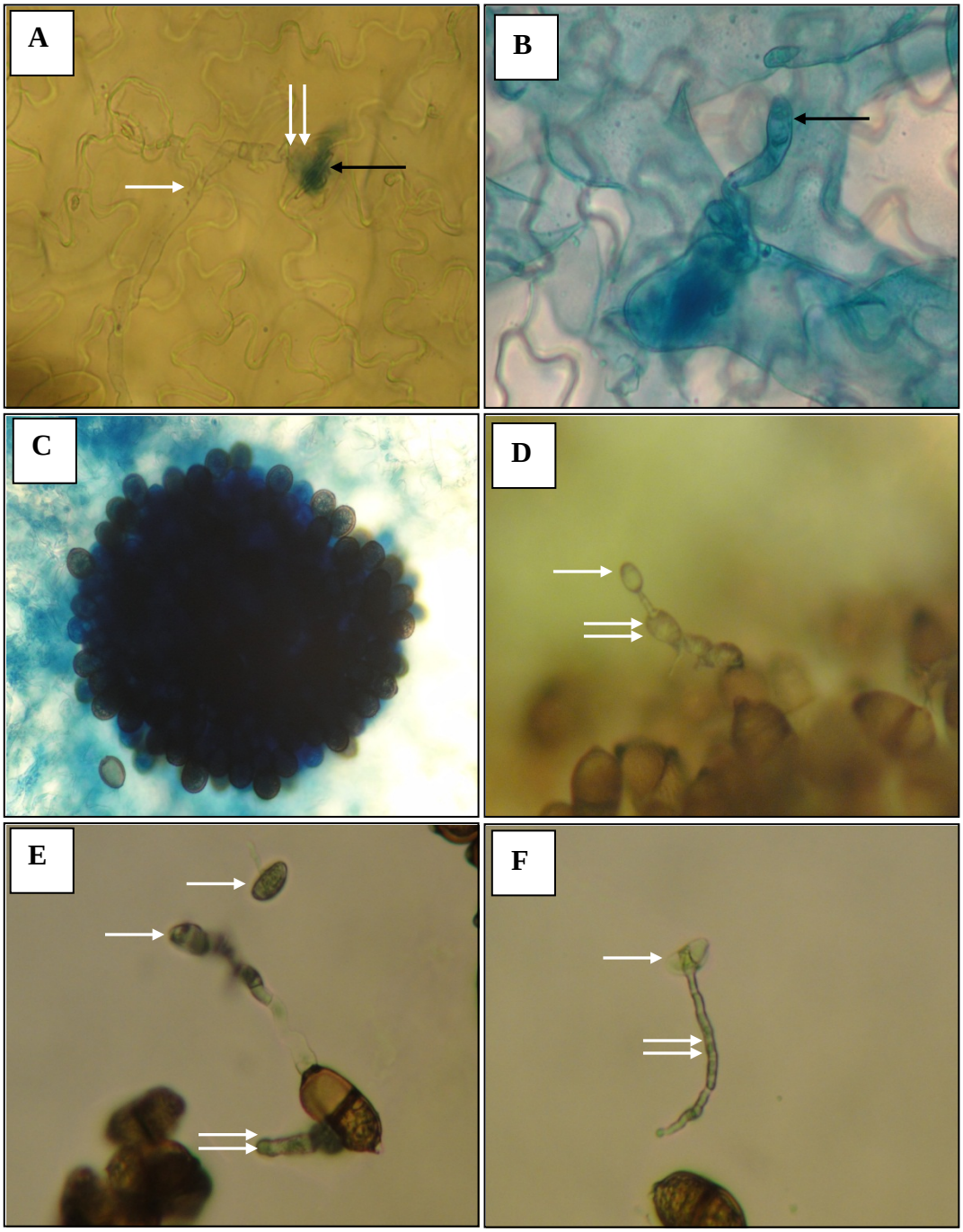
spore germination, penetration and intercellular development (Brusseze and Hasan, 1983) (plate 6.2).

6.12 Further considerations

6.12.1 Impact studies

For any biological control agent to be considered for release, the agent should have an impact on the target species preferably measured under controlled conditions in a series of replicated tests against uninoculated controls. It is a fact that few studies evaluate the impact of a biological control agent pre-release and often the biological control researcher relies on field observations in the plants native range to justify impacts. Indeed, it is often these observations that are used initially to select potential biological control agents for further consideration. In this case, an assessment of a different variety of the rust, which attacks *I. parviflora*, is informative. In mainland Europe, *P. komarovii* can incur high levels of plant mortality within populations of *I. parviflora*, in some cases up to 100% (Bacigálová *et al.*, 1998). Infection of the aecial stem stage has been shown to reduce biomass and fecundity when compared to uninfected plants (Piskorz and Klimko, 2006). These results are consistent with observations of infection on *I. glandulifera* in the Himalayas (Pers. obs., Author).

Plate 6.2 Microscopic symptoms and spore germination of *Puccinia komarovii*



A Appressoria formation over stomata. The single white arrow shows the germ tube of the urediniospores (out of picture) and the formation of the appressoria (double white arrows) over the stomata (black single arrow) (x200)
B Hyphae growth within plant cell (*Impatiens glandulifera*) (black arrow) (x400)
C Pustule formation (x200)
D Teliospore germination with formation of basidium (double white arrow) and basidiospores (single white arrow)
E Germinating teliospore. Teliospore forming initial germ tube (double white arrow) and basidiospores (single white arrow)
F Germinating basidiospore (single white arrow) with germ tube (double white arrow)

Impact studies for the biological control of *I. glandulifera* should be conducted in two stages. Firstly, tests should be conducted, if feasible, on the mortality of plants to stem infection, in controlled replicated experiments against uninoculated controls. Secondly, as the urediniospores stage of *P. komarovii* would infect individual plants in the natural environment repeatedly over a period of time, repeated inoculations are required to mimic the natural infection process in the native range. Such tests should be conducted on young plants with vigour, growth, biomass and fecundity recorded.

6.12.2 Genetic diversity of introduced populations

It is often quoted that levels of genetic variation in introduced species are relatively low but this does not hold true for all introduced species. Jahodova *et al.* (2007) found considerable diversity in populations of *H. mantegazzianum*, which lead them to conclude that there had been multiple introductions of the plant into Europe. A recent study conducted on the genetic variation in *I. glandulifera* populations in the UK, by Walker *et al.* (2008), showed substantial variation in populations of *I. glandulifera* within and between catchments in the UK. A certain degree of genetic variation would be expected in a sexually reproducing annual species, as opposed to a predominantly asexual perennial species like *F. japonica*. Therefore, for *I. glandulifera*, high levels of genetic variation in the UK does not necessarily mean there have been multiple introductions from the native range and introgression may be the real cause. Historic information on the exact localities from where our UK biotypes were collected in the native range as lacking, as is information on whether *I. glandulifera* was collected from numerous locations in the native range with repeated introductions. We cannot assume that populations of *I. glandulifera* in the UK all came from the same region and are thus genetically similar and equally susceptible with the rust pathogen. In order to confirm

compatibility with populations nationwide, field collected seed from different localities and subsequent infection studies are required.

6.12.3 Climatic matching

Relating the life cycle and the climatic parameters, which trigger each spore stage, to that of the climatic conditions in the proposed area of release, is essential to determine the likelihood of the rust pathogen establishing and completing its life cycle in the introduced range. Although the Himalayas and the UK have similar seasons, the limits are considerably different with colder winters and hotter summers in the Himalayas. It is interesting to note, however, that *P. komarovii* persists throughout mainland Europe on *I. parviflora* (Piskorz and Klimko, 2006) and thus this provides an indication to suggest the rust will persist here in the UK on *I. glandulifera*.

6.12.4 Dissemination of knowledge

One of the final hurdles in the biological control of *F. japonica* was a public consultation that consisted of both workshops and presentations, and web based responses. Shaw *et al.* (2011) advocate the early and consistent sharing of information on all aspects of a biological control programme. Providing the public and stakeholders with information and being prepared to answer their questions and concerns, and disseminating information in a variety of formats, both scientific publications and easy to read fact-sheets, can go some way to engaging with the wider community. Fowler *et al.* (2000) highlighted the importance of addressing conflicts of interest in biocontrol and in section 1.17 it was highlighted that not all sectors of the public consider *I. glandulifera* an unwanted addition to the UK. Beekeepers should be engaged with at the earliest opportunity in order to inform them what the potential release of a biological control agent against *I. glandulifera* will and will not achieve. For example, biological

control aims to reduce the occurrence of the target species to a lower threshold where it will no longer be an ecological problem, thus *I. glandulifera* will still be present in the UK for bee populations to exploit the nectar.

6.12.5 Licencing procedures

A clear pathway has now been established to regulate, licence and release an invertebrate biological control agent in the UK (Shaw *et al.*, 2011). However, this process does not apply for micro-organisms. Seier (2005) highlights the only legal document to cover the regulation of fungal biological control agents in the EU was the EU directive 91/414 (1991) which has recently been updated as the EC regulation No. 1107/2009 entitled 'concerning the placing of plant protection products on the market'. Even within the title of the regulation discrepancies appear with regard to regulating and licencing a classical biological control agent, which are neither plant protection 'products' nor are they for placing on the market. Indeed, the release of a classical biological control agent, in general, would not be a commercial enterprise as often a single release is conducted for the benefit of all (Sheppard *et al.*, 2006).

6.12.6 Release and monitoring

Only once the agent has passed all the tests, and host specificity has been confirmed would it be recommended for release, but final authorisation will depend on the risk assessment made by the local responsible organisation. Prior to release, as seen with the biological control of *F. japonica*, the UK government expected a monitoring programme to be in place, which included containment and control, should any non-target or adverse impacts become known. Ideally, caged field tests would follow host specificity testing in quarantine, in order to determine the behaviour of the proposed agent under a more natural setting (Tanner, 2008). Although feasible with larger

arthropod biological control agents, further consideration is needed if regulations require this for microorganisms, more elaborate containment may be planned, though this would indeed come with an increased cost though geographical isolation may suffice. The cost of caged field experiments was one of the reasons why a biological control programme against *Pteridium aquilinum* L. in the UK, was abandoned (McFadyen, 1998).

Classical biological control of non-native invasive plant species come with the benefit that a single release may control and reduce the vigour, occurrence and impact of the invasive population on a geographical scale that would be difficult to achieve with more traditional methods. However, there are risks involved but many of these can be minimised by thorough host specificity testing. Indirect risks associated with the release of a non-native organism are harder to establish in the laboratory and best guesses often have to suffice. On the downside, biological control will not eradicate a weed population (Shaw, 2003) and even a high level of control may take many years to achieve as the population of the agent builds in the new environment (Fowler and Holden, 1994). When successful, a classical biological control programme can save countries £-millions in lost revenue and an unquantifiable figure in terms of ecosystem preservation (Tomley and Evans, 2004). As the more traditional control methods are failing to suppress *I. glandulifera* on a national and local scale, biological control offers an alternative approach, which could reduce the occurrence of *I. glandulifera* to an acceptable level, making it more amenable to traditional control, in an economical and ecologically sound way. Chemicals which were once widely available to control non-native invasive plants, such as Diquat®, have now been withdrawn from sale in many European countries, and the scale of occurrence and rate of spread of some invasive riparian weeds, now present in Europe, demands a catchment scale control approach.

However, this is often unachievable with traditional methods due mainly to the sheer scale of the infestation.

7 General Discussion

Increased trade and travel, land-use changes, and the desire from the public for novel floral additions to gardens and parks, have contributed to non-native invasive plant species becoming a global issue (Pyšek *et al.*, 2010). The impacts of non-native invasive plant species are far reaching and include economic influences (Pimentel *et al.*, 2005; Williamson *et al.*, 2010), effects on infrastructure (Defra, 2010), degradation of natural ecosystems (Vilá *et al.*, 2011), and displacement of species (Topp *et al.*, 2008). Understanding and addressing the impacts of non-native invasive plant species is an essential component to promote habitat restoration, post removal. At present in the UK more focus is given to the control and eradication of some of our more widespread non-native invasive plant species compared to researching the impacts of these invasive weeds on the ecosystems they invade. Indeed, control and the potential eradication of non-native invasive plant species is a desirable objective. However, in order to promote habitat restoration, and establish communities resilient to subsequent plant invasions it is essential to understand the ecosystem influences the invasive plant species has on the invaded habitat. With an understanding of the growth attributes of *I. glandulifera* and how the species invades, and alters the invaded ecosystem, we can then aim to remediate these impacts to promote native species recovery.

In section 1.22 I detail the aims and hypothesis I aim to address in this thesis which relate to the areas of research I consider are currently lacking in the current literature (Table 1.2). In chapter 2, I set out to test the hypothesis that the presence of *I. glandulifera* has a negative impact on the above-ground invertebrate community by displacing functional groups within the invertebrate community structure. From the results detailed in section 2.3.1 it is evident that *I. glandulifera* significantly affects the

invertebrate community by reducing the abundance of taxonomic groups within the invaded stands, compared to the uninvaded stands. The negative impact, expressed as a lower abundance of invertebrates in the invaded stands was higher in the foliage-dwelling invertebrate community compared to the leaf litter invertebrate community. As suggested in section 2.4, this is due to *I. glandulifera* being a poor food source for invertebrate species in the introduced range. The decrease in invertebrate prey species had a knock-on effect for predator species, where spiders were significantly reduced in abundance in the invaded stands. This has implications for the conservation of invertebrate populations when *I. glandulifera* invades a habitat, as invaded stands harbour a reduced abundance of invertebrates. Equally important is the potential detrimental impact on the ecosystem functioning in the invaded sites. The displacement of key invertebrate groups, such as detritivores and predators, may impact on the productivity of the ecosystem and the services the habitat provides which are of human benefit (Dangles *et al.*, 2002; Eisenhauer *et al.*, 2011b; Milcu *et al.*, 2010).

The significant decrease in the percentage cover of *I. glandulifera* in the invaded sites at Harmondsworth Moor during 2008 facilitated the testing of the hypothesis that the occurrence and abundance of invertebrate groups within the community seasonal related to fluctuations in the occurrence of *I. glandulifera*, either negatively or positively. The foliage and leaf litter invertebrate community failed to respond significantly to the reduced percentage cover of *I. glandulifera* in the second year of the study, suggesting that there is a natural lag-phase for the recovery of the invertebrate community (sections 2.3.1 and 2.3.2). This implies that long-term ecosystem management over a number of seasons, is required to restore the invertebrate community once *I. glandulifera* has been removed.

In chapter 3, I tested the hypothesis that *I. glandulifera* has a negative impact on the ground beetle community on exposed riverine sediments (ERS). As *I. glandulifera* was shown to increase the abundance of generalist carabid species in the invaded ERS, even though the diversity of carabid species was similar between invaded, uninvaded and mixed vegetation ERS (section 3.3.2), the hypothesis was rejected. Even though the presence of *I. glandulifera* attracts a higher numbers of generalist ground beetles, this acts to degrade the conservation value of ERS. Those ground beetle species dependent to the ERS habitat for all or part of their life cycle, incur increased stress not only from the presence of *I. glandulifera* reducing the available niches, but also from interspecific competition between generalist ground beetle species moving into the ERS.

In addition, I hypothesised that ERS specialist ground beetle species will be more strongly negatively affected by the presence of *I. glandulifera* than generalist ground beetle species. This hypothesis was accepted, as all ground beetle species significantly associated with ERS invaded with *I. glandulifera* were generalist species. The overall increased abundance of ground beetles, coupled with increased species richness, does not necessarily imply a healthy ecosystem with high conservation value (Christian *et al.*, 2009). Rather, it is the equilibrium, and species composition, within the community that promotes a healthy ecosystem (Díaz *et al.*, 2005). These results suggest that it is important to evaluate the impact of invasive weeds at a species level.

It is now well known that the above and below-ground invertebrate communities are intrinsically linked (van der Putten *et al.*, 2009). When evaluating the impact of *I. glandulifera* at a community level it is essential to contrast the impacts of the invasion to the above- and below-ground invertebrate community. The below-ground invertebrate community was significantly affected by *I. glandulifera*, but only in 2007,

and the response was positive, compared to the uninvaded sites (section 4.3.1). This response was driven by a significant influx of Collembola into the invaded sites ($P < 0.05$). However, invertebrate groups within the soil, appeared to be more buffered by the presence of *I. glandulifera* (section 4.3.1). Therefore, the hypothesis that the presence of *I. glandulifera* has a negative impact on the below-ground invertebrate community by displacing functional groups within the invertebrate community structure was rejected. In fact, none of the invertebrate groups were negatively associated with the invaded stands. Interestingly, Collembola were positively associated with the invaded stands compared to that of the uninvaded stands. However, the increased abundance of Collembola in the below-ground community into invaded sites can have potential impacts on the productivity of the ecosystem. The influx of Collembola into an invaded system can have direct effects on the native plant species within the invaded stands (Masters, 2004).

Hulme and Bremner (2006) highlighted that the removal of *I. glandulifera* may lead to other non-native invasive plant species colonising the managed areas, thereby failing the desired conservation goals. Unfortunately, the authors did not go further by suggesting any underlying reasons for this and therefore did not consider any remedial actions that could be adopted to prevent the recolonisation of non-native plant species into the managed areas. In chapter 4, I set out to test one hypothesis that may suggest why non-native plant species readily colonise previously invaded sites, compared to native plant species, and this is due to most of our non-native plant species being weakly associated with AM fungi. The hypothesis I tested was that *I. glandulifera* will affect the soil AM community and this will be expressed in a decreased percentage colonisation on the roots of plants grown in soil from under invaded stands. In addition, I hypothesised that native plants grown in the invaded soils will have reduced performance, expressed as

plant biomass, when grown in soil from under invaded stands compared to uninvaded vegetation and this will be related to reduced AM colonisation on the roots.

All three plant species tested showed a decrease in the percentage colonisation of AM fungi when grown in soil from under stands of *I. glandulifera* compared to soil from under stands of native vegetation (Figure 4.4). Two of the three plant species tested, namely *Plantago lanceolata* and *Lotus corniculatus*, showed an increased above-ground biomass, which was related to the percentage colonisation of AM fungi when grown in soil from under native stands compared to plants grown in soil from under stands of *I. glandulifera* (see figure 4.6). In addition, both plant species had decreased performance (expressed as above-ground biomass) when grown in soil from an invaded area. The percentage colonisation of AM fungi and plant biomass were positively correlated (Figure 4.6). This suggests that those native plant species that are dependent on AM for their performance can be significantly affected by depleted levels of AM fungi because of *I. glandulifera* colonisation. This has implications for the management of *I. glandulifera* and the restoration of the community post-removal. Those native species, which are dependent on AM for their colonisation, may require an AM inoculum added to the soil to aid their establishment (Allen *et al.*, 2005; Thiet and Boerner, 2007). When considering restoration of an invaded site it is also worth considering the planting of more aggressive native AM dependent plant species which may act to replenish the AM community faster than less aggressive plant species.

Whether there is a 'tipping point' to the invasion by *I. glandulifera*, where the impacts manifest as the invasion progresses over time, requires further research on a longer time scale, preferably studied from the time of the initial invasion (Dogra *et al.*, 2010; Pawson *et al.*, 2010). This is important from a management perspective, not only for *I.*

glandulifera, but also for other non-native plant species in the UK, as in the fact that often management is only applied to those populations where the infestation is visibly high. However, if an intermediate occurrence of *I. glandulifera* has negative impacts on the invertebrate community, and/or, if the transmission of an intermediate population to a monocultures occurs over a short timescale from a conservation stand-point, management should be timed to control intermediate populations. The studies into the impact of *I. glandulifera* on invertebrate populations, both above and below ground would also benefit from studying the plant over a longer period to evaluate the effect of natural vegetation fluctuations and their effect on invertebrate populations (see sections 2.4 and 4.4).

Understanding the traits and processes which can lead to a plant species becoming an invasive species has been a hot topic for debate over many years, where some consider that this area of ecology tells us very little about which species will become invasive (Thomson and Davis, 2011). However, I would suggest that traits are an important component of understanding why some non-native invasive plant species become so successful, though traits should not be considered in isolation. It is clear that part of the success of *I. glandulifera* in its introduced range is due to the prolific seed production and the habitats where the plant grows which have facilitated the spread of the species throughout the UK. However, this alone does not explain the success of the species.

In chapter 5, I set out to test the hypothesis that when released from the pressure of host specific natural enemies, *I. glandulifera* populations in the introduced range will have increased growth (both height and total leaf area) compared to those in the native range. The study showed that in general, *I. glandulifera* was smaller in height, and had a lower total leaf surface area in the native range compared to the introduced range, and the

percentage damage by natural enemies was significantly higher in the native range compared to the introduced range. However, I do not consider that the lack of natural enemies is the only explanation to why *I. glandulifera* is more successful in the introduced range compared to the native range. It was interesting to see that in the introduced range, there was a negative relationship when comparing height, total leaf area, and above-ground biomass to AM colonisation. Whereas in the native range there was no relationship, suggesting that plants in the introduced range are associated with possibly the wrong type/species of AM fungi.

Therefore, invasion ecology can benefit from studying plants in their native and introduced ranges (Ebeling *et al.*, 2008; Erfmeier and Bruelheide, 2004; Widmer *et al.*, 2007). Plant species, which are highly regulated in their native range, and display tendencies to become weedy despite this, should be carefully considered before being introduced into new geographic region, where regulation may potentially be significantly lower. Whether this is feasibly realistic due to the scale of such studies, and the cost involved remains to be seen.

Davis *et al.* (2011) suggest there is a bias, in both the scientific community and now the public, against non-native species where often the threats and impacts cited for intense control and management are unjustified. The authors go on to suggest that we should worry far less about the changing flora of many regions as little can now be accomplished to restore the habitat to its former state (Vince, 2011). However, I do not share this point of view. With sensitive management and habitat restoration, it is possible to at least reduce the monocultures of *I. glandulifera* which are inundating our river systems throughout the UK (Tanner, 2008). Indeed, if our attitude remains focused towards control and eradication of *I. glandulifera*, using traditional, expensive

methods, repeated year on year, we are fighting a losing battle. As environmentalists, ecologists, botanists, and biological control scientists we surely need to combine our disciplines into more novel approaches where we understand the impacts of the invasion and the species.

Understandably, at a government and policy level, resource allocation for the control and management of non-native invasive species has been led by economics - the costs of a particular species to the economy. The annual predicted cost of *F. japonica* in Britain, being just over £165-million, justifies the investment into research and control costs (Williamson *et al.*, 2010). The annual cost of *I. glandulifera* is estimated to be in the region of £1-million to the British economy, though as discussed in section 1.15, this figure is likely to be underestimated (Williamson *et al.*, 2010). Putting a cost on the extinction of a single carabid species, due to invasion of *I. glandulifera* on ERS, or understanding a unit cost of increased abundance of Collembola into an invaded field, is somewhat difficult when considering the impact in isolation.

It is encouraging that the UK is leading the way in Europe when it comes to providing funds for new management and control techniques. Indeed, although classical biological control is certainly not a novel tool for the control of non-native invasive plant species globally, it still is in Europe. Past failures, sometimes on a spectacular scale, have led to scepticism of the adoption of biological control as a management practice for the control of invasive species (Tanner and Shaw, 2008). As discussed in section 6.12.4, and in Shaw *et al.* (2011), educating the public, interested stakeholders, and funding bodies, using a variety of information platforms, can go some way to updating the community on the advances, and the scientific protocol, in the field of biological control.

The research into using *Puccinia komarovii* as a potential biological control agent is progressing and early indications suggest the rust is highly host specific to *I. glandulifera*. Therefore, the hypothesis that some of the natural enemies found in the plant's native range can be utilised as classical biocontrol agents in the introduced range is supported. The implementation of classical biological control programmes, as a tool to combat non-native invasive plants, has been shown to provide significant results, which provide benefits to agriculture and native biodiversity (Van Driesche *et al.*, 2010). Although a biological control programme requires significant funds during the research and monitoring phase of the programme (Shaw, 2003; Shaw *et al.*, 2011), the benefits have been shown to far outweigh the costs (van Wilgen *et al.*, 2004). In Australia, the cost benefit ratio of a biological control programme against Patterson's curse (*Echium* species) has been predicted to increase over time, where at the beginning of the release phase the cost: benefit ratio is estimated to be 14:1, rising to 47:1 35 years after release (Nordblum *et al.*, 2001). As classical biological control is in its infancy in Europe current cost: benefit data is absent. However, with a successful European biological control programme we should see similar returns on the investment, over time, to that of other countries.

As discussed in section 6.12.6, classical biological control does not aim to eradicate the host, but instead aims to reduce the invasive population to a threshold where it has a lower ecological impact compared to that before the biological control agent has been applied (McFadyen, 1998). Biological control has its drawbacks, as it is not a quick fix solution (Shaw, 2003). Any impacts on the target weed may take time as the classical biological control agent establishes, adapts to its new environment, and builds up the population and disperses. However, biological control may act to facilitate the natural regeneration of native plant species as they may be able to compete better with the

weakened host, due to being re-associated with their co-evolved host specific natural enemies.

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Appendix 1

Plant species identified at Harmondsworth Moor

Site type	uninvaded		invaded		uninvaded		invaded		uninvaded		invaded		uninvaded		invaded	
Site number	1		1		2		2		3		3		4		4	
Year	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
Species																
Agrostis stolonifera L.	16.8	0.5			1.5	0.5			2.2	0.7			2.2	10.3	1.8	
Angelica sylvestris L.												4.5				
*Apium nodiflorum*L.				4.7												
Caltha palustris L.		3.0		3.7										5.2		5.8
Calystegia sepium (L.) R.Br.			1.5	14.0		10.2		3.5	4.2		1.2	12.7				0.5
Cerastium arvense L.		0.3		2.7	2.7	2.5			0.7		0.7	0.7		7.5		1.5
Cerastium fontanum Baumg.					1.0							1.0	5.0	2.5	4.3	
Chamerion angustifolium L.					0.5									0.3		
Daucus carota L.	1.2				3.8		0.7									
Epilobium hirsutum L.		0.3				7.5						15.2				
Filipendula ulmaria (L.) Maxim.	1.2	12.0														
Galium aparine L.			1.2	12.5		13.2	3.7	24.2	6.5	1.0	1.3	25.8		13.3	1.8	11.7
Geranium dissectum L.						1.7								1.3		
Holcus lanatus L.	16.3	1.7			3.2	4.5	2.2		5.8	2.5		0.3	9.7	7.8		2.3
Holcus mollis L.		40.2		2.5	1.2	12.7	9.2		5.2		16.8		21.7	15.0	0.3	3.7
Hypericum perforatum L.	0.8												0.3			
Impatiens capensis Meerb.					0.5			0.8		0.5	1.0	2.3				
Impatiens glandulifera Royle			99.7	33.3		3.8	84.7	24.7	7.3	1.2	68.3	18.5	0.5		85.8	31.7
Juncus inflexus L.	39.3	16.2			57.2	13.5		1.0						0.8		0.7
Lamium album L.														0.8		
Lotus corniculatus L.	2.0				0.8								0.2			
Luzula pilosa (L.) Wild.	11.5				7.5	4.2										
Lythrum salicaria L.	0.5	1.3			0.5									0.5		
Malva sylvestris L.						1.5								3.3		

Site type	uninvaded		invaded		uninvaded		invaded		uninvaded		invaded		uninvaded		invaded	
Site number	1		1		2		2		3		3		4		4	
Year	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
Species																
Persicaria hydropiper (L.) Delabre		0.8				0.8							0.3	0.3		
Persicaria amphibia (L.) A.Gray	0.8	2.0			5.7	1.0	1.2						2.2	0.7		
Persicaria maculosa L.				0.3	1.0							1.8		0.2		
Phalaris arundinacea L.	18.5	12.5		21.0	11.8			29.2	12.3	33.3		5.3	12.5			
Plantago lanceolata L.													1.7	1.2		
Plantago major L.													0.3	1.7		
Poa annua L.		4.3				0.5			14.2	1.5			16.5	6.7	1.5	0.2
Potentilla erecta L.	0.3												0.2			
Ranunculus acris L.						0.5							0.3	1.3		
Ranunculus repens L.	0.5	9.7			0.5	2.3					0.7					
Rorippa amphibia (L.) Bess.													6.7	0.8	6.3	29.2
Rubus fruiticosus L.	0.5			1.3	2.8		2.3		24.5	46.3	6.8	2.5		0.2		
Rumex crispus L.		4.0				0.8						0.7	0.8	1.7		0.8
Rumex obtusifolius L.				2.7	2.5	0.7								0.5		
Rumex acetosella L.	0.2				0.3								0.3	0.3		0.3
Rumex conglomeratus Murray					0.3								1.0	0.8		
Salix alba L.	0.8										10.2	2.5				0.8
Trifolium dubium Sibth.													0.5	0.2		
Trifolium pratense L.	1.2				1.3								0.3	0.5		
Urtica dioica L.			0.5	9.5		26.8	2.7	28.5	30.3	15.5	0.7	18.3	26.8	25.0	4.7	17.5

Site type	uninvaded		invaded		uninvaded		invaded		uninvaded		invaded	
Site number	5		5		6		6		7		7	
Year	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
Species												
Agrostis stolonifera L.								2.3	0.7	1.0		
Angelica sylvestris L.								1.3				
*Apium nodiflorum*L.												
Caltha palustris L.										3.0		
Calystegia sepium (L.) R.Br.	0.2	1.8	0.7		2.3		0.8		0.8	6.0	3.3	2.5
Cerastium arvense L.						1.0	0.5					
Cerastium fontanum Baumg.												
Chamerion angustifolium L.												
Daucus carota L.												
Epilobium hirsutum L.								15.8				
Filipendula ulmaria (L.) Maxim.												
Galium aparine L.	2.2	2.5	4.7	11.8	8.3	6.7	5.0	2.7	26.7	14.5	3.2	7.3
Geranium dissectum L.												
Holcus lanatus L.			2.2						0.5	1.2		
Holcus mollis L.		6.2	4.2	26.0				9.3		1.2	2.2	
Hypericum perforatum L.												
Impatiens capensis Meerb.			0.5	0.7					1.7			
Impatiens glandulifera Royle		0.3	80.7	37.8			76.2	35.0	5.7	0.5	84.2	25.5
Juncus inflexus L.												
Lamium album L.												
Lotus corniculatus L.												
Luzula pilosa (L.) Wild.												
Lythrum salicaria L.							0.8					
Malva sylvestris L.												

Site type	uninvaded		invaded		uninvaded		invaded		uninvaded		invaded	
Site number	5		5		6		6		7		7	
Year	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
Species												
Persicaria hydropiper (L.) Delabre									1.3	1.7		
Persicaria amphibia (L.) A.Gray												
Persicaria maculosa L.								0.7				
Phalaris arundinacea L.	1.8	2.5	1.0				11.5	1.3	8.2	2.0	4.0	59.5
Plantago lanceolata L.												
Plantago major L.												
Poa annua L.								2.2		6.8		
Potentilla erecta L.				3.2								
Ranunculus acris L.			0.2									
Ranunculus repens L.			0.7	5.8				0.5		2.0	0.3	0.5
Rorippa amphibia (L.) Bess.									2.5			
Rubus fruiticosus L.			1.3		3.2	12.3	4.0	6.3	10.2	24.2		
Rumex crispus L.												
Rumex obtusifolius L.				4.2			2.7		1.2	3.8		
Rumex acetosella L.				0.5								
Rumex conglomeratus Murray												
Salix alba L.												
Trifolium dubium Sibth.												
Trifolium pratense L.												
Urtica dioica L.	98.7	92.7	6.8	13.7	92.5	90.7	7.2	34.7	39.8	37.3	8.3	12.8

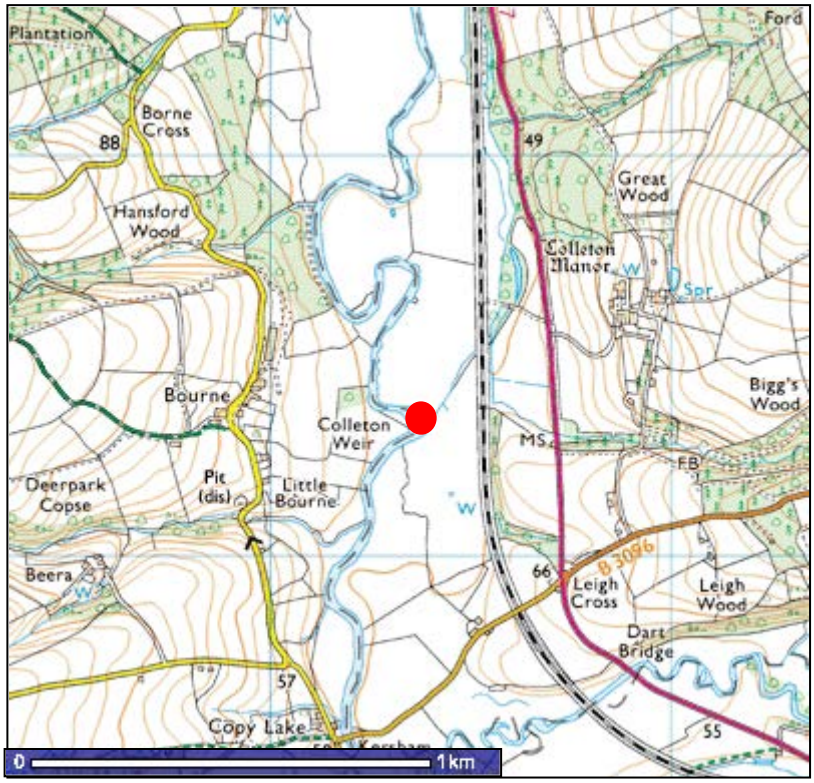
Site type	uninvaded		invaded		uninvaded		invaded	
Site number	8		8		9		9	
Year	2007	2008	2007	2008	2007	2008	2007	2008
Species								
Agrostis stolonifera L.								
Angelica sylvestris L.								
*Apium nodiflorum*L.								
Caltha palustris L.								0.8
Calystegia sepium (L.) R.Br.	1.2		1.0	2.5	2.3	7.5	2.3	5.0
Cerastium arvense L.	1.0				1.3		0.3	0.5
Cerastium fontanum Baumg.								
Chamerion angustifolium L.								
Daucus carota L.								
Epilobium hirsutum L.				2.8				
Filipendula ulmaria (L.) Maxim.								
Galium aparine L.	13.2	4.2	7.3	27.5	7.3	32.5	4.5	11.7
Geranium dissectum L.								
Holcus lanatus L.	0.5	0.8					0.5	0.7
Holcus mollis L.	1.2	0.8					1.3	1.0
Hypericum perforatum L.								
Impatiens capensis Meerb.								
Impatiens glandulifera Royle	1.2	81.7	95.0	33.3	3.2	1.2	84.3	37.2
Juncus inflexus L.							1.7	0.3
Lamium album L.								
Lotus corniculatus L.								
Luzula pilosa (L.) Wild.								
Lythrum salicaria L.								
Malva sylvestris L.								

Site type	uninvaded		invaded		uninvaded		invaded	
Site number	8		8		9		9	
Year	2007	2008	2007	2008	2007	2008	2007	2008
Species								
Persicaria hydropiper (L.) Delabre			0.5					
Persicaria amphibia (L.) A.Gray		0.3						
Persicaria maculosa L.					0.8			
Phalaris arundinacea L.								0.3
Plantago lanceolata L.								
Plantago major L.								
Poa annua L.	0.2							
Potentilla erecta L.								
Ranunculus acris L.			0.3					
Ranunculus repens L.								1.7
Rorippa amphibia (L.) Bess.								
Rubus fruiticosus L.	2.7	0.8		7.5	0.8	3.3		
Rumex crispus L.	0.7						0.3	
Rumex obtusifolius L.		4.2			0.5			
Rumex acetosella L.								
Rumex conglomeratus Murray								
Salix alba L.		5.3					2.0	1.7
Trifolium dubium Sibth.								
Trifolium pratense L.								
Urtica dioica L.	87.5	5.8	4.8	14.2	87.3	61.7	14.8	27.7

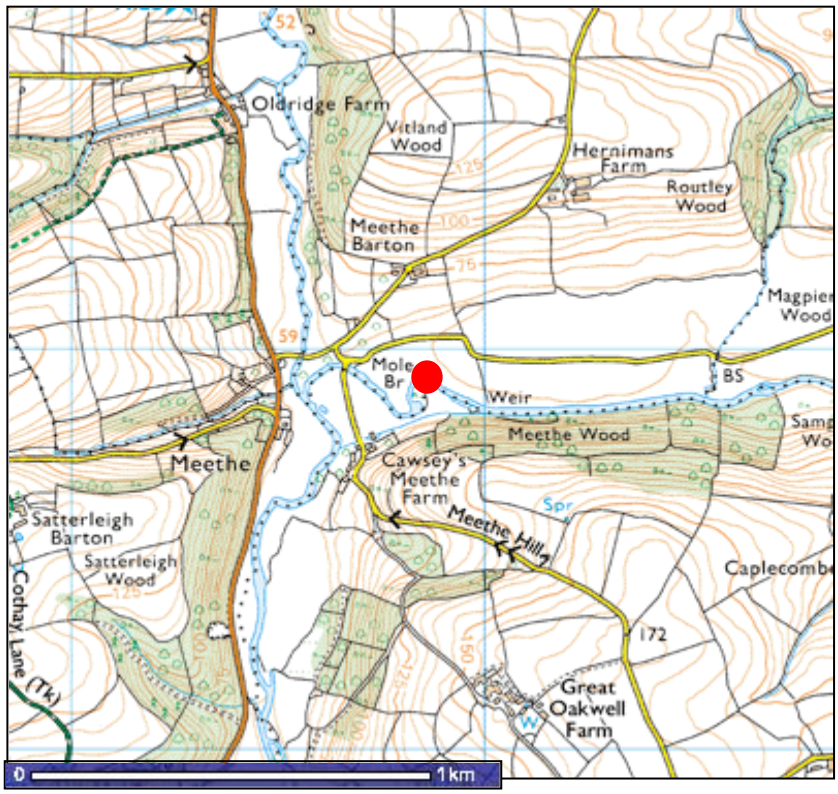
Appendix 2

Photographs and local maps of the nine exposed riverine sediments

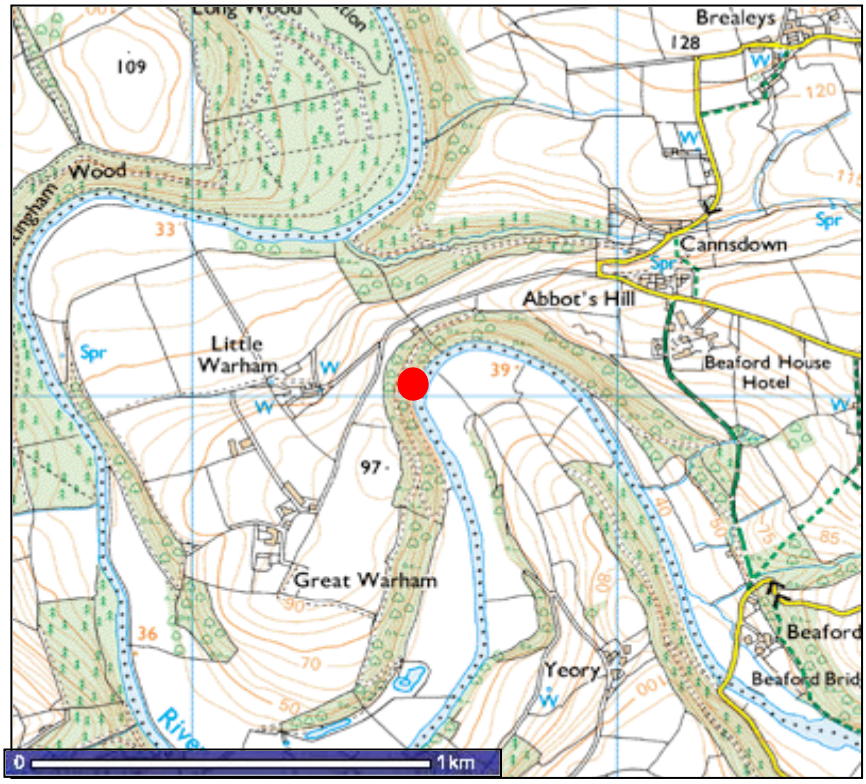
(Note: red dot on map indicates the location of the exposed riverine sediment)



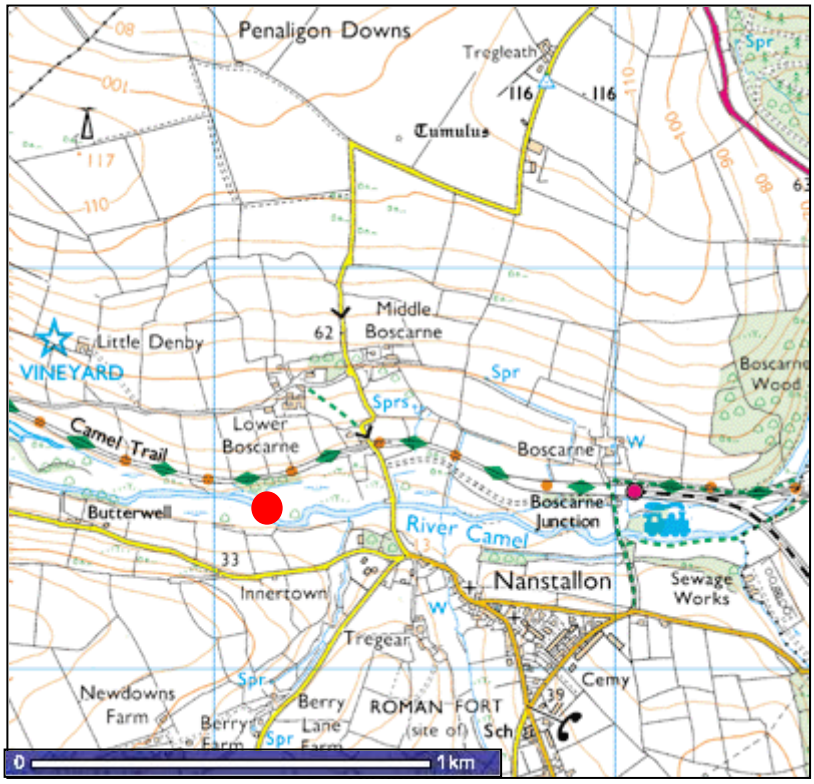
ERS 1: Mid bar (Mixed vegetation) Colleton Weir, North Devon, UK.



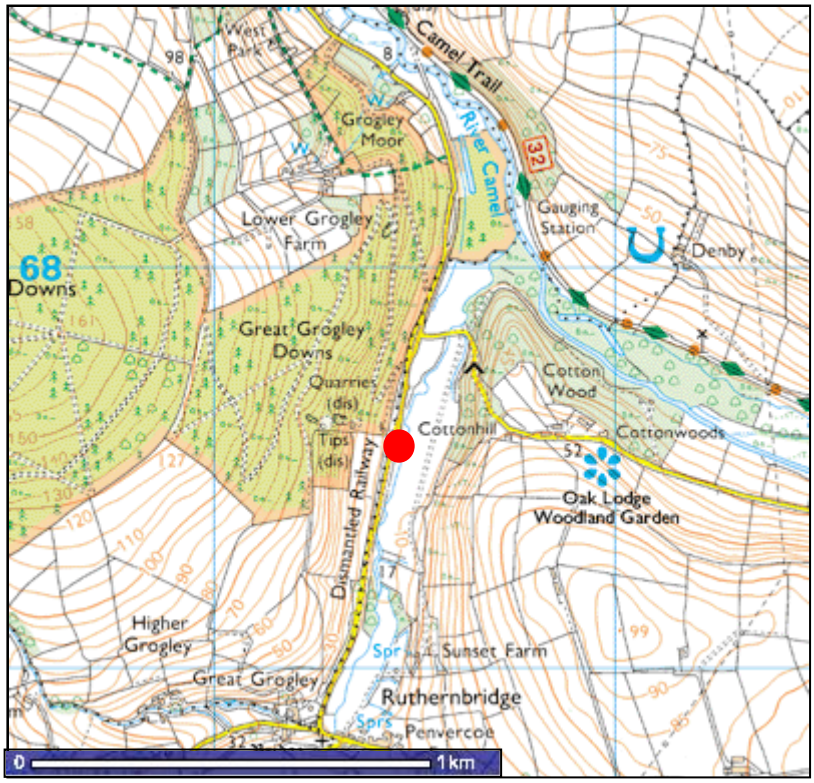
ERS 2: Side bar (Invaded) Meethe Bridge, North Devon, UK.



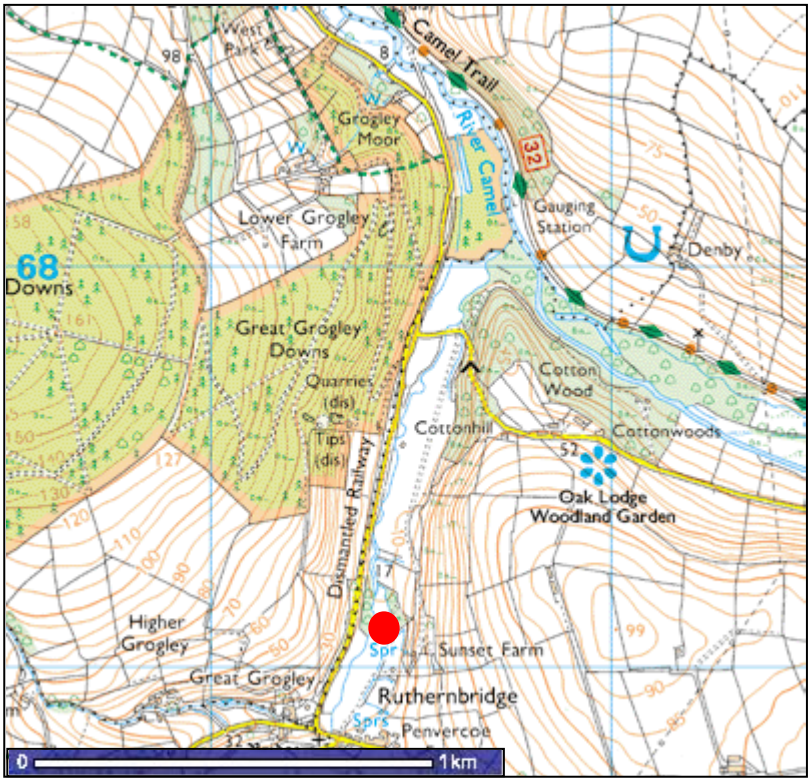
ERS 3: Side bar (Uninvaded) River Torridge, near Torrington, Devon, UK.



ERS 4: Side bar (Mixed vegetation) Boscarne, Cornwall, UK.



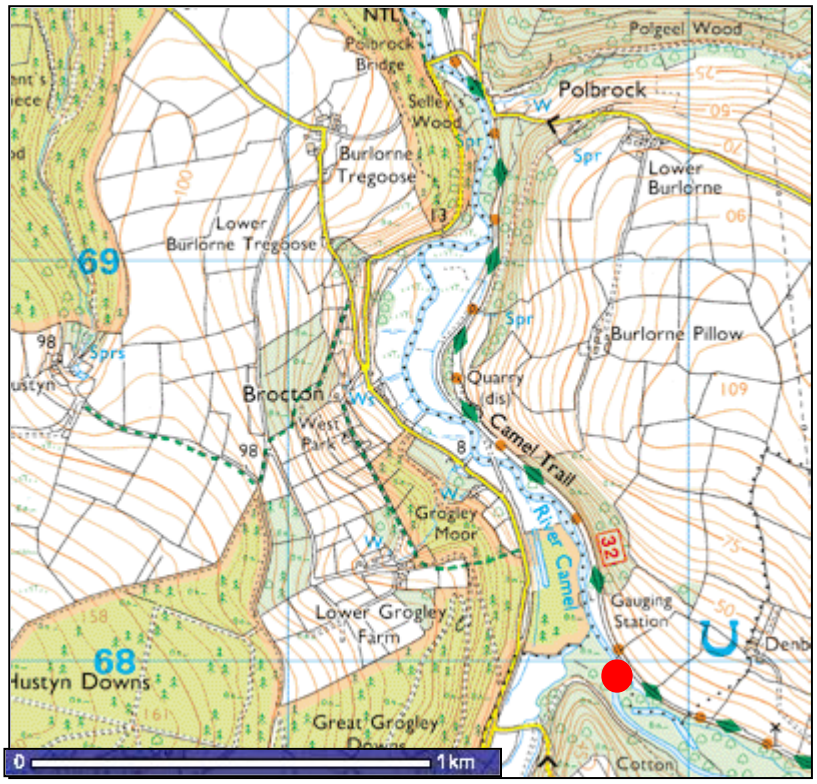
ERS 5: Side bar (Uninvaded) Near Ruthern Bridge, Cornwall, UK.



ERS 6: Side bar (Uninvaded) Near Ruthern Bridge, Cornwall, UK.

ERS 7: Side bar (Invaded) Grogley Moor, Cornwall, UK.

ERS 8: Mid bar (Mixed vegetation) Grogley Moor, Cornwall, UK.



ERS 9: Side bar (Invaded) Grogley Moor, Cornwall, UK.

Appendix 3

Plant species identified on the exposed riverine sediment sites

Site type	Mixed	Invaded	Uninvaded	Mixed	Uninvaded	Uninvaded	Invaded	Mixed	Invaded
Site label	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Species									
Ajuga reptans L.			0.3		0.8				
Allium ursinum L.	1.5				3.8				
Anthoxanthum odoratum L.	6.7				1.2				
Anthriscus sylvestris Hoffm.					21.7			45.0	
Arum maculatum L.				0.3					
Buddleja davidii Franch.	2.0								
Calystegia sepium (L.) R.Br.	1.7	1.7	0.8	0.3					
Cardamine flexuosa With.	0.7		0.7	1.3		1.2			8.3
Carex echinata Murray					1.7	0.3			
Carex remorta L.					5.8				
Carex pendula Huds.					1.7				
Carex viridula Michaux.	2.5								
Centaurea scabiosa L.					1.3				
Cerastium fontanum Baumg.			1.0						
Cerastium glomeratum Thuill.			0.8			0.8			
Equisetum arvense L.	3.3								
Festuca rubra L.	0.8				1.7				
Galium aparine L.	0.8	7.8		10.7	4.2	3.3	0.5		20.0
Heracleum sphondylium L.	1.7		1.7	0.8	0.3				
Holcus lanatus L.	6.7		0.5						
Impatiens glandulifera Royle	25.0	50.8	0.2	32.5			93.3	44.2	50.0
Juncus effusus L.									
Montia sibirica L.			1.8	1.3	4.3	16.7		1.5	

Site type	Mixed	Invaded	Uninvaded	Mixed	Uninvaded	Uninvaded	Invaded	Mixed	Invaded
Site label	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Species									
Oenanthe crocata L.				0.3		4.2			
Percicaria hydropiper L.		3.3	4.0		0.8		0.2		1.7
Percicaria lapthafolium L.	2.5	5.8							
Plantago major L.					0.8	0.3			
Poa trivialis L.	9.3			9.2					
Primula veris L.	0.7				5.0	0.5			
Ranunculus ficaria L.				0.3	1.7	1.7			
Ranunculus repens L.				5.0	0.8	0.8			
Rubus fruticosus L.		0.7	1.3	0.8	3.3	0.8			
Rumex obtusifolius L.	7.5	0.5			5.0			2.0	
Rumex sanguineus L.			0.8						
Rumex acetosa L.		1.3	2.7	1.7					
Rumex crispus L.	0.8	2.5	1.0	2.5	1.5	5.3			
Salix alba L.	16.7								
Silene dioica (L.) Clairv.	0.7		1.2	0.3	8.3	1.7		2.5	
Stellaria holostea L.	2.5				0.8				
Urtica dioica L.	2.3	3.3	2.2	12.5		11.7	0.4	0.8	18.3
Veronica hederifolia L.				4.5	0.7	0.8			
Veronica montana L.					0.8	7.5			

Appendix 4

The proposed test plant list for the biological control of *Impatiens glandulifera* Royle

Where status: I- Introduced species to the UK; N- Native species to the UK O- Ornamental species to the UK; E- Economically important species in the UK

Order	Family	Genus	species	Status
Ericales	Balsaminaceae	*Impatiens*	*glandulifera*	I
Ericales	Balsaminaceae	*Impatiens*	*namchabarwensis*	O
Ericales	Balsaminaceae	*Impatiens*	*balfourii*	O
Ericales	Balsaminaceae	*Impatiens*	*balsamina*	O
Ericales	Balsaminaceae	*Impatiens*	*capensis*	I
Ericales	Balsaminaceae	*Impatiens*	*walleriana*	O
Ericales	Balsaminaceae	*Impatiens*	*hawkeri*	O
Ericales	Balsaminaceae	*Impatiens*	*auricoma*	O
Ericales	Balsaminaceae	*Impatiens*	*noli-tangere*	N
Ericales	Balsaminaceae	*Impatiens*	*textori*	O
Ericales	Balsaminaceae	*Impatiens*	*parviflora*	I
Ericales	Balsaminaceae	*Impatiens*	*sodenii*	O
Ericales	Balsaminaceae	*Impatiens*	*arguta*	O
Ericales	Balsaminaceae	*Impatiens*	*omeiana*	O
Ericales	Balsaminaceae	*Impatiens*	*rothii*	O
Ericales	Balsaminaceae	*Impatiens*	*repens*	O
Ericales	Balsaminaceae	*Impatiens*	*kilimanjaro* x *pseudoviola*	O
Ericales	Balsaminaceae	*Impatiens*	*kerriae*	O
Ericales	Balsaminaceae	*Impatiens*	*stenantha*	O
Ericales	Balsaminaceae	*Impatiens*	*puberula*	O
Ericales	Balsaminaceae	*Impatiens*	*uniflora*	O
Ericales	Balsaminaceae	*Impatiens*	*niamniamensis*	O
Ericales	Balsaminaceae	*Impatiens*	*apiculata*	O
Ericales	Balsaminaceae	*Impatiens*	*flanaganae*	O
Ericales	Balsaminaceae	*Impatiens*	*tinctoria*	O
Ericales	Balsaminaceae	*Impatiens*	*keilii*	O
Ericales	Balsaminaceae	*Impatiens*	*gomphophylla*	O
Ericales	Balsaminaceae	*Impatiens*	*parasitica*	O
Ericales	Balsaminaceae	*Impatiens*	*scabrida*	O
Ericales	Balsaminaceae	*Impatiens*	*pseudoviola*	O

Order	Family	Genus	Species	Status
Ericales	Balsaminaceae	*Impatiens*	*tuberosa*	O
Ericales	Balsaminaceae	*Impatiens*	*platypetala*	O
Ericales	Balsaminaceae	*Impatiens*	*sutherlandii*	O
Ericales	Balsaminaceae	*Impatiens*	*langbianensis*	O
Ericales	Polemoniaceae	*Polemonium*	*caeruleum*	N
Ericales	Polemoniaceae	*Phlox*	*divaricata*	O
Ericales	Primulaceae	*Primula*	*veris*	N
Ericales	Primulaceae	*Primula*	*vulgaris*	N
Ericales	Primulaceae	*Anagallis*	*arvensis*	N
Ericales	Myrsinaceae	*Ardisia*	*japonica*	O
Ericales	Theaceae	*Camellia*	*japonica* var. Tama no ura	O
Ericales	Symplocaceae	*Symplocos*	*sawafutagi*	O
Ericales	Diapensiaceae	*Shortia*	*uniflora*	O
Ericales	Actinidiaceae	*Actinidia*	*kolomikta*	O
Ericales	Actinidiaceae	*Actinidia*	*deliciosa*	E
Ericales	Clethraceae	*Clethra*	*alnifolia*	O
Ericales	Cyrillaceae	*Cyrilla*	*parvifolia*	O
Ericales	Ericaceae	*Erica*	*ciliaris*	N
Ericales	Ericaceae	*Calluna*	*vulgaris*	N
Ericales	Ericaceae	*Andromeda*	*polifolia*	N
Ericales	Ericaceae	*Vaccinium*	*oxycoccos*	N
Ericales	Ericaceae	*Vaccinium*	*myrtillus*	N
Ericales	Ericaceae	*Rhododendron*	var. Percy Wiseman	O
Ericales	Ericaceae	*Vaccinium*	*corymbosum* var. Bluetta	E
Ericales	Ericaceae	*Vaccinium*	*macrocarpon* var. Howes	E
Ericales	Theaceae	*Camellia*	*sinensis*	E
Genaniales	Geraniaceae	*Geranium*	*robertianum*	N
Rosales	Urticaceae	*Urtica*	*dioica*	N
Rubiales	Rubiaceae	*Galium*	*aparine*	N
Rosales	Rosaceae	*Rubus*	*fruticosus*	N